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Elevated CO_2 Induces Root Defensive Mechanisms in Tomato Plants When Dealing with Ammonium Toxicity

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An adequate carbon supply is fundamental for plants to thrive under ammonium stress. In this work, we studied the mechanisms involved in tomato (*Solanum lycopersicum* L.) response to ammonium toxicity when grown under ambient or elevated CO_2 conditions (400 or 800 p.p.m. CO_2). Tomato roots were observed to be the primary organ dealing with ammonium nutrition. We therefore analyzed nitrogen (N) and carbon (C) metabolism in the roots, integrating the physiological response with transcriptomic regulation. Elevated levels of CO_2 preferentially stimulated root growth despite the high ammonium content. The induction of anaplerotic enzymes from the tricarboxylic acid (TCA) cycle led to enhanced amino acid synthesis under ammonium nutrition. Furthermore, the root transcriptional response to ammonium toxicity was improved by CO_2-enriched conditions, leading to higher expression of stress-related genes, as well as enhanced modulation of genes related to signaling, transcription, transport and hormone metabolism. Tomato roots exposed to ammonium stress also showed a defense-like transcriptional response according to the modulation of genes related to detoxification and secondary metabolism, involving principally terpenoid and phenolic compounds. These results indicate that increasing C supply allowed the co-ordinated regulation of root defense mechanisms when dealing with ammonium toxicity.

Keywords: Nitrogen assimilation • Pathogenesis • Secondary metabolism • *Solanum lycopersicum* • Stress • Transcriptome.

Abbreviations: ABC, ATP-binding cassette; ACC, 1-aminocyclopropane-1-carboxylate; AS, asparagine synthetase; CA, carbonic anhydrase; CYP, cytochrome P450 monooxygenases; FDR, false discovery rate; GABA, γ-aminobutyric acid; GDH, glutamate dehydrogenase; GOGAT, glutamate synthase; Grx, glutaredoxin; GS, glutamine synthetase; GST, glutathione S-transferase; GT, glycosyltransferase; ICDH, isocitrate dehydrogenase; ICL, isocitrate lyase; LRR, leucine-rich repeat; MDH, malate dehydrogenase; MS, malate synthase; NAD-ME, NAD-malic enzyme; NADP-ME, NADP-malic enzyme; NRT, nitrate transporter; PAL, phenylalanine ammonia lyase; PEPC, phosphoenolpyruvate carboxylase; PR, pathogenesis-related; RLK, reeptor-like protein kinase; ROS, reactive oxygen species; TCA, tricarboxylic acid.

Introduction

Plants can absorb inorganic nitrogen (N) from soil in the form of nitrate (NO_3^-) or ammonium (NH_4^+), although crops are generally used to nitric or mixed nutrition. However, several environmental problems can arise from the use of nitric fertilizers, leading to water, soil and air pollution caused by nitrate leaching or nitrogenous gas emissions (Xu et al. 2016). In order to avoid these negative effects, the use of ammonium-based fertilizers has been proposed as an alternative practice in agriculture, even though it may induce a change in nutrition and metabolism which the plant will have to overcome. In general, NH_4^+ should be the preferred N source for plants, as it requires less energy to absorb and is a key intermediate in many metabolic reactions. Nevertheless, when ammonium is the unique N source it can prove toxic to the growth of most plants (Britto and Kronzucker 2002). The symptoms displayed by the plants are collectively known as 'ammonium syndrome' and include leaf chlorosis, changes in root:shoot ratio, a decrease in net photosynthesis, cation uptake inhibition and rhizosphere acidification; all together they provoke a decrease in plant yield (Britto and Kronzucker 2002, Esteban et al. 2016, Liu and von Wirén 2017). However, the appearance of these symptoms of toxicity depends on a certain ammonium threshold, which varies between species and even cultivars (Cruz et al. 2011, Sarasketa et al. 2014), and also depends on environmental growth conditions, such as CO_2 atmospheric concentration (Vega-Mas et al. 2015, Rubio-Asensio and Bloom 2016) or pH (Sarasketa et al. 2016, F. Wang et al. 2016). In natural soils, ammonium concentration can increase due to atmospheric N deposits in the form of NH_3 and cause shifts in vegetation patterns (Xu et al. 2016). In addition, such a high N input can lead to soil acidification, exacerbating the deleterious effects of ammonium toxicity (Bobbink et al. 2010, Fowler et al. 2013).

Many efforts have attempted to reveal the physiological and molecular mechanisms underlying ammonium toxicity, but

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there is no general consensus regarding which traits are responsible for plant tolerance to ammonium (Britto and Kronzucker 2002, Bittsánszky et al. 2015, Esteban et al. 2016). The role played by the roots as the first ammonium-responsive organ has been highlighted in the literature. Indeed, ammonium nutrition leads to changes in root system development (Rogato et al. 2010, Giehl and von Wirén 2014, Li et al. 2014) and in the primary metabolism of this organ (Setién et al. 2014), which, together with ammonium flux/efflux through root cells, has been shown to be essential for plant response under ammonium stress (Coskun et al. 2013, Esteban et al. 2016). Indeed, the root's ability to store NH_4^+, presumably in the vacuole, is said to be a good strategy for maintaining cytosolic NH_4^+ homeostasis, while simultaneously preventing its transport to the shoots, where the presence of NH_4^+ is more critical (Britto and Kronzucker 2002). At the same time, the efficient assimilation of NH_4^+ into amino acids, primarily through glutamine synthetase (GS) activity, is also necessary to confer tolerance to the plant (Guan et al. 2016). Ammonium assimilation is closely linked to carbon (C) metabolism, since the supply of organic acids, maintained by the tricarboxylic acid (TCA) cycle, is indispensable in amino acid synthesis (Gauthier et al. 2010, Sweetlove et al. 2010). Thus, a high availability of carbon skeletons is essential for the plant to deal with ammonium nutrition. In this sense, an increase in ammonium tolerance was reported after adding inorganic C to the root zone (Viktor and Cramer 2003, Bialczyk et al. 2005, Roosta and Schjoerring 2008) or by increasing irradiance to stimulate photosynthesis (Setién et al. 2013).

Enriching atmospheric CO_2 has also been evaluated as an environmental growth condition that could potentially alleviate ammonium toxicity (Vega-Mas et al. 2015, Rubio-Asensio and Bloom 2016). Indeed, atmospheric CO_2 concentration has been increasing at an accelerated rate in the recent centuries due to anthropogenic activities, and its rise is predicted to continue (IPCC 2014). Several studies have therefore focused on this issue with respect to plant growth, development and crop productivity (Ainsworth and Rogers 2007, Reddy et al. 2010, Xu et al. 2014, Coskun et al. 2016). The increased carboxylation rate of Rubisco under CO_2-enriched conditions enhances the net photosynthetic rate, at least in the short term; while long-term exposure can be associated with photosynthetic acclimation, usually in relation to a N limitation (Ainsworth and Rogers 2007, Vicente et al. 2016). Given that plants need to absorb and assimilate enough N to maintain their dry weight increase, N availability is key in developing sinks for increased photoassimilate formation and, therefore, produces enhanced growth under elevated CO_2 conditions (Ainsworth and Rogers 2007).

Moreover, the form of the applied N (nitrate or ammonium source) influences the plant's metabolic adaptation to elevated CO_2 concentrations, although the precise response to this adaptation varies between species and for different growth conditions (Sato and Yanagisawa 2014, Takatani et al. 2014, Noguchi et al. 2015, Coskun et al. 2016, Rubio-Asensio and Bloom 2016). In the case of tomato (cv. Agora Hybrid F1), improved plant growth due to elevated levels of CO_2 has been reported previously (Vega-Mas et al. 2015). However, the authors showed that the plant's specific response to elevated CO_2 levels depended on the N source and dose supplied. For instance, at a moderate NH_4^+ supply, stomatal closure at elevated CO_2 concentrations prevented NH_4^+ accumulation in tissue, so the tomato plant did not display any symptoms of toxicity in terms of biomass production. In contrast, with a high NH_4^+ supply, whole-plant biomass was restricted, providing evidence of toxic conditions (Vega-Mas et al. 2015). Nevertheless, the precise mechanisms used to cope with ammonium stress have not yet been studied.

In this context, the present work was conceived under the hypothesis that the metabolic processes which handle ammonium toxicity depend on carbon availability, especially at the root level, since this organ is apparently of vital importance when plants are under ammonium stress. To study this theory, we grew Agora Hybrid F1 tomato plants under a toxic ammonium supply (15 mM) and at two different concentrations of atmospheric CO_2 (400 and 800 p.p.m.). These were compared with plants grown with 15 mM nitrate concentration. An integrative approach was followed, first measuring plant biomass and NH_4^+ content, and then determining the root N and C metabolites and enzyme activities. Lastly, a transcriptomic analysis of the tomato root was carried out in order to investigate the complexity of molecular events underlying its response to ammonium nutrition and elevated CO_2.

Results

Tomato plant biomass production and NH_4^+ content

The shoots of tomato plants grown with a high dose of ammonium (15 mM) as the N source produced less biomass than those grown with nitrate, at both ambient (400 p.p.m.) and elevated (800 p.p.m.) levels of atmospheric CO_2 (**Fig. 1A**). The root biomass was also reduced under ammonium nutrition at ambient CO_2 conditions. At elevated CO_2, in contrast, ammonium-fed root biomass equalled that of nitrate-fed roots (**Fig. 1A**).

A greater accumulation of NH_4^+ was observed in the roots of plants grown under ammonium nutrition compared with plants grown on nitrate under both CO_2 regimes (**Fig. 1B**). Furthermore, at elevated CO_2, the NH_4^+ content even increased in the roots of plants grown with ammonium and it also accumulated in the leaves (**Fig. 1B**). Allocating biomass to the roots under conditions of high C availability (800 p.p.m. CO_2) indicated that the plant was investing lots of resources in this organ that eventually allowed high NH_4^+ accumulation. To understand the mechanism used by the root to face ammonium stress, we explored the metabolic and transcriptomic responses of this organ further.

C and N metabolism in tomato roots

The predominant amino acids in the roots were asparagine (Asn), glutamine (Gln) and glutamate (Glu) (**Fig. 2A**; Supplementary Table S1). High levels of Asn and Gln accumulated under ammonium nutrition (**Fig. 2A**). In contrast, Glu

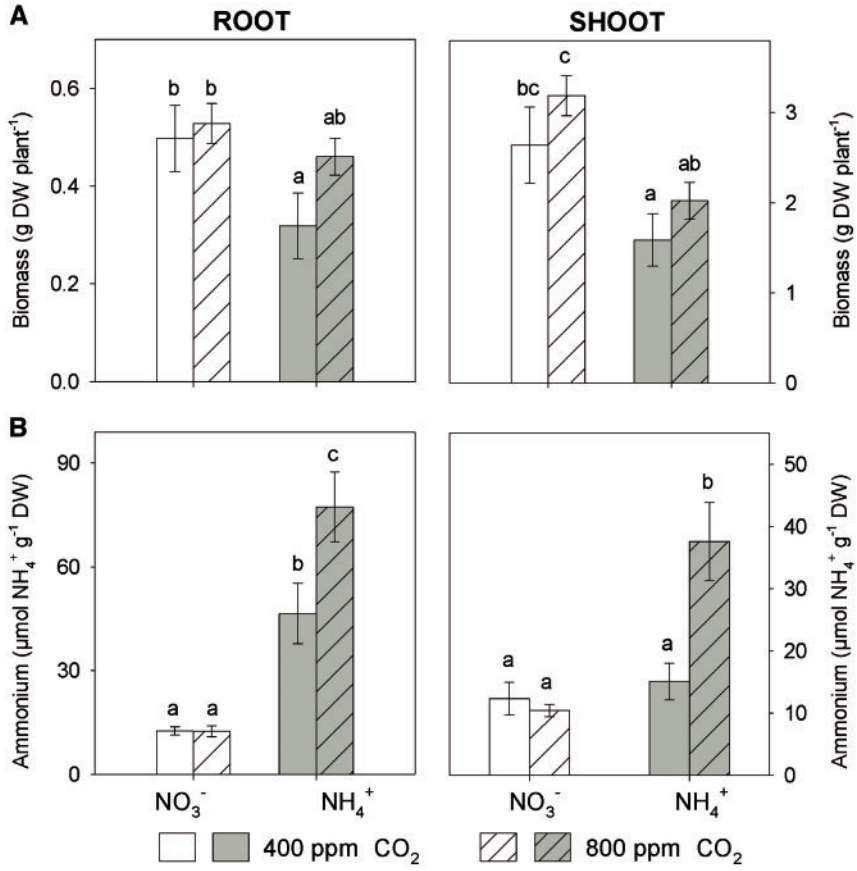

Fig. 1 Biomass and ammonium contents in tomato plants. (A) Root and shoot (leaves and stem) biomass and (B) root and leaf ammonium content in tomato plants grown under nitrate (white bars) or ammonium (gray bars) nutrition at ambient (400 p.p.m., plain bars) or elevated (800 p.p.m., striped bars) CO_2 concentration. Data represent mean values ± SE ($n = 9$). Letters represent significant differences between treatments analyzed by Duncan's test ($P < 0.05$).

levels remained the same regardless of N and CO_2 conditions (**Fig. 2A**). In general, total amino acid content was higher in ammonium-fed roots with respect to nitrate-fed roots for both CO_2 concentrations (**Fig. 2B**). The soluble protein content did not reveal any differences between the CO_2 and N treatments (**Fig. 2C**). Both cytosolic (GS1) and plastidic (GS2) glutamine synthetase (GS) polypeptides were detected in the tomato roots, although GS1 was the predominant isoform (Supplementary Fig. S1). Neither the activity (**Fig. 2D**) nor the protein content of GS showed any difference between the N and CO_2 treatments. In contrast, GDH activity was significantly higher under ammonium nutrition, but only at elevated CO_2 (**Fig. 2E**). A single GDH polypeptide was detected in the tomato roots, remaining practically constant independent of the growing conditions (Supplementary Fig. S1).

The soluble carbohydrate content in the roots was higher under ammonium nutrition compared with nitrate nutrition at ambient CO_2 (**Fig. 3A**) as a result of increased fructose content (Supplementary Table S2). The starch content was lower in the roots of ammonium-fed plants grown at elevated levels of CO_2 with respect to ambient conditions (**Fig. 3B**). Regarding TCA anaplerotic enzymes (**Fig. 3C–G**), malate dehydrogenase (MDH), NADP-malic enzyme (NADP-ME) and isocitrate dehydrogenase (ICDH) activities were induced in ammonium-fed roots at elevated CO_2. In nitrate-fed roots, on the other hand, ICDH was induced at ambient CO_2.

Root transcriptomic analysis

Changes in the whole transcriptome of roots from plants grown under different N sources and CO_2 concentrations were analyzed by a significance-based comparison [false discovery rate (FDR < 0.05)]. The number of genes whose expression was altered based on different growth conditions was represented with Venn diagrams (**Fig. 4A, B**).

Genes differentially expressed in response to atmospheric CO_2 concentration. The genes differentially modulated by CO_2 within each N nutrition were classified into functional categories modified with MapMan software (Supplementary Tables S3, S4). Twenty-seven root genes changed their levels of expression in response to different atmospheric CO_2 conditions; nine were observed under nitrate nutrition and 19 under ammonium nutrition (**Fig. 4A**). Of the 27 genes regulated by CO_2, only one was common to both N sources (**Fig. 4A**) and encoded a protein of unknown function (Solyc11g056660.1.1) (Supplementary Tables S3, S4). In ammonium-fed roots, six out of the 19 genes that responded to the change in CO_2 conditions belonged to the functional category of transporters (Supplementary Table S4). Out of these six genes, three corresponded to nitrate

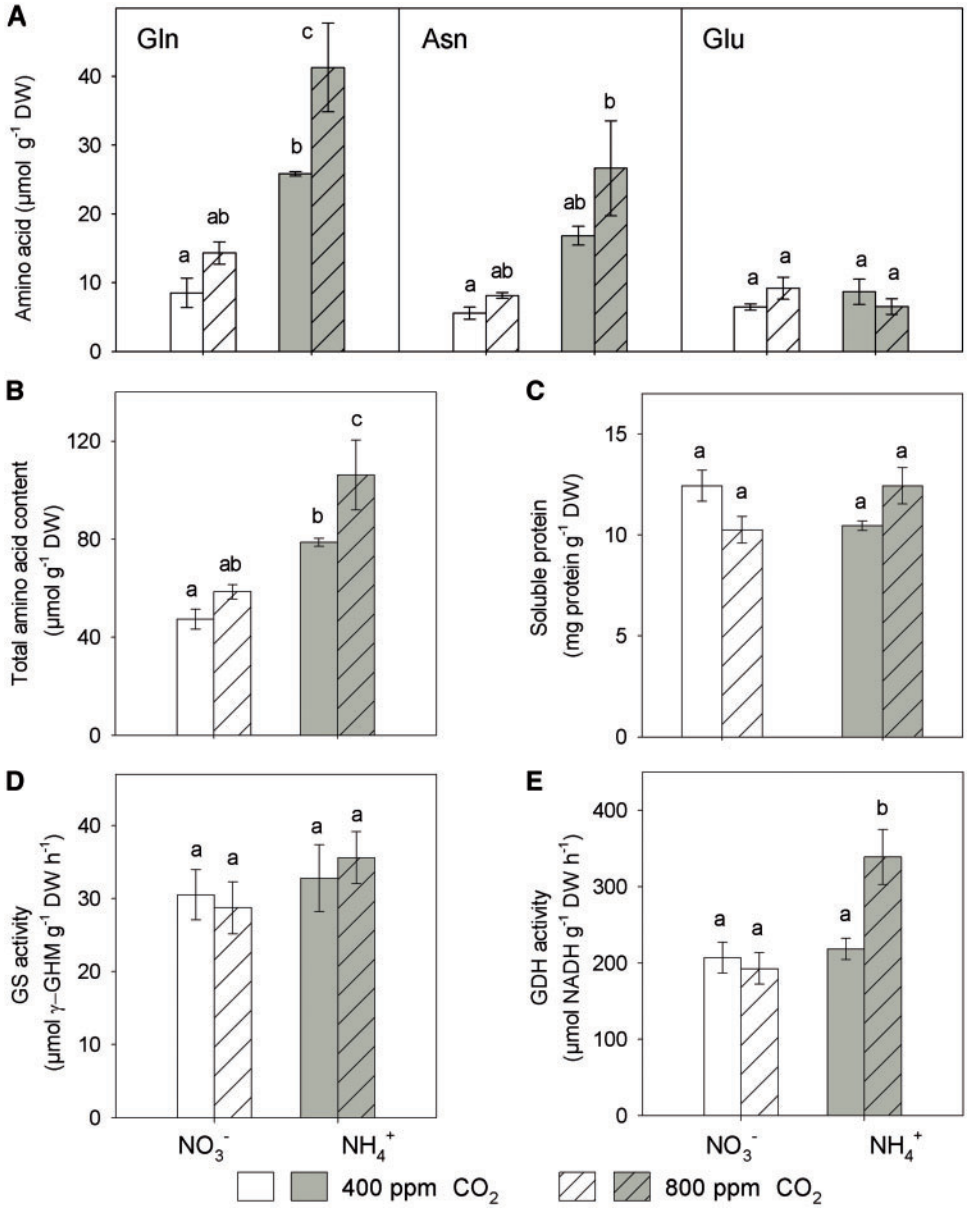

Fig. 2 Metabolites and enzyme activities related to N metabolism in tomato roots. (A) Individual contents of Gln, Asn and Glu, (B) total amino acid content, (C) soluble protein content, (D) glutamine synthetase (GS) activity and (E) glutamate dehydrogenase (GDH) activity in roots of tomato plants grown under nitrate (white bars) or ammonium (gray bars) nutrition at ambient (400 p.p.m., plain bars) or elevated (800 p.p.m., striped bars) CO_2 concentration. Values represent the mean ± SE (n = 3–9). Letters represent significant differences between treatments analyzed by Duncan's test ($P < 0.05$).

transporters (Solyc11g069740.1.1 encoding NRT2.3, and Solyc11g069760.1.1 and Solyc06g010250.2.1 encoding NRT2.4) and were less expressed under elevated levels of CO_2.

Genes differentially expressed in response to N source. The response of the root transcriptome to the N source was greatly modulated at elevated CO_2 concentrations compared with atmospheric CO_2 (**Fig. 4B**). In fact, the expression of just 42 genes was affected by the N source at ambient CO_2, while 585 genes were differentially expressed at elevated levels of CO_2. Of all these genes, only 34 were commonly modulated by the N source under both CO_2 regimes tested, and all of them changed in the same direction at both CO_2 concentrations. For most of the commonly expressed genes, the magnitude of the change was higher at 800 p.p.m. CO_2 than at 400 p.p.m. CO_2. Interestingly, among the 34 common genes, 15 appeared in the list of genes most differentially expressed by ammonium at elevated CO_2 levels (**Table 1**, indicated with an asterisk). This applies to the genes encoding vetispiradiene synthase 1-like (Solyc01g101190.2.1), abscisic stress-ripening protein 2-like (Solyc04g071580.2.1) or carbonic anhydrase (CA; Solyc02g067750.2.1), which showed much higher expression under ammonium nutrition. On the other hand, two genes encoding nitrate NRT2.4 and NRT2.3 transporters (Solyc11g069760.1.1 and Solyc11g069740.1.1, respectively) were the least expressed in response to ammonium as the only source of N.

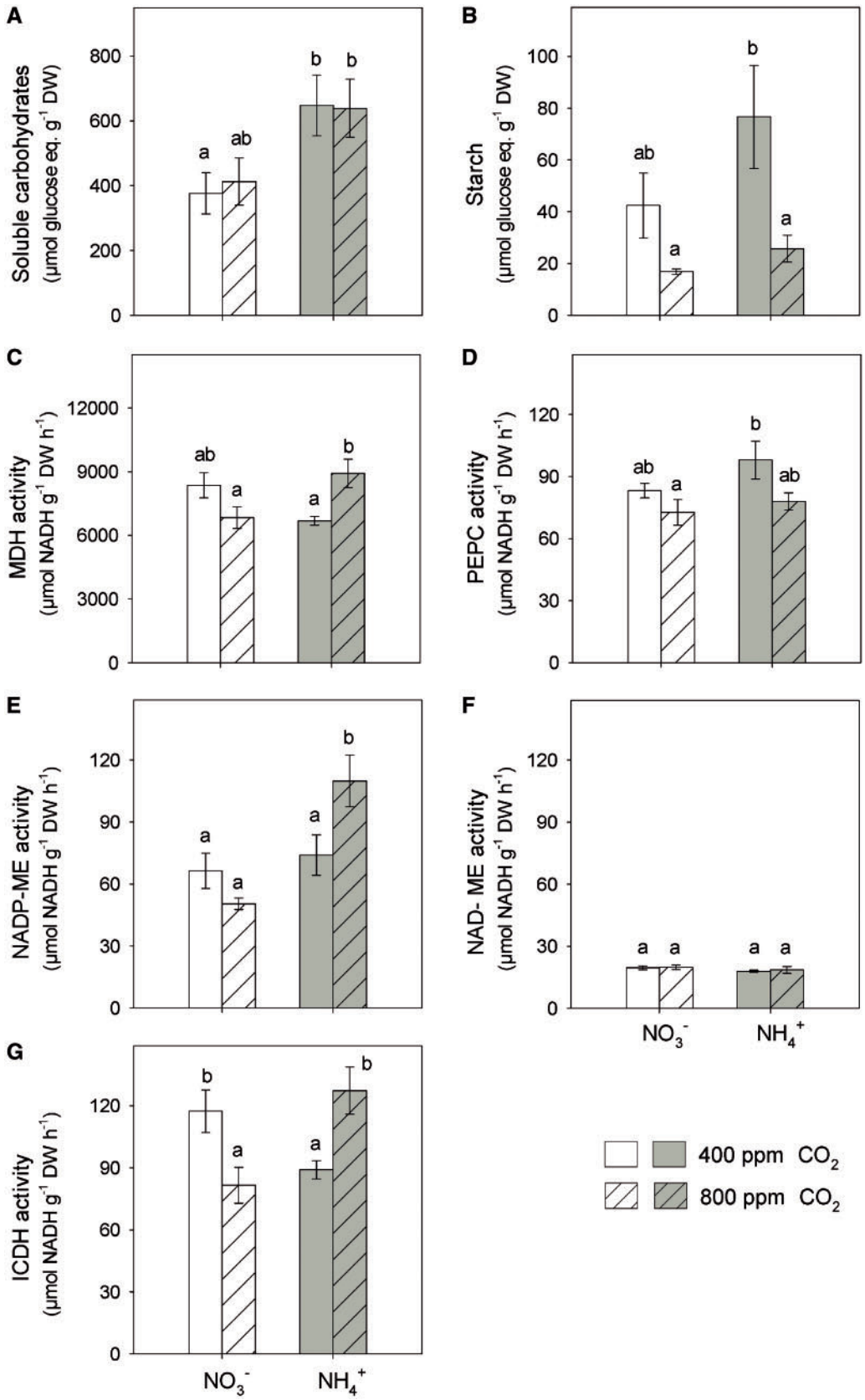

Fig. 3 Carbohydrates, starch and TCA anaplerotic enzyme activities in tomato roots. (A) Soluble carbohydrates and (B) starch content (expressed as glucose equivalents), and activities of (C) phosphoenolpyruvate carboxylase (PEPC), (D) malate dehydrogenase (MDH), (E) NADP-malic enzyme (NADP-ME), (F) NAD-malic enzyme (NAD-ME) and (G) isocitrate dehydrogenase (IDCH) in roots of tomato plants grown under nitrate (white bars) or ammonium (gray bars) nutrition at ambient (400 p.p.m., plain bars) or elevated (800 p.p.m., striped bars) CO_2 concentration. Values represent the mean ± SE ($n = 9$). Statistical analysis was as described in **Fig. 1**. Letters represent significant differences between treatments analyzed by Duncan's test ($P < 0.05$).

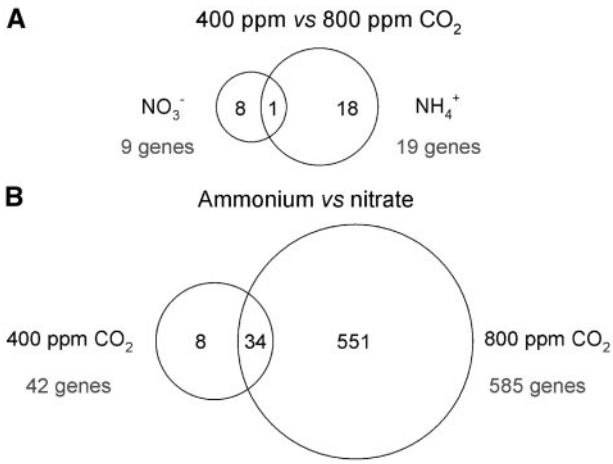

Fig. 4 Transcriptomic analysis of tomato roots. Venn diagrams representing the number of genes differentially expressed in response to (A) the CO_2 condition under nitrate or ammonium nutrition and (B) the N source at ambient (400 p.p.m.) or elevated (800 p.p.m.) CO_2 conditions.

At ambient CO_2, the categories most influenced by N nutrition were stress, which contained four pathogenesis-related (PR) proteins, transport and secondary metabolism (**Fig. 5**; Supplementary Table S5). In addition, these genes were commonly regulated by ammonium under both CO_2 growth conditions (as indicated in dark gray in **Fig. 5**). Contrastingly, at elevated CO_2 concentrations, we observed a huge specific response to N source, with 551 different genes from those modulated by the N source at ambient levels of CO_2 (**Fig. 4B**). The most affected categories were stress, signaling, RNA, transport and hormone metabolism (**Fig. 5**). Among the genes modulated by N source exclusively at elevated CO_2, 78% showed a higher level of expression under ammonium nutrition compared with nitrate nutrition, many of which belonged to biotic stress response, UDP glycosyltransferase and signaling categories (Supplementary Table S6).

Discussion

Elevated CO_2 favored root growth as the key organ when faced with ammonium toxicity

Biomass integrates plant performance under certain environmental growth conditions and it can therefore be considered a good parameter for determining the long-term plant response to stress conditions such as high ammonium levels (Ariz et al. 2011, Sarasketa et al. 2014, Setién et al. 2014). Biomass reduction provoked by ammonium toxicity is generally associated with an accumulation of NH_4^+ in the tissues of different plant species, including tomato roots (Cruz et al. 2006, Setién et al. 2014). NH_4^+ accumulation also indicates an imbalance between NH_4^+ uptake and assimilation processes. The roots of Agora Hybrid F1 tomatoes were previously shown to assimilate NH_4^+ efficiently under a moderate ammonium concentration (7.5 mM) and the biomass production did not reveal any symptoms of toxicity (Vega-Mas et al. 2015). In contrast, at a high ammonium dose (15 mM), the enhanced accumulation of NH_4^+ in the roots reflects the organ's insufficient or inefficient ability to assimilate NH_4^+, which under ambient CO_2 conditions translated into root growth inhibition (**Fig. 1A, B**). As expected, elevated levels of CO_2 stimulated whole-plant growth regardless of the N source (Vega-Mas et al. 2015). Interestingly, under CO_2-enriched conditions, NH_4^+ content was still higher in ammonium-fed roots and was even translocated to the leaf (**Fig. 1B**), indicating that the root's capacity to assimilate ammonium had been exceeded. However, under CO_2-enriched conditions, the classic growth inhibition effect provoked by ammonium toxicity was observed exclusively in the shoots, while increasing CO_2 concentration enhanced ammonium-fed root growth (**Fig. 1A**). Such biomass reallocation to the root, in spite of NH_4^+ accumulation, denotes the plasticity of tomato Agora Hybrid roots as a crucial organ when coping with ammonium stress. To understand this behavior, we studied the metabolic and transcriptomic response of the root further.

The accumulation of NH_4^+ in roots was concomitant with the enhanced synthesis of amino acids (**Fig. 2B**), mainly stored as Gln and Asn (**Fig. 2A**), which act as principal N transport molecules. The assimilation of NH_4^+ in higher plants occurs mainly through the GS/glutamate synthase (GOGAT) cycle, and an enhanced NH_4^+ assimilating capacity is usually accompanied by an increase in GS activity (Cruz et al. 2006, Guan et al. 2016; F. Wang et al. 2016). In our study, we did not observe an increase in GS activity (**Fig. 2D**), thus suggesting the collaboration of accessory ammonium-assimilation pathways such as asparagine synthetase (AS) and/or glutamate dehydrogenase (GDH). The high Asn accumulation in ammonium-fed plants (**Fig. 2A**) could be indicative of the AS activity favoring NH_4^+ assimilation into Asn (Ohashi et al. 2015), especially under CO_2-enriched conditions.

On the other hand, although it is established that the primary role of GDH under non-stress conditions is Glu catabolism (Tercé-Laforgue et al. 2013), several authors advocate that the enzyme plays an assimilating role under stress conditions, which implies high levels of NH_4^+ accumulation (Tercé-Laforgue et al. 2004, Skopelitis et al. 2007, Betti et al. 2014, Pérez-Delgado et al. 2015). So the induction of root GDH activity at elevated CO_2 concentrations (**Fig. 2E**) could act as a metabolic mechanism in an attempt to alleviate NH_4^+ accumulation (**Fig. 1B**) whenever carbon skeletons are not limiting. In this sense, the higher concentration of root soluble carbohydrates, at the expense of starch (**Fig. 3A, B**), would support the role of GDH in the synthesis of Glu at elevated CO_2. Besides GDH, other pathways such as GS/GOGAT or even GABA (γ-aminobutyric acid) synthesis may also contribute to a constant Glu content in ammonium-fed roots (Forde and Lea 2007). Indeed, higher expression levels of the root gene coding for glutamate decarboxylase, an enzyme involved in the synthesis of GABA from Glu, were observed in response to ammonium nutrition at elevated CO_2 concentrations (Supplementary Table S6). Additionally, the increased expression of eight genes involved in the degradation of histidine (His), a route that yields Glu, could also play a role in Glu homeostasis (Supplementary Table S6).

Table 1 Highly modulated genes in response to N source at elevated CO_2

Probe name	Transcript ID	Description	FC (log_2)
Genes most expressed under ammonium nutrition vs. nitrate nutrition			
CUST_8902_PI429680070	Solyc03g020030.2.1	Proteinase inhibitor type-2 CEVI57-like	8.47
CUST_4137_PI429680070	Solyc01g101190.2.1	Vetispiradiene synthase 1-like	7.78*
CUST_11146_PI429680070	Solyc03g116190.1.1	Chitin-binding lectin 1-like	7.41
CUST_13628_PI429680070	Solyc04g071580.2.1	Abscisic stress-ripening protein 2-like	7.35*
CUST_6210_PI429680070	Solyc02g067750.2.1	Carbonic anhydrase CA3	7.16*
CUST_9233_PI429680070	Solyc03g034320.2.1	Protein NtpR-like	6.85*
CUST_7596_PI429680070	Solyc02g085660.1.1	Anthocyanidin 3-*O*-glucosyltransferase 5-like	6.80*
A_96_P137462	Solyc00g174340.1.1	Pathogenesis-related protein	6.44*
CUST_24802_PI429680070	Solyc09g007020.1.1	Pathogenesis-related protein	6.42*
CUST_8868_PI429680070	Solyc03g019690.1.1	Serine protease inhibitor 1-like	6.38*
CUST_5867_PI429680070	Solyc02g062300.2.1	BURP domain-containing protein 3-like	6.36
CUST_26372_PI429680070	Solyc09g066400.1.1	Premnaspirodiene oxygenase-like	6.33
CUST_3692_PI429680070	Solyc01g096720.2.1	Protein zinc induced facilitator-like 1-like	6.33*
CUST_29956_PI429680070	Solyc11g007490.1.1	Cyanidin-3-O-glucoside 2-O-glucuronosyltransferase-like	6.29*
CUST_1243_PI429680070	Solyc01g008620.2.1	β-1,3-Glucanase	6.28
CUST_31831_PI429680070	Solyc11g069880.1.1	Kiwellin-like	6.21
CUST_21336_PI429680070	Solyc07g052370.2.1	Premnaspirodiene oxygenase-like	6.18*
CUST_23803_PI429680070	Solyc08g074260.2.1	Cytochrome P450 71D7-like	6.18
A_96_P207784	Solyc02g086300.2.1	Uncharacterized protein	6.16*
A_96_P145311	Solyc09g006010.2.1	Pathogenesis-related leaf protein 4-like	6.15*
Genes least expressed under ammonium nutrition vs. nitrate nutrition			
CUST_31819_PI429680070	Solyc11g069760.1.1	High affinity nitrate transporter 2.4-like	−8.44*
CUST_31817_PI429680070	Solyc11g069740.1.1	NRT2;3 protein	−8.17*
CUST_17393_PI429680070	Solyc06g010250.2.1	High affinity nitrate transporter 2.4-like	−6.58
CUST_19051_PI429680070	Solyc06g072350.2.1	CASP-like protein RCOM_1206790-like	−6.58
CUST_17083_PI429680070	Solyc06g006110.2.1	Vacuolar cation/proton exchanger 3-like	−6.42
A_96_P040931	Solyc03g117250.2.1	Hop-interacting protein THI116	−6.34
CUST_7473_PI429680070	Solyc02g084410.2.1	Lactoylglutathione lyase-like	−6.23
CUST_26402_PI429680070	Solyc09g072690.1.1	Cation/calcium exchanger 1-like	−6.22
CUST_26708_PI429680070	Solyc09g082760.2.1	Aspartic proteinase oryzasin-1-like	−6.13
CUST_21956_PI429680070	Solyc07g063690.1.1	Calcium-binding protein PBP1-like	−6.06
CUST_10848_PI429680070	Solyc03g113210.2.1	Mitochondrial outer membrane protein porin of 36 kDa-like	−5.93
CUST_8528_PI429680070	Solyc03g005280.2.1	Aspartic proteinase A1-like	−5.82
A_96_P179814	Solyc06g068040.2.1	Probable polygalacturonase At1g80170	−5.63
A_96_P227554	Solyc03g111120.2.1	Malate synthase, glyoxysomal-like	−5.61
CUST_15491_PI429680070	Solyc05g015350.2.1	Cytochrome P450 78A4-like	−5.52
CUST_27451_PI429680070	Solyc10g007600.2.1	Glycolate oxidase	−5.45
A_96_P046531	Solyc01g006070.2.1	Uncharacterized protein	−5.40
A_96_P046531	Solyc04g014520.1.1	Transmembrane protein 45B-like	−5.40
CUST_25500_PI429680070	Solyc09g018200.1.1	Transcription repressor OFP1	−5.40
CUST_21347_PI429680070	Solyc07g052480.2.1	isocitrate lyase	−5.26

A list and description of the 20 most differentially expressed and 20 least differentially expressed genes in tomato root in response to N source (ammonium vs. nitrate) at elevated CO_2 (FDR < 0.05) is given.
The fold change (FC) value is given as log_2.
Asterisks indicate genes commonly modulated by the N source at both ambient and elevated CO_2.

Anaplerotic carbon activities showed N source-dependent versatility at elevated CO_2

In addition to the classical TCA cycle flux mode for energy production, cells can accomplish non-cyclic TCA fluxes under specific metabolic and physiological conditions (Tcherkez et al. 2009, Gauthier et al. 2010, Sweetlove et al. 2010). Thus, when C skeletons are removed from the TCA cycle to cover NH_4^+ assimilation, cycle intermediates have to be replenished through anaplerotic processes (Sweetlove et al. 2010), especially in the

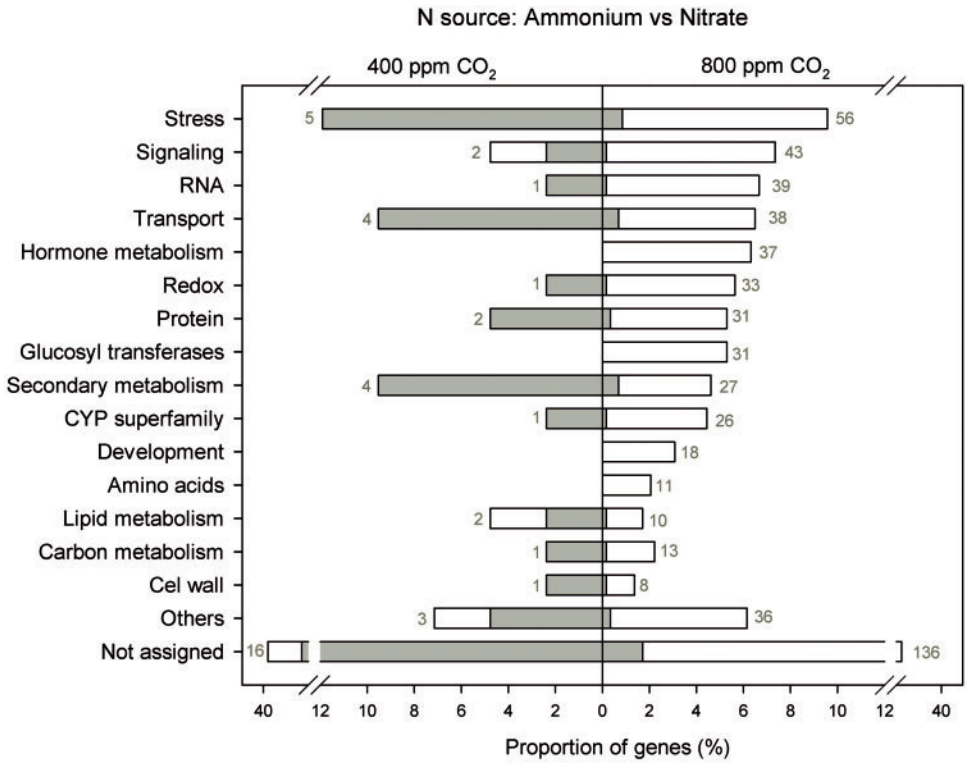

Fig. 5 Functional categories of genes modulated by N source in tomato roots. Root genes differentially expressed by the N source for each CO_2 atmospheric condition classified into functional categories, modified from MapMan software. The abundance of genes for each category is expressed on the *x*-axis as a percentage of the total genes, 42 and 585, modulated by N nutrition at ambient (400 p.p.m.) and elevated CO_2 (800 p.p.m.), respectively. Figures next to each bar indicate the number of genes within each category. Dark gray indicates the percentage of the genes commonly modulated by N nutrition at both ambient and elevated CO_2 conditions (34 genes in total). Some genes were classified in more than one category. Functional categories with < 8 modulated genes are grouped into the 'Others' category.

presence of a high NH_4^+ content (Setién et al. 2014, Vega-Mas et al. 2015). The high availability of soluble carbohydrates under CO_2-enriched conditions, along with the induction of root MDH, NADP-ME and ICDH anaplerotic activities (**Fig. 3**), would provide the supply of carbon skeletons for subsequent NH_4^+ assimilation into Asn and Gln (**Fig. 2A**). MDH may also contribute to pH homeostasis (Gerendás and Ratcliffe 2000), possibly in collaboration with other enzymes such as CA, which is involved in the interconversion between CO_2 and bicarbonate ion (HCO_3^-). Moreover, CA is known to participate in the recycling of the respired CO_2 in heterotrophic organs (Diamantopoulos et al. 2013). In this sense, the very high expression of CA3, which encodes a cytosolic β-type CA isoform (**Table 1**; Supplementary Tables S5, S6), suggests that this enzyme may be involved in promoting C metabolism to face ammonium toxicity, especially at elevated levels of CO_2. Thus, the higher expression of the CA cytosolic isoform could contribute, providing phosphoenolpyruvate carboxylase (PEPC) activity with HCO_3^-. However, the lower expression of a gene coding for phosphoenolpyruvate carboxylase kinase 2 (PPCK2) (Supplementary Table S6), which is responsible for the post-translational activation of PEPC (O'Leary et al. 2011), could explain the lack of enhanced PEPC activity at an elevated CO_2 content (**Fig. 3D**).

In contrast to ammonium nutrition, elevated CO_2 concentrations did not induce the performance of TCA anaplerotic activities in nitrate-fed roots, and CO_2-enriched conditions even reduced ICDH activity (**Fig. 3G**). Therefore, carbon skeletons could have been diverted from TCA to other C-metabolizing pathways. In fact, two genes coding for malate synthase (MS) and one for isocitrate lyase (ICL) showed higher expression levels in response to nitrate nutrition at enriched CO_2 conditions (Supplementary Table S6), as they are among the genes with the highest fold change (**Table 1**). ICL converts isocitrate to succinate and glyoxylate, which is condensed with acetyl-CoA by MS to form malate, in the so-called glyoxylate cycle which bypasses the two decarboxylation steps of the conventional flux mode of TCA (Eprintsev et al. 2015). So additional malate synthesis through the glyoxylate cycle could be occurring under nitrate nutrition at elevated levels of CO_2.

Elevated CO_2 magnified the transcriptomic response of roots in order to deal with ammonium stress

Transcriptomic analyses have already shown that besides modulating genes directly involved in N metabolism, ammonium nutrition also modulates a wide range of genes involved in processes such as signaling, transcription, cell wall development, transport, biotic stress, hormone and secondary metabolism in different species, highlighting the importance of the N source in terms of whole-cell regulation (Patterson et al. 2010, Ruzicka et al. 2010, W. Wang et al. 2016). In the present work,

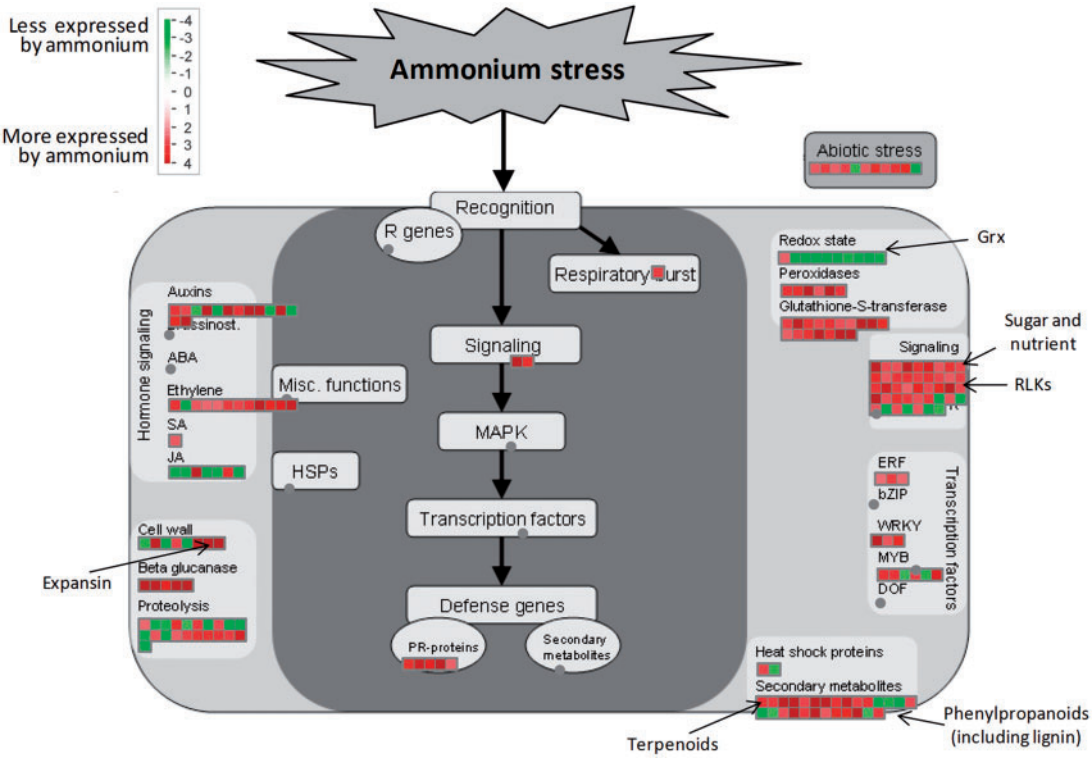

Fig. 6 An overview of general root metabolism and stress response modified from MapMan software for genes modulated by N source (ammonium vs. nitrate) at elevated CO_2 condition. The color of the squares indicates the direction of the fold change, with higher (red) or lower (green) expression produced by ammonium nutrition compared with nitrate. Certain key genes of gene families have been indicated in the figure. Grx, glutaredoxins; RLKs, receptor-like kinases.

the transcriptomic modulation of tomato roots due to ammonium nutrition reflects the cell response to a stressful situation by modulating stress, transport and secondary metabolism gene expression, regardless of atmospheric CO_2 concentration (**Fig. 5**). Nevertheless, the intensification of this transcriptomic response upon increasing CO_2 availability compared with ambient CO_2 (**Fig. 4B**) is noteworthy and it led to an extensive modulation of stress markers (**Figs. 5**, **6**). Elevated levels of CO_2 have been shown to induce important changes in the gene expression of photosynthetic tissues (Ainsworth et al. 2006, Fukayama et al. 2009, Takatani et al. 2014). However, there was little evidence that the CO_2 concentration itself acted as a modulator of the tomato root transcriptome (**Fig. 4A**).

The amplified transcriptomic modulation of ammonium-fed roots at elevated CO_2 (**Fig. 5**) may be driven by the modulation of a high number of transcription factors and signaling components (**Fig. 6**; Supplementary Table S6). Although it is still unclear which signaling pathways contribute to ammonium response, it has been proposed that NH_4^+ acts as a signal molecule (Patterson et al. 2010, Li et al. 2014, Liu and von Wirén 2017). Additionally, modifying atmospheric CO_2 levels is known to affect the expression of not just primary metabolism genes, but also of genes related to signaling processes (Ainsworth et al. 2006, Fukayama et al. 2009). In the present study, the increased expression of 28 genes encoding receptor-like protein kinases (RLKs) under CO_2-enriched conditions (**Fig. 6**; Supplementary Table S6), mainly corresponding to G-type lectin S-receptor-like serine/threonine protein kinases and leucine-rich repeat (LRR) RLKs, highlights that the capacity of tomato roots to respond to a stressful situation is enhanced by increased CO_2 concentrations. The modulation of G-type lectin S-receptor-like has been previously described in response to salt and drought (Sun et al. 2013), while the LRR RLKs modulated in our tomato roots are known to respond to oxidative stress and pathogen attack (Osakabe et al. 2013). On the other hand, the higher levels of expression of genes involved in sugar and nutrient sensing (such as two genes encoding glutamate 2.7 receptors) caused by ammonium nutrition at elevated CO_2 concentrations (**Fig. 6**; Supplementary Table S6) could be related to the increased N status (**Fig. 2A, B**) and the high content of soluble carbohydrates (**Fig. 3A**) since sugars also behave as signaling molecules (Kaplan et al. 2012). Furthermore, the cross-talk between sugar and hormonal signals, suggested to be a key aspect in crop yield improvement at elevated levels of CO_2 (Kaplan et al. 2012), could explain the large modulation of hormone-related genes observed exclusively under CO_2-enriched conditions (**Figs. 5**, **6**). In this regard, the modulation of a large number of auxin-responsive genes and the higher expression of several ethylene-responsive transcription factors (ERFs) by ammonium suggest that these two phytohormones are drivers of the stress response in ammonium-fed roots at elevated levels of CO_2. In fact, the biosynthesis of ethylene must surely has been enhanced, as indicated by the increased expression of 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase genes

(Supplementary Table S6), and ethylene production is well known to be induced by auxin signaling (Tsuchisaka and Theologis 2004).

Defense response and detoxification systems increased in light of ammonium toxicity in tomato roots

Interestingly, ammonium nutrition, especially under CO_2-enriched conditions, provoked the increased expression of a large number of genes in the stress category that are related to pathogen infection, including multiple PR genes (**Fig. 5**; Supplementary Tables S5, S6). This is also the case for genes coding for chitinases and β-1,3-glucanases, two hydrolytic enzymes that mediate the response to fungal infections in tomato roots (Zeng, 2015) or genes coding for osmotin- and thaumatin-like proteins, which are involved in plant defense and the response to osmotic stress (Kumar et al. 2015). Similarly, Patterson et al. (2010) also observed that Arabidopsis roots activate pathogenesis-related transcriptional responses upon exposure to NH_4^+. Both biotic and abiotic stresses are known to share common elements in their signaling pathways (Fujita et al. 2006), so we can hypothesize that the induction of defense genes in tomato roots could be promoting the plant immune response. Pathogen attack is often associated with the appearance of ROS (reactive oxygen species) in the cell, and ammonium stress is also known to trigger ROS overproduction and antioxidant defense systems (Patterson et al. 2010, W. Wang et al. 2016). In fact, ammonium-fed tomato plants have been shown to display an increased resistance to virulent *Pseudomonas syringae* (Fernández-Crespo et al. 2015). The authors suggested that ROS accumulation induced by ammonium stress and hormone signaling regulation were important for ammonium-induced pathogen resistance. Along this line, our transcriptomic analyses revealed that many genes from the redox category, such as peroxidases, were modulated by ammonium nutrition at elevated levels of CO_2 (**Figs. 5**, **6**), suggesting the tomato roots could be suffering an oxidative imbalance.

Promoting ROS-scavenging systems is a common plant strategy to thrive under stress conditions (Apel and Hirt 2004, You and Chan 2015), where glutathione is one of the key components in the plant antioxidant and detoxification system (Roxas et al. 2000, Noctor 2002). Most of the genes identified in the redox category at elevated CO_2 concentrations were related to glutathione metabolism [17 glutathione *S*-transferase (GST) genes and nine glutaredoxin (Grx) genes] (Supplementary Table S6). The Grxs are responsible for reducing protein disulfide bonds coupled to the oxidation of glutathione, and so they also act as antioxidant enzymes (Noctor et al. 2012, You and Chan, 2015). Grx genes were less expressed in ammonium-fed tomato roots (**Fig. 6**), in line with the Grx gene expression induction observed in Arabidopsis plants under nitrate nutrition (Patterson et al. 2016). In contrast, GSTs, which conjugate reduced glutathione to xenobiotics to transform them into less or non-toxic derivatives, showed greater expression levels under ammonium supply (**Fig. 6**). Glutathione conjugates can be transported into the vacuole where they would be metabolized further (Noctor et al. 2012). Accordingly, the higher expression of several ATP-binding cassette (ABC) transporters under ammonium nutrition and elevated levels of CO_2 (Supplementary Table S6) could be involved in transporting glutathione conjugates formed via GST into the vacuole (Klein et al. 2006). Interestingly, Cyt P450 monooxygenases (CYP superfamily), which can also act along with GSTs and ABC transporters in the detoxification of different xenobiotics (Yuan et al. 2007), also showed higher expression levels under ammonium nutrition. Such co-ordinated regulation of GSTs, ABC transporters and CYP genes might indicate a detoxifying mechanism to protect against ammonium stress through the accumulation of N compounds in the root vacuole. Additionally, the expression of a vacuolar pyrophosphatase gene (AVP1 gene; Supplementary Table S6), which may play an indirect role in NH_4^+ vacuolar storage (Wood et al. 2006), was also enhanced, highlighting the role of tomato root vacuole when dealing with ammonium toxicity.

Modulation of secondary metabolism to protect the root against ammonium toxicity

Elevated CO_2 concentrations significantly promoted the transcriptomic modulation of secondary metabolism in response to ammonium nutrition (**Fig. 5**), as was the case for several genes from phenylpropanoid metabolism involved in lignin biosynthesis (**Fig. 6**). The stimulation of lignin biosynthesis in ammonium-fed roots would be promoted due to its interconnection with enhanced C and amino acid availability at elevated CO_2 conditions (**Fig. 2**). Although lignin itself does not contain nitrogen, the first step in the phenylpropanoid pathway, driven by phenylalanine ammonia lyase (PAL), requires phenylalanine (Phe). Interestingly, three PAL genes were more expressed under ammonium nutrition at elevated CO_2 (Supplementary Table S6). The NH_4^+ released from Phe by PAL is further reassimilated by the cytosolic GS isoform (GS1) (Vanholme et al. 2010). In tomato roots, the gene encoding GS1 showed higher expression levels under ammonium supply at elevated CO_2 (Supplementary Table S6). Indeed, overlapping co-ordinated expression of both PAL and GS1 genes has previously been reported in Arabidopsis in response to pathogen attack (Sakurai et al. 2001, Seifi et al. 2013). In addition, the higher expression of cationic peroxidases coding for cationic peroxidase 1-like protein) (Supplementary Table S6) could be promoting the synthesis of lignin in tomato roots (Quiroga et al. 2000). Along with these changes, other genes related to cell wall components were also modulated by ammonium, mainly under CO_2-enriched conditions. In particular, we observed a higher expression of β-expansin-encoding genes (expansin-like B1 protein) (**Fig. 6**; Supplementary Table S6). Since β-expansins are involved in cell wall loosening (Cosgrove 2000), the greater cell wall expansion might facilitate lignin deposition. The increased lignin biosynthesis might play an important role in protecting the root against NH_4^+ stress (W. Wang et al. 2016) and could help to maintain root biomass production under CO_2-enriched conditions. For example, lignin biosynthesis influences the ability of the Casparian strip to regulate solute influx (Barberon et al. 2016), and thus may help restrict excess ammonium uptake.

The role of secondary metabolism in the response to ammonium stress was also highlighted by the higher modulation of the CYP superfamily at elevated levels of CO_2 (**Fig. 5**; Supplementary Table S6). CYP superfamily members participate in a broad range of detoxification or biosynthetic pathways of primary and secondary metabolism, such as those related to phenylpropanoids, alkaloids, terpenoids, lipids and even those associated with plant hormone metabolism (Xu et al. 2015). In particular, terpenoid synthesis seems to play a common role in the secondary metabolism response of tomato roots to ammonium nutrition regardless of CO_2 conditions (**Fig. 6**; Supplementary Tables S5, S6). Crop plants, including tomato, are known to emit a wide variety of volatile terpenes (van Schie et al. 2007, Falara et al. 2011). Notably, the vetispiradiene synthase gene, one of the most differentially expressed genes (**Table 1**; Supplementary Table S6), is involved in the synthesis of sesquiterpene phytoalexins, which participate in plant defense against bacterial and fungal pathogens (Takahashi et al. 2007). Moreover, some CYP members, such as the premnaspirodiene oxygenase-like gene which is highly expressed under ammonium nutrition (**Table 1**), are also involved in the synthesis of phytoalexin terpenes (Takahashi et al. 2007). Therefore, these results point to a notable role for phytoalexins in the root response to ammonium stress.

Lastly, UDP-glycosyltransferases (GTs) also represent a large group of genes subject to transcriptional modulation under ammonium nutrition and elevated CO_2 conditions (**Fig. 5**). GTs play important roles in plant stress responses, by glycosylating hormones and secondary metabolites (e.g. Tiwari et al. 2015). Most of the GT genes modulated in tomato roots, even at ambient CO_2, were related to phenolic metabolism, especially anthocyanin metabolism (Supplementary Table S6). Indeed, a gene coding for anthocyanidin 3-*O*-glucosyltransferase 5-like was among the genes most modulated by ammonium supply (**Table 1**). Anthocyanins are flavonoid pigments that play a role in ROS detoxification and often accumulate during biotic or abiotic stress (Kovinich et al. 2014), including ammonium stress (Marino et al. 2016). Similarly, GTs also participate in the plant defense, e.g. in the synthesis of phenyl glycosides with antiviral and antioxidant properties or in the synthesis of phytoalexins (Tiwari et al. 2015). Indeed some specific GTs more expressed in ammonium-fed roots at elevated levels of CO_2, such as scopoletin glucosyltransferase (Supplementary Table S6), are also involved in the synthesis of phytoalexins (Sun et al. 2014). To summarize, these results indicate that the increased C resources at elevated CO_2 concentrations are invested on the biosynthesis of several secondary metabolites to promote a defense-like response in roots subjected to ammonium toxicity.

Conclusions

Plant growth restriction provoked by a toxic ammonium dose was counteracted in Agora Hybrid F1 tomato roots by the CO_2-enriched conditions, despite the high accumulation of ammonium in the tissues. The pathways for C skeleton production showed N source-dependent versatility in tomato roots. Under ammonium nutrition, the promotion of TCA anaplerotic reactions at elevated levels of CO_2, together with the potential contribution of CA, helps fuel C skeleton production for NH_4^+ assimilation. Indeed, increased carbon availability enhanced ammonium assimilation into amino acids.

The root's key role in coping with ammonium stress was also underlined by the organ's transcriptomic response, which was significantly promoted by elevated CO_2 concentrations. Ammonium toxicity led to increased expression of stress-responsive genes compared with nitrate nutrition; it also modulated the expression of several genes involved in signaling (RLKs), transcription, transport and hormone metabolism. Interestingly, an enhanced CO_2 supply produced a defense-like transcriptomic response to cope with ammonium stress, which included a higher expression of PR genes as well as genes involved in cell redox status and secondary metabolism. On the one hand, the higher expression of GST genes under ammonium nutrition indicates a possible detoxification mechanism in which the vacuole would play a central role. On the other hand, the modulation of genes involved in the biosynthesis of terpenoids and phenolic compounds, together with the participation of GTs and CYP family members, suggests that these secondary metabolites also play a role in the defense response to ammonium stress. In particular, the enhanced expression of lignin biosynthetic genes indicates that more C resources are invested in the cell wall structures, which could strengthen the root growth. Such a response, together with the production of defense compounds, such as phytoalexins, may confer on the root more resistance to ammonium toxicity at elevated levels of CO_2.

The improvement in the response of the root when tomato plants grew at elevated CO_2 concentrations points to a beneficial role for the predictable future CO_2-enriched atmospheric conditions when dealing with ammonium excess. Therefore, this study also offers a new insight into crop yield response under an ammonium-based N fertilization management in the context of climate change.

Materials and Methods

Growth conditions and harvesting

Tomato plants (*Solanum lycopersicum* L. cv. Agora Hybrid F1, Vilmorin®) were grown in 2.7 liter pots with perlite : vermiculite (1 : 2; v/v) in two controlled environment chambers (Phytotron Service, SGIker, UPV/EHU). The environmental conditions inside the chambers were a light intensity of 500 µmol photon m^{-2} s^{-1}, 24°C/18°C and 60% and 70% relative humidity during light (14 h) and dark (10 h) periods, respectively. Pots were irrigated three times per week with a nutrient solution described in Setién et al. (2014), containing 15 mM N supplied as 7.5 mM $Ca(NO_3)_2$ or 7.5 mM $(NH_4)_2SO_4$. The solution was buffered with $CaCO_3$ and the pH adjusted to 5.8 on each irrigation day. Three plants were grown in each pot for 4 weeks. Eighteen pots per N treatment were distributed over time in three consecutive trials for each CO_2 condition, under either 400 p.p.m. CO_2 (ambient) or 800 p.p.m. CO_2 (elevated), in two identical chambers. Within each chamber, pots were randomly distributed and their position was changed three times a week. To prevent a chamber bias, the CO_2 concentration in each chamber was switched between trials. The three plants in the same pot were always harvested together. For biomass determinations, nine pots per treatment were harvested in total ($n = 9$), and shoots and

roots were dried at 80°C. For biochemical and physiological analyses, the nine remaining pots were harvested during the first 3 h from the onset of the light period ($n = 9$). The two youngest fully expanded leaves were harvested after the main vein was removed for metabolic determinations. Root and leaf samples were frozen in liquid nitrogen, ground to a fine powder and stored at −80°C until further determinations. For some determinations (amino acids, GS and GDH polypeptide contents, and transcriptomic analysis) plants from three pots were pooled per treatment ($n = 3$).

Determination of metabolites

Ammonium content was determined in aqueous extractions from 10 mg of lyophilized root and leaf samples (Sarasketa et al. 2014). Soluble sugars were measured in hydroalcoholic extractions from 10 mg of lyophilized root samples by an enzymatic assay according to the manufacturer's protocols (Enzytec™ fluid D-glucose/D-fructose or sucrose, Scil). Glucose, fructose and sucrose contents were expressed as glucose equivalents. Starch was analyzed from the pellet of the hydroalcoholic extracts after enzymatic hydrolysis with amyloglucosidase and amylase in 0.2 M sodic acetate buffer (pH 4.8) and determined as glucose equivalents. Amino acids were extracted from 150 mg of frozen root samples with 1 M HCl and determined by capillary electrophoresis (Beckman Coulter PA-800, Beckman Coulter Inc.) as in Marino et al. (2016).

Enzymatic assays and GS and GDH protein contents

Soluble protein content, GS and GDH activities were determined in roots as described in Setién et al. (2014). The polypeptides for GS and GDH were quantified by SDS–PAGE in the presence of specific antibodies under the conditions established by Setién et al. (2014). The activities of the TCA cycle anaplerotic enzymes PEPC, MDH, NADP-ME, NAD-dependent malic enzyme (NAD-ME) and ICDH were determined as described in Sarasketa et al. (2016).

Statistical analysis

Data analyses were carried out using IBM SPSS 20.0 software (IBM Corp.). Statistical analysis of normality and homogeneity of variance were analyzed by Kolmogorov–Smirnov and Levene tests. Analysis of significant differences within each treatment included one-way analysis of variance (ANOVA) and comparison of means (Duncan's test). Details of statistical analyses and significance levels are presented in the figure legends.

RNA extraction, microarray hybridization and data analysis

Total RNA extraction and purification was performed from 100 mg of root frozen samples using an RNeasy® Plant Mini kit (Qiagen) following the manufacturer's protocol. Three independent biological replicates were used for the transcriptomic analysis. Total RNA was quantified spectrophotometrically by NanoDrop ND-1000 and the integrity of the RNA was estimated using the RNA 6000 Nano Assay (Bioanalyzer 2100).

The tomato transcriptome was obtained by hybridization of fluorescently (Cy3 dye) labeled cDNA samples to DNA microarrays. Microarray slides were designed and produced through Agilent eArray (Agilent Technologies; http://www.agilent.com) using both custom-designed and pre-designed probe sets specifically developed for *Solanum lycopersicum*. An aliquot of 50 ng of total RNA of each sample was processed using One-Color Microarray-Based Gene Expression Analysis (Low Input Quick Amp Labeling) (v.6.5; Agilent Technologies) following the manufacturer's instructions. Labeled cDNA was generated from RNA using AffinityScript Reverse Transcriptase (Agilent Technologies) in the presence of Cy3-CTP fluorophore, and manual hybridization was carried out in a SureHyb hybridization chamber (Agilent Technologies). Finally, the microarrays were scanned on a G2565CA DNA Microarray Scanner (Agilent Technologies). The raw images were processed by Agilent Feature Extraction Software (ver. 10.7.3.1) (Agilent Technologies), and data analysis was performed by GeneSpring GX 12.6 (Agilent Technologies). Data were normalized using the function rma of the limma package (Linear Models for Microarray Data, v. 2.10.5) of bioconductor (Smyth 2004). Differentially expressed genes were determined using Rank products (Breitling et al. 2004) with FDR correction (FDR < 0.05). Minimum Information About a Microarray Experiment (MIAME)-compliant data were deposited at Array Express (http://www.ebi.ac.uk/arrayexpress) as E-MTAB-5558. The differentially expressed genes were visualized and classified into functional categories based on the MapMan program (Usadel et al. 2005). Annotation of gene functional descriptions was complemented based on homology searches (BlastX).

Supplementary data

Supplementary are available at PCP online.

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Disclosures

The authors have no conflicts of interest to declare.

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