Plant Physiology and Biochemistry 211 (2024) 108666

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Peculiarity of the early metabolomic response in tomato after urea, ammonium or nitrate supply

Arianna Lodovici [a], Sara Buoso [a], Begoña Miras-Moreno [b,1], Luigi Lucini [b], Pascual Garcia-Perez [b], Nicola Tomasi [a,*], Roberto Pinton [a], Laura Zanin [a]

[a] Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Via delle Scienze 206 - 33100, Udine, Italy
[b] Department for Sustainable Food Process, Research Centre for Nutrigenomics and Proteomics, Università Cattolica del Sacro Cuore, Piacenza, Italy

ARTICLE INFO

Handling Editor: Dr K Kees Venema

Keywords:
Fertilizer
Gene expression
Metabolic pathway
Multiomic
Nitrogen form
Nitrogen assimilation
Root uptake

ABSTRACT

Nitrogen (N) is the nutrient most applied in agriculture as fertilizer (as nitrate, Nit; ammonium, A; and/or urea, U, forms) and its availability strongly constrains the crop growth and yield. To investigate the early response (24 h) of N-deficient tomato plants to these three N forms, a physiological and molecular study was performed.

In comparison to N-deficient plants, significant changes in the transcriptional, metabolomic and ionomic profiles were observed. As a probable consequence of N mobility in plants, a wide metabolic modulation occurred in old leaves rather than in young leaves. The metabolic profile of U and A-treated plants was more similar than Nit-treated plant profile, which in turn presented the lowest metabolic modulation with respect to N-deficient condition. Urea and A forms induced some changes at the biosynthesis of secondary metabolites, amino acids and phytohormones. Interestingly, a specific up-regulation by U and down-regulation by A of carbon synthesis occurred in roots. Along with the gene expression, data suggest that the specific N form influences the activation of metabolic pathways for its assimilation (cytosolic GS/AS and/or plastidial GS/GOGAT cycle). Urea induced an up-concentration of Cu and Mn in leaves and Zn in whole plant.

This study highlights a metabolic reprogramming depending on the N form applied, and it also provide evidence of a direct relationship between urea nutrition and Zn concentration. The understanding of the metabolic pathways activated by the different N forms represents a milestone in improving the efficiency of urea fertilization in crops.

1. Introduction

Nitrogen (N) is one of the most required macronutrients by crops and its availability is the major limiting factor for plant growth and productivity (Marschner, 2012; Vidal et al., 2020). To sustain the current rate of crop production, roughly 110 million tons of N fertilizers are used globally (FAOSTAT, 2021). Due to the low adsorption of N in the soil, only a fraction of N applied by fertilization is taken up by plants. Indeed, it has been estimated that only 25–50% of N applied in agriculture is used by crops, whereas the remaining fraction contributes to environmental pollution through greenhouse gas emission like nitrous oxide and ammonia volatilization, as well as eutrophication of groundwater by nitrate leaching (Raun and Johnson, 1999; Gutiérrez, 2012; Omara et al., 2019). Therefore, the overuse of N fertilizers can negatively impact the environment, in addition to represent a cost for crop production.

In order to improve the sustainability of agronomical practices, a reduction of the chemical inputs for crop production is needed (United Nations, 2015 http://sustainabledevelopment.un.org). This goal could be achieved by increasing the N-use efficiency in crops. Therefore, in the last years, the study of the mechanisms behind using different N forms by plants has increased relevance (Zhang et al., 2015; Zanin et al., 2015a; Vidal et al., 2020; Buoso et al., 2021a).

Plants prefer to sustain their N needs through the acquisition of inorganic N sources (over the organic ones) naturally present in soils or conferred as fertilizer (Marschner, 2012; Farzadfar et al., 2021). In agricultural soils, the N sources preferentially taken up by plant roots are nitrate (NO_3^-) and ammonium (NH_4^+), two inorganic forms (Lauter, 1996; Wang and Macko, 2011; Kiba and Krapp, 2016). However, other N

* Corresponding author.
E-mail addresses: arianna.lodovici@uniud.it (A. Lodovici), sara.buoso@uniud.it (S. Buoso), mariabegona.miras@um.es (B. Miras-Moreno), luigi.lucini@unicatt.it (L. Lucini), pasgarcia@uvigo.gal (P. Garcia-Perez), nicola.tomasi@uniud.it (N. Tomasi), roberto.pinton@uniud.it (R. Pinton), laura.zanin@uniud.it (L. Zanin).
[1] Present address: University of Murcia, Spain.

https://doi.org/10.1016/j.plaphy.2024.108666
Received 16 February 2024; Received in revised form 18 April 2024; Accepted 24 April 2024
Available online 25 April 2024

Abbreviations

A	(ammonium treatment)
ABA	(abscisic acid or its relative compounds)
AMTs	(ammonium transporters)
AS	(asparagine synthetase)
Asn	(asparagine)
BRs	(brassinostreoids or their relative compounds)
Ca	(calcium)
CKs	(cytokinin's or their relative compounds
Cu	(copper)
Cys	(cysteine)
DUR3	(urea transporter)
EF1	(elongation factor 1)
Fe	(iron)
Gln	(glutamine)
Glu	(glutamate)
GOGAT	(glutamine oxoglutarate aminotransferase or glutamate synthetase)
GS	(glutamine synthetase)
Glu-SA	(glutamate 5-semialdehyde)
HATS	(high affinity nitrate transporter)
K	(potassium)
LATS	(low affinity nitrate transporter)
Met	(methionine)
Mg	(magnesium)
Mn	(manganese)
N	(nitrogen)
Nit	(nitrate treatment)
Na	(sodium)
NAR2.1	(nitrate transporter accessory protein)
Nii or NiR	(nitrite reductase)
NR	(nitrate reductase)
NRTs	(nitrate transporters)
OL	(old leaves)
P	(phosphorous)
PEP	(phosphoenolpyruvate)
Phe	(phenylalanine)
Pro	(proline)
R	(roots)
S	(sulphur)
U	(urea treatment)
Ubi	(ubiquitin)
YL	(young leaves)
Zn	(zinc)

sources (such as amino acids, small peptides and urea) can also play a role in plant N nutrition (Ikeda and Tan, 1998; Zanin et al., 2015a, 2015b; Zhang et al., 2019; Buoso et al., 2021a, 2021b). In the last decades, mainly due to its low production cost and high N content, urea has become the N-fertilizer form most applied worldwide in agriculture (Glibert et al., 2006; Zanin et al., 2014; IFA, https://www.ifastat.org/). As N availability is often a limiting growth factor, plants have developed specific mechanisms to acquire N by combining the activity of High- and Low-Affinity Transport Systems (HATS and LATS, respectively, Kiba and Krapp, 2016). In particular, nitrate is acquired by specific transporters belonging to NPF/NRT1 and NRT2 multigene families (NRT1.1, NRT1.2, NRT2.1 and NRT2.2 in *Arabidopsis*). Nitrate is then converted to nitrite by nitrate reductase (NR) encoded by the *SlNIA* gene in tomato (*AtNIA1* and *AtNIA2* in *Arabidopsis*), and nitrite is converted to ammonium by nitrite reductase (NiR) encoded by *NIR* gene (Daniel-Vedele et al., 1989; Tsay et al., 2011).

In plants, ammonium can derive from catabolic and assimilatory reactions (nitrite reduction) or by its direct acquisition from the soil solution. Regarding the root uptake of ammonium, at least three AMT transporters are known to mediate the HATS of ammonium acquisition in tomato, AMT1.1, 1.2 and 1.3 (homologous to AMT1.1, 1.2, 1.3, 1.5 in *Arabidopsis*) while AMT2.1, ammonium facilitator-type-transporters, potassium channels and aquaporins mediate the ammonium LATS (Yuan et al., 2007; Duan et al., 2018; Filiz and Akbudak, 2020). Ammonium can be the substrate of different assimilatory enzymes, such as plastidic enzymes (glutamine synthetase 2, GS2, and glutamine oxoglutarate aminotransferase, GOGAT, of the GS/GOGAT cycle) and cytosolic enzymes (as glutamine synthetase, GS; Forde and Lea, 2007; Bernard and Habash, 2009). In several plants such as Arabidopsis, tomato and potato, changes in plant biomass production, depending on the inorganic N source supplied, have been observed (Heeb et al., 2005; Cambui et al., 2011; Qiqige et al., 2017).

Plants acquire urea through high- and low-affinity transport systems, mediated by DUR3 transporter and aquaporins, respectively (Kojima, 2007; Wang, 2008; Zanin et al., 2014; Liu et al., 2015). Although the acquisition of nitrate and ammonium has been extensively studied (in tomato Aci et al., 2021; for review see Hachiya and Sakakibara, 2017), the urea acquisition in crops has been less investigated (e.g. under hydroponic system: Peet et al., 1985; Ikeda and Tan, 1998; Mérigout et al., 2008a; Horchani et al., 2010; Arkoun et al., 2012; Wang et al., 2012; Zanin et al., 2015a; Buoso et al., 2021a–c; under soil condition: Arkoun et al., 2012; Beier et al., 2019; Bauer and von Wirén, 2020). A better comprehension of urea nutritional pathway in plants would be extremely useful to identify new solution for the optimization of urea acquisition in crops. In tomato it has been demonstrated that urea is absorbed and also translocated by the plant as urea (Tan et al., 2000). In wheat, N assimilation pattern depends on the N form supplied to the nutrient solution, as stated by Garnica et al. (2010a). Criddle and his co-workers (1988) provided evidence of a reciprocal interaction among different forms of N in wheat, as the acquisition of a particular form was influenced by the one previously applied to the nutrient solution. In maize, the use of different N forms (including urea), determined changes on plant biomass production, root morphology, and also on N-containing compounds (Safdarian et al., 2014, Buoso et al., 2021a,c). An increase in the total N content of plants treated with NH_4NO_3 has been observed in comparison to urea treated plants and accompanied by the rise of NUpE (N Uptake Efficiency), on the other hand, urea seems to have a remarkable efficiency compared to nitrate or ammonium source in terms of dry matter production per unit of N taken-up (Mérigout et al., 2008a). Moreover, at the transcriptional level, the N form available for the root uptake significantly modulates the gene expression profile of crops (Zanin et al., 2015a; Lupini et al., 2017; Buoso et al., 2021a, 2021b; Khalil et al., 2024).

The present work aims to characterize the early response of tomato plants to urea nutrition in comparison to the inorganic N forms, nitrate or ammonium. Thus, the ionomic, metabolomic and transcriptional profiles of tomato plants were analysed 24 h after N resupply to investigate the nutritional pathways activated by the availability of the three different N forms: urea, nitrate or ammonium.

2. Materials and methods

2.1. Plant growth

Tomato seeds (*Solanum lycopersicum* L., cv "Marmande" from DOTTO Spa, Udine, Italy) were germinated for 7 days on filter paper moistened with 0.5 mM $CaSO_4$ and then tomato seedlings were grown under hydroponic conditions as previously described by Pinton et al. (1997).

Twentyone-day-old plants were then transferred to the following nutrient solution (mM: 0.70 K_2SO_4, 0.1 KCl, 2.00 $Ca(NO_3)_2$, 0. 50 $MgSO_4$, 0.10 KH_2PO_4; μM: 10.00 H_3BO_3, 0.50 $MnSO_4$, 0.50 $ZnSO_4$, 0.20 $CuSO_4$, 0.07 Na_2MoO_4, 100 Fe-EDTA) adjusted to pH 6.0 with KOH 1 M. After 14-days tomato plants (35-day-old plants) were transferred to an N-free nutrient solution (mM: 0.7 K_2SO_4, 0.1 KCl, 1.0 $CaSO_4$, 0.5 $MgSO_4$, 0.1 KH_2PO_4; μM: 10 H_3BO_3, 0.50 $MnSO_4$, 0.50 $ZnSO_4$, 0.20 $CuSO_4$, 0.07 Na_2MoO_4, 100 Fe-EDTA), with this nutrient condition, plants were grown for 7 days and the pH was buffered using 1.5 mM MES-BTP (pH 6.0). The nutrient solution was renewed every 3 days.

At the end of the growing period, (42-day-old, vegetative stage) plants were treated for 24 h with different N forms. Therefore, plants were transferred to an N-free nutrient solution containing 2 mM total N, supplied in form of: nitrate (Nit treatment), urea (U treatment) or ammonium (A treatment; applied as potassium nitrate, urea or ammonium sulphate, respectively; Supplementary Table S1). As a control some plants were maintained in N-free nutrient solution (-N treatment). At the end of the experiment roots (R), young leaves (YL) or old leaves (OL) were sampled for transcriptional, ionomic and metabolomic analyses. Before sampling, the tomato roots were washed three times in deionized water. Moreover, the fresh weight (FW), dry weight (DW), SPAD values (SPAD-502, Minolta, Osaka, Japan) and plant length of R and shoots (S) were measured.

During the whole growing period, the controlled climatic conditions were the following: 16/8 (day/night) photoperiod; 220 μmol m^{-2} s^{-1} light intensity; 25/20 °C (day/night) temperature and 70–80% relative humidity. The light transmittance of leaves was monitored using the SPAD instrument (SPAD-502, Minolta, Osaka, Japan).

2.2. Ionomic analyses

The element concentrations of macro- and micro-nutrients in tomato samples were determined by Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP-OES 5800, Agilent Technologies, Santa Clara, USA) and CHN analyser (CHN IRMS Isoprime 100 Stable Isotope Ratio Mass Spectrometer, Elementar, Como, Italy).

For ICP analyses, plant samples were oven dried for 72 h at 60–80 °C and grinded. For each sample, around 100 mg of grinded powder was digested with concentrated ultrapure HNO_3 using a microwave oven (ETHOS EASY, Milestone Srl, Sorisole, BG, Italy) accordingly to the USEPA 3052 method "Plant Xpress" (USEPA, 1995). Element quantifications were carried out using certified multi-element standards.

For CHN analyses, R and S samples were dried, and their total N and carbon contents were determined by CHN-IRMS (CHN IRMS Isoprime 100 Stable Isotope Ratio Mass Spectrometer, Elementar).

2.3. Metabolomic analysis

Samples of R, YL and OL (four replicates of each organ per treatment) were ground in liquid N using a pestle and mortar. According to the method previously reported (Paul et al., 2019), 1.0 g of each plant sample was extracted in 10 mL of a hydroalcoholic solution (80:20 *v/v* methanol:water), acidified with 0.1% formic acid, using an Ultra Turrax (Polytron PT, Switzerland). The extracts were then centrifuged (6000 g, 10 min at 4 °C) and the supernatants filtered through 0.22 μm cellulose syringe filters in vials for analysis. The untargeted metabolomic analysis was performed using a quadrupole-time-of-flight mass spectrometer (6550 iFunnel, Agilent Technologies, Santa Clara, USA) coupled to an ultra-high-pressure liquid chromatograph (UHPLC, 1290 series, Agilent Technologies, Santa Clara, USA) via a JetStream Electrospray ionization system, under previously optimized analytical conditions (Paul et al., 2019). Two injections were performed for each extract, resulting in a total of 8 analytical replicates (N = 8). Briefly, 6 μL of each sample were injected and a reverse phase chromatographic separation was achieved by using a C18 column (Agilent Zorbax eclipse plus; 50 mm × 2.1 mm, 1.8 μm) and a water-acetonitrile binary gradient (from 6 to 94 % organic in 32 min). The mass spectrometer worked in positive FULL SCAN mode (range 100–1200 m/z, 0.8 spectra s^{-1}, 30,000 full widths at half maximum nominal resolution). Compound identification was achieved through the 'find-by-formula' algorithm setting a sensitivity detection threshold of 10,000 counts for annotation using the software Profinder B.07 (Agilent Technologies), and the Plant Metabolic Network PlantCyc 9.6 database (Plant Metabolic Network, http://www.plantcyc.org; Filiz and Akbudak, 2020). The whole isotope pattern (i.e., monoisotopic mass, isotopic spacing, and isotopic ratio) was used for annotation, considering 5 ppm for mass accuracy, according to a level 2 of confidence (Salek et al., 2015). The raw metabolomic dataset was carefully checked to remove artifacts/synthetic metabolites and isobaric compounds and further extrapolated from the software Mass Profiler Professional B.12.06 (Agilent Technologies) after post-acquisition data filtering (compounds do not present in 100% of the replications within at least one treatment were discarded), baselining and normalization as previously reported (Paul et al., 2019).

2.4. Gene expression analysis

Tomato roots were grinded in liquid N. Total RNA was extracted from approximately 70 mg of powder using the Spectrum Plant Total RNA Kit (Sigma Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions (protocol A). RNA quantity and quality were inspected using a NanoDrop device (NanoDrop Technologies, Wilmington, Delaware, USA) and by migration in agarose gel, respectively. Afterward, 1 μg of extracted RNA was retrotranscribed into cDNA adding: 1 μl of 70 μM Oligo-d (T), 1 μl dNTP (10 mM), 20 U RNase inhibitors, 200 U M-MLV Reverse Transcriptase (M-MLV Reverse Transcriptase Sigma Aldrich) according to the manufacturer's instruction. Using primer3 software (version 4.0.1) primers were designed and synthetized by Merck (MERCK KGAA, Darmstadt, Germany; Supplementary Table S3). Real time RT-PCR analyses were performed with CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Data were referred to the averaged expression of two housekeeping genes *SlEF1* and *SlUbi* (Supplementary Table S3). Data were normalized using the $2^{-\Delta\Delta CT}$ accordingly to Livak and Schmittgen (2001) method. The efficiency of each set of primer was estimated using the qPCR package for statistical analysis by R software (R version 2.9.1, www.dr-spiess.de/qpcR.html) as indicated by Ritz and Spiess (2008; R Core Team, 2021).

2.5. Statistical analyses

Physiological, ionomic, gene expression and morphometric analysis were performed on three independent experiments (three biological replicates, N = 3), and for each replicate a pool from three tomato plants was used. Statistical analyses were performed by SigmaPlot 14.0 (SigmaPlot Software, CA, USA), using one-way ANOVA with a Holm-Sidak's test as post hoc test for multiple comparisons (p-value <0.05, N = 3).

Metabolomic analyses were performed using 8 analytical replicates (N = 8). The metabolomic dataset was processed using the Agilent Mass Profiler Professional B.12.06 and interpreted as previously reported (García-Pérez et al., 2021). Specifically, raw abundance data were transformed to $\log_2$ values, normalized to the 75th percentile, and baselined to the median of all samples. Hierarchical cluster analysis (HCA, Euclidean distance, Ward's linkage) and Volcano plot analysis ($P < 0.01$, Bonferroni multiple testing correction; fold-change FC ≥ 2) were obtained from the Mass Profiler Professional B.12.06. The unsupervised HCA was applied with a dual aim, providing a broad perspective on the grouping of samples according to their similarities attributed to their metabolome-wide profile and to assess the homogeneity of replicates, thus enabling the removal of eventual outliers within experimental groups (Pinto, 2017). The Volcano plot analysis allowed us to extrapolate the differential compounds that were then interpreted using the PlantCyc Pathway Tool (Hawkins et al., 2021).

Moreover, the raw metabolomic and ionomic datasets were exported

into SIMCA 16 (Umetrics, Malmo, Sweden) software for principal component analysis (PCA) and orthogonal projection to latent structures discriminant analysis (OPLS-DA) supervised modeling. OPLS-DA was cross-validated statistically by means of cross-validation analysis of variance (CV-ANOVA; $p < 0.001$), inspected for outliers, overfitting was excluded (permutation testing, $N = 200$), and then R^2Y (goodness-of-fit) and Q^2Y (goodness-of-prediction) were recorded. Regarding gene expression analysis the heatmap was generated using ClustVis (https://biit.cs.ut.ee/clustvis/; Metsalu and Vilo, 2015) webtool using the fold parameters. The significance of the clustering observed in the PCA was assessed by PERMANOVA test using 5000 permutations performed with R version 4.3.0 (vegan package; Oksanen et al., 2014).

3. Results

3.1. Morphological observations and ionomic analysis

After 24 h of treatment with different N-forms, no significant changes of SPAD, shoot and root heights, fresh weights, and dry weights were measured among the treatments (Supplementary Fig. S1, Supplementary Fig. S2, Supplementary Fig. S3). Nevertheless, significant changes in the profile of macro- and micro-nutrient concentrations were determined, especially under U (Fig. 1, Supplementary Table S2). Indeed, the performed PCA show that U samples are separated from the other ones (Supplementary Fig. S4). U treatment induced the up concentration of Zn in whole plant organs (YL, OL, and R), of Mg, Mn, P, and Cu in YL, of K, Mn, Cu in OL, and of N in R. On the other hand, only few changes in the elemental profiles were observed by ammonium or nitrate in comparison to –N (A: induced a significant up-concentration of K in OL and N in R; Nit induced a significant up-concentration of Mg in YL, of K and Mg in OL and of N in R). Concerning plants' carbon content, no differences could be observed across treatments (Supplementary Table S2).

3.2. Metabolomic analysis

Overall, 3534 chemical entities were putatively annotated using the comprehensive database PlantCyc 12.6. The annotated compounds and composite mass spectra (mass and abundance combinations) are provided in Supplementary Table S4. The Hierarchical cluster analysis (HCA) was performed firstly with non-averaged data to evaluate the homogeneity of replicates within experimental groups and remove outliers (data not shown), and further with averaged data to determine the similarities between treatments according to their metabolome-wide profile. The heatmap-based clustering showed two sub-clusters in both young and old leaves (Fig. 2A and C) and roots (Fig. 2E), clearly separating U and A from Nit and –N samples. The subsequent supervised model OPLS-DA separated all the samples in the score plot according to the treatments for both young and old leaves (Fig. 2B and D) and roots

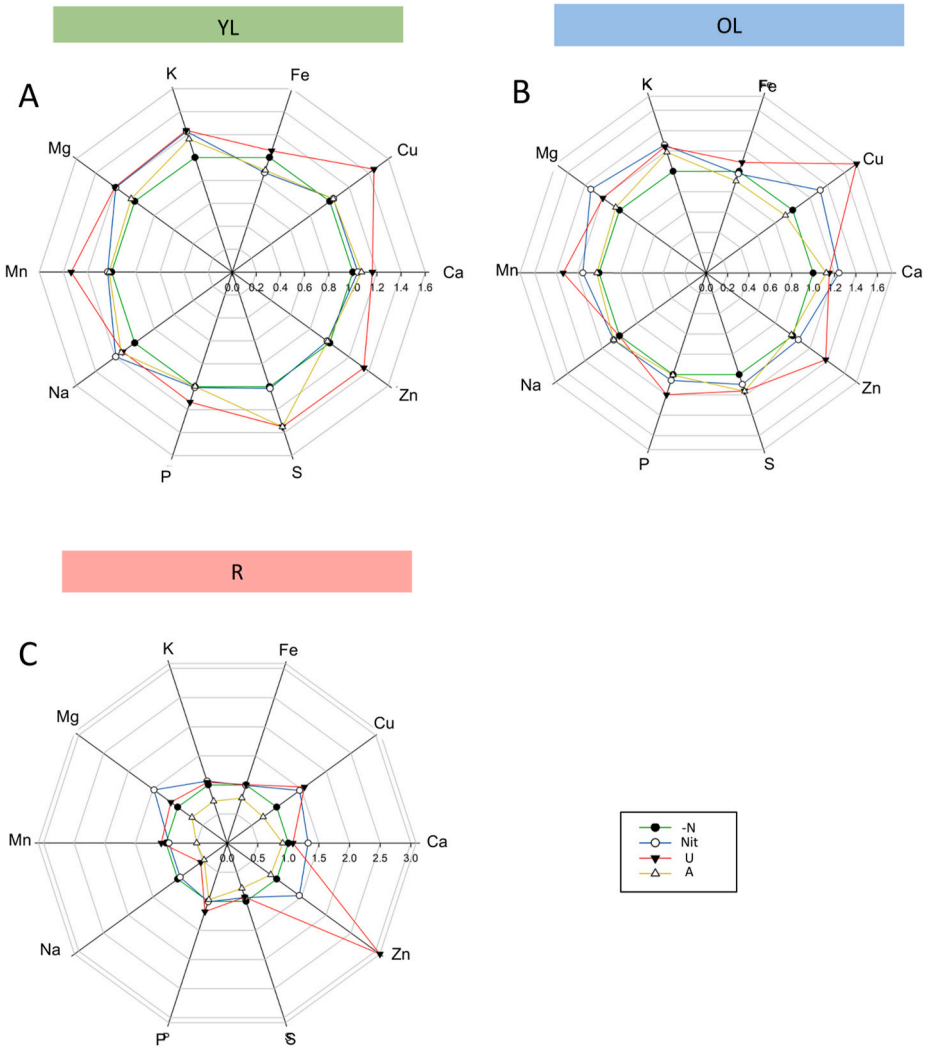

Fig. 1. Ionomic analysis of tomato plants after 24 h of treatment with different N sources. In panels A, B and C, the elemental concentrations in young leaves (YL), old leaves (OL) and roots (R) are shown as radar plots, respectively. For each element the concentration values were scaled to average values of –N samples (value 1.0).

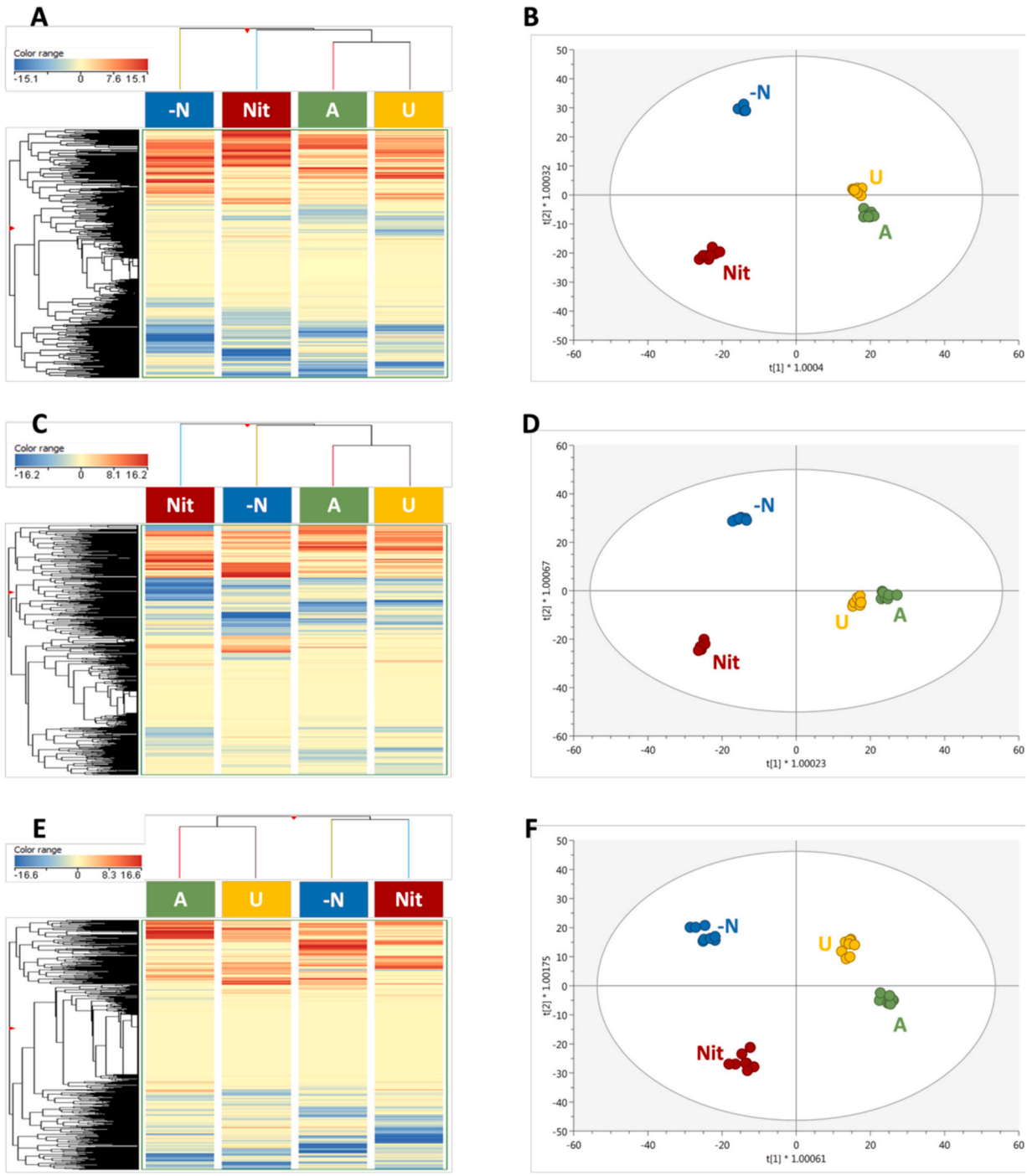

Fig. 2. Unsupervised hierarchical cluster analysis carried out from ultra-performance liquid chromatography electrospray ionization quadrupole time-of-flight mass spectrometry (UHPLC-ESI/QTOF-MS) metabolomics analysis of young leaves (A), old leaves (C) and roots (E) after N supply. The fold-change-based heat map was used to build hierarchical clusters (linkage rule: Ward; distance: Euclidean). Score plot of orthogonal projection to latent structures discriminant analysis (OPLS-DA) supervised modeling carried out on untargeted metabolomics profiles of young leaves (B), old leaves (D) and roots (F) after N supply. "-N", refers to N-free condition; "Nit", refers to nitrate treatment; "U" refers to urea treatment; "A", refers to ammonium treatment.

(Fig. 2D). This supervised discriminant analysis provided a clear insight into the separation between –N and Nit-treated plants *vs* U- and A-treated plants, in all the organs considered in agreement with HCA (Fig. 2).

For each N treatment and for each organ (OL, YL and R), the metabolites whose abundance significantly differed from –N treatment ($P < 0.01$, Bonferroni multiple testing correction; fold-change FC ≥ 2) according to Volcano analysis. These compounds were further incorporated into the metabolic pathway analysis in order to comprehensively represent the impact of N forms (A, U, and Nit) on the biosynthetic metabolism of tomato plants with respect to limiting N conditions (-N).

In Fig. 3 (D-F) the biosynthetic pathways significantly modulated in OL, YL and R by N treatments with respect to –N conditions are shown. Similar trends in metabolite accumulation were found after applying organic or inorganic N, involving both primary and secondary biosynthetic pathways, where an alteration was reported in the entire plant when N was applied. In all cases, a general down-regulation of

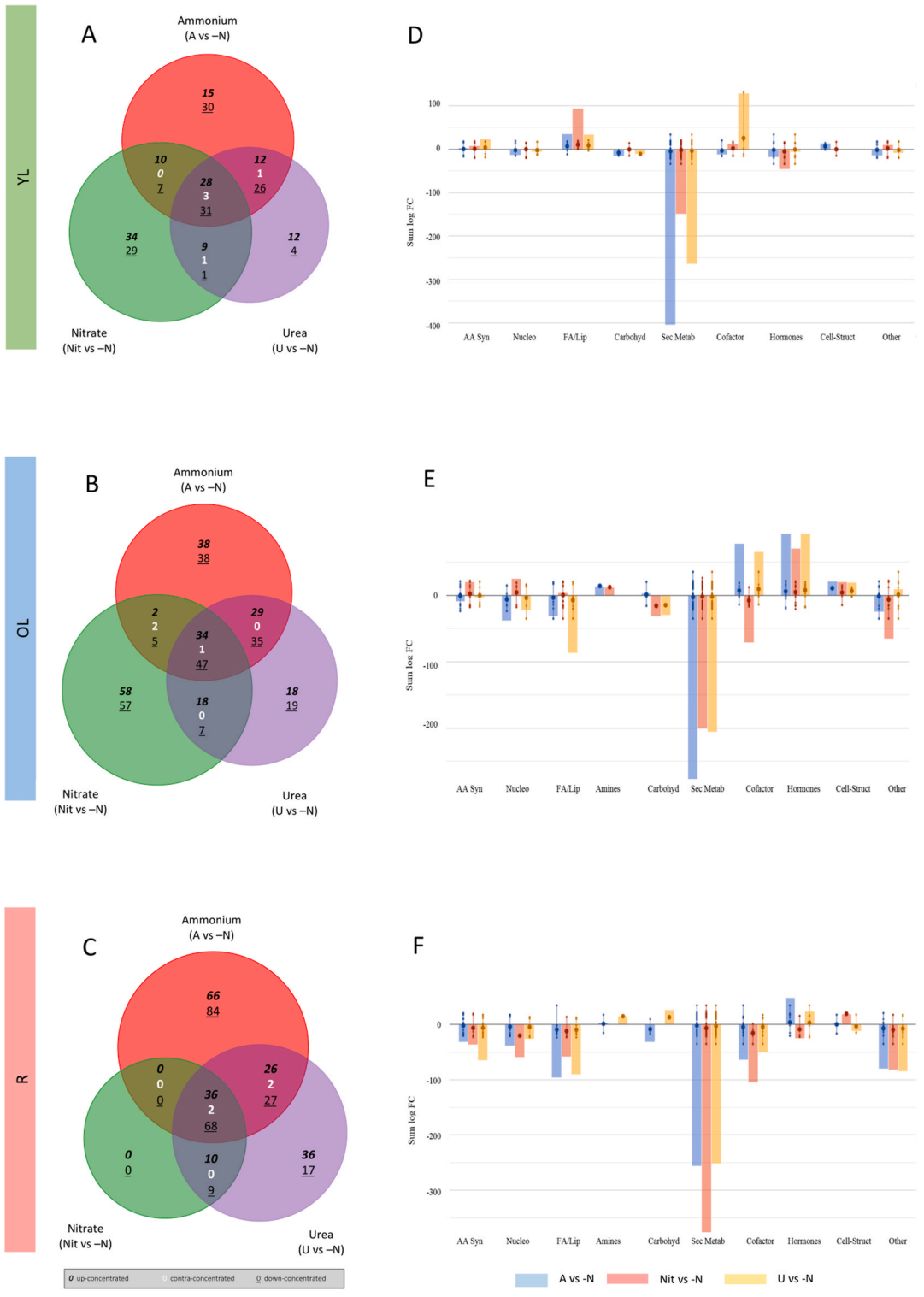

Fig. 3. Metabolomic profiles in tomato young leaves (YL, panels A and D), old leaves (OL, panels B and E), roots (panels C and F). Venn diagram showing the significant compounds involved in the response to ammonium in comparison to control (A *vs* –N), to urea (U *vs* –N) and to nitrate (Nit *vs* –N) referred to each organ (YL, panel A; OL, panel B; and R, panel C). Biosynthetic pathways impaired by N-forms in YL, OL and R (panels D, E, and F, respectively). Significant metabolites and their fold-change values were uploaded into the Omic Viewer Dashboard of the PlantCyc pathway Tool software (www.pmn.plantcyc.com). The large dots represent the average (mean) of all log Fold-change (FC) for metabolites, and the small dots represent the individual log FC for each metabolite. The x-axis represents each set of subcategories, while the y-axis corresponds to the cumulative log FC. AA Syn: amino acids biosynthesis; Nucleo: nucleosides and nucleotides biosynthesis; FA/Lip: fatty acids and lipids biosynthesis; Amines: amines and polyamines biosynthesis; Carbohyd: carbohydrates biosynthesis; Sec Metab: secondary metabolites biosynthesis; Cofactors: cofactors, prosthetic groups, electron carriers, and vitamins biosynthesis; Hormones: hormones biosynthesis; Cell-struct: cell structure biosynthesis ($P < 0.01$, Bonferroni multiple testing correction; $FC \geq 2$).

secondary metabolism was reported with respect to –N conditions, although substantial differences were found depending on the plant tissue. As expected, the biosynthesis of N-containing compounds was significantly modulated under N supply. The biosynthesis of amino acids, in particular, those related to the GS-GOGAT pathway, seemed to be strongly affected by N supply. An increased abundance of Asn was observed in YL and OL under all three N treatments in comparison to –N, which was combined with the accumulation of glutamine (Gln) and a concomitant reduction of glutamate (Glu) and aspartate (Asp) in A-treated plants. Nevertheless, several differences were found according to leave stage since several derivatives of Glu and Asp were distinctively found in OL, whereas the levels of one derivate of Asp (*N*-carbamoyl-L-aspartate) were strongly reduced by all N treatments in comparison to –N, coupled with an increase in the levels of one derivate of Glu (*N*-(4-hydroxybenzoyl)-L-glutamate), mediated by U and A. Similarly to leaves, Asn abundance was also found increased in R under any N form in comparison to –N. Moreover, the levels of one Asp derivative and several Glu derivatives mainly decreased by A and U treatments (Table 1).

The biosynthesis of phytohormones was also modulated, especially after U and A supply with respect to –N. Regarding CKs, *cis*-zeatin and *trans*-zeatin riboside levels were increased in response to all the treatments in YL. In OL, a decrease of *trans*-zeatin abundance was observed in all the treatments while an increase of isopentenyladenine-7-*N*-glucoside levels occurred when A was supplied. In R, zeatin precursors, especially isopentenyladenine-9-*N*-glucoside and of isopentenyladenine-7-*N*-glucoside showed increased levels in A and U, the latter showing also increased levels of dihydrozeatin (Supplementary Table S5). Regarding auxin biosynthetic pathways, an increase in 2-*trans*- abscisic acid abundance was observed in A-treated OL (Supplementary Table S5).

3.3. Gene expression analyses

Differences in the expression of key genes involved in N acquisition were analysed by real-time RT-PCR in tomato roots after 24 h of treatment. These analyses evaluated the expression of fourteen genes coding for: nitrate transporters (*SlNRT2.2, SlNRT1.5, NPF6.3),* an accessory protein of nitrate transporters *(SlNAR2.1*), urea transporter *(SlDUR3),* ammonium transporters *(SlAMT1-1, SlAMT1-2)* and N assimilatory enzymes *(SlNii1, SlNiR, SlGS2, SlGS1, SlNR, SlGOGAT, SlAS)*.

Regarding the effect of Nit treatment, the genes *SlNRT2.2, SlNAR2.1, SlGS2, SlNii1, SlNiR* were significantly upregulated by Nit in comparison to other N treatments. This behavior is also well visualized by heatmap clustering analyses (Fig. 4). On the other hand, the presence of this N form in the nutrient solution downregulated the expression of *SlNRT1.5, NPF6.3, SlDUR3, SlAMT1-1* and *SlGS1* if compared to the –N treatment. When ammonium was supplied to the nutrient solution, a significant upregulation of *SlNR* and *SlGOGAT* genes was observed in comparison to –N. In contrast, *SlNRT2.2, SlNAR2.1, SlNRT1.5, NPF6.3, SlDUR3, SlNii1, SlNiR* and *SlGS2* genes were downregulated when A was conferred as N source in comparison to the –N treatment. Concerning the effect of U supplied as a N source, *SlAMT1-2* and *SlAS1* were significantly upregulated by U compared to other N treatments (Fig. 4).

4. Discussion

In the literature, an increase in plant biomass and SPAD values has been reported when plants are grown for a prolonged period under N sufficiency (days or weeks, Aci et al., 2021). In the present study, as expected, the short exposure of tomato plants to N treatments (24 h) did not affect plant morphometry or chlorophyll content (SPAD, Supplementary Fig. S3). However, a short exposure to N treatments induced changes in the ionomic, metabolomic, and transcriptional profiles. Untargeted metabolomic analysis revealed both a common and a specific modulation dependent on the N form applied to the nutrient

Table 1
Amino acids involved in the redistribution of N inside plants and related compounds. List and relative p-value and Log FC of amino acids related to the GS-GOGAT cycle and their related compounds per each organ (OL, YL and R) and per each N treatment *vs* the N-depleted condition (-N): Nit *vs* –N, U *vs* –N, A *vs* –N. The empty spaces mean no significant differences between treatments.

	Nit vs –N		U vs –N		A vs –N	
Young leaves (YL)	Log FC	p-value	Log FC	p-value	Log FC	p-value
glutamine					2.65	0.0006
glutamate					−1.57	0.0005
asparagine	17.53	0.0002	17.02	0.0002	16.53	0.0002
N-(4-hydroxybenzoyl)-L-glutamate			10.45	0.0070	10.92	0.0047
N-carbamoyl-L-aspartate	−17.55	0.0005	−12.92	0.0100	−15.37	0.0020
Old leaves (OL)	Log FC	p-value	Log FC	p-value	Log FC	p-value
glutamine					1.95	0.0014
glutamate					−1.51	0.0013
asparagine	20.24	0.0002	19.87	0.0002	16.90	0.0002
aspartate					−1.50	0.0091
gamma-thiomethyl glutamate	−15.28	0.0002	−20.61	0.0002	−20.67	0.0002
(6*S*)-5-formyl-tetrahydrofolate mono-L-glutamate			−1.44	0.0024		
N-(4-hydroxybenzoyl)-L-glutamate			11.60	0.0016	14.28	0.0003
erythro-4-hydroxy-L-glutamate					−1.52	0.0003
N-carbamoyl-L-aspartate	−17.86	0.0003	−16.28	0.0006	−13.97	0.0029
aspartate 4-semialdehyde			−1.01	0.0008	−1.34	0.0002
(3-hydroxy-2-oxindol-3-yl)acetyl-L-aspartate					−2.87	0.0010
Root (R)	Log FC	p-value	Log FC	p-value	Log FC	p-value
asparagine	19.91	0.0002	12.39	0.0004	19.15	0.0002
5,10-methylenetetrahydropteroyl mono-L-glutamate					−35.02	0.0002
gamma-thiomethyl glutamate			−17.48	0.0002	−17.71	0.0002
10-formyl-tetrahydrofolate mono-L-glutamate					−15.59	0.0006
(6*S*)-5-formyl-tetrahydrofolate mono-L-glutamate	−14.86	0.0007	−15.18	0.0005	−15.59	0.0004
erythro-4-hydroxy-L-glutamate					4.18	0.0002
N-(4-hydroxybenzoyl)-L-glutamate			13.62	0.0002	13.37	0.0002
(indol-3-yl)acetyl-L-aspartate					−15.47	0.0002

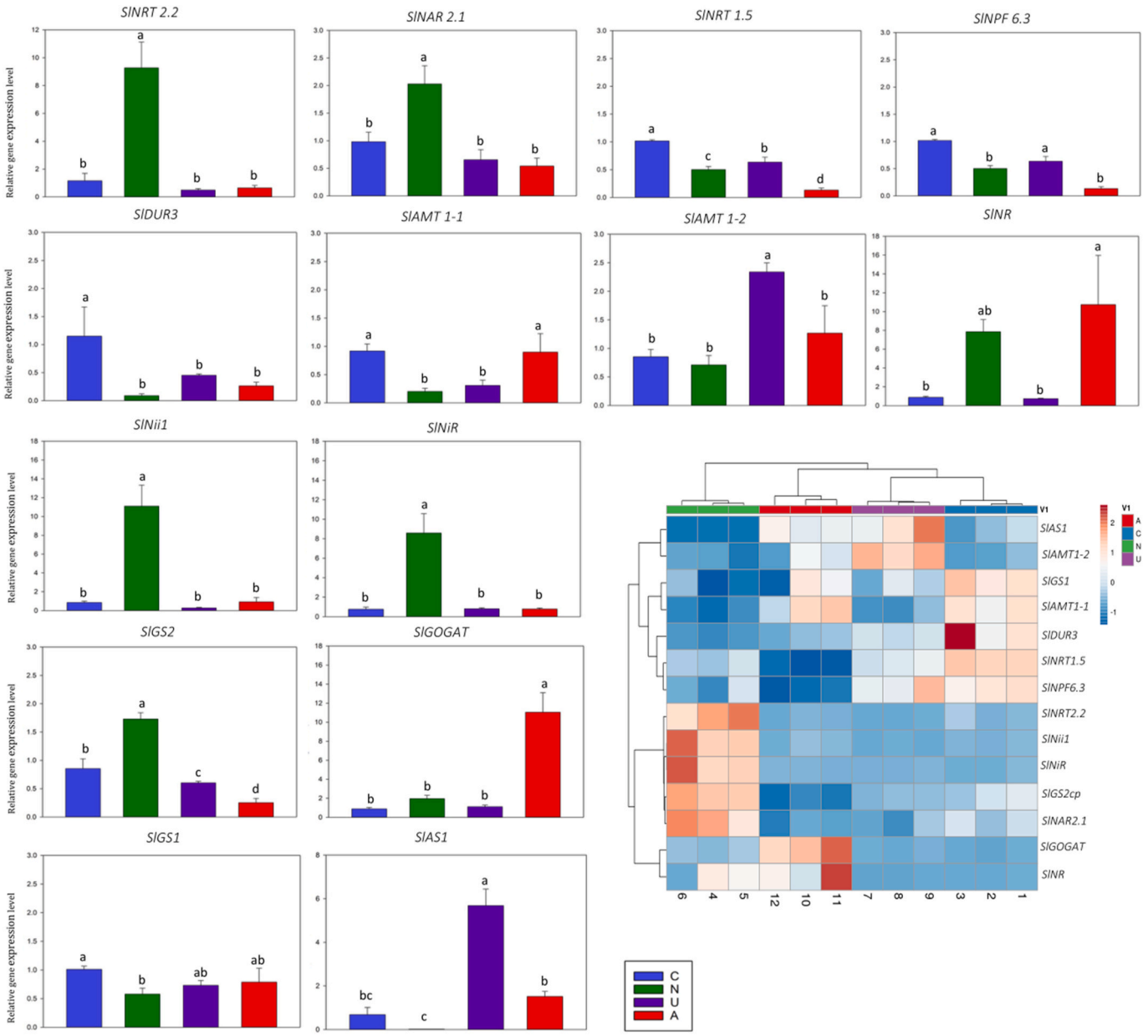

Fig. 4. Relative gene expression level of tomato roots after 24 h of treatment with different N-forms. Data were referred to the averaged expression of two housekeeping genes *SlEF1* and *SlUbi*. Relative changes in gene transcripts levels were referred to the average transcript level of housekeeping genes in N-free control roots (-N, relative gene expression = 1). In heatmap, a clustering of relative gene expression levels and samples in tomato roots is shown. A, refers to ammonium treatment (red); –N, refers to control (N-free condition, blue); Nit, refers to nitrate treatment (green); U refers to urea treatment (purple). In the histograms, letters refer to statistical significance differences (Holm-Sidak ANOVA, N = 3, p-value< 0.05).

solution. As suggested by the multivariate statistics (HCA and OPLS-DA), forms A and U had a more significant impact on plant metabolism with respect to –N conditions than Nit, following a plant-organ dependence (Fig. 2). Interesting changes were observed in amino acid, secondary metabolite, and hormone biosynthesis. (Fig. 3, Supplementary Table S5).

4.1. Amino acid biosynthesis

Regarding the amino acids directly involved in the assimilation and translocation of the macronutrient, changes in the pattern of Glu, Gln and Asn were observed in plants and especially A induced an increase of Gln and a decrease of Glu in leaves (OL and YL, Table 1). The increase of Gln after A supply has been previously observed in several studies in tomato and cucumber, while in other plant species (i.e., wheat, rice, soybean, and pea) a supply of this N form led to a predominant increase of Asn over Gln, suggesting a difference in the amino acids concentration among plant species in response to A supply (Koga and Ikeda, 2000; Ikeda et al., 2004; Ikeda et al., 2005; Ueda et al., 2008; Ariz et al., 2013). In our study, under all N supply conditions, Asn was found increased in all three organs compared to N depletion conditions (Table 1, Supplementary Table S5). This data taken together with the non-significant difference in total N among treated plants (Supplementary Table S2) indicates that, at least in the short-term, tomato plants are able to use urea as a N form. In a recent paper, Huang and coworkers (2022) define Asn synthetase as a marker to identify genotypes characterized by a high N use efficiency under low inputs of the macronutrient. It is interesting to note that the gene expression of this enzyme (*SlAS1*) in roots was stimulated by urea (U) more than ammonium (A) or nitrate (Nit, Fig. 4) although lower abundance of Asn was observed in U-treated roots in

comparison to the inorganic N forms. The transcriptional induction of *AS* by urea was found in other plant species such as maize and *Arabidopsis* (Mérigout et al., 2008b; Zanin et al., 2015a).

In agreement with the literature, the expression of some genes involved in the nitrate transport and assimilation was induced by Nit treatment, as indicated by a significant up-regulation in tomato roots of some genes related to the transport of nitrate and its reduction into ammonium (*SlNRT2.2, SlNAR2.1, SlNii1, SlNiR, SlGS2)* in comparison to the other N treatments (U and A, Fig. 4). Based on sequence similarity with the *Arabidopsis* isoforms, it is plausible to suppose that these Nit-responsive genes (*SlNii1, SlNiR,* and *SlGS2*) have a plastidial localization. On the other hand, ammonium (directly taken up or released by urea hydrolysis) should preferentially be substrate of cytosolic GS isoform that operate the N assimilation along with the cytosolic AS enzyme (GS1, AS; Lam et al., 1996; Ishiyama et al., 2004a; 2004b; Cruz et al., 2006; Liu and von Wirén, 2017; Buoso et al., 2021a). Buoso and coworkers (2021a) provided evidence in maize roots that this latter assimilatory pathway was already transcriptionally induced after 24 h of treatment with urea and/or ammonium. In agreement with this observation, our data suggest the induction by urea of this cytosolic pathway as *SlAS1* was found upregulated (Fig. 4).

More than other N treatments, the application of ammonium to nutrient solution strongly induced the expression of NADH-dependent glutamate synthetase isoform (*SlGOGAT*; Fig. 4). This behavior might be linked to the overabundance of its substrate (Gln) and depression in the levels of its product (Glu) after the A treatment (Table 1). This result agrees with previous evidence in *Arabidopsis*, where NADH-GOGAT was strongly induced by exogenous ammonium supply (Liu and von Wirén, 2017). Under A treatment, the results suggest a fast accumulation of Gln in leaves that may be consequence of a prompt assimilation of this N form to avoid ammonium toxicity.

Regarding the urea cycle and the arginine biosynthesis, argininosuccinate (a precursor of arginine) was found increased in Nit-treated OL, whereas its abundance was decreased in U- or A-treated OL (Supplementary Table S5) with respect to –N conditions. In senescing leaves, an accumulation of arginine (as well as an increase in arginase activities) is expected and it has been shown to increase with the leaf age (Diaz et al., 2005).

In plants, proline is an important metabolite which is accumulated under a broad range of stress conditions like osmotic and oxidative stresses, high light intensity, extreme temperatures, nutrient deficiencies, high levels of heavy metals, air pollution (Claussen, 2002; Rehman et al., 2021). Pérez-Alfocea and coworkers (1993) showed that in tomato the proline concentration contributes less in terms of quantity to the total fraction of osmotically active solutes than other osmolytes, hence playing other roles than water-stress tolerance including N cycling (Zhang and Becker, 2015). Proline is linked to N cycling processes as its catabolism releases glutamate, which in turn is the substrate of N assimilatory enzymes (as GS1) for the biosynthesis of amino acids (Zhang and Becker, 2015). Claussen (2002) provided evidence that, under hydroponic conditions, tomato plants increased the proline concentration in leaves when ammonium, more than nitrate, was the predominant N form supplied to the nutrient solution. Under our short-term experiments, proline levels increased in OL and R almost regardless of the N form applied (in R the highest proline levels were induced by A; Supplementary Table S5). Along with proline, also a precursor of its biosynthesis (glutamate 5-semialdehyde, Glu-SA) was found to be increased in roots independently of the N form applied compared to –N conditions. In plants, Glu-SA is an intermediate of a glutamate dependent pathway for the synthesis of proline (Hu et al., 1992; Verbruggen and Hermans, 2008). Thus, within 24 h of treatment, the N resupply seemed to be perceived by all treated tomato plants. These data suggests that the proline degradation in source organs for the N cycling was repressed when N was applied to nutrient solution (24 h treatment) leading to an over accumulation of this amino acid in source organs (OL and R) and this early response was observed when urea, ammonium or nitrate were applied to nutrient solution (Supplementary Table S5).

Overall, all three N forms seem to downregulate the biosynthesis of S-containing amino acids (like Cys and Met) in roots, whereas they were induced by Nit in shoots, compared to –N conditions. Similarly, the U treatment increased adenosine-3′,5′-bisphosphate levels in OL (Supplementary Table S5). Urea is not reported to directly affect the levels of this metabolite; despite it can indirectly affect various cellular processes in plants, including N metabolism and protein synthesis, which may have downstream effects on the levels of certain nucleotides (Mérigout et al., 2008b; Zanin et al., 2015a; Wang, 2008). In chickpea, the catabolism of allantoate derived from purine degradation releases urea (Muñoz et al., 2001). Thus, it is plausible to suppose that urea concentration may have, in turn, a regulatory effect on this pathway.

The phenylalanine biosynthesis and that of other aromatic amino acids (Tyr and Trp) were found to be generally downregulated in R by all three N forms with respect to –N condition (Supplementary Table S5). In agreement with the literature data, this result confirms that when N becomes available for root uptake, a metabolic shift from the accumulation of carbon-based secondary metabolites to N-rich compounds is activated by plants, especially after a period of N deficiency. Therefore, an overall downregulation of the phenylpropanoid pathway is expected (Leja et al., 2010; Kováčik et al., 2007; Ravazzolo et al., 2020; Xun et al., 2020) and this trend was induced by U as well as by A or Nit treatments. Interestingly, the higher abundance reported for shikimate 3-phosphate in OL by U and A, and not by Nit, along with the lower levels of phosphoenolpyruvate by all the N treatments might suggest different levels of inhibition of the shikimate pathway depending on the N form applied (Supplementary Table S5).

4.2. Biosynthesis of secondary metabolites

A significant portion of the metabolic changes in all three organs was related to secondary metabolisms. Several metabolites involved in the phenylpropanoid pathway exhibited alterations in their abundance. The consumption of phenylalanine, which releases ammonium, is the first step of the phenylpropanoid pathway, whose activation is a marker of low N availability (Yang et al., 2011). Interestingly, some metabolites of the phenylpropanoid pathway were responsive to the N form applied to the nutrient solution. Data indicates that the N supply to the nutrient solution, especially in form of urea or ammonium, induced the reprogramming of secondary metabolism with the de-induction of the phenylpropanoid pathway. Especially in R, the levels of shikimate, pelargonidin, bergaptol, cinnamate, quercetin, bis-noryangonin, pseudoisoeugenol were mostly increased in A and U with respect to –N conditions. On the other hand, in the same organ, the abundance of isoarctigenin, 7-4′-dimethylquercetin, 3,7-dimethylquercetin, kievitone, and taxifolin was increased only by U (Supplementary Table S5. Also in OL, some phenylpropanoid derivatives were differentially altered, showing a rather similar response due to U and A; instead, baicalein 7-*O*-glucuronide and cyanidin 3-*O*-(6″-*O*-malonyl)-β-D-glucoside were specifically responsive to urea. These results suggest that elicitation of flavons' and flavonoids' by urea treated plants seems to be a peculiar pattern linked to the N form applied. The biosynthesis and release of flavonoids into the rhizosphere are known components of the nutritional responses activated to promote the Fe and P uptake in plants or the symbiosis under N deficiency in Fabaceae plants and increase the plant resilience to nutrients deficiency (Biała-Leonhard et al., 2021; Buoso et al., 2022; Chai and Schachtman, 2022).

Changes at the metabolic and transcriptional levels occurred along with changes also at the ionomic level. In tomato, the concentration of macro- and micro-elements may vary due to phenological state, varieties, and the N form supplied (Borgognone et al., 2013; Alfosea-Simón et al., 2022). In our results, the ionomic analysis indicated that urea acquisition induced an accumulation of Zn and Cu in plants more than in the presence of inorganic N forms (Fig. 1, Supplementary Table 2). For the first time, these results provide clear evidence of a direct relationship

between N forms and zinc (Zn) concentration that was only suggested in previous works (Ozanne, 1955; Viets Jr et al., 1957; Pal et al., 2019). In maize, the addition of urea and a urease inhibitor modulated the expression of several genes related to Zn and Fe transport and storage (Zanin et al., 2016). This observation can suggest the occurrence of an interplay between urea nutrition pathway and the micronutrient acquisition in plants probably due to the important role of divalent cations as cofactors of some N enzymes (e.g. urease, GS, GOGAT). Thus, the use of urea fertilizer may find application in the Zn biofortification of crops. However, additional research is required to identify the rhizodeposition under urea nutrition and to establish a correlation between its composition and crop resilience to metal stresses.

4.3. Phytohormones biosynthesis

N can act as a signal triggering a signal cascade that induces or represses genes involved in the nutrient uptake, reduction, and assimilation, leading to changes in the N status of the plant (Li et al., 2014). In particular, the role of nitrate to act as signal molecule in the transcriptional and metabolomic reprogramming has been well reported in plants (Stitt, 1999; Gutiérrez et al., 2007; Krouk et al., 2011; Vidal et al., 2015; Krouk, 2016; O'Brien et al., 2016; Vidal et al., 2020; Lamig et al., 2022). In our study, all the N forms led to modulate the expression profile of some genes related to N acquisition and they also led to a reprogramming of metabolism, and this modulation was more evident under ammonium nutrition (A treatment).

Although nitrate signaling has been deeply studied, ammonium has also been proposed to act as a signal molecule for morphological and physiological plant responses (Liu and von Wirén, 2017; Ueda et al., 2017). All these multifactorial changes seemed to be regulated by complex crosstalk between N and phytohormone signaling pathways (Kiba et al., 2011; Krouk et al., 2011; Vega et al., 2019). Moreover, it has been described that the form of N can modulate the phytohormone concentration in plants (Bauer and von Wirén, 2020; Buoso et al., 2021c, 2023; Khalil et al., 2024). In our study, we observed a clear modulation of hormonal biosynthetic pathway in all organs under all N treatments, being more evident under ammonium treatment (Fig. 3).

Regarding the ABA synthesis, an increase of 2-*trans* abscisic acid levels was observed under A and U in OL with respect to –N. Previous studies provide evidence that ammonium nutrition can trigger some ABA-mediated responses, such as: the enhancement of stomatal closing coupled with a reduction of water loss via transpiration, the increase of resistance to pathogen infection H_2O_2-dependent, and the modulation of plant adaptive responses to environmental changes (Garnica et al., 2010b; Fernández-Crespo et al., 2015; Liu and von Wirén, 2017; Ganz et al., 2022). Our data (transcriptional and metabolomic analysis) confirm that the N assimilation process is active in roots when reductive N forms were applied to the nutrient solution. Under these conditions, the pathways involved in N recycling and remobilization in old leaves (known to be ABA-mediated processes, Watanabe et al., 2014), were probably still active 24 h after treatment.

Cytokinins (as isopentenyl adenine-type, *trans*-zeatin, *cis*-zeatin and dihydrozeatin) regulate nutrient uptake and translocation in plants (Takei et al., 2001; Sakakibara, 2003; Sakakibara et al., 2006). In response to N nutritional status, plants have developed several systems to regulate the biosynthesis of CKs in roots, especially of precursors of active CKs (Gu et al., 2018; Mandal et al., 2022). Usually, a prolonged N deficiency reduces CK production while a supply of the nutrient increases the overall CK content in plants (Takei et al., 2001, 2004; Lacuesta et al., 2018). In agreement with literature, in our study YL of all

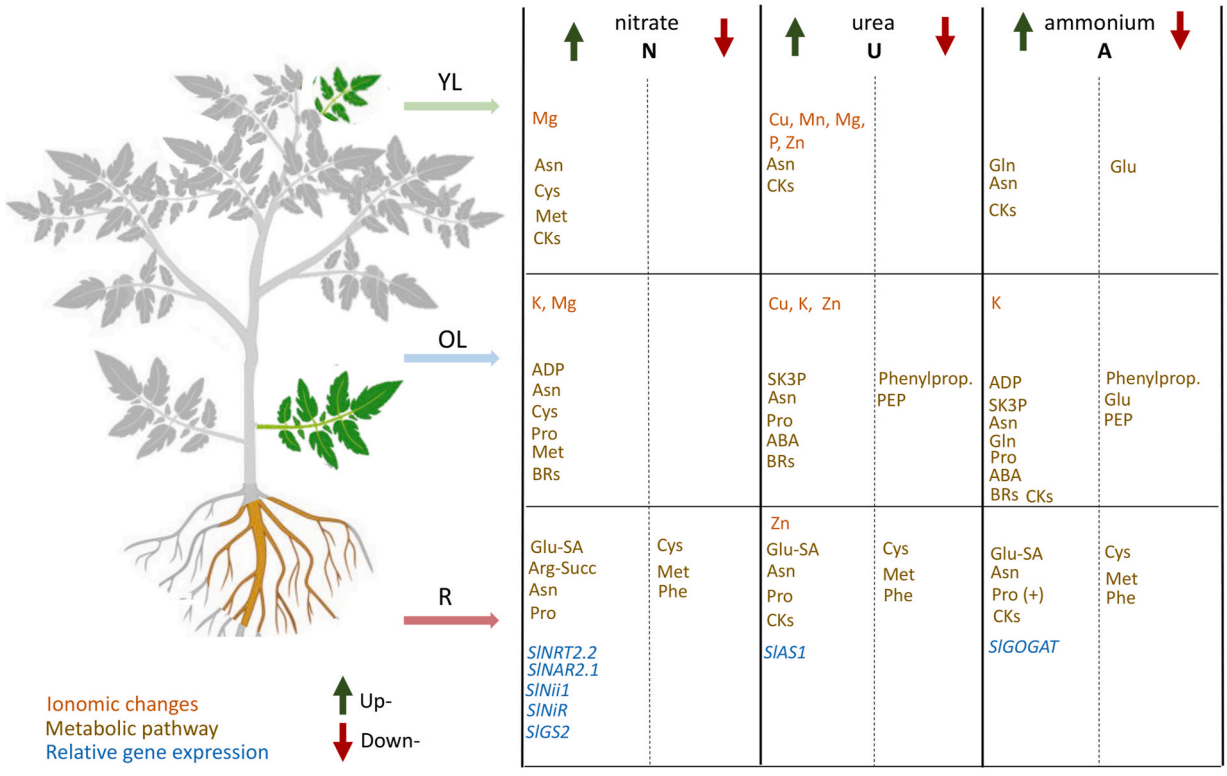

Fig. 5. Schematic representation of the main changes occurred at the ionomic, metabolomic, and transcriptional level in tomato cited in the Discussion. Red and green arrows refer to up-concentrated/regulated metabolite (or nutrient)/transcripts and down-concentrated/regulated metabolite (or nutrient)/transcripts, respectively. Nit, refers to nitrate treatment; U refers to urea treatment; A, refers to ammonium treatment. YL (Young Leaves), OL (Old Leaves), R (Roots), Cu (Copper), Fe (Iron), Mn (Manganese), P (Phosphorous), Zn (Zinc), Asn (Asparagine), Cys (Cysteine), Gln (Glutamine), Glu (Glutamate), Met (Methionine), Phe (Phenylalanine), Pro (Proline), Glu-SA (glutamate 5-semialdehyde), ADP (adenosine 3′-5′ biphosphate), Arg-Succ (arginosuccinate), PEP (phosphoenolpyruvate), Phenylprop. (phenylpropanoids), ABA (Abscisic acid or its relative compounds), BRs (Brassinostreoids or their relative compounds), CKs (cytokinin's or their relative compounds), SK3P (Shikimate 3-phosphate; picture modified from www.istockphoto.com).

treated plants showed increased levels of *cis*-zeatin (cZ) with respect to –N condition. The occurrence of cZ type in plants has been proposed to be relevant in the maintenance of basal level of CK activity to overcome abiotic stresses (Schäfer et al., 2015). Interestingly, only the reductive forms of N (U and A) displayed an enhancement of CK precursors levels in roots that might allow a prompt adaptation of plant growth to environmental changes (Supplementary Table S5). Moreover, dihydrozeatin showed an increased abundance only in the roots of U-treated plants. These results confirm the relationship between CK and N nutrition and moreover suggest that the presence of active CKs depend on the supplied N form.

In comparison to IAA, CK or ABA, the interplay between N and other signaling pathway, such as brassinosteroids (BRs), remains still poorly characterized. In literature fragmentary information is available about the role of BRs for N nutrition. The biosynthesis of this hormonal class is positively regulated by ammonium (Wei and Li, 2020). Other works underline the involvement of the second messenger Ca^{2+} in the crosstalk among nitrate nutrition and BR biosynthesis (Du and Poovaiah, 2005; Riveras et al., 2015). In a recent review (Han et al., 2023), the role of BRs in regulating the plant response under low N availability has been emphasized. In contrast to N sufficiency, mild N deficiency induces the synthesis of BRs and their signaling to promote N foraging response. Specifically, BRs promote the expression of *BES1*, a protein that interacts with LBD37, reducing the capacity of this latter transcription factor to negatively regulate the N-foraging response (Han et al., 2023). In our data, an accumulation of BRs was observed in OL independently to the N form applied (Fig. 5, Supplementary Table S5). Therefore, urea, as well as the inorganic forms of N, induced the accumulation of this group of plant steroid hormones suggesting an interaction between BR pathway and the macronutrient N itself, rather than its specific form.

In conclusion, this study indicates that the recovery of tomato plants from a mild-N deficiency triggers a strong plant's early response involving ionomic, metabolomic and transcriptional changes linked to the N-form supplied to the plants (for a schematic representation of the main discussed results see Fig. 5). Specifically in roots, *SlNii1, SlNiR, SlGS2* were induced by nitrate, whereas *SlGOGAT* was induced by ammonium and the *SlAS* was induced by urea treatment. The activation of the N assimilatory pathway in roots by all three N forms is supported also by the increase of Asn concentration in plants. The patterns of Asn, Glu, Gln and Asp in tomato plants suggest the occurrence of the transport of N from senescent tissues to sink organs to sustain the plant growth. Moreover, the biosynthesis of fatty acids, sterols and cofactors was more intense in YL while decreased in OL and R. A cross talk between urea acquisition and micronutrients in plants is underlined by an increase of the Zn and Cu concentrations in plants as well as a specific induction of the cofactor biosynthetic pathway in YL. Finally, the N form applied suggests a differential involvement of CKs, ABA and BRs in the activation of the early response to the recovery of tomato plants to the mild N-deficiency (Fig. 5). This work provides new hints for improving N use efficiency on crops, paving the way towards the adjustment of guidelines for sustainable fertilization management.

CRediT authorship contribution statement

Arianna Lodovici: Writing – original draft, Methodology, Formal analysis, Data curation. **Sara Buoso:** Writing – review & editing, Supervision, Conceptualization. **Begoña Miras-Moreno:** Formal analysis, Data curation. **Luigi Lucini:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis, Conceptualization. **Pascual Garcia-Perez:** Writing – review & editing, Visualization, Data curation. **Nicola Tomasi:** Writing – review & editing, Supervision, Data curation. **Roberto Pinton:** Writing – review & editing, Supervision, Conceptualization. **Laura Zanin:** Writing – review & editing, Writing – original draft, Supervision, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

as supplementary material I provide all the data.

Acknowledgements

This study was conducted withing the Agritech National Research Center and received funding by the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 June 17, 2022, CN00000022) and by the Departmental Strategic Plan (PSD) of the University of Udine - Interdepartmental Project on Artificial Intelligence (2020-25). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2024.108666.

References

Aci, M.M., Lupini, A., Mauceri, A., Sunseri, F., Abenavoli, M.R., 2021. New insights into N-utilization efficiency in tomato (Solanum lycopersicum L.) under N limiting condition. Plant Physiol. Biochem. 166, 634–644.

Alfosea-Simón, M., Simón-Grao, S., Zavala-Gonzalez, E.A., Navarro-Morillo, I., Martínez-Nicolás, J.J., Alfosea-Simón, F.J., García-Sánchez, F., et al., 2022. Ionomic, metabolic and hormonal characterization of the phenological phases of different tomato genotypes using omics tools. Sci. Hortic. 293, 110697.

Ariz, I., Asensio, A.C., Zamarreño, A.M., García-Mina, J.M., Aparicio-Tejo, P.M., Moran, J.F., 2013. Changes in the C/N balance caused by increasing external ammonium concentrations are driven by carbon and energy availabilities during ammonium nutrition in pea plants: the key roles of asparagine synthetase and anaplerotic enzymes. Physiol. Plantarum 148 (4), 522–537.

Arkoun, M., Sarda, X., Jannin, L., Laîné, P., Etienne, P., Garcia-Mina, J.M., Yvin, J.-C., Ourry, A., 2012. Hydroponics versus field lysimeter studies of urea, ammonium and nitrate uptake by oilseed rape (Brassica napus L.). J. Exp. Bot. 63, 5245–5258.

Bauer, B., von Wirén, N., 2020. Modulating tiller formation in cereal crops by the signalling function of fertilizer nitrogen forms. Sci Rep - UK 10.

Beier, M.P., Fujita, T., Sasaki, K., Kanno, K., Ohashi, M., Tamura, W., et al., 2019. The urea transporter DUR3 contributes to rice production under nitrogen-deficient and field conditions. Physiol. Plantarum 167 (1), 75–89.

Bernard, S.M., Habash, D.Z., 2009. The importance of cytosolic glutamine synthetase in nitrogen assimilation and recycling. New Phytol. 182, 608–620.

Biała-Leonhard, W., Zanin, L., Gottardi, S., de Brito Francisco, R., Venuti, S., Valentinuzzi, F., et al., 2021. Identification of an isoflavonoid transporter required for the nodule establishment of the Rhizobium-Fabaceae symbiotic interaction. Front. Plant Sci. 12, 758213.

Borgognone, D., Colla, G., Rouphael, Y., Cardarelli, M., Rea, E., Schwarz, D., 2013. Effect of nitrogen form and nutrient solution pH on growth and mineral composition of self-grafted and grafted tomatoes. Sci. Hortic. 149, 61–69. Amsterdam.

Buoso, S., Tomasi, N., Said-Pullicino, D., Arkoun, M., Yvin, J.C., Pinton, R., Zanin, L., et al., 2021a. Characterization of physiological and molecular responses of Zea mays seedlings to different urea-ammonium ratios. Plant Physiol. Biochem. (Issy les Moulineaux, Fr.) 162, 613–623.

Buoso, S., Tomasi, N., Said-Pullicino, D., Arkoun, M., Yvin, J.C., Pinton, R., Zanin, L., 2021b. Responses of hydroponically grown maize to various urea to ammonium ratios: physiological and molecular data. Data Brief 36, 107076.

Buoso, S., Tomasi, N., Arkoun, M., Maillard, A., Jing, L., Marroni, F., Zanin, L., et al., 2021c. Transcriptomic and metabolomic profiles of Zea mays fed with urea and ammonium. Physiol. Plantarum 173, 935–953.

Buoso, S., Zamboni, A., Franco, A., Commisso, M., Guzzo, F., Varanini, Z., Zanin, L., 2022. Nodulating white lupins take advantage of the reciprocal interplay between N and P nutritional responses. Physiol. Plantarum 174 (1), e13607.

Buoso, S., Lodovici, A., Salvatori, N., Tomasi, N., Arkoun, M., Maillard, A., Alberti, G., Peressotti, A., Fabio Marroni, F., Pinton, R., Zanin, L., 2023. Nitrogen nutrition and xylem sap composition in Zea mays: effect of urea, ammonium and nitrate on ionomic and metabolic profile. Plant Sci. 336, 111825.

Cambui, C.A., Svennerstam, H., Gruffman, L., Nordin, A., Ganeteg, U., Näsholm, T., 2011. Patterns of plant biomass partitioning depend on nitrogen source. PLoS One 6, e19211.
Chai, Y.N., Schachtman, D.P., 2022. Root exudates impact plant performance under abiotic stress. Trends Plant Sci. 27 (1), 80–91.
Claussen, W., 2002. Growth, water use efficiency, and proline content of hydroponically grown tomato plants as affected by nitrogen source and nutrient concentration. Plant Soil 247, 199–209.
Criddle, R.S., Ward, M.R., Huffaker, R.C., 1988. Nitrogen uptake by wheat seedlings, interactive effects of four nitrogen sources: NO3−, NO2−, NH4+, and urea. Plant Physiol. 86, 166–175.
Cruz, C., Bio, A.F.M., Domínguez-Valdivia, M.D., Aparicio-Tejo, P.M., Lamsfus, C., Martins-Loução, M.A., 2006. How does glutamine synthetase activity determine plant tolerance to ammonium? Planta 223, 1068–1080.
Daniel-Vedele, F., Dorbe, M.F., Caboche, M., Rouzé, P., 1989. Cloning and analysis of the tomato nitrate reductase-encoding gene: protein domain structure and amino acid homologies in higher plants. Gene 85, 371–380.
Diaz, C., Purdy, S., Christ, A., Morot-Gaudry, J.F., Wingler, A., Masclaux-Daubresse, C., 2005. Characterization of markers to determine the extent and variability of leaf senescence in Arabidopsis. A metabolic profiling approach. Plant Physiol. 138, 898–908.
Du, L., Poovaiah, B.W., 2005. Ca^{2+}/calmodulin is critical for brassinosteroid biosynthesis and plant growth. Nature 437, 741–745.
Duan, F., Giehl, R.F., Geldner, N., Salt, D.E., von Wirén, N., 2018. Root zone–specific localization of AMTs determines ammonium transport pathways and nitrogen allocation to shoots. PLoS Biol. 16, e2006024.
FAOSTAT, 2021. Inorganic Fertilizers 1961–2019, vol. 27. FAOSTAT Analytical Brief Series No, Rome.
Farzadfar, S., Knight, J.D., Congreves, K.A., 2021. Soil organic nitrogen: an overlooked but potentially significant contribution to crop nutrition. Plant Soil 462, 7–23.
Fernández-Crespo, E., Scalschi, L., Llorens, E., García-Agustín, P., Camañes, G., 2015. NH_4^+ protects tomato plants against Pseudomonas syringae by activation of systemic acquired acclimation. J. Exp. Bot. 6621, 6777–6790.
Filiz, E., Akbudak, M.A., 2020. Ammonium transporter 1 (AMT1) gene family in tomato (Solanum lycopersicum L.): bioinformatics, physiological and expression analyses under drought and salt stresses. Genomics 112, 3773–3782.
Forde, B.G., Lea, P.J., 2007. Glutamate in plants: metabolism, regulation, and signaling. J. Exp. Bot. 58, 2339–2358.
Ganz, P., Porras-Murillo, R., Ijato, T., Menz, J., Straub, T., Stührwohldt, N., Neuhäuser, B., et al., 2022. Abscisic acid influences ammonium transport via regulation of kinase CIPK23 and ammonium transporters. Plant Physiol. 190, 1275–1288.
García-Pérez, P., Miras-Moreno, B., Lucini, L., Gallego, P.P., 2021. The metabolomics reveals intraspecies variability of bioactive compounds in elicited suspension cell cultures of three Bryophyllum species. Ind. Crops Prod. 163, 113322.
Garnica, M., Houdusse, F., Zamarreño, A.M., Garcia-Mina, J.M., 2010a. Nitrate modifies the assimilation pattern of ammonium and urea in wheat seedlings. J. Sci. Food Agric. 90, 357–369.
Garnica, M., Houdusse, F., Zamarreño, A.M., Garcia-Mina, J.M., 2010b. The signal effect of nitrate supply enhances active forms of cytokinins and indole acetic content and reduces abscisic acid in wheat plants grown with ammonium. J. Plant Physiol. 167, 1264–1272.
Glibert, P.M., Harrison, J., Heil, C., Seitzinger, S., 2006. Escalating worldwide use of urea–a global change contributing to coastal eutrophication. Biogeochemistry 77, 441–463.
Gu, J., Li, Z., Mao, Y., Struik, P.C., Zhang, H., Liu, L., Yang, J., 2018. Roles of nitrogen and cytokinin signals in root and shoot communications in maximizing of plant productivity and their agronomic applications. Plant Sci. 274, 320–331.
Gutiérrez, R.A., Gifford, M.L., Poultney, C., Wang, R., Shasha, D.E., Coruzzi, G.M., Crawford, N.M., 2007. Insights into the genomic nitrate response using genetics and the Sungear Software System. J. Exp. Bot. 58 (9), 2359–2367.
Gutiérrez, R.A., 2012. Systems biology for enhanced plant nitrogen nutrition. Science 336, 1673–1675.
Hachiya, T., Sakakibara, H., 2017. Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. J. Exp. Bot. 68, 2501–2512.
Han, C., Wang, L., Lyu, J., Shi, W., Yao, L., Fan, M., Bai, M.Y., 2023. Brassinosteroid signaling and molecular crosstalk with nutrients in plants. J Gen Genom. 50, 541–553.
Hawkins, C., Ginzburg, D., Zhao, K., Dwyer, W., Xue, B., Xu, A., et al., 2021. Plant Metabolic Network 15: a resource of genome-wide metabolism databases for 126 plants and algae. J. Integr. Plant Biol. 63, 1888–1905.
Heeb, A., Lundegårdh, B., Ericsson, T., Savage, G.P., 2005. Nitrogen form affects yield and taste of tomatoes. J. Sci. Food Agric. 85, 1405–1414.
Horchani, F., Hajri, R., Aschi-Smiti, S., 2010. Effect of ammonium or nitrate nutrition on photosynthesis, growth, and nitrogen assimilation in tomato plants. J. Plant Nutr. Soil Sci. 173, 610–617.
Hu, C.A., Delauney, A.J., Verma, D.P., 1992. A bifunctional enzyme (delta 1-pyrroline-5-carboxylate synthetase) catalyzes the first two steps in proline biosynthesis in plants. Proc. Natl. Acad. Sci. 89, 9354–9358.
Huang, Y., Wang, H., Zhu, Y., Huang, X., Li, S., Wu, X., Zhao, Y., Bao, Z., Qin, L., Jin, Y., Cui, Y., Ma, G., Xiao, Q., Wang, Q., Wang, J., Yang, X., Liu, H., Lu, X., Larkins, B.A., Wang, W., Wu, Y., 2022. THP9 enhances seed protein content and nitrogen-use efficiency in maize. Nature 612, 292–300.
IFA Short-Term Fertilizer Outlook 2022 – 2023. IFA Market Intelligence Service. https://www.ifastat.org/databases.
Ikeda, H., Tan, X., 1998. Urea as an organic nitrogen source for hydroponically grown tomatoes in comparison with inorganic nitrogen sources. J. Soil Sci. Plant Nutr. 44, 609–615.
Ikeda, M., Takeshi, K., Nobuhisa, K., 2004. Carbon skeletons for amide synthesis during ammonium nutrition in tomato and wheat roots. Soil Sci. Plant Nutr. 50, 141–147.
Ikeda, M., Ueda, S., Yamakawa, T., 2005. Preferential provision of carbon skeletons for amide synthesis during ammonium nutrition in the roots of various plants. In: Li, C. J., et al. (Eds.), Plant Nutrition for Food Security, Human Health and Environmental Protection. Tsinghua University Press, Beijing, pp. 190–191.
Ishiyama, K., Inoue, E., Tabuchi, M., Yamaya, T., Takahashi, H., 2004a. Biochemical background and compartmentalized functions of cytosolic glutamine synthetase for active ammonium assimilation in rice roots. Plant Cell Physiol. 45, 1640–1647.
Ishiyama, K., Inoue, E., Watanabe-Takahashi, A., Obara, M., Yamaya, T., Takahashi, H., 2004b. Kinetic properties and ammonium-dependent regulation of cytosolic isoenzymes of glutamine synthetase in Arabidopsis. J. Biol. Chem. 279 (16), 16598–16605.
Khalil, S., Strah, R., Lodovici, A., Vojta, P., Berardinis, F.D., Ziegler, J., et al., 2024. The activation of iron deficiency responses of grapevine rootstocks is dependent to the availability of the nitrogen forms. BMC Plant Biol. 24, 218.
Kiba, T., Kudo, T., Kojima, M., Sakakibara, H., 2011. Hormonal control of nitrogen acquisition: roles of auxin, abscisic acid, and cytokinin. J. Exp. Bot. 62, 1399–1409.
Kiba, T., Krapp, A., 2016. Plant nitrogen acquisition under low availability: regulation of uptake and root architecture. Plant Cell Physiol. 57, 707–714.
Koga, N., Ikeda, M., 2000. Enhancement of glucose utilization in provision of carbon skeletons for ammonium assimilation in wheat roots. Fae. Agr. Kyushu Univ. 45, 7–13.
Kojima, S., 2007. AtDUR3 represents the major transporters for high-affinity urea transport across the plasma membrane of nitrogen-deficient Arabidopsis roots: high-affinity urea uptake in plants. Plant J. 52, 30–40.
Krouk, G., 2016. Hormones and nitrate: a two-way connection. Plant Mol. Biol. 91, 599–606.
Krouk, G., Ruffel, S., Gutiérrez, R.A., Gojon, A., Crawford, N.M., Coruzzi, G.M., Lacombe, B., 2011. A framework integrating plant growth with hormones and nutrients. Trends Plant Sci. 16, 178–182.
Kováčik, J., Klejdus, B., Bačkor, M., Repčák, M., 2007. Phenylalanine ammonia-lyase activity and phenolic compounds accumulation in nitrogen-deficient Matricaria chamomilla leaf rosettes. Plant Sci. 172, 393–399.
Lacuesta, M., Saiz-Fernández, I., Podlešáková, K., Miranda-Apodaca, J., Novák, O., Doležal, K., De Diego, N., 2018. The trans and cis zeatin isomers play different roles in regulating growth inhibition induced by high nitrate concentrations in maize. Plant Growth Regul. 85, 199–209.
Lam, H.M., Coschigano, K.T., Oliveira, I.C., Melo-Oliveira, R., Coruzzi, G.M., 1996. The molecular genetics of nitrogen assimilation into amino acids in higher plants. Annu. Rev. Plant Biol. 47, 569–593.
Lamig, L., Moreno, S., Álvarez, J.M., Gutiérrez, R.A., 2022. Molecular mechanisms underlying nitrate responses in plants. Curr. Biol. 32, 433–439.
Lauter, F.R., 1996. Preferential expression of an ammonium transporter and of two putative nitrate transporters in root hairs of tomato. Proc. Natl. Acad. Sci. 93, 8139.
Leja, M., Wyżgolik, G., Mareczek, A., 2010. Phenolic compounds of red cabbage as related to different forms of nutritive nitrogen. Folia Hort. Ann. 22, 19–24.
Li, Y., Krouk, G., Coruzzi, G.M., Ruffel, S., 2014. Finding a nitrogen niche: a systems integration of local and systemic nitrogen signalling in plants. J. Exp. Bot. 65, 5601–5610.
Liu, G.W., Sun, A.L., Li, D.Q., Athman, A., Gilliham, M., Liu, L.H., 2015. Molecular identification and functional analysis of a maize (Zea mays) DUR3 homolog that transports urea with high affinity. Planta 241, 861–874.
Liu, Y., von Wirén, N., 2017. Ammonium as a signal for physiological and morphological responses in plants. J. Exp. Bot. 68, 2581–2592.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods 25, 402–408.
Lupini, A., Princi, M.P., Araniti, F., Miller, A.J., Sunseri, F., Abenavoli, M.R., 2017. Physiological and molecular responses in tomato under different forms of N nutrition. J. Plant Physiol. 216, 17–25.
Mandal, S., Ghorai, M., Anand, U., Samanta, D., Kant, N., Mishra, T., Dey, A., et al., 2022. Cytokinin and abiotic stress tolerance-What has been accomplished and the way forward? Front. Genet. 13.
Marschner, P., 2012. Marschner's Mineral Nutrition of Higher Plants, third ed. Academic Press, London; Waltham, MA.
Mérigout, P., Gaudon, V., Quilleré, I., Briand, X., Daniel-Vedele, F., 2008a. Urea use efficiency of hydroponically grown maize and wheat. J. Plant Nutr. 31, 427–443.
Mérigout, P., Lelandais, M., Bitton, F., Renou, J.P., Briand, X., Meyer, C., Daniel-Vedele, F., 2008b. Physiological and transcriptomic aspects of urea uptake and assimilation in Arabidopsis plants. Plant Physiol. 147, 1225–1238.
Metsalu, T., Vilo, J., 2015. ClustVis: a web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. Nucleic Acids Res. 43, 566–570.
Muñoz, A., Piedras, P., Aguilar, M., Pineda, M., 2001. Urea is a product of ureidoglycolate degradation in chickpea. Purification and characterization of the ureidoglycolate urea-lyase. Plant Physiol. 125, 828–834.
O'Brien, J.A., Vega, A., Bouguyon, E., Krouk, G., Gojon, A., Coruzzi, G., Gutiérrez, R.A., 2016. Nitrate transport, sensing, and responses in plants. Mol. Plant 9, 837–856.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2014. Vegan: Community ecology package. R Package Version 2, 2, 0. http://CRAN.Rproject.org/package=vegan.

Omara, P., Aula, L., Oyebiyi, F., Raun, W.R., 2019. World cereal nitrogen use efficiency trends: review and current knowledge. Agrosyst. geosci. environ. 2, 1–8.
Ozanne, P.G., 1955. The effect of nitrogen on zinc deficiency in subterranean clover. Aust. J. Biol. 8, 47–55.
Pal, V., Singh, G., Dhaliwal, S.S., 2019. Agronomic biofortification of chickpea with zinc and iron through application of zinc and urea. Commun. Soil Sci. Plant Anal. 50, 1864–1877.
Paul, K., Sorrentino, M., Lucini, L., Rouphael, Y., Cardarelli, M., Bonini, P., et al., 2019. A combined phenotypic and metabolomic approach for elucidating the biostimulant action of a plant-derived protein hydrolysate on tomato grown under limited water availability. Front. Plant Sci. 10.
Peet, M.M., Raper Jr, C.D., Tolley, L.C., Robarge, W.P., 1985. Tomato responses to ammonium and nitrate nutrition under controlled root-zone pH. J. Plant Nutr. 8, 787–798.
Pérez-Alfocea, F., Estan, M.T., Cruz, A.S., Bolarin, M.C., 1993. Effects of salinity on nitrate, total nitrogen, soluble protein and free amino add levels in tomato plants. J. Hortic. Sci. 68, 1021–1027.
Pinto, R.C., 2017. Chemometrics methods and strategies in metabolomics. Metabolomics: from Fundamentals to Clinical Applications, pp. 163–190.
Pinton, R., Cesco, S., De Nobili, M., Santi, S., Varanini, Z., 1997. Water-and pyrophosphate-extractable humic substances fractions as a source of iron for Fe-deficient cucumber plants. Biol. Fertil. Soils 26, 23–27.
Qiqige, S., Jia, L., Qin, Y., Chen, Y., Fan, M., 2017. Effects of different nitrogen forms on potato growth and development. J. Plant Nutr. 40, 1651–1659.
Ravazzolo, L., Trevisan, S., Forestan, C., Varotto, S., Sut, S., Dall'Acqua, S., et al., 2020. Nitrate and ammonium affect the overall maize response to nitrogen availability by triggering specific and common transcriptional signatures in roots. Int. J. Mol. Sci. 21 (2), 686.
Riveras, E., Alvarez, J.M., Vidal, E.A., Oses, C., Vega, A., Gutiérrez, R.A., 2015. The calcium ion is a second messenger in the nitrate signaling pathway of Arabidopsis. Plant Physiol. 169, 1397–1404.
R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project.org/.
Raun, W.R., Johnson, G.V., 1999. Improving nitrogen use efficiency for cereal production. J. Agron. 91, 357–363.
Rehman, A.U., Bashir, F., Ayaydin, F., Kóta, Z., Páli, T., Vass, I., 2021. Proline is a quencher of singlet oxygen and superoxide both in in vitro systems and isolated thylakoids. Physiol. Plantarum 172, 7–18.
Ritz, C., Spiess, A.-N., 2008. qpcR: an R package for sigmoidal model selection in quantitative real-time polymerase chain reaction analysis. Bioinformatics 24, 1549–1551.
Safdarian, M., Razmjoo, J., Dehnavi, M.M., 2014. Effect of nitrogen sources and rates on yield and quality of silage corn. J. Plant Nutr. 37, 611–617.
Sakakibara, H., 2003. Nitrate-specific and cytokinin-mediated nitrogen signaling pathways in plants. J. Plant Res. 116, 253–257.
Sakakibara, H., Takei, K., Hirose, N., 2006. Interactions between nitrogen and cytokinin in the regulation of metabolism and development. Trends Plant Sci. 11, 440–448.
Salek, R.M., Neumann, S., Schober, D., Hummel, J., Billiau, K., Kopka, J., et al., 2015. COordination of Standards in MetabOlomicS (COSMOS): facilitating integrated metabolomics data access. Metabolomics 11, 1587–1597.
Schäfer, M., Brütting, C., Meza-Canales, I.D., Großkinsky, D.K., Vankova, R., Baldwin, I. T., Meldau, S., 2015. The role of cis-zeatin-type cytokinins in plant growth regulation and mediating responses to environmental interactions. J. Exp. Bot. 66 (16), 4873–4884.
Stitt, M., 1999. Nitrate regulation of metabolism and growth. Curr. Opin. Plant Biol. 2, 178–186.
Takei, K., Sakakibara, H., Taniguchi, M., Sugiyama, T., 2001. Nitrogen-dependent accumulation of cytokinins in root and thetranslocation to leaf: implication of cytokinin species that induces geneexpression of maize responseregulator. Plant Cell Physiol. 42, 85–93.
Takei, K., Ueda, N., Aoki, K., Kuromori, T., Hirayama, T., Shinozaki, K., Sakakibara, H., et al., 2004. AtIPT3 is a key determinant of nitrate-dependent cytokinin biosynthesis in Arabidopsis. Plant Cell Physiol. 45, 1053–1062.
Tan, X.W., Ikeda, H., Oda, M., 2000. The absorption, translocation, and assimilation of urea, nitrate or ammonium in tomato plants at different plant growth stages in hydroponic culture. Sci. Hortic. 84 (3–4), 275–283.
Tsay, Y.F., Ho, C.H., Chen, H.Y., Lin, S.H., 2011. Integration of nitrogen and potassium signaling. Annu. Eev. Plant Biol 62, 207–226.
Ueda, S., Ikeda, M., Yamakawa, T., 2008. Provision of carbon skeletons for amide synthesis in non-nodulated soybean and pea roots in response to the source of nitrogen supply. Soil Sci. Plant Nutr. 54 (5), 732–737.
Ueda, Y., Konishi, M., Yanagisawa, S., 2017. Molecular basis of the nitrogen response in plants. Soil Sci. Plant Nutr. 63, 329–341.
United Nations, 2015. Transforming Our World: the 2030 Agenda for Sustainable Development. https://sustainabledevelopment.un.org. (Accessed 10 April 2023).
USEPA, 1995. EPA Method 3052: microwave assisted acid digestion of siliceous and organically based matrices. In: Test Methods for Evaluating Solid Waste, third ed. Washington, DC.
Vega, A., O'Brien, J.A., Gutiérrez, R.A., 2019. Nitrate and hormonal signalling crosstalk for plant growth and development. Curr. Opin. Plant Biol. 52, 155–163.
Verbruggen, N., Hermans, C., 2008. Proline accumulation in plants: a review. Amino Acids 35, 753–759.
Vidal, E.A., Álvarez, J.M., Moyano, T.C., Gutiérrez, R.A., 2015. Transcriptional networks in the nitrate response of Arabidopsis thaliana. Curr. Opin. Plant Biol. 27, 125–132.
Vidal, E.A., Alvarez, J.M., Araus, V., Riveras, E., Brooks, M.D., Krouk, G., Gutiérrez, R.A., et al., 2020. Nitrate in 2020: thirty years from transport to signaling networks. Plant Cell 32, 2094–2119.
Viets Jr, F.G., Boawn, L.C., Crawford, C.L., 1957. The effect of nitrogen and types of nitrogen carrier on plant uptake of indigenous and applied zinc. Soil Sci. Soc. Am. J. 21, 197–201.
Wang, L., Macko, S.A., 2011. Constrained preferences in nitrogen uptake across plant species and environments. Plant Cell Environ. 34, 525–534.
Wang, W.-H., 2008. Molecular and physiological aspects of urea transport in higher plants. Plant Sci. 175, 467–477.
Wang, W.H., Köhler, B., Cao, F.Q., Liu, G.W., Gong, Y.Y., Sheng, S., Liu, L.H., 2012. Rice DUR3 mediates high-affinity urea transport and plays an effective role in improvement of urea acquisition and utilization when expressed in Arabidopsis. New Phytol. 193, 432–444.
Watanabe, S., Matsumoto, M., Hakomori, Y., Takagi, H., Shimada, H., Sakamoto, A., 2014. The purine metabolite allantoin enhances abiotic stress tolerance through synergistic activation of abscisic acid metabolism. Plant Cell Environ. 37, 1022–1036.
Wei, Z., Li, J., 2020. Regulation of brassinosteroid homeostasis in higher plants. Front. Plant Sci. 11, 583622.
Xun, Z., Guo, X., Li, Y., Wen, X., Wang, C., Wang, Y., 2020. Quantitative proteomics analysis of tomato growth inhibition by ammonium nitrogen. Plant Physiol. Biochem. 154, 129–141.
Yang, X.S., Wu, J., Ziegler, T.E., Yang, X., Zayed, A., Rajani, M.S., et al., 2011. Gene expression biomarkers provide sensitive indicators of in planta nitrogen status in maize. Plant Physiol. 157, 1841–1852.
Yuan, L., Loque, D., Kojima, S., Rauch, S., Ishiyama, K., Inoue, E., von Wirén, N., 2007. The organization of high-affinity ammonium uptake in Arabidopsis roots depends on the spatial arrangement and biochemical properties of AMT1-type transporters. Plant Cell 19, 2636–2652.
Zanin, L., Tomasi, N., Wirdnam, C., Meier, S., Komarova, N.Y., Mimmo, T., Pinton, R., et al., 2014. Isolation and functional characterization of a high affinity urea transporter from roots of Zea mays. BMC Plant Biol. 14, 1–15.
Zanin, L., Tomasi, N., Zamboni, A., Varanini, Z., Pinton, R., 2015b. The urease inhibitor NBPT negatively affects DUR3-mediated uptake and assimilation of urea in maize roots. Front. Plant Sci. 6, 1007.
Zanin, L., Venuti, S., Tomasi, N., Zamboni, A., De Brito Francisco, R.M., Varanini, Z., Pinton, R., 2016. Short-term treatment with the urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) alters urea assimilation and modulates transcriptional profiles of genes involved in primary and secondary metabolism in maize seedlings. Front. Plant Sci. 7, 845.
Zhang, P., Dumroese, R.K., Pinto, J.R., 2019. Organic or inorganic nitrogen and rhizobia inoculation provide synergistic growth response of a leguminous forb and tree. Front. Plant Sci. 10, 1308.
Zanin, L., Zamboni, A., Monte, R., Tomasi, N., Varanini, Z., Cesco, S., Pinton, R., 2015a. Transcriptomic analysis highlights reciprocal interactions of urea and nitrate for nitrogen acquisition by maize roots. Plant Cell Physiol. 56, 532–548.
Zhang, L., Becker, D.F., 2015. Connecting proline metabolism and signaling pathways in plant senescence. Front. Plant Sci. 6, 552.
Zhang, X., Davidson, E.A., Mauzerall, D.L., Searchinger, T.D., Dumas, P., Shen, Y., 2015. Managing nitrogen for sustainable development. Nature 528, 51–59.