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Running title: Plant primary metabolism and plant diseases

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Plant primary metabolism regulated by nitrogen contributes to plant-pathogen interactions

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Abstract

Nitrogen contributes to plant defence responses by the regulation of plant primary metabolism during plant-pathogen interactions. Based on biochemical, physiological, bioinformatic and transcriptome approaches, we investigated how different nitrogen forms (ammonium vs. nitrate) regulate the physiological response of cucumber (Cucumis sativus) to Fusarium oxysporum f. sp. cucumerinum (FOC) infection. The metabolic profile revealed that nitrate-grown plants accumulated more organic acids, while ammonium-grown plants accumulated more amino acids; FOC infection significantly increased levels of both amino acids and organic acids in the roots of ammonium-grown plants. Transcriptome analysis showed that genes related to carbon metabolism were mostly up-regulated in plants grown with nitrate, whereas in ammonium-grown plants the upregulated genes were mostly those that related to primary nitrogen metabolism. Root FOC colonization and disease incidence were positively correlated with levels of root amino acids and negatively correlated with levels of root organic acids. In conclusion, organic acids metabolism and related genes expression increased under nitrate, whereas ammonium increased amino acids level and expression of related genes; these altered levels of organic acids and amino acids resulted in different tolerances to FOC infection depending on the nitrogen forms supplied.

Keywords: nitrogen, plant primary metabolism, plant-pathogen interactions, Fusarium wilt

Abbreviations**:** FOC, Fusarium oxysporum f. sp. cucumerinum; FA, fusaric acid; DI, disease incidence; A, ammonium-grown plants; N, nitrate-grown plants; AI, ammonium-grown plants inoculated with FOC; NI, nitrate-grown plants inoculated with FOC; FDR, false discovery rate.

Introduction

Plant metabolism and pathogen infection are closely linked (Berger et al. 2007, Fagard et al. 2014). Pathogens require nutrition from the host for successful colonization (Snoeijers et al. 2000), and this availability of nutrition can be affected by plant metabolism (Fagard et al. 2014, Rojas et al. 2014). Moreover, plant defence responses to pathogen requires energy supply, which is mainly derived from primary metabolic processes (Bolton 2009). Primary metabolites also function as signalling molecules to trigger defence responses (Rojas et al. 2014), following pathogen recognition and signal transduction processes (Dangl and Jones 2001, Kachroo and Robin 2013). The regulation of primary metabolites, i.e. carbohydrates, amino acid and lipids, is therefore a key response to infection by pathogens (Rojas et al. 2014).

Pathogen infection has a strong impact on both primary and secondary metabolism in plants, and this affects plant growth and yield (Gupta et al. 2013). Firstly, the energy required by the defence response is intensive, increasing the demand for assimilates (Bednarek and Osbourn 2009, Swarbrick et al. 2006). Secondly, pathogens often manipulate plant metabolism, by withdrawing nutrients and further increasing the demand for assimilation (Lanoue et al. 2010). Finally, photosynthesis decreases as development of chlorotic and necrotic tissue after pathogen infection occurs, resulting in a change in the sugar accumulation and sink metabolism (Berger et al. 2007, Swarbrick et al. 2006).

Nitrogen plays an essential role in plant-pathogen interactions (Gupta et al. 2013, Kusano et al. 2011, Thalineau et al. 2016). Depending on plant species and pathogen strains, nitrogen can affect the resistance and susceptibility of plant to diseases by regulating plant growth and physiology, affecting pathogen growth and virulence, and modifying the rhizosphere environment (Huber and Thompson 2007, Fagard et al. 2014). Nitrogen also affects the mechanical strength of cell walls by changing the amount of plant cellulose and lignin (Talbot and Treseder 2011), which prevents pathogen penetration and limits disease development. Many plant constituents (i.e., amino acids, organic acids, phenolic compounds, sugar, nucleic acids, total nitrogen,

proteins and C/N ratio) that are altered by nitrogen supply (Sakakibara et al. 2006), are correlated with resistance or susceptibility to disease (Berger et al. 2007, Rojas et al. 2014).

Ammonium and nitrate are the major inorganic nitrogen forms absorbed by higher plants. Plants could regulate their nitrogen and carbon metabolism in response to nitrogen availability and environmental conditions, nitrogen and carbon metabolism are tightly linked in the fundamental biochemical (Coruzzi and Bush, 2001; Gutierrez et al., 2007). Although the effects of different nitrogen forms on plant-pathogen interactions have been examined in several studies (Gupta et al. 2013, Lopez-Berges et al. 2010, Snoeijers et al.2000, Thalineau et al. 2016), their underlying mechanisms remain largely unknown. In particular, it is unclear how different nitrogen forms affect plant defence mechanisms through changes to primary metabolic pathways. In our previous study, we found that different nitrogen forms could affect cucumber Fusarium wilt disease by regulating citrate acid exudation (Wang et al. 2016). However, the effects of different nitrogen forms on plant organic acids and amino acids synthesis and its relationship with cucumber Fusarium wilt are largely unknown. The aim of this study was to investigate the roles of nitrogen and carbon metabolism on plant disease development when regulated by different nitrogen forms. We focused on the influences of ammonium and nitrate on the responses of cucumber to Fusarium oxysporum f. sp. cucumerinum (FOC) infection. Cucumber was chosen because it is easy to work with, susceptible to FOC infection, and the amino acid synthesis pathway is already understood (Buchanan et al., 2000, see Fig S2). Individual plants were grown in pots, supplied with either ammonium or nitrate, and either inoculated or not inoculated with FOC. Nitrogen uptake and transport, primary plant nitrogen and carbon metabolism and related transcriptome regulation were all recorded, as well as disease incidence, severity and the production of the strongly phytotoxic secondary metabolite fusaric acid.

Results

Influence of nitrogen forms and FOC infection on plant metabolism

Wilt symptoms were found in ammonium-grown plants after FOC infection (Fig. 1). Nitrate-grown cucumber plants, which were more tolerant to Fusarium wilt, had lower disease index and root FOC colonization (Fig. S1).

The source of nitrogen and FOC infection affected both carbon and nitrogen metabolism. In the leaves, levels of total carbon and soluble sugars were higher in ammonium-grown plants (Fig. 2a, e), while total nitrogen and soluble protein were reduced in infected ammonium-grown plants (Fig. 2b, d). Overall, there was little effect on the C:N ratio (Fig. 2c). In the stems, the major difference between the plants was that soluble protein and total nitrogen were increased in infected ammonium-grown plants (Fig. 2b, d). In the roots, total carbon, total nitrogen and levels of soluble protein were higher in ammonium-grown plants. The different nitrogen forms could regulate the plant metabolism and further affect pathogen infection and colonization. The metabolite content of nitrate-grown plants were not affected by FOC infection, and were resistance to it. By contrast, the metabolite contents of ammonium-grown plants were significantly changed by pathogen infection, to which the plants were susceptible.

Effects of nitrogen forms and FOC infection on the organic acid and amino acid levels of cucumber plants

Organic acids and amino acids are the potentially significant carbon and nitrogen source for the growth of pathogen. A more detailed look at plant metabolism showed that, under non-inoculated conditions, the leaf and root organic acid (oxalic, malic, citrate, succinic and fumaric) levels in nitrate-grown plants were higher than those of the ammonium-grown plants (Fig. 3a, b), especially those of oxalic and malic. The organic acid levels in nitrate-grown plants were not affected by FOC infection, whereas the levels of leaf malic, citrate and succinic acids and root succinic acid were markedly increased in ammonium-grown plants after FOC infection.

In contrast to those of organic acids, most of the amino acid levels in ammonium-grown plants were markedly higher than those of nitrate-grown plants (Fig. 4). Ser, His, Lys, Pro, Cys, Met and Arg contents in the leaves of ammonium-grown plants were significantly higher than those in nitrate-grown plants under non-inoculated conditions (Fig. 4a), whereas the leaf Ala, Thr, Glu and Gly contents were markedly lower than

those in nitrate-grown plants. FOC infection significantly increased the Ala and Glu contents and decreased the Thr, Asp, Ser and Arg contents in the leaves of ammonium-grown plants but decreased the leaf Ala, Thr, Lys and Tyr contents in nitrate-grown plants. The levels of all of the amino acids (except for Tyr and Cys) in the roots of ammonium-grown plants were markedly higher than those of nitrate-grown plants (Fig. 4b). FOC infection significantly increased the levels of all of the amino acids in the roots of ammonium-grown plants. Different nitrogen forms could affect the organic acids and amino acids content in cucumber plants, which may attribute to the resistance or susceptibility of cucumber plants to FOC infection.

Transcriptional regulation of central carbon and nitrogen metabolism in response to different nitrogen forms and FOC infection

As plants regulate their nitrogen and carbon metabolism in response to nitrogen availability and environmental stresses, the expression of genes encoding enzymes involved in central carbon and nitrogen metabolism in cucumber plants was analysed using Illumina sequencing technology. In the leaves, genes encoding for isocitrate dehydrogenase (IDH1 and IDH5), nitrate reductase (NR2, NR8, NR9 and NR10), nitrite reductase (NiR2) and glutamate synthase (GOGAT1) in nitrate-grown plants were significantly up-regulated compared to those in ammonium-grown plants (Fig. 5), whereas the expression of genes encoding for malate dehydrogenase (MDH10) and glutamine synthetase (GS3) were down-regulated in nitrate-grown plants. After FOC infection, the NR2 and GOGAT1 expression levels were markedly up-regulated and that of MDH10 was down-regulated in the leaves of ammonium-grown plants, while the NR2 and NR3 expression levels were down-regulated in the leaves of nitrate-grown plants.

In the roots, the expression of genes encoding for pyruvate dehydrogenase (PDH2), malate dehydrogenase (MDH6 and MDH10), nitrate reductase (NR6, NR8 and NR9), nitrite reductase (NiR3) and glutamine synthetase (GS1) were markedly up-regulated compared to those in ammonium-grown plants (Fig. 5), while the IDH1, IDH5 (encoding for isocitrate dehydrogenase), NR2, NR10, GS2, GS3 and GOGAT1 (encoding for glutamate synthase) expression levels were down-regulated. FOC

infection significantly induced MDH5, NR3 and GS4 expression and inhibited NR6 and NR9 expression in the roots of ammonium-grown plants, while the MDH1, NR2 and GOGAT1 expression levels were markedly up-regulated and the CS3, NR10 and NiR3 expression levels were down-regulated in the roots of nitrate-grown plants. The expression of genes encoding enzymes involved in central carbon and nitrogen metabolism in cucumber plants were regulated by different nitrogen forms and FOC infection.

Transcriptional regulation of amino acids in response to different nitrogen forms and FOC infection

An overview of amino acid biosynthesis in cucumber plants is presented in Figure S2 [modified from Buchanan et al. (2000)]. In the leaves, the GDH2 (encoding for glutamate dehydrogenase), GS3 (encoding for glutamine synthetase), AS3, AS4 (encoding for asparagine synthetase), TDH2 (encoding for threonine dehydratase), ALT2 (encoding for alanine transaminase), SAT4 (encoding for serine O-acetyltransferase) and TPS1 (encoding for tryptophan synthase) expression levels in nitrate-grown plants were significantly down-regulated, and the GS4, ACOAT2 (encoding for acetylornithine aminotransferase), TDH4, TDH5, ALS2 (encoding for acetolactate synthase), OASS6 [encoding for O-acetylserine (thiol) lyase], and SK1 (encoding for shikimate kinase) expression levels were up-regulated compared to those in ammonium-grown plants (Fig. 6). FOC infection markedly increased ACOAT2, AspAT4 (encoding for aspartate aminotransferase), AS6, TDH4 and OASS6 expression and decreased AOD2 (encoding for acetylornithine deacetylase), TDH1, TDH2, ALT2, CM3 (encoding for chorismate mutase), TPS1 and TPS5 (encoding for tryptophan synthase) expression in the leaves of ammonium-grown plants. The AS4, HSDH1 (encoding for homoserine dehydrogenase) and SAT4 expression levels were up-regulated and the AS3 and TDH4 expression levels were down-regulated in leaves of nitrate-grown plants after FOC infection.

In the roots, the GS1 (encoding for glutamine synthetase), ASS1 (encoding for argininosuccinate synthase), SAT2 (encoding for serine O-acetyltransferase) and OASS6 [encoding for O-acetylserine (thiol) lyase] expression levels were induced and

the GDH2 (encoding for glutamate dehydrogenase), GS2, GS3, P5CS1 (encoding for delta-1-pyrroline-5-carboxylate synthetase), AANA8 (encoding for amino-acid N-acetyltransferase), ACOAT1 (encoding for acetylornithine aminotransferase), AOD2, AOD3 (encoding for acetylornithine deacetylase), ASS2 (encoding for argininosuccinate synthase), AS1, AS3, AS6 (encoding for asparagine synthetase), TDH1, TDH2, TDH3 (encoding for threonine dehydratase), ALT2 (encoding for alanine transaminase), GHMT4 (encoding for glycine hydroxymethyltransferase) and RPK2 (encoding for ribose-phosphate pyrophosphokinase) expression levels were suppressed in nitrate-grown plants compared to those in ammonium-grown plants (Figure 6). After FOC infection, the AOD3, AS2 and TDH3 expression levels were up-regulated and the P5CS1, AS1, AS3, AS6 and RPK2 expression levels were down-regulated in roots of ammonium-grown plants. In contrast, the P5CS1, ACOAT1, AOD2, TDH2 and TPS1 (encoding for tryptophan synthase) expression levels were induced and the GS4 expression level was suppressed in nitrate-grown plants after FOC infection. As compared to nitrate-grown plants, amino acids metabolism and related gene expression were increased in ammonium-grown plants.

Primary nitrogen and carbon metabolism of cucumber regulated by different nitrogen forms during Fusarium oxysporum infection

Changes in nitrogen and carbon metabolism of cucumber plants in response to different nitrogen forms during FOC infection were summarized in Fig. 7 and Fig. S3. Leaf citrate, malate, succinic acids, and root succinic acid contents were significantly increased in ammonium grown plants after FOC infection (Fig. S3a), while no significant difference was found in nitrate grown plants (Fig. S3b). After FOC infection, most of amino acids in roots were markedly increased in both ammonium and nitrate grown plants (Fig. S3), corresponded with up-regulated related gene expression. Most of organic acids in nitrate grown plants were higher than ammonium grown plants after FOC infection (Fig. 7), whereas most of amino acids were lower than ammonium grown plants which corresponded with down-regulated related gene expression. The primary nitrogen and carbon metabolism of cucumber plants could be affected by different nitrogen forms and FOC infection.

Effects of root amino acids and organic acids on cucumber Fusarium wilt

The observed differences in amino and organic acid levels were associated with disease outcomes. The spearman correlational analysis showed that root amino acids were significantly positively correlated with root FOC number and disease incidence (DI) (Fig. 8a), whereas root organic acids, such as malic and citrate, were significantly negatively correlated with root FOC number and DI. The root His, Asp, Phe and Pro were largely contributed to the root FOC number and DI (Fig. 8b), which have the relative influence of about 70% to 80%. FOC sporulation and fusaric acid (FA) production were slightly increased by most of amino acids and organic acids (Fig. 9a, b), whereas FA production was markedly induced by Val, Cys, Met, Gly, His, Leu and Phe, about 2-7 times of that in control. FOC colonization and toxin production were induced by amino acids which resulted in high disease incidence.

Discussion

Nitrogen is one of the most important mineral elements that modulates the plant-pathogen interaction. Due to differences in nitrogen forms, plant species, pathogen type and the developmental stage of nitrogen application, our current understanding of the effect of nitrogen on disease development is incomplete (Huber and Thompson, 2007; Gupta et al., 2013). In our previous study, we found that nitrate application significantly decreased the Fusarium oxysporum f. sp. cucumerinum (FOC) colonization and disease incidence compared with ammonium application (Fig. 1 and S1) (Wang et al. 2016). A similar result was obtained from Gupta et al. (2013), in which the nitrate feeding of tobacco enhanced disease resistance to Pseudomonas syringae pv. phaseolicola compared to ammonium by increasing salicylic acid (SA) and nitric oxide (NO) accumulation, as well as pathogenesis-related protein 1 (PR1) gene expression. In contrast, ammonium nutrition induced tomato resistance against P. syringae pv tomato DC3000 through the activation of systemic acquired acclimation (Fernández-Crespo et al. 2015).

Carbon and nitrogen metabolism are tightly coordinated in the fundamental biochemical pathway in plants. Host chemical traits influencing pathogen infection include tissue nitrogen chemistry and carbohydrate composition, such as total nitrogen and carbon concentrations, C/N ratio, protein and amino acid concentrations, total carbohydrates and soluble fraction components (Roberts and Paul 2006). Phaeocryptopus gaeumannii, the causal agent of Swiss needle cast disease in Douglas fir, responds to host nutrient status; high nitrogen and carbon availability inside Douglas fir needles is associated with increased fungal fruiting and thus disease severity (El-Hajj et al. 2004). In the current study, the root total carbon and nitrogen contents of ammonium-grown plants were significant higher than nitrate-grown plants (Fig. 2a, b), which may provide carbon and nitrogen for FOC infection and colonization. During plant-pathogen interactions, carbohydrates play the role of a double-edged sword, in which act not only as nutrition for the pathogen but also involved in metabolic reactions associated with host defence responses (Herbers et al. 1996). In current study, the soluble sugar content did not change in nitrate-grown plants after FOC infection (Fig. 2e), which showed high resistance to FOC infection. However, the soluble sugar content of root and stem, in which FOC colonized, were markedly decreased in ammonium-grown plants after FOC infection (Fig. 2e), which may attribute to FOC colonization and nutrition consumption. While the leaf soluble sugar content increased in ammonium-grown plants after FOC infection may function as priming signals contributing to immune response against pathogens (Berger et al. 2007, Rojas et al. 2014). Plants modulate their tissue sugar content as a signal to initiate subsequent defence reactions in response to pathogen infection.

Carbon and nitrogen sensing and signalling are critical for regulating metabolism and development in response to the environment (Coruzzi and Zhou 2001). Nitrate addition increased organic acid synthesis to provide carbon skeletons for amino acid synthesis and to act as counter anions and prevent alkalinization (Coruzzi and Bush 2001, Stitt 1999). In tobacco, nitrate initiates a coordinated increase in the expression of several genes involved in organic acid synthesis, such as phosphoenolpyruvate carboxylase (PPC), cytosolic pyruvate kinase (PK), citrate synthase (CS) and NADP-

isocitrate dehydrogenase (ICDH-1) (Scheible et al. 1997, Stitt 1999). Our results showed that nitrate nutrition increased the contents of oxalic, malic, citrate, succinic and fumaric acids both in the leaves and roots of cucumber plants (Fig. 3), corresponding with the higher IDH (encoding for isocitrate dehydrogenase) and MDH (encoding for malate dehydrogenase) gene expression in leaves and roots, respectively (Fig. 5). Pathogen infection significantly increased the contents of malic, citrate and succinic acids in leaf, as well as root succinic acid under ammonium nutrition, but there was no effect under nitrate nutrition (Fig. 3), suggesting that pathogens try to manipulate plant metabolism for their own advantages regarding nutrient acquisition (Berger et al. 2007, Fagard et al. 2014).

Amino acid metabolism regulated by different nitrogen forms, amino acid levels in ammonium-grown plants were higher than those in nitrate-grown plants (Fig. 4), corresponding to lower leaf TS (encoding for threonine synthase), SAT (encoding for serine O-acetyltransferase), ALT (encoding for alanine transaminase), AS (encoding for asparagine synthetase), and GDH (encoding for glutamate dehydrogenase) expression levels and root GHMT (encoding for glycine hydroxymethyltransferase), RPK (encoding for ribose-phosphate pyrophosphokinase), ALT, AS, TDH (encoding for threonine dehydratase), GDH, P5CS (encoding for delta-1-pyrroline-5-carboxylate synthetase), AANA (encoding for amino-acid N-acetyltransferase), ACOAT (encoding for acetylornithine aminotransferase), and AOD (encoding for acetylornithine deacetylase) expression levels (Fig. 6). Ammonium is a fundamental substrate for amino acid synthesis, similar result was obtained by Gupta et al. (2013), the total amino acid levels increased in ammonium-fed tobacco, probably related to carbon metabolism, which provides carbon skeleton for ammonium assimilation in roots. Amino acid metabolism varies significantly in response to pathogen attack (Bais et al. 2005, Fagard et al. 2014), the network of amino acid metabolic pathways establish integral parts of the plant immune system (Monteoliva et al. 2014). In Arabidopsis leaves, the levels of several free amino acids, such as Val, Leu, Ile, Phe, Tyr, Trp and Lys, significantly increased after inoculation with SAR-inducing P. syringae bacteria, whereas the amounts of Asp decreased (Zeier 2013). Although several studies have investigated the

role of nitrogen on plant-pathogen interactions (Fagard et al. 2014, Fernández-Crespo et al. 2015, Gupta et al. 2013, Lopez-Berges et al. 2010, Mur et al. 2017, Snoeijers et al.2000, Thalineau et al. 2016, Wang et al. 2016), the interface between nitrogen assimilation and the plant defence response remains largely unknown. Our results revealed that amino acids levels markedly increased in the roots of ammonium-grown plants after FOC infection (Fig. 4a), this phenomenon was correlated with different expression levels of amino-acid-related genes (Fig. 6), indicating that the expression of genes related to amino acid metabolism varies with pathogen infection.

In plant-pathogen interactions, metabolic reprogramming is a consequence of both the defence response (secondary metabolites accumulation) and the requirement for carbon and nitrogen sources by pathogen. Metabolic changes after pathogen infection include defence-associated and disease-associated metabolites, which are supported by carbohydrate, amino acid and lipid metabolic pathways (Rojas et al. 2014). The integrate network of carbon and nitrogen metabolism in cucumber plants regulated by different nitrogen forms and FOC infection were summarized in Figure 7 and S3, which show that nitrate increased the organic acid metabolism and related gene expression, whereas ammonium increased the amino acid metabolism and related gene expression. Carbon and nitrogen metabolism were changed by FOC infection, especially in ammonium grown plants. Organic acids and amino acids are major metabolisms in higher plants which are involved in plant-microbe interactions. In present study, spearman correlational analysis showed that root malic and citrate acids were negatively correlated with FOC number and disease index (DI), while amino acids were positively correlated with FOC number and DI (Fig. 8a). Root His, Asp, Phe and Pro largely contributed to FOC number and DI (Fig. 8b). Amino acids metabolic pathways constitute integral parts of the plant immune system and are essential for plant-pathogen interactions (Fabro et al. 2004, Hwang et al. 2011, Navarova et al. 2012). For example, rapid activation of asparagine synthetase in susceptible tomato plants plays a dual role in promoting Botrytis cinerea virulence by both facilitating pathogen-induced host senescence and providing a rich nitrogen sources to support pathogen growth (Seifi et al. 2014), which is consistent with present study that amino acids were positively

correlated with FOC number and DI (Fig. 8a).

Nitrogen can regulate plant-pathogen interactions through an effect on pathogen virulence (Zhou et al. 2017), the preferred nitrogen source ammonium was found to repress the virulence-related functions of Fusarium oxysporum, such as penetration, vegetative hyphal fusion, or root adhesion, via protein kinase TOR and bZIP protein MeaB (Lopez-Berges et al. 2010). Fusaric acid (FA) was supposed to be critical for FOC infection and disease development (Wang et al. 2013), thus effects of different amino acids and organic acids on FOC sporulation and FA production were investigated. These results showed that although FOC sporulation was induced by most of amino acids and organic acids, FA production was higher in amino acids as compared to organic acids (Fig. 9b), suggesting that amino acids were preferred nitrogen sources for FOC to produce FA. The possible mechanism is that the expression of genes (e.g. FUB genes) involved in FA production may be induced by amino acids (Brown et al. 2012; Brown et al. 2015; Niehaus et al. 2014). However, the inner-mechanism is largely unknown, especially genes involved in toxin production are needed for further study.

In conclusion, primary nitrogen and carbon metabolism in cucumber plants were changed by FOC infection depending on the source of nitrogen. Nitrate increased organic acid metabolism and related gene expression, whereas ammonium increased amino acid metabolism and related gene expression. The increase of ammonium/nitrate ratio regulated the genes expression involved in carbon and nitrogen metabolisms, and induced amino acids accumulation in cucumber plants which further stimulated FA production and FOC sporulation, thus increased disease incidence of cucumber Fusarium wilt (Fig. 10). The organic and amino acids metabolism regulated by different nitrogen forms contributes to cucumber Fusarium wilt tolerance.

Materials and methods

Plant material and growth condition

Seeds of cucumber (Cucumis sativus L.), cultivar ‘Jingyan 4’, susceptible to Fusarium oxysporum f. sp. cucumerinum, were germinated in sterile quartz sand and transplanted to nutrient solution containing either NO_3^- or NH_4^+ when the first leaf

emerged. The composition of the nutrient solution was as follows: 2.5 mmol L^{-1} $(NH_4)_2SO_4$ or $Ca(NO_3)_2$, 2.5 mmol L^{-1} K_2SO_4, 1.0 mmol L^{-1} KH_2PO_4, 2.0 mmol L^{-1} $MgSO_4$, 35.8 μmol L^{-1} Fe-EDTA, 57.8 μmol L^{-1} H_3BO_3, 11.4 μmol L^{-1} $MnCl_2$, 0.96 μmol L^{-1} $ZnSO_4$, 0.4 μmol L^{-1} $CuSO_4$, and 0.48 μmol L^{-1} H_2MoO_4. Ca in the ammonium-containing nutrient solution was included by the addition of $CaCl_2$. A nitrification inhibitor (DCD) was added to each nutrient solution to prevent the oxidation of ammonium. To maintain the pH at 6.80 ± 0.20 during culture, $CaCO_3$ was added to the nutrient solution.

In the pot experiment, cucumber seedlings were transplanted to pots containing 3 kg of continuously-cropped cucumber soil when the first leaf emerged. Nitrogenous fertilizer containing $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ with dicyandiamide (DCD) was added to the soil before transplanting.

Pathogen incubation and infection

Pathogenic fungi of Fusarium oxysporum f. sp. cucumerinum (FOC) were first incubated on potato dextrose agar (PDA) medium in Petri dishes in the dark at 28 °C for 7 days. A conidial suspension was leached from the plate-grown mycelium by adding sterile water and gently stirring. For inoculation, the roots of four-week-old cucumber plants were immersed in a FOC conidial suspension (10^6 conidia ml^{-1}). The roots of control plants were immersed in sterilized water.

Determination of total carbon and nitrogen contents

Plant samples were dried at 75 °C for 3 days, and then powdered. The powdered oven-dried samples were analysed using an automatic elemental analyser (Vario EL III, Elementar, Germany), and the C/N ratio was calculated (Watanabe et al. 2008).

Determination of soluble protein and soluble sugar content

For soluble protein content measurement, fresh samples (0.5 g) were homogenized in a pre-chilled mortar and pestle placed on ice with 5 ml of 0.05 M phosphate buffer (pH 6.7). The homogenate was centrifuged at 10,000 g at 4 °C for 10 min, and the supernatant was used for protein analysis. Protein was quantified according to the Bradford method (Bradford 1976). Each 0.1 ml protein sample was mixed with 5 ml of protein reagent [containing 0.01% (w/v) coomassie brilliant blue G-250, 4.7% (w/v)

ethanol and 8.5% (w/v) phosphoric acid]. The absorbance was measured at 595 nm after 2 min. The protein content was calculated by comparison with the standard curve prepared form a bovine serum albumin (BSA) solution.

To measure the soluble sugar content, oven-dried plant material (0.3 g) was extracted with 20 ml of H_2O at 100 °C for 10 min. The extracts were filtered and analysed for soluble sugar content using the anthrone-sulphuric acid method (Shin et al. 2000). The reaction consisted of 1 ml of the extract mixed with 1 ml of H_2O, 0.5 ml of anthrone reagent (1 g of anthrone and 50 ml of ethyl acetate) and 5 ml of H_2SO_4 and was heated in a boiling water bath for 1 min. After cooling to room temperature, the samples were read at 630 nm using a spectrophotometer (T6, Beijing Purkinje General Instrument Co., Ltd., Beijing, China). A calibration curve with sucrose was used as a standard.

Organic acid extraction and identification

The organic acids in root tissue were extracted by the methods described by De la Fuente et al. (1997) and Lanoue et al. (2010) with some modifications. For this extraction, 500 mg frozen samples were grounded in a mortar with 2 ml of methanol:water (80:20, v/v). The plant material and solvent were shaken at 1200 rpm for 3 min and centrifuged at 12, 000 g for 5 min. The supernatant was used for high-performance liquid chromatography (HPLC) analyses.

The standard organic acid compounds that were used for HPLC were oxalic, malic, citrate, succinic and fumaric acids. The compounds were identified using an HPLC system (Agilent 1200, USA) with an XDB-C18 column (4.6×250 mm, Agilent, USA) according to the method of Wang et al. (2016). The analytical conditions were as follows: temperature of column: 40 °C, detector wavelength: 210 nm, and injection volume: 20 μl. The mobile phase consisted of 5 mmol L^{-1} H_2SO_4 (A) and methanol (B) with a gradient elution. The compositions of the gradients were as follows: 0 min, 95% A plus 5% B at a flow rate of 0.4 ml min^{-1} → 10 min, 90% A plus 10% B at a rate of 0.4 ml min^{-1}→15 min, 90% A plus 10% B at a rate of 0.4 ml min^{-1}→16 min, 90% A plus 10% B at a rate of 0.5 ml min^{-1}→20 min, 90% A plus 10% B at a rate of 0.5 ml min^{-1}→stop. All of the chemicals were of high purity, and the solvents were HPLC

spectral grade. Major peaks were identified by comparing the retention time with that of the matching standard.

Free amino acid extraction and identification

Amino acids in cucumber plants were extracted by the methods described by Kim (2009). Plant samples (0.5 g) were rapidly frozen in liquid nitrogen and ground to powder using a mortar and pestle. The frozen powder was diluted in 3% trichloroacetic acid for 1 h and centrifuged at 10, 000 rpm for 15 min. The supernatant was filtered through 0.45 μm Millipore membrane filters and analysed in a Biochrom 30 amino acid analyser (Biochrom, Cambridge, UK).

Transcriptome analysis of cucumber plants infected with FOC under different nitrogen forms

Transcriptome analysis of cucumber seedlings from different treatments was performed at 8 days post inoculation. In order to eliminate the variation between individual plants, roots from five different cucumber plants were mixed to prepare the pooled RNA sample for RNA-Seq. Poly(A)-containing mRNA was isolated using magnetic beads with oligo (dT) and fragmented into short pieces by fragmentation buffer. Then, cDNA was synthesized using the mRNA fragments as templates. After purification and end repair, the cDNA fragments were ligated to the sequencing adapters. The suitable fragments were selected for the PCR amplification as templates after agarose gel electrophoresis. During the quality control (QC) steps, an Agilent 2100 Bioanalyser and ABI Step One Plus Real-Time PCR System were used for the quantification and qualification of the sample library. Finally, the library was sequenced using Illumina HiSeq^{TM} 2000.

Reads that contained adapters, more than 5% unknown bases, and low-quality reads (the percentage of low-quality bases of quality value ≤10 was greater than 30% in a read) were removed, and all of the clean reads were aligned to the cucumber genome (downloaded from ftp://www.icugi.org/pub/genome/cucumber/Chinese_long/v2/) using SOAPaligner/ SOAP2 (Li et al. 2009) with no more than five mismatches. The gene expression level was calculated using the RPKM (reads per kilobase transcriptome per million mapped

reads) method (Mortazavi et al. 2008). The RPKM method is able to eliminate the influence of different gene lengths and sequencing discrepancies from the calculation of gene expression. Corrections for false positive and false negative errors were performed by calculating the FDR (false discovery rate) value (Benjamini and Yekutieli 2001). The DEGs (differentially expressed genes) were selected using FDR ≤ 0.001 and the absolute value of $Log_2Ratio \geq 1$ as threshold values.

Quantitative detection of FOC and disease index (DI)

Plant samples (100 mg) were ground into a fine power in liquid nitrogen using a mortar and pestle. Genomic DNA was extracted according to Lin et al. (2009). The isolated DNA samples were then used as templates for polymerase chain reactions using the FOC-specific SCAR primers, designed by Lievens et al. (2007). RT-qPCR was performed using a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA), according to the methods of Wang et al. (2016).

Disease indices were recorded from days 6 to 9 after inoculation with FOC and expressed on a scale of 0 – 4 as follows: 0, the entire plant was healthy; 1, ＜ 25% of leaves were wilted; 2, 25% - 50% of leaves were wilted; 3, 50% - 75% of leaves were wilted; and 4, 75% - 100% of leaves were wilted. The disease index was calculated using the following formula:

Disease index = [∑(rating × number of plants rated) / (highest rating × total number of plants)] ×100

Effects of organic acids and amino acids on FOC sporulation and fusaric acid (FA) production

Sporulation and FA production tests were performed in Bilai's medium (1 g KNO_3, 1 g KH_2PO_4, 0.5 g KCl, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.2 g starch, 0.2 g glucose, 0.2 g sucrose per 1 L) and Czapek-Dox medium (3 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g KCl, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$, 30g sucrose per 1 L), respectively. Amino acid could use as both carbon and nitrogen sources for microbe growth, while organic acid only use as carbon sources, then the carbon sources in the culture mediums were replaced by different amino acids and organic acids. The carbon sources of starch, glucose and sucrose in Bilai's medium and sucrose in Czapek-Dox medium were

replaced by different amino acids and organic acids. All cultures were inoculated with 2×10^7 conidia ml^{-1}, and cultivated on a rotary shaker at 180 rpm for 7 days at 28 °C in the dark. FOC sporulation in the Bilai's medium were counted in a hemocytometer.

FA in the Czapek-Dox medium was extracted using the method described by Wang et al. (2013). The medium were first filtrated with 0.45 mm membrane filters to exclude mycelium and microconidia, and acidified with 2 M HCl to pH 2.5. Then, the acidified supernatant was extracted sequentially three times using ethyl acetate, the ethyl acetate supernatants from both extraction steps were combined and evaporated to dryness under vacuum at 40 °C on a rotary evaporator. The residue was dissolved in methanol and analyzed using high-performance liquid chromatography (HPLC) according to Wang et al. (2016).

HPLC analyses were performed in an Agilent 1200 Series HPLC system (Agilent technologies, USA) equipped with an Agilent Zorbax Eclipse XDB-C18 column (4.6×250 mm, 5 μm). The temperature was set at 50 °C. The samples (10 μl) were eluted with methanol : 0.43% o-phosphoric acid (68%:32%) over a period of 15 min. The retention time was approximately 5.8 min, with a mobile-phase flow rate of 1 ml min^{-1}. FA was detected by monitoring the UV A_{271}. The samples were quantified against a standard curve of synthetic FA (Sigma).

Statistical analysis

The Spearman correlation coefficient (a non-parametric measure of correlation coefficient) was calculated using SPSS 16.0 to investigate the possible correlation between the root amino acids/organic acids and FOC number/disease incidence (DI), and visualized using the corrplot package in R. Aggregated boosted tree (ABT) analysis, a statistical learning method giving both accurate prediction and explanation (De'ath 2007), was carried out using the gbmplus package (with 500 trees used for the boosting, 0.02-folds shrinkage rate and three-way interactions) to quantitatively and visually evaluate the relative influence of amino acids and organic acids on FOC number and DI (relative variable importance plot).

The experiments were repeated three times. A one-way analysis of variance (ANOVA) was applied to assess differences in each parameter among treatments using

532 the SPSS 16.0 software. The means and standard deviation were presented from three
533 independent experiments. Significant differences ($P<0.05$) between treatments are
534 indicated by different letters.

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Disclosures

The authors have no conflicts of interest to declare.

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Figure legends

Figure 1 Effects of different nitrogen forms on cucumber Fusarium wilt in the pot experiment. Cucumber plants were supplied with ammonium (A) or nitrate (N) and were either not inoculated or inoculated with FOC (FOC-inoculated ammonium-grown plants are labeled AI; FOC-inoculated nitrate-grown plants are labeled NI).

Figure 2 Metabolite contents of cucumber plants under different nitrogen forms after FOC infection. a, Total carbon content; b, total nitrogen content; c, carbon to nitrogen (C:N) ratio; d, soluble protein content; e, soluble sugar content. All of the measurements were conducted at 8 days post inoculation. Cucumber plants were supplied with ammonium (A) or nitrate (N) and either non-inoculated or inoculated with FOC (FOC-inoculated ammonium-grown plants are labelled AI; FOC-inoculated nitrate-grown plants are labelled NI). The results represent the means ± SD of five replicates.

Figure 3 Organic acids in cucumber plants affected by different nitrogen forms and FOC infection. The oxalic, malic, citrate, succinic and fumaric acid levels (μmol g^{-1} FW) in the leaves (a) and roots (b) of cucumber plants were analysed at 8 days post inoculation. Cucumber plants were supplied with ammonium (A) or nitrate (N) either inoculated with water (non-inoculated) or inoculated with FOC (FOC-inoculated ammonium-grown plants as AI; FOC-inoculated nitrate-grown plants as NI). The results represent the means ± SD of five replicates, and significant differences ($P<0.05$) among the different treatments are indicated by different letters.

Figure 4 Heatmaps of the amino acids in cucumber plants affected by different nitrogen forms and FOC infection. The leaf (a) and root (b) amino acid levels were determined at 8 days post inoculation. Amino acids were clustered via correlation distance and hierarchical agglomerative clustering. Red denotes high content, blue denotes low content. Cucumber plants were supplied with ammonium (A) or nitrate (N) either inoculated with water (non-inoculated) or inoculated with FOC (FOC-inoculated ammonium-grown plants as AI; FOC-inoculated nitrate-grown plants as NI). The results represent the means ± SD of five replicates, and the significant differences

708 ($P<0.05$) among different treatments are indicated by different letters (Unit: μmol g^{-1}
709 FW).
710
711 Figure 5 Transcriptional regulation of central carbon and nitrogen metabolism in
712 response to different nitrogen forms and FOC infection. Cucumber plants were supplied
713 with ammonium (A) or nitrate (N) and either non-inoculated or inoculated with FOC
714 (FOC-inoculated ammonium-grown plants as AI; FOC-inoculated nitrate-grown plants
715 as NI). Transcriptome analysis of cucumber seedlings from different treatments was
716 performed at 8 days post inoculation. The expression of genes encoding enzymes
717 involved in central carbon and nitrogen metabolism in cucumber plants were regulated
718 by different nitrogen forms and FOC infection. The full description of the genes, exact
719 fold-change values, and false detection rate are presented in Table S1. Abbreviations:
720 PDH, pyruvate dehydrogenase; CS, citrate synthase; ACO, aconitate hydratase; IDH,
721 isocitrate dehydrogenase; OGDH, 2-oxoglutarate dehydrogenase; SAS, succinyl-CoA
722 synthetase, SDH, succinate dehydrogenase; FH, fumarate hydratase; MDH, malate
723 dehydrogenase; NR, nitrate reductase; NiR, nitrite reductase; GS, glutamine synthetase;
724 and GOGAT, glutamate synthase. Corrections for false positive and false negative
725 errors were performed by calculating the FDR (false discovery rate) value. Genes that
726 had a log twofold change greater than or equal to ±1 and FDR < 0.001 were considered
727 differentially expressed.
728
729 Figure 6 Amino acid biosynthesis regulated by different nitrogen forms and FOC
730 infection in cucumber plants. Heatmap representation of amino acid biosynthetic
731 pathway gene expression under different nitrogen forms during FOC infection.
732 Cucumber plants were supplied with ammonium (A) or nitrate (N) and either non-
733 inoculated or inoculated with FOC (FOC-inoculated ammonium-grown plants as AI;
734 FOC-inoculated nitrate-grown plants as NI). The full description of the genes, exact
735 fold-change values, and false detection rate are presented in Table S2. Corrections for
736 false positive and false negative errors were performed by calculating the FDR (false
737 discovery rate) value. Genes that had a log twofold change greater than or equal to ±1

and FDR < 0.001 were considered differentially expressed.

Figure 7 Changes in nitrogen and carbon metabolism in response to Fusarium oxysporum infection in cucumber plants under different nitrogen forms. The amino acid levels, organic acid levels and related genes expression were present the ratio of NI to AI (NI/AI). The coloured bar limits show 6-fold up- or down-regulation (blue indicates low content or expression; red indicates high content or expression). After FOC infection, the levels of most of amino acids and the expression of related genes were higher in ammonium-grown plants, while the levels of most of the organic acids and the expression of related gene were higher in nitrate-grown plants.

Figure 8 Effects of root amino acids and organic acids on FOC number and disease index (DI) of cucumber plants. (a) Spearman correlational analysis between the root amino acids/organic acids and FOC number/disease incidence (DI). The heat map displays the effect size measure is represented by the type and intensity of the colour (red is negative, blue is positive), whereas the statistical significance of the analysis is represented by the asterisk in the rectangle (* $P < 0.05$ and ** $P < 0.01$). (b) Relative influence of amino acids and organic acids on FOC number and DI. Amino acids and organic acids which are statistical significance with FOC number and DI in Fig. 8a were choose.

Figure 9 Effects of amino acids and organic acid on sporulation (a) and fusaric acid (FA) production (b) of FOC. The original Bilai's and Czapek-Dox medium were defined as CK. Asterisk (*) indicate significant difference to CK ($P < 0.05$). The results represent the means ± SD of five replicates.

Figure 10 Model of different nitrogen forms on cucumber Fusarium wilt disease. The increase of ammonium/nitrate regulated the genes expression involved in carbon and nitrogen metabolisms, and increased amino acids in cucumber plants which stimulated

767 FA production and FOC sporulation, thus increased disease incidence of cucumber
768 Fusarium wilt.
769

Supplement Data

Table S1 The expression of genes involved in the central carbon and nitrogen metabolism of cucumber plants. Corrections for false positive and false negative errors were performed by calculating the FDR (false discovery rate) value. Genes that had a log twofold change greater than or equal to ±1 and FDR < 0.001 were considered differentially expressed. Red indicates up-regulation, and green indicates down-regulation.

Table S2 The expression of genes involved in amino acid biosynthesis in cucumber plants. Corrections for false positive and false negative errors were performed by calculating the FDR (false discovery rate) value. Genes that had a log twofold change greater than or equal to ±1 and FDR < 0.001 were considered differentially expressed. Red indicates up-regulation, and green indicates down-regulation.

Figure S1 Effects of different nitrogen forms on disease index of Fusarium wilt and root Fusarium oxysporum f. sp. cucumerinum (FOC) colonization. Cucumber plants were supplied with ammonium or nitrate, and inoculated with FOC for eight days. FOC-inoculated ammonium-grown plants are labelled AI; FOC-inoculated nitrate-grown plants are labelled NI. The results represent the means ± SD of five replicates.

Figure S2 Overview of amino acid biosynthesis in higher plants (Modified from Buchanan et al., 2000).

Figure S3 Changes in nitrogen and carbon metabolism in response to Fusarium oxysporum infection in cucumber plants under different nitrogen forms. The amino acid levels, organic acid levels and related genes expression were compared between different nitrogen forms and FOC infection. Panels a and b present the ratio of AI to A (AI/A) and NI to N (NI/N), respectively. The coloured bar limits show 6-fold up- or down-regulation (blue indicates low content or expression; red indicates high content or expression).

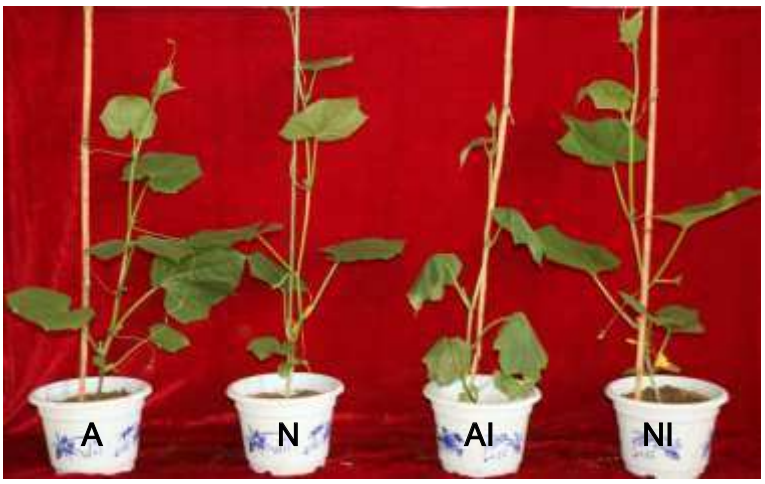


Figure 1 Effects of different nitrogen forms on cucumber Fusarium wilt in the pot experiment. Cucumber plants were supplied with ammonium (A) or nitrate (N) and were either not inoculated or inoculated with FOC (FOC-inoculated ammonium-grown plants are labeled AI; FOC-inoculated nitrate-grown plants are labeled NI).

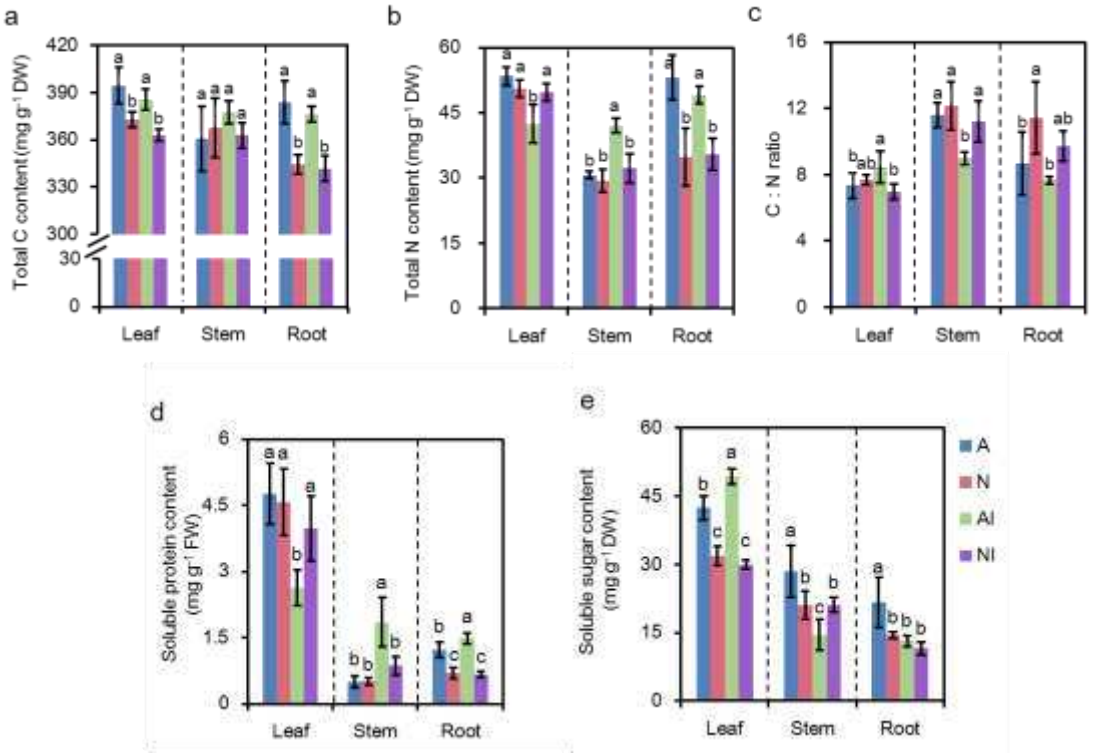


Figure 2 Metabolite contents of cucumber plants under different nitrogen forms after FOC infection. a, Total carbon content; b, total nitrogen content; c, carbon to nitrogen (C:N) ratio; d, soluble protein content; e, soluble sugar content. All of the measurements were conducted at 8 days post inoculation. Cucumber plants were supplied with ammonium (A) or nitrate (N) and either non-inoculated or inoculated with FOC (FOC-inoculated ammonium-grown plants are labelled AI; FOC-inoculated nitrate-grown plants are labelled NI). The results represent the means ± SD of five replicates.

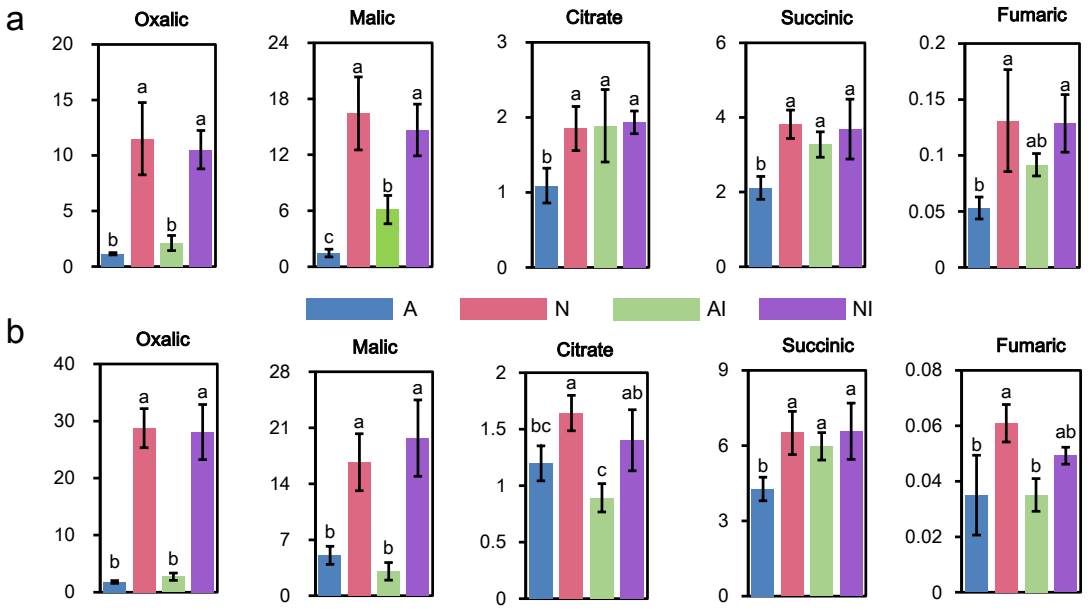
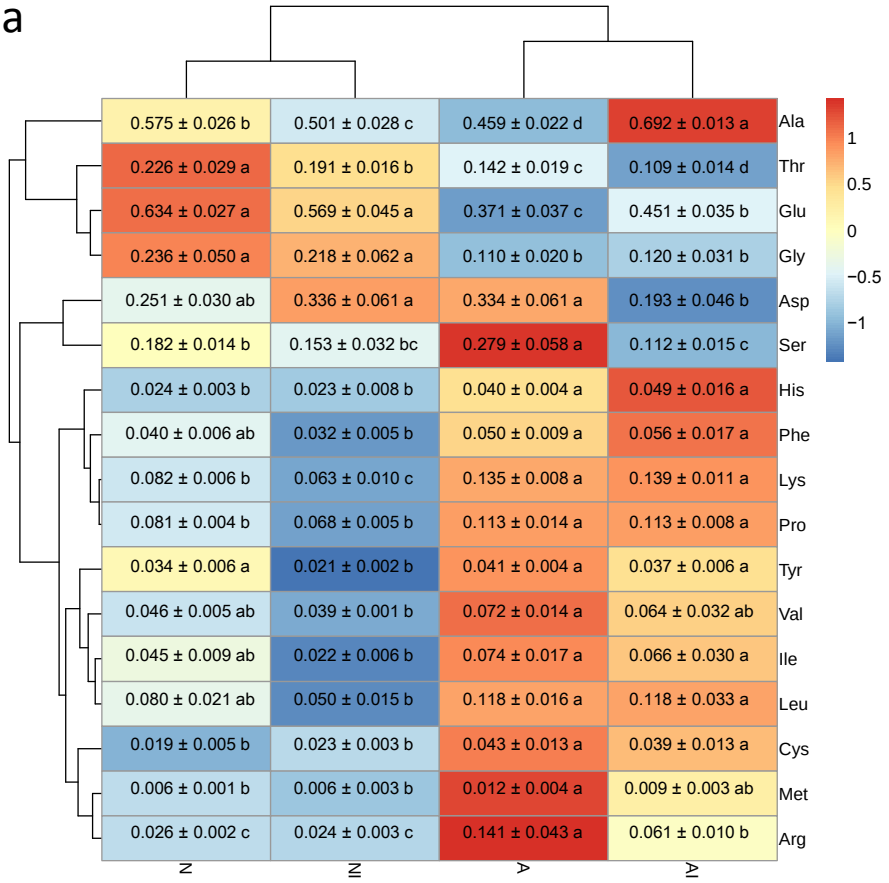


Figure 3 Organic acids in cucumber plants affected by different nitrogen forms and FOC infection. The oxalic, malic, citrate, succinic and fumaric acid levels (μmol g^{-1} FW) in the leaves (a) and roots (b) of cucumber plants were analysed at 8 days post inoculation. Cucumber plants were supplied with ammonium (A) or nitrate (N) either inoculated with water (non-inoculated) or inoculated with FOC (FOC-inoculated ammonium-grown plants as AI; FOC-inoculated nitrate-grown plants as NI). The results represent the means ± SD of five replicates, and significant differences ($P<0.05$) among the different treatments are indicated by different letters.

a


0.575 ± 0.026 b
0.501 ± 0.028 c
0.459 ± 0.022 d
0.692 ± 0.013 a
Ala
0.226 ± 0.029 a
0.191 ± 0.016 b
0.142 ± 0.019 c
0.109 ± 0.014 d
Thr
0.634 ± 0.027 a
0.569 ± 0.045 a
0.371 ± 0.037 c
0.451 ± 0.035 b
Glu
0.236 ± 0.050 a
0.218 ± 0.062 a
0.110 ± 0.020 b
0.120 ± 0.031 b
Gly
0.251 ± 0.030 ab
0.336 ± 0.061 a
0.334 ± 0.061 a
0.193 ± 0.046 b
Asp
0.182 ± 0.014 b
0.153 ± 0.032 bc
0.279 ± 0.058 a
0.112 ± 0.015 c
Ser
0.024 ± 0.003 b
0.023 ± 0.008 b
0.040 ± 0.004 a
0.049 ± 0.016 a
His
0.040 ± 0.006 ab
0.032 ± 0.005 b
0.050 ± 0.009 a
0.056 ± 0.017 a
Phe
0.082 ± 0.006 b
0.063 ± 0.010 c
0.135 ± 0.008 a
0.139 ± 0.011 a
Lys
0.081 ± 0.004 b
0.068 ± 0.005 b
0.113 ± 0.014 a
0.113 ± 0.008 a
Pro
0.034 ± 0.006 a
0.021 ± 0.002 b
0.041 ± 0.004 a
0.037 ± 0.006 a
Tyr
0.046 ± 0.005 ab
0.039 ± 0.001 b
0.072 ± 0.014 a
0.064 ± 0.032 ab
Val
0.045 ± 0.009 ab
0.022 ± 0.006 b
0.074 ± 0.017 a
0.066 ± 0.030 a
Ile
0.080 ± 0.021 ab
0.050 ± 0.015 b
0.118 ± 0.016 a
0.118 ± 0.033 a
Leu
0.019 ± 0.005 b
0.023 ± 0.003 b
0.043 ± 0.013 a
0.039 ± 0.013 a
Cys
0.006 ± 0.001 b
0.006 ± 0.003 b
0.012 ± 0.004 a
0.009 ± 0.003 ab
Met
0.026 ± 0.002 c
0.024 ± 0.003 c
0.141 ± 0.043 a
0.061 ± 0.010 b
Arg
1
0.5
0
−0.5
−1
N
N
A
AI

b

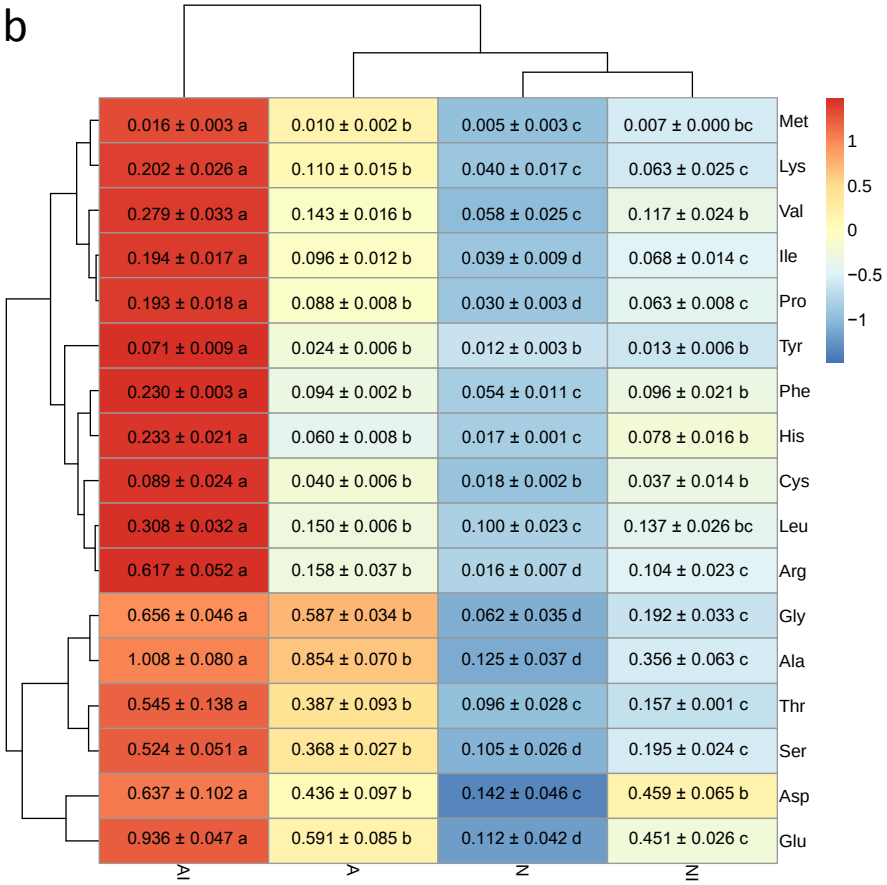

0.016 ± 0.003 a
0.010 ± 0.002 b
0.005 ± 0.003 c
0.007 ± 0.000 bc
Met
0.202 ± 0.026 a
0.110 ± 0.015 b
0.040 ± 0.017 c
0.063 ± 0.025 c
Lys
0.279 ± 0.033 a
0.143 ± 0.016 b
0.058 ± 0.025 c
0.117 ± 0.024 b
Val
0.194 ± 0.017 a
0.096 ± 0.012 b
0.039 ± 0.009 d
0.068 ± 0.014 c
Ile
0.193 ± 0.018 a
0.088 ± 0.008 b
0.030 ± 0.003 d
0.063 ± 0.008 c
Pro
0.071 ± 0.009 a
0.024 ± 0.006 b
0.012 ± 0.003 b
0.013 ± 0.006 b
Tyr
0.230 ± 0.003 a
0.094 ± 0.002 b
0.054 ± 0.011 c
0.096 ± 0.021 b
Phe
0.233 ± 0.021 a
0.060 ± 0.008 b
0.017 ± 0.001 c
0.078 ± 0.016 b
His
0.089 ± 0.024 a
0.040 ± 0.006 b
0.018 ± 0.002 b
0.037 ± 0.014 b
Cys
0.308 ± 0.032 a
0.150 ± 0.006 b
0.100 ± 0.023 c
0.137 ± 0.026 bc
Leu
0.617 ± 0.052 a
0.158 ± 0.037 b
0.016 ± 0.007 d
0.104 ± 0.023 c
Arg
0.656 ± 0.046 a
0.587 ± 0.034 b
0.062 ± 0.035 d
0.192 ± 0.033 c
Gly
1.008 ± 0.080 a
0.854 ± 0.070 b
0.125 ± 0.037 d
0.356 ± 0.063 c
Ala
0.545 ± 0.138 a
0.387 ± 0.093 b
0.096 ± 0.028 c
0.157 ± 0.001 c
Thr
0.524 ± 0.051 a
0.368 ± 0.027 b
0.105 ± 0.026 d
0.195 ± 0.024 c
Ser
0.637 ± 0.102 a
0.436 ± 0.097 b
0.142 ± 0.046 c
0.459 ± 0.065 b
Asp
0.936 ± 0.047 a
0.591 ± 0.085 b
0.112 ± 0.042 d
0.451 ± 0.026 c
Glu
1
0.5
0
−0.5
−1
AI
A
N
N

Figure 4 Heatmaps of the amino acids in cucumber plants affected by different nitrogen forms and FOC infection. The leaf (a) and root (b) amino acid levels were determined at 8 days post inoculation. Amino acids were clustered via correlation distance and hierarchical agglomerative clustering. Red denotes high content, blue denotes low content. Cucumber plants were supplied with ammonium (A) or nitrate (N) either inoculated with water (non-inoculated) or inoculated with FOC (FOC-inoculated ammonium-grown plants as AI; FOC-inoculated nitrate-grown plants as NI). The related amino acid contents were presented. The results represent the means ± SD of five replicates, and the significant differences ($P<0.05$) among different treatments are indicated by different letters (Unit: μmol g^{-1} FW).

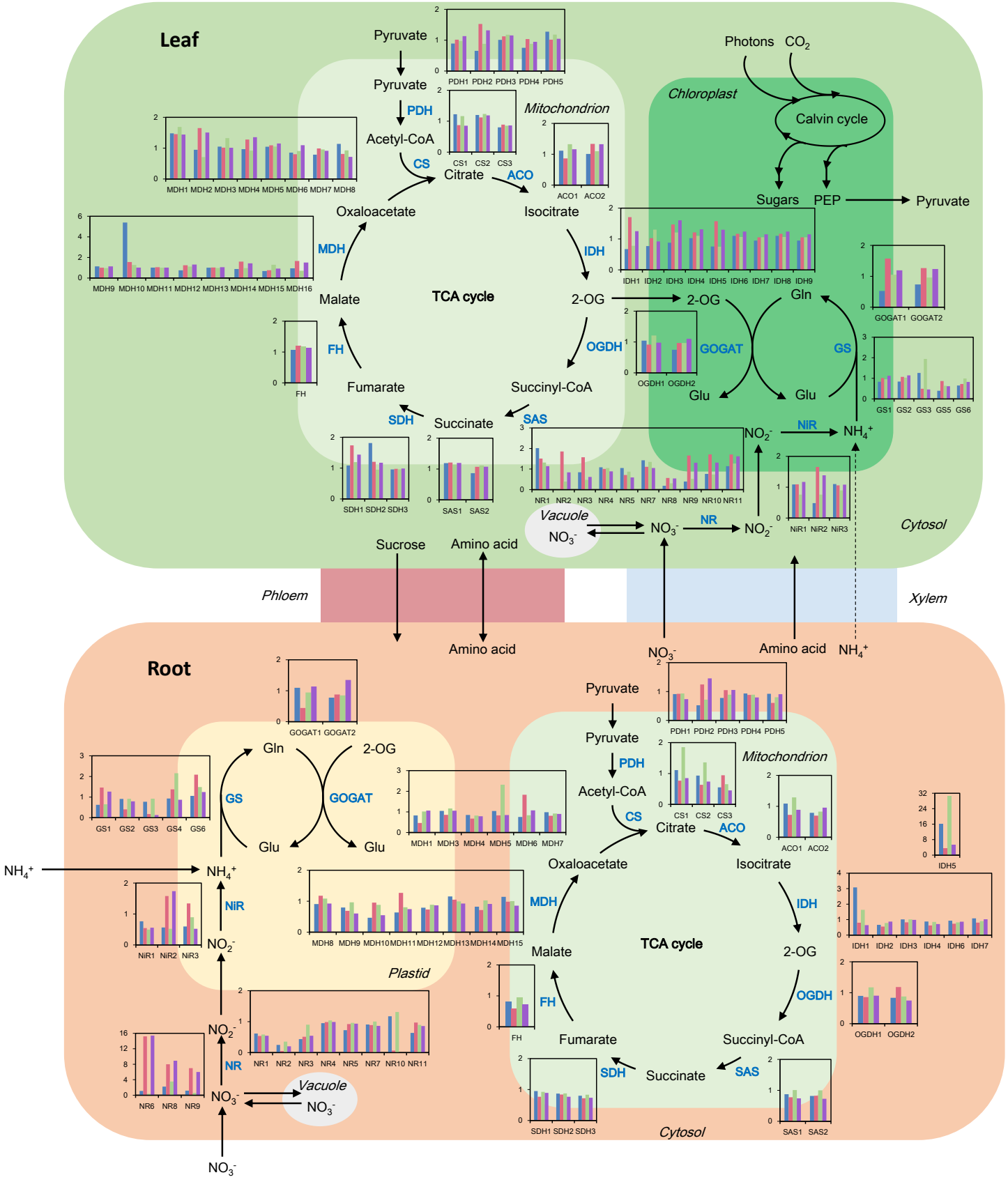

Leaf
Pyruvate
Pyruvate
PDH
Acetyl-CoA
CS
Citrate
ACO
Oxaloacetate
Isocitrate
MDH
IDH
Malate
TCA cycle
2-OG
FH
OGDH
Fumarate
Succinyl-CoA
SDH
Succinate
SAS
Mitochondrion
Chloroplast
Photons
CO2
Calvin cycle
Sugars
PEP
Pyruvate
Gln
GOGAT
GS
Glu
NO2-
NiR
NH4+
NR
NO3-
Vacuole
Cytosol
Sucrose
Amino acid
Phloem
Xylem
Root
Plastid
NH4+
NO3-

Figure 5 Transcriptional regulation of central carbon and nitrogen metabolism in response to different nitrogen forms and FOC infection. Cucumber plants were supplied with ammonium (A) or nitrate (N) and either non-inoculated or inoculated with FOC (FOC-inoculated ammonium-grown plants as AI; FOC-inoculated nitrate-grown plants as NI). Transcriptome analysis of cucumber seedlings from different treatments was performed at 8 days post inoculation. The expression of genes encoding enzymes involved in central carbon and nitrogen metabolism in cucumber plants were regulated by different nitrogen forms and FOC infection. The full description of the genes, exact fold-change values, and false detection rate are presented in Table S1. Abbreviations: PDH, pyruvate dehydrogenase; CS, citrate synthase; ACO, aconitate hydratase; IDH, isocitrate dehydrogenase; OGDH, 2-oxoglutarate dehydrogenase; SAS, succinyl-CoA synthetase, SDH, succinate dehydrogenase; FH, fumarate hydratase; MDH, malate dehydrogenase; NR, nitrate reductase; NiR, nitrite reductase; GS, glutamine synthetase; and GOGAT, glutamate synthase. Corrections for false positive and false negative errors were performed by calculating the FDR (false discovery rate) value. Genes that had a log twofold change greater than or equal to ±1 and FDR < 0.001 were considered differentially expressed.

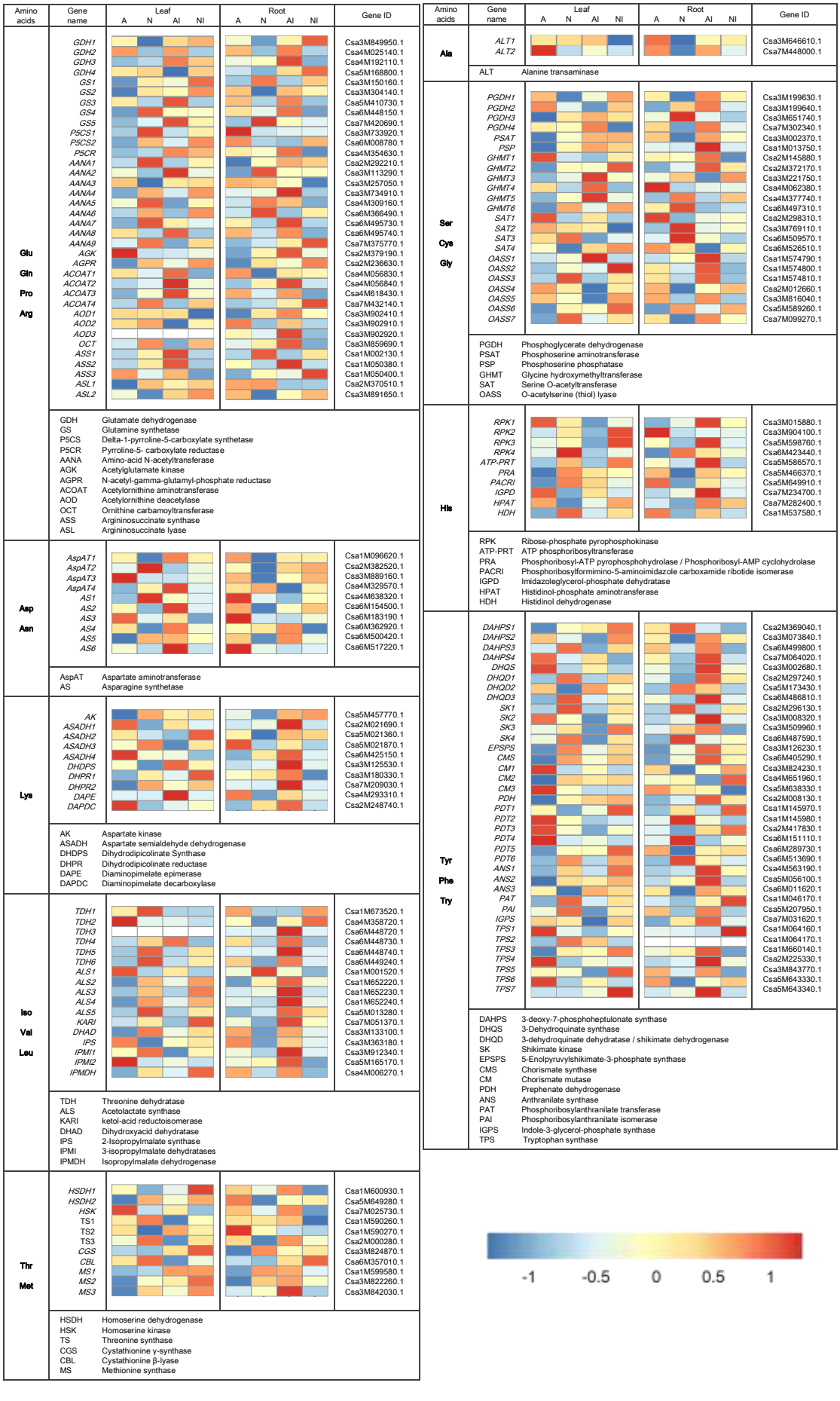

Amino acids
Gene name
Leaf
A N AI NI
Root
A N AI NI
Gene ID
Glu
Gln
Pro
Arg
GDH1 GDH2 GDH3 GDH4 GS1 GS2 GS3 GS4 GS5 P5CS1 P5CS2 P5CR AANA1 AANA2 AANA3 AANA4 AANA5 AANA6 AANA7 AANA8 AANA9 AGK AGPR ACOAT1 ACOAT2 ACOAT3 ACOAT4 AOD1 AOD2 AOD3 OCT ASS1 ASS2 ASS3 ASL1 ASL2
Csa3M849950.1 Csa4M025140.1 Csa4M192110.1 Csa5M168800.1 Csa3M150160.1 Csa3M304140.1 Csa5M410730.1 Csa6M448150.1 Csa7M420690.1 Csa3M733920.1 Csa6M008780.1 Csa4M354630.1 Csa2M292210.1 Csa3M113290.1 Csa3M257050.1 Csa3M734910.1 Csa4M309160.1 Csa6M366490.1 Csa6M495730.1 Csa6M495740.1 Csa7M375770.1 Csa2M379190.1 Csa2M236630.1 Csa4M056830.1 Csa4M056840.1 Csa4M618430.1 Csa7M432140.1 Csa3M902410.1 Csa3M902910.1 Csa3M902920.1 Csa3M859690.1 Csa1M002130.1 Csa1M050380.1 Csa1M050400.1 Csa2M370510.1 Csa3M891650.1
GDH Glutamate dehydrogenase
GS Glutamine synthetase
P5CS Delta-1-pyrroline-5-carboxylate synthetase
P5CR Pyrroline-5- carboxylate reductase
AANA Amino-acid N-acetyltransferase
AGK Acetylglutamate kinase
AGPR N-acetyl-gamma-glutamyl-phosphate reductase
ACOAT Acetylornithine aminotransferase
AOD Acetylornithine deacetylase
OCT Ornithine carbamoyltransferase
ASS Argininosuccinate synthase
ASL Argininosuccinate lyase
Asp
Asn
AspAT1 AspAT2 AspAT3 AspAT4 AS1 AS2 AS3 AS4 AS5 AS6
Csa1M096620.1 Csa2M382520.1 Csa3M889160.1 Csa4M329570.1 Csa4M638320.1 Csa6M154500.1 Csa6M183190.1 Csa6M362920.1 Csa6M500420.1 Csa6M517220.1
AspAT Aspartate aminotransferase
AS Asparagine synthetase
Lys
AK ASADH1 ASADH2 ASADH3 ASADH4 DHDPS DHPR1 DHPR2 DAPE DAPDC
Csa5M457770.1 Csa2M021690.1 Csa5M021360.1 Csa5M021870.1 Csa6M425150.1 Csa3M125530.1 Csa3M180330.1 Csa7M209030.1 Csa4M293310.1 Csa2M248740.1
AK Aspartate kinase
ASADH Aspartate semialdehyde dehydrogenase
DHDPS Dihydrodipicolinate Synthase
DHPR Dihydrodipicolinate reductase
DAPE Diaminopimelate epimerase
DAPDC Diaminopimelate decarboxylase
Iso
Val
Leu
TDH1 TDH2 TDH3 TDH4 TDH5 TDH6 ALS1 ALS2 ALS3 ALS4 ALS5 KARI DHAD IPS IPMI1 IPMI2 IPMDH
Csa1M673520.1 Csa4M358720.1 Csa6M448720.1 Csa6M448730.1 Csa6M448740.1 Csa6M449240.1 Csa1M001520.1 Csa1M652220.1 Csa1M652230.1 Csa1M652240.1 Csa5M013280.1 Csa7M051370.1 Csa3M133100.1 Csa3M363180.1 Csa3M912340.1 Csa5M165170.1 Csa4M006270.1
TDH Threonine dehydratase
ALS Acetolactate synthase
KARI ketol-acid reductoisomerase
DHAD Dihydroxyacid dehydratase
IPS 2-Isopropylmalate synthase
IPMI 3-isopropylmalate dehydratases
IPMDH Isopropylmalate dehydrogenase
Thr
Met
HSDH1 HSDH2 HSK TS1 TS2 TS3 CGS CBL MS1 MS2 MS3
Csa1M600930.1 Csa5M649280.1 Csa7M025730.1 Csa1M590260.1 Csa1M590270.1 Csa2M000280.1 Csa3M824870.1 Csa6M357010.1 Csa1M599580.1 Csa3M822260.1 Csa3M842030.1
HSDH Homoserine dehydrogenase
HSK Homoserine kinase
TS Threonine synthase
CGS Cystathionine γ-synthase
CBL Cystathionine β-lyase
MS Methionine synthase
Amino acids
Gene name
Leaf
A N AI NI
Root
A N AI NI
Gene ID
Ala
ALT1 ALT2
Csa3M646610.1 Csa7M448000.1
ALT Alanine transaminase
Ser
Cys
Gly
PGDH1 PGDH2 PGDH3 PGDH4 PSAT PSP GHMT1 GHMT2 GHMT3 GHMT4 GHMT5 GHMT6 SAT1 SAT2 SAT3 SAT4 OASS1 OASS2 OASS3 OASS4 OASS5 OASS6 OASS7
Csa3M199630.1 Csa3M199640.1 Csa3M651740.1 Csa7M302340.1 Csa3M002370.1 Csa1M013750.1 Csa2M145880.1 Csa2M372170.1 Csa3M221750.1 Csa4M062380.1 Csa4M377740.1 Csa6M497310.1 Csa2M298310.1 Csa3M769110.1 Csa6M509570.1 Csa6M526510.1 Csa1M574790.1 Csa1M574800.1 Csa1M574810.1 Csa2M012660.1 Csa3M816040.1 Csa5M589260.1 Csa7M099270.1
PGDH Phosphoglycerate dehydrogenase
PSAT Phosphoserine aminotransferase
PSP Phosphoserine phosphatase
GHMT Glycine hydroxymethyltransferase
SAT Serine O-acetyltransferase
OASS O-acetylserine (thiol) lyase
His
RPK1 RPK2 RPK3 RPK4 ATP-PRT PRA PACRI IGPD HPAT HDH
Csa3M015880.1 Csa3M904100.1 Csa5M598760.1 Csa6M423440.1 Csa5M586570.1 Csa5M466370.1 Csa5M649910.1 Csa7M234700.1 Csa7M282400.1 Csa1M537580.1
RPK Ribose-phosphate pyrophosphokinase
ATP-PRT ATP phosphoribosyltransferase
PRA Phosphoribosyl-ATP pyrophosphohydrolase / Phosphoribosyl-AMP cyclohydrolase
PACRI Phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase
IGPD Imidazoleglycerol-phosphate dehydratase
HPAT Histidinol-phosphate aminotransferase
HDH Histidinol dehydrogenase
Tyr
Phe
Try
DAHPS1 DAHPS2 DAHPS3 DAHPS4 DHQS DHQD1 DHQD2 DHQD3 SK1 SK2 SK3 SK4 EPSPS CMS CM1 CM2 CM3 PDH PDT1 PDT2 PDT3 PDT4 PDT5 PDT6 ANS1 ANS2 ANS3 PAT PAI IGPS TPS1 TPS2 TPS3 TPS4 TPS5 TPS6 TPS7
Csa2M369040.1 Csa3M073840.1 Csa6M499800.1 Csa7M064020.1 Csa3M002680.1 Csa2M297240.1 Csa5M173430.1 Csa6M486810.1 Csa2M296130.1 Csa3M008320.1 Csa3M509960.1 Csa6M487590.1 Csa3M126230.1 Csa6M405290.1 Csa3M824230.1 Csa4M651960.1 Csa5M638330.1 Csa2M008130.1 Csa1M145970.1 Csa1M145980.1 Csa2M417830.1 Csa6M151110.1 Csa6M289730.1 Csa6M513690.1 Csa4M563190.1 Csa5M056100.1 Csa6M011620.1 Csa1M046170.1 Csa5M207950.1 Csa7M031620.1 Csa1M064160.1 Csa1M064170.1 Csa1M660140.1 Csa2M225330.1 Csa3M843770.1 Csa5M643330.1 Csa5M643340.1
DAHPS 3-deoxy-7-phosphoheptulonate synthase
DHQS 3-Dehydroquinate synthase
DHQD 3-dehydroquinate dehydratase / shikimate dehydrogenase
SK Shikimate kinase
EPSPS 5-Enolpyruvylshikimate-3-phosphate synthase
CMS Chorismate synthase
CM Chorismate mutase
PDH Prephenate dehydrogenase
ANS Anthranilate synthase
PAT Phosphoribosylanthranilate transferase
PAI Phosphoribosylanthranilate isomerase
IGPS Indole-3-glycerol-phosphate synthase
TPS Tryptophan synthase
-1 -0.5 0 0.5 1

Figure 6 Amino acid biosynthesis regulated by different nitrogen forms and FOC infection in cucumber plants. Heatmap representation of amino acid biosynthetic pathway gene expression under different nitrogen forms during FOC infection. Cucumber plants were supplied with ammonium (A) or nitrate (N) and either non-inoculated or inoculated with FOC (FOC-inoculated ammonium-grown plants as AI; FOC-inoculated nitrate-grown plants as NI). The full description of the genes, exact fold-change values, and false detection rate are presented in Table S2. Corrections for false positive and false negative errors were performed by calculating the FDR (false discovery rate) value. Genes that had a log twofold change greater than or equal to ±1 and FDR < 0.001 were considered differentially expressed.

NI/AI

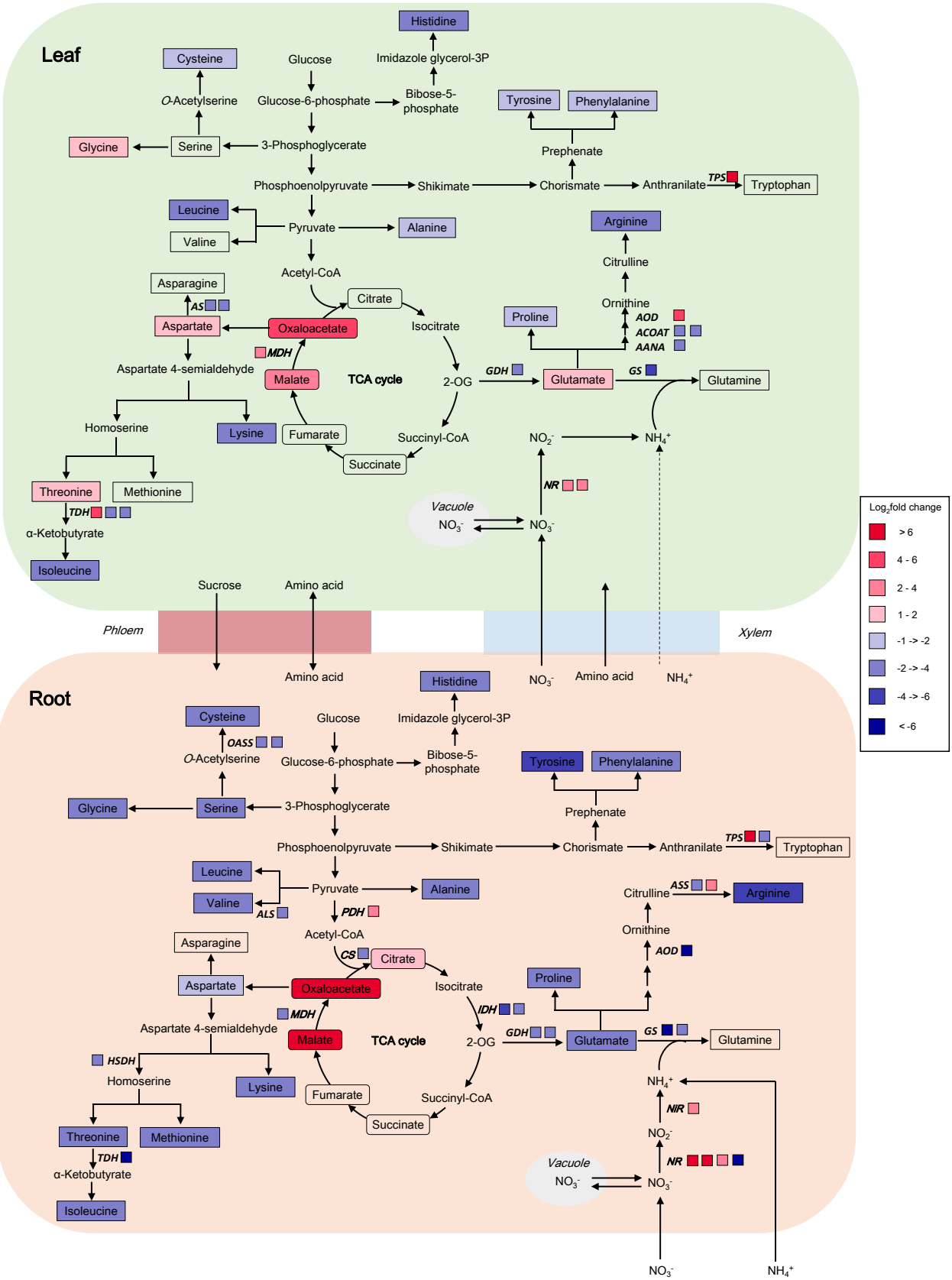

Leaf
Histidine
Imidazole glycerol-3P
Cysteine
Glucose
O-Acetylserine
Glucose-6-phosphate
Bibose-5-phosphate
Tyrosine
Phenylalanine
Glycine
Serine
3-Phosphoglycerate
Prephenate
Phosphoenolpyruvate
Shikimate
Chorismate
Anthranilate
TPS
Tryptophan
Leucine
Valine
Pyruvate
Alanine
Arginine
Citrulline
Acetyl-CoA
Asparagine
AS
Citrate
Ornithine
Proline
AOD
ACOAT
AANA
Aspartate
Oxaloacetate
Isocitrate
MDH
Aspartate 4-semialdehyde
Malate
TCA cycle
2-OG
GDH
Glutamate
GS
Glutamine
Homoserine
Lysine
Fumarate
Succinyl-CoA
Succinate
NO2-
NH4+
NR
Threonine
Methionine
TDH
Vacuole
NO3-
α-Ketobutyrate
Isoleucine
Sucrose
Amino acid
Phloem
Xylem
Root
OASS
HSDH
ALS
PDH
CS
IDH
NIR
Log2fold change
> 6
4 - 6
2 - 4
1 - 2
-1 -> -2
-2 -> -4
-4 -> -6
< -6

Figure 7 Changes in nitrogen and carbon metabolism in response to Fusarium oxysporum infection in cucumber plants under different nitrogen forms. The amino acid levels, organic acid levels and related genes expression were present the ratio of NI to AI (NI/AI). The coloured bar limits show 6-fold up- or down-regulation (blue indicates low content or expression; red indicates high content or expression). After FOC infection, the levels of most of amino acids and the expression of related genes were higher in ammonium-grown plants, while the levels of most of the organic acids and the expression of related gene were higher in nitrate-grown plants.

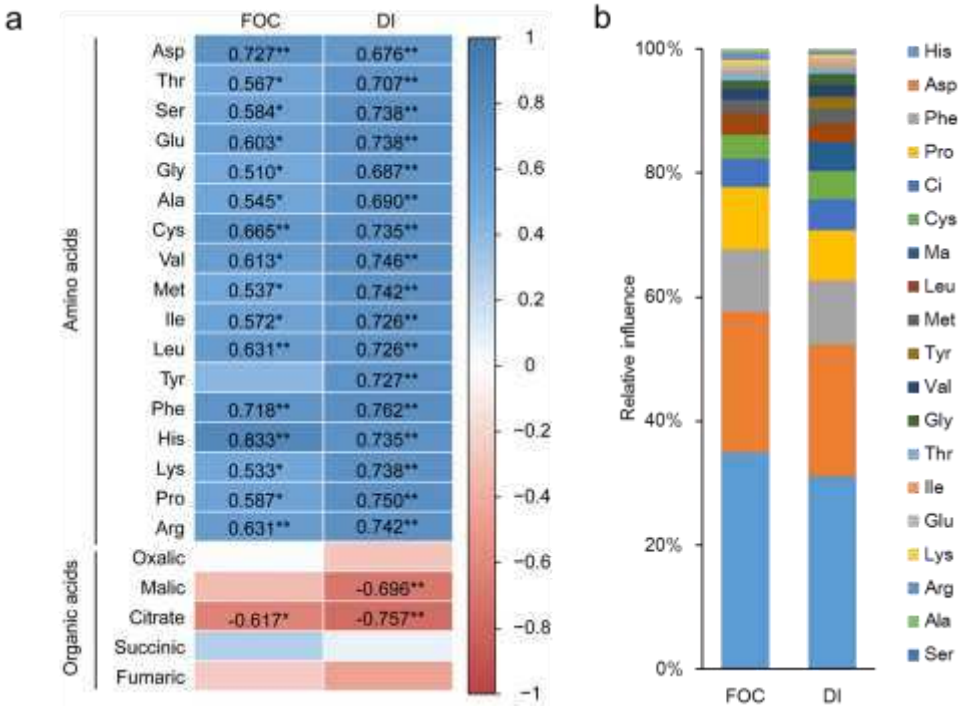


Figure 8 Effects of root amino acids and organic acids on FOC number and disease index (DI) of cucumber plants. (a) Spearman correlational analysis between the root amino acids/organic acids and FOC number/disease incidence (DI). The heat map displays the effect size measure is represented by the type and intensity of the colour (red is negative, blue is positive), whereas the statistical significance of the analysis is represented by the asterisk in the rectangle (* $P < 0.05$ and ** $P < 0.01$). (b) Relative influence of amino acids and organic acids on FOC number and DI. FOC number in cucumber plant was quantitatively detected by RT-qPCR. Amino acids and organic acids which are statistical significance with FOC number and DI in Fig. 8a were choose.

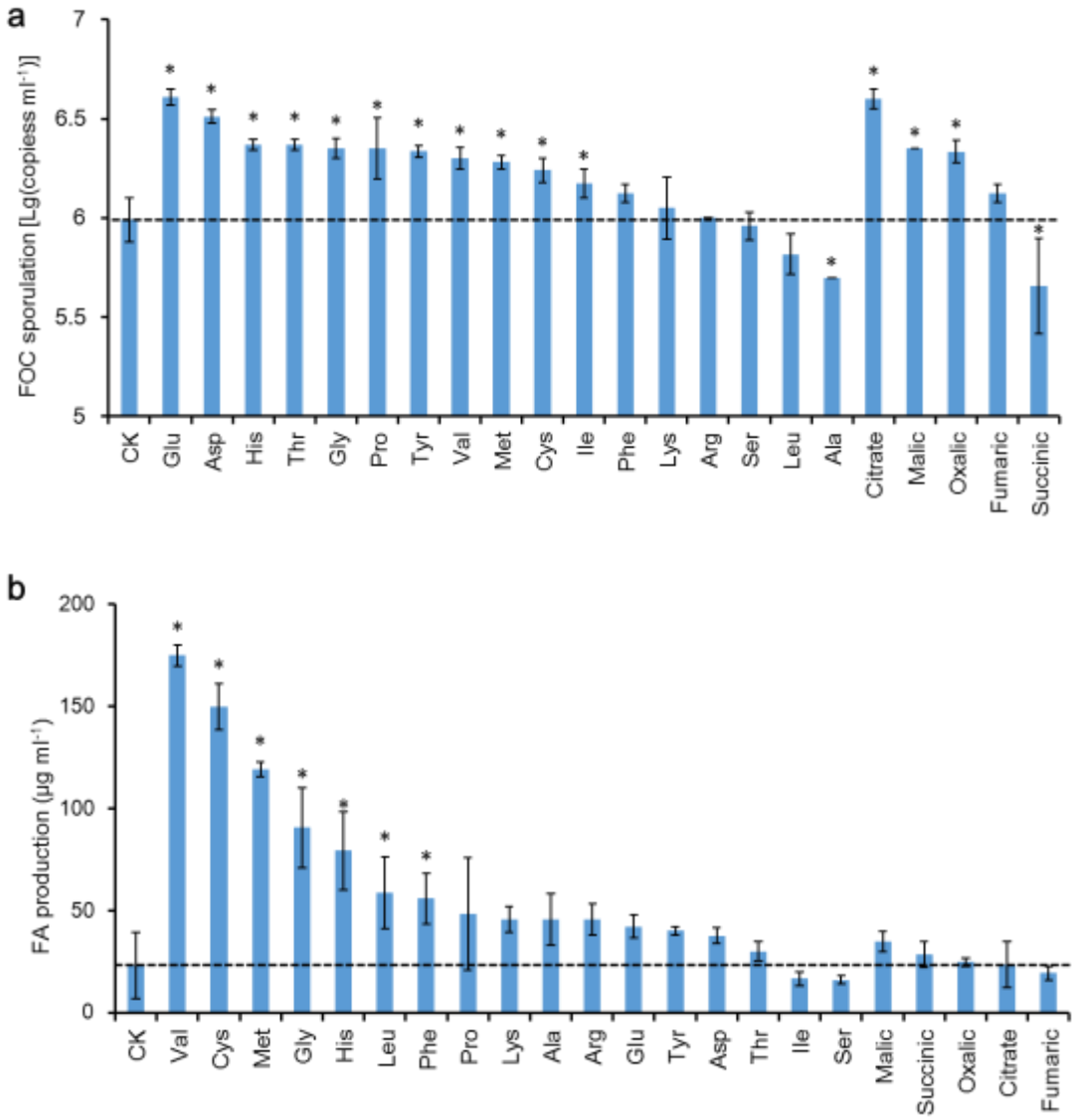


Figure 9 Effects of amino acids and organic acid on sporulation (a) and fusaric acid (FA) production (b) of FOC. The original Bilai's and Czapek-Dox medium were defined as CK. Asterisk (*) indicate significant difference to CK ($P < 0.05$). The results represent the means ± SD of five replicates.

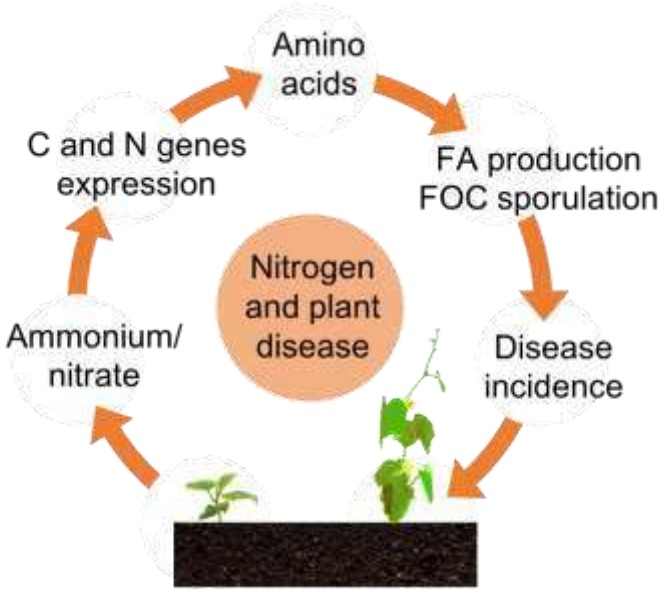


Figure 10 Model of different nitrogen forms on cucumber Fusarium wilt disease. The increase of ammonium/nitrate regulated the genes expression involved in carbon and nitrogen metabolisms, and increased amino acids in cucumber plants which stimulated FA production and FOC sporulation, thus increased disease incidence of cucumber Fusarium wilt.

Table S2 The expression of genes involved in amino acid biosynthesis in cucumber plants. Corrections for fals FDR (false discovery rate) value. Genes that had a log twofold change greater than or equal to ±1 and FDR < 0 regulation, and green indicates down-regulation.

						Leaf	
Amino acids	**Gene name**			**geneID**	**log_2(N/A)**	**log_2(AI/A)**	**log_2(NI/N)**
Glu Gln Pro Arg	GDH	Glutamate dehydrogenase [EC: 1.4.1.4]	GDH1	Csa3M849950.1	-0.07949	0.017175	0.108333
			GDH2	Csa4M025140.1	-1.22111	-0.02122	0.085201
			GDH3	Csa4M192110.1	0.023146	0.19579	0.132137
			GDH4	Csa5M168800.1	0.061492	-0.65889	0.012556
	GS	Glutamine synthetase [EC: 6.3.1.2]	GS1	Csa3M150160.1	0.284783	0.200576	0.146763
			GS2	Csa3M304140.1	0.345118	0.290667	0.092315
			GS3	Csa5M410730.1	-1.35449	0.619969	-0.10357
			GS4	Csa6M448150.1	1.136658	0.687993	-0.4949
			GS5	Csa7M420690.1	0.154279	0.609451	0.189498
	P5CS	Delta-1-pyrroline-5-carboxylate synthetase [EC: 2.7.2.11 1.2.1.41]	P5CS1	Csa3M733920.1	0.628983	0.065832	-0.28358
			P5CS2	Csa6M008780.1	0.792957	0.308864	0.09214
	P5CR	Pyrroline-5-carboxylate reductase [EC: 1.5.1.2]	P5CR	Csa4M354630.1	0.07798	0.500172	0.363053
	AANA	Amino-acid N-acetyltransferase [EC: 2.3.1.1]	AANA1	Csa2M292210.1	0.381953	-0.02221	-0.20405
			AANA2	Csa3M113290.1	-0.11973	0.245644	0.100789
			AANA3	Csa3M257050.1	-0.21535	-0.02728	0.200859
			AANA4	Csa3M734910.1	0.623562	0.214947	0.036912
			AANA5	Csa4M309160.1	0.471356	-0.42756	-0.34939
			AANA6	Csa6M366490.1	0.547107	-0.07564	0.043362
			AANA7	Csa6M495730.1	0.331629	0.191795	-0.23025
			AANA8	Csa6M495740.1	-0.78341	0.345093	-0.09324
			AANA9	Csa7M375770.1	0.39885	-0.17881	-0.12587
	AGK AGPR	Acetylglutamate kinase [EC: 2.7.2.8] N-acetyl-gamma-glutamyl-phosphate	AGK	Csa2M379190.1	-0.16476	-0.16016	0.029
			AGPR	Csa2M236630.1	0.989035	0.582527	0.084669
	ACOAT	Acetylornithine aminotransferase [EC: 2.6.1.11]	ACOAT1	Csa4M056830.1	-0.2058	0.150027	-0.16069
			ACOAT2	Csa4M056840.1	1.520924	4.060185	0.749521
			ACOAT3	Csa4M618430.1	-	-	0.012556
			ACOAT4	Csa7M432140.1	0.488745	-0.04818	-0.10575
	AOD	Acetylornithine deacetylase [EC: 3.5.1.16]	AOD1	Csa3M902410.1	-0.00515	-0.00174	-0.16386
			AOD2	Csa3M902910.1	0.183889	-2.29031	-0.39407
			AOD3	Csa3M902920.1			
	OCT	Ornithine carbamoyltransferase	OCT	Csa3M859690.1	0.413793	-0.16225	-0.05895
Asp Asn	ASS	Argininosuccinate synthase [EC: 6.3.4.5]	ASS1	Csa1M002130.1	0.29477	0.578262	-0.29873
			ASS2	Csa1M050380.1	0.388473	0.678991	-0.43996
			ASS3	Csa1M050400.1	-0.10426	-0.01266	-0.06184
	ASL	Argininosuccinate lyase [EC: 4.3.2.1]	ASL1	Csa2M370510.1	0.450801	0.361862	-0.01667
			ASL2	Csa3M891650.1	-0.15251	0.091085	0.291657
	AspAT	Aspartate aminotransferase [EC: 2.6.1.1]	AspAT1	Csa1M096620.1	-0.15623	0.077379	0.157364
			AspAT2	Csa2M382520.1	0.213957	-0.06418	-0.1997
			AspAT3	Csa3M889160.1	-0.25424	-0.2618	0.04661
			AspAT4	Csa4M329570.1	0.935961	1.470973	-0.42673
	AS	Asparagine synthetase [EC: 6.3.5.4]	AS1	Csa4M638320.1	0.835824	0.378265	-0.62287
			AS2	Csa6M154500.1	-0.41115	0.341773	0.191061
			AS3	Csa6M183190.1	-1.14994	-0.45254	-1.13978
			AS4	Csa6M362920.1	-1.649	0.326359	1.234948

			AS5	Csa6M500420.1	0.20035	0.200302	-0.06428
			AS6	Csa6M517220.1	-0.81554	1.216648	0.118166
	AK	Aspartate kinase [EC: 2.7.2.4]	AK	Csa5M457770.1	0.399974	0.313065	-0.05395
			ASADH1	Csa2M021690.1	-0.84413	-0.23239	0.300252
	ASADH	Aspartate semialdehyde dehydrogenase [EC: 1.2.1.11]	ASADH2	Csa5M021360.1	-0.15715	-0.21992	0.27559
			ASADH3	Csa5M021870.1	0.058555	-0.0691	-0.03475
			ASADH4	Csa6M425150.1	-0.35051	-0.38863	-0.24465
Lys	DHDPS	Dihydrodipicolinate Synthase [EC: 4.2.1.52]	DHDPS	Csa3M125530.1	-0.04314	0.042585	0.049432
			DHPR1	Csa3M180330.1	0.403931	0.2057	0.243881
	DHPR	Dihydrodipicolinate reductase [EC: 1.3.1.26]	DHPR2	Csa7M209030.1	0.788619	0.510224	-0.05493
	DAPE	Diaminopimelate epimerase [EC: 5.1.1.7]	DAPE	Csa4M293310.1	-0.00476	0.137258	0.003233
	DAPDC	Diaminopimelate decarboxylase [EC:	DAPDC	Csa2M248740.1	-0.20326	-0.15416	0.079492
			TDH1	Csa1M673520.1	0.935961	-	-
			TDH2	Csa4M358720.1	-2.23396	-2.1972	-0.98744
	TDH	Threonine dehydratase [EC: 4.3.1.19]	TDH3	Csa6M448720.1	-	-	-
			TDH4	Csa6M448730.1	1.105886	1.432154	-1.57241
			TDH5	Csa6M448740.1	3.636401	0.709688	-0.62899
			TDH6	Csa6M449240.1	0.389754	0.045963	-0.16433
			ALS1	Csa1M001520.1	-0.43194	-0.17983	-0.01167
Iso Val Leu	ALS	Acetolactate synthase [EC: 2.2.1.6]	ALS2	Csa1M652220.1	1.067827	0.621332	0.070058
			ALS3	Csa1M652230.1	0.724854	0.06063	0.150059
			ALS4	Csa1M652240.1	0.662811	0.298426	-0.02602
			ALS5	Csa5M013280.1	0.386595	0.017426	-0.1658
	KARI	Ketol-acid	KARI	Csa7M051370.1	0.071573	-0.02247	0.066096
	DHAD	Dihydroxyacid	DHAD	Csa3M133100.1	0.422062	0.19326	-0.09494
	IPS	2-isopropylmalate	IPS	Csa3M363180.1	-0.42308	-0.1738	0.315641
	IPMI	3-isopropylmalate dehydratases [EC: 4.2.1.33]	IPMI1	Csa3M912340.1	0.071188	-0.09234	-0.06453
			IPMI2	Csa5M165170.1	-0.3244	-0.26156	-0.00422
	IPMDH	Isopropylmalate	IPMDH	Csa4M006270.1	0.324313	0.138221	0.137511
			HSDH1	Csa1M600930.1	-0.71612	-0.23878	1.249595
	HSDH	Homoserine dehydrogenase [EC: 1.1.1.3]	HSDH2	Csa5M649280.1	0.716245	0.466246	0.007198
	HSK	Homoserine kinase [EC: 2.7.1.39]	HSK	Csa7M025730.1	-0.2338	-0.11457	-0.03128
			TS1	Csa1M590260.1	0.040249	-0.09588	-0.05684
	TS	Threonine synthase [EC: 4.2.99.2]	TS2	Csa1M590270.1	-0.2278	0.04258	0.208392
Thr Met			TS3	Csa2M000280.1	0.18033	-0.16922	-0.09461
	CGS	Cystathionine γ-synthase [EC: 2.5.1.48]	CGS	Csa3M824870.1	-0.02284	0.141307	0.250531
	CBL	Cystathionine β-lyase [EC: 4.4.1.8]	CBL	Csa6M357010.1	0.022144	-0.04735	-0.04048
			MS1	Csa1M599580.1	0.017988	0.147396	0.130091
	MS	Methionine synthase [EC: 2.1.1.14]	MS2	Csa3M822260.1	0.394814	0.477813	0.294665
			MS3	Csa3M842030.1	0.558405	0.381031	0.099972
			ALT1	Csa3M646610.1	0.012598	0.031317	-0.23869
Ala	ALT	Alanine transaminase [EC: 2.6.1.2]	ALT2	Csa7M448000.1	-2.34151	-1.27737	0.242038
			PGDH1	Csa3M199630.1	-0.22195	-0.09393	0.238788
	PGDH	Phosphoglycerate dehydrogenase [EC: 1.1.1.95]	PGDH2	Csa3M199640.1	-0.10196	-0.17057	0.073766
			PGDH3	Csa3M651740.1	0.61295	0.722334	0.019311
			PGDH4	Csa7M302340.1	-0.02751	0.113585	-0.24328
	PSAT	Phosphoserine	PSAT	Csa3M002370.1	0.186344	0.293013	0.111849
	PSP	Phosphoserine phosphatase [EC:	PSP	Csa1M013750.1	0.235521	0.331176	0.059861
			GHMT1	Csa2M145880.1	-0.32823	-0.39313	0.161203
		Glycine	GHMT2	Csa2M372170.1	0.488009	0.448821	0.324089

Ser Cys Gly	GHMT	Glycine hydroxymethyltransferase [EC: 2.1.2.1]	GHMT3	Csa3M221750.1	0.093674	0.31245	0.036878
			GHMT4	Csa4M062380.1	-0.56529	0.237076	-0.14481
			GHMT5	Csa4M377740.1	0.282066	0.610758	-0.06452
			GHMT6	Csa6M497310.1	0.312871	0.142254	0.011164
	SAT	Serine O-acetyltransferase [EC: 2.3.1.30]	SAT1	Csa2M298310.1	-0.31764	-0.12702	-0.01383
			SAT2	Csa3M769110.1	-0.03058	-0.0511	-0.19469
			SAT3	Csa6M509570.1	0.050654	-0.14772	-0.12959
			SAT4	Csa6M526510.1	-2.38597	0.458149	3.100019
	OASS	O-acetylserine [thiol] lyase [EC: 2.5.1.47]	OASS1	Csa1M574790.1	0.151085	0.550417	-0.18098
			OASS2	Csa1M574800.1	0.14493	0.089271	0.241329
			OASS3	Csa1M574810.1	0.833391	-0.04149	-0.30937
			OASS4	Csa2M012660.1	-0.08198	-0.33822	0.002542
			OASS5	Csa3M816040.1	-0.18769	-0.60633	0.236331
			OASS6	Csa5M589260.1	1.623046	1.491322	0.589279
			OASS7	Csa7M099270.1	0.182285	0.058719	-0.05923
	RPK	Ribose-phosphate pyrophosphokinase [EC: 2.7.6.1]	RPK1	Csa3M015880.1	-0.06149	-0.15986	-0.03314
			RPK2	Csa3M904100.1	0.195102	-0.09088	0.181036
			RPK3	Csa5M598760.1	0.071709	-0.14537	0.195838
			RPK4	Csa6M423440.1	0.287828	-0.07247	-0.26983
His	ATP-PRT	ATP phosphoribosyltransferase [EC:	ATP-PRT	Csa5M586570.1	0.355523	-0.00347	0.073163
	PRA-PH	Phosphoribosyl-AMP	PRA-PH	Csa5M466370.1	0.048748	0.037395	-0.01895
	PRA-CH	Phosphoribosyl-AMP	PRA-CH	Csa5M466370.1	0.048748	0.037395	-0.01895
	PACRI	Phosphoribosylformimino-	PACRI	Csa5M649910.1	0.282884	0.228736	-0.16289
	IGPD	imidazoleglycerol-	IGPD	Csa7M234700.1	-0.16431	-0.05359	0.039264
	HPAT	Histidinol-phosphate	HPAT	Csa7M282400.1	-0.01673	-0.00901	0.016656
	HDH	aminotransferase [EC: Histidinol dehydrogenase [EC:	HDH	Csa1M537580.1	0.596323	0.164441	-0.16697
	DAHPS	3-Deoxy-7-phosphoheptulonate synthase [EC: 2.5.1.54]	DAHPS1	Csa2M369040.1	0.389821	0.475073	0.270074
			DAHPS2	Csa3M073840.1	-0.14929	-0.29489	0.196149
			DAHPS3	Csa6M499800.1	0.357277	-0.14041	-0.1163
			DAHPS4	Csa7M064020.1	-0.40711	-0.15489	-0.17261
	DHQS	3-Dehydroquinate	DHQS	Csa3M002680.1	-0.17116	-0.08879	-0.00513
	DHQD	3-Dehydroquinate dehydratase / shikimate dehydrogenase [EC: 4.2.1.10/1.1.1.25]	DHQD1	Csa2M297240.1	0.291858	0.017738	-0.07375
			DHQD2	Csa5M173430.1	-0.42704	0.047248	0.293798
			DHQD3	Csa6M486810.1	0.348344	0.084932	-0.12974
	SK	Shikimate kinase [EC: 2.7.1.71]	SK1	Csa2M296130.1	1.117601	-0.03874	-0.39018
			SK2	Csa3M008320.1	-0.2083	-0.48504	0.038334
			SK3	Csa3M509960.1	0.052416	-0.07549	0.04886
			SK4	Csa6M487590.1	0.312751	-0.29031	-0.15629
	EPSPS	3-Enolpyruvylshikimate-3-phosphate synthase	EPSPS	Csa3M126230.1	0.368798	0.165968	-0.06517
	CMS	Chorismate synthase [EC: 4.2.3.5]	CMS	Csa6M405290.1	0.302758	0.18278	-0.02617
	CM	Chorismate mutase [EC: 5.4.99.5]	CM1	Csa3M824230.1	-0.35769	-0.20967	-0.00891
			CM2	Csa4M651960.1	0.709685	0.644235	-0.01492
			CM3	Csa5M638330.1	-0.71513	-1.26525	-0.52702
Tyr Phe Try	PDH	Prephenate dehydrogenase [EC:	PDH	Csa2M008130.1	0.308302	0.384914	0.106082
	PDT	Prephenate dehydratase [EC: 4.2.1.51]	PDT1	Csa1M145970.1	-0.22274	-0.05765	0.393646
			PDT2	Csa1M145980.1	-0.17803	-0.30651	-0.08249
			PDT3	Csa2M417830.1	-0.26291	-0.47833	0.003454
			PDT4	Csa6M151110.1	-0.64361	-0.52105	-0.05675
			PDT5	Csa6M289730.1	0.375441	0.389057	0.305884
			PDT6	Csa6M513690.1	0.70676	0.127169	-0.04097

ANS	Anthranilate synthase [EC: 4.1.3.27]	ANS1	Csa4M563190.1	0.394521	0.106834	0.12218
		ANS2	Csa5M056100.1	0.225793	0.19971	0.02809
		ANS3	Csa6M011620.1	-0.7537	0.186847	0.865714
PAT	Phosphoribosylanthranilate transferase [EC:	PAT	Csa1M046170.1	0.15781	0.036025	-0.05083
PAI	Phosphoribosylanthranilate isomerase [EC:	PAI	Csa5M207950.1	0.264076	-0.2319	-0.16082
IGPS	Indole-3-glycerol-phosphate synthase [EC:	IGPS	Csa7M031620.1	-0.03075	-0.24567	0.031624
TPS	Tryptophan synthase [EC: 4.2.1.20]	TPS1	Csa1M064160.1	-1.06404	-1.76424	0.27559
		TPS2	Csa1M064170.1	0.782487	0.630803	-0.69526
		TPS3	Csa1M660140.1	0.136652	0.123203	0.067271
		TPS4	Csa2M225330.1	-0.28245	-0.18717	0.063146
		TPS5	Csa3M843770.1	-0.21604	-2.34921	0.930094
		TPS6	Csa5M643330.1	-0.78154	-0.36725	0.064817
		TPS7	Csa5M643340.1	9.14E-05	0.09072	0.413806

e positive and false negative errors were performed by calculating the
.001 were considered differentially expressed. Red indicates up-

Root

log_2(NI/AI)	log_2(N/A)	log_2(AI/A)	log_2(NI/N)	log_2(NI/AI)
0.011668	-0.149608	0.0383251	0.315719	0.1277861
-1.1147	-1.803773	0.1898996	0.2846394	-1.709033
-0.04051	0.1182741	0.6423747	-0.587234	-1.111335
0.732938	-0.240786	0.4887319	0.9478867	0.2183687
0.23097	1.2236658	0.0556184	-0.205956	0.9620912
0.146765	-1.182306	0.0067152	0.9756168	-0.213404
-2.07803	-2.103541	0.2488248	-0.498187	-2.850552
-0.04623	-0.75596	0.4312817	-1.611081	-2.798322
-0.26567	0.9792869	0.4903436	-0.74308	-0.254137
0.279575	-4.910537	-3.071219	1.7812368	-0.058082
0.576232	-0.790933	-0.881887	0.7037865	0.7947401
-0.05914	-0.054116	0.1121228	-0.18312	-0.349359
0.200112	0.0978191	0.1467328	0.0350821	-0.013832
-0.26458	0.4203321	0.2181443	-0.209896	-0.007708
0.012789	-0.020341	-0.24249	-0.772544	-0.550395
0.445527	0.0455969	0.3702165	-0.157824	-0.482443
0.54953	0.4206285	0.3381723	-0.570923	-0.488467
0.66611	0.3924316	-0.070467	-0.207956	0.2549427
-0.09042	-0.393037	0.3555258	0.0101378	-0.738425
-1.22174	-2.11853	0.1682473	-	-
0.451792	0.2171319	0.580145	0.6837538	0.3207407
0.0244	0.0973199	0.1732937	-0.033671	-0.109645
0.491177	0.7199923	0.533009	0.17798	0.3649634
-0.51652	-1.320375	-0.212733	1.0048778	-0.102765
-1.78974	0.5163635	0.8938876	-0.347402	-0.724927
-1.34613	-0.652124	0.0406455	-0.434492	-1.127262
0.431173	0.0674317	0.3404034	0.5131462	0.2401745
-0.16726	0.239084	0.3170806	-0.048714	-0.12671
2.080131	-4.823074	-0.303636	1.848351	-2.671087
	-1.170998	1.0668702	-	-
0.517084	0.1488185	0.3543097	-0.333496	-0.538987
-0.58222	1.7095233	-0.147684	-0.503237	1.3539706
-0.73047	-1.100608	1.0162442	0.125885	-1.990968
-0.15344	0.1104919	0.0746435	0.110878	0.1467263
0.072272	0.0063719	-0.191755	-0.192109	0.0060174
0.048065	-0.553586	-0.145892	0.5530736	0.14538
-0.07625	-0.568599	-0.124997	0.4371117	-0.00649
0.078432	-0.226239	0.0641355	0.3234241	0.0330494
0.054171	-0.822701	-0.223676	0.828677	0.2296521
-0.96174	0.0373769	-0.238534	-0.069944	0.2059665
-0.16531	-1.055269	-1.580162	0.3515793	0.8764724
-0.56186	-0.645428	-0.218517	0.6112114	0.1843
-1.83717	-1.979272	-2.286503	0.6153316	0.9225617
-0.74041	0.0513947	0.141775	-0.315625	-0.406005

-0.06423	-0.404143	0.0660913	0.2311308	-0.239103
-1.91402	-1.460963	-1.984142	-0.244332	0.2788466
0.032961	-0.31248	0.3015943	0.6474588	0.0333841
-0.31149	0.0111627	0.0798238	-0.012296	-0.080957
0.338364	-0.630076	0.146012	0.7378145	-0.038273
0.092903	-0.668241	-0.264331	0.0093307	-0.394579
-0.20654	0.2459171	0.3804831	-0.137224	-0.27179
-0.03629	0.0415076	0.3360358	0.0033424	-0.291186
0.442112	0.0513947	0.1439997	-0.226417	-0.319022
0.223468	-0.019282	0.3507623	0.0037524	-0.366292
-0.13878	-0.475303	-0.102902	0.1802453	-0.192156
0.030386	0.2063072	0.3013509	-0.15817	-0.253214
-	-	-	-	-
-1.02421	-2.270533	0.1537477	3.0613447	0.6370637
-	-	10.898505	-	-6.065874
-1.89867	-0.427397	0.1871707	-0.061899	-0.676467
2.297722	-	-	-	-
0.179458	-0.096688	0.0269065	0.0335486	-0.090046
-0.26378	0.0961826	0.0027905	-0.151701	-0.058309
0.516553	0.6242235	0.9570484	-0.148109	-0.480934
0.814284	0.1751301	0.9613822	-0.215943	-1.002195
0.338364	-0.178392	0.7760405	0.203669	-0.750764
0.203365	0.074452	0.3177746	0.146165	-0.097158
0.160142	-0.092208	0.1465727	0.1092806	-0.1295
0.133859	-0.268696	1.2E-05	0.1133319	-0.155376
0.066356	-0.98608	-0.13231	0.4134631	-0.440306
0.098997	-0.12724	0.3178897	0.0756507	-0.369479
-0.06706	0.0361701	0.1632058	-0.151499	-0.278535
0.323603	-0.26811	0.024159	-0.057265	-0.349534
0.772261	-0.485633	0.1818302	-0.891189	-1.558652
0.257197	-0.936915	-0.319742	0.6756541	0.0584818
-0.1505	-0.150343	0.0353598	-0.109847	-0.29555
0.079291	-0.005521	0.0257566	-0.21329	-0.244568
-0.06199	-0.196266	-0.306839	-0.134374	-0.023801
0.254936	-0.456768	0.086396	0.3206024	-0.222562
0.086384	0.8294475	0.5648412	-0.180547	0.0840595
0.029016	-0.038184	0.1480848	0.2693018	0.0830331
0.000683	0.3822081	0.3690674	-0.2441	-0.230959
0.211666	0.2200371	0.2474293	-0.071794	-0.099186
0.277346	0.0714226	0.2623736	-0.106463	-0.297414
-0.2574	-0.613927	-0.206799	0.4352464	0.0281179
-0.8221	-1.151944	-0.049759	0.5726159	-0.529569
0.110769	-0.393989	0.1495875	0.3795556	-0.164021
0.142368	0.345942	0.4260424	-0.217671	-0.297772
-0.09007	0.493441	-0.058705	-0.720037	-0.167891
-0.38438	-0.711566	0.1746735	0.3724313	-0.513808
0.00518	-0.431271	0.0440426	0.3029124	-0.172401
-0.03579	-0.032507	0.3592102	0.0122424	-0.379474
0.2261	-0.017905	0.0782919	-0.115044	-0.211241
0.363276	0.0627596	0.2115807	0.0687983	-0.080023

-0.1819	0.1262931	-0.185462	0.0457322	0.3574869
-0.94718	-1.231387	-0.921402	0.3219378	0.0119529
-0.39321	0.7698167	0.0920325	-0.133284	0.5445
0.181782	0.4127943	-0.724236	-0.900587	0.236443
-0.20445	-0.686186	-0.427781	0.3086722	0.0502669
-0.17418	1.4625458	0.4878225	-0.772146	0.202577
0.068788	0.3539575	0.0688805	-0.423157	-0.13808
0.255902	-0.155825	-0.920562	-0.105502	0.6592343
-0.58031	0.2723247	0.7074721	-0.292082	-0.727229
0.296988	-0.620551	0.4389352	-0.44933	-1.508816
0.56551	-0.899696	0.4061907	-0.232569	-1.538455
0.258776	-0.985412	-0.278256	0.9620609	0.2549045
0.654972	-0.420976	0.0348598	-0.000355	-0.456191
0.721002	1.5380032	0.772357	-0.054889	0.7107574
0.064331	-0.280119	0.0816202	0.2201211	-0.141619
0.065229	0.1193948	0.3569879	0.0489449	-0.188648
0.467015	-2.691371	-2.023454	0.791295	0.1233785
0.412918	-0.133998	0.1897423	0.064849	-0.258891
0.090464	0.4661598	0.3494603	-0.233198	-0.116498
0.432156	-0.05128	0.1205387	-0.011614	-0.183432
-0.0076	-0.602762	-0.011037	0.4525163	-0.139209
-0.0076	-0.602762	-0.011037	0.4525163	-0.139209
-0.10874	-0.232535	-0.071219	0.0843462	-0.07697
-0.07145	0.0403932	0.1763942	-0.020107	-0.156108
0.008929	-0.125737	0.3066367	0.3594131	-0.072961
0.264908	-0.303786	-0.055906	0.0545004	-0.19338
0.184822	0.2528057	-0.329842	-0.807304	-0.224656
0.341757	-0.151422	0.0627867	0.152852	-0.061357
0.381394	-0.331706	0.0895111	-0.133769	-0.554987
-0.42483	-0.215809	0.1977809	0.059602	-0.353988
-0.08749	-0.20217	0.3506201	0.21489	-0.3379
0.200368	-0.256407	0.4117245	0.4360014	-0.232131
-0.18049	0.3087025	0.2410839	-0.290793	-0.223174
0.133674	-0.195561	0.3774317	0.1194134	-0.45358
0.766161	0.5329777	-0.005226	-0.224272	0.3139318
0.315072	0.0805411	0.3711607	-0.016532	-0.307152
0.176764	0.2475372	0.3489372	-0.433028	-0.534428
0.44677	0.4418071	0.1388211	-0.235354	0.0676323
0.137655	0.1708406	0.3711	0.0707386	-0.129521
0.093803	0.1510441	0.348368	0.0479422	-0.149382
-0.15693	-0.29728	-0.080541	0.3362572	0.1195184
0.050525	0.0549221	-0.274244	0.2254207	0.5545866
0.0231	-0.054743	-0.077708	-0.191631	-0.168666
0.02947	0.1088294	0.4290012	0.1569545	-0.163217
0.22856	0.5039069	0.1005131	0.2209748	0.6243686
0.04599	0.5996733	0.0736574	-0.827753	-0.301737
0.218879	-0.361046	0.3827082	0.9424037	0.1986499
-0.17931	0.7854907	-0.250302	-0.636374	0.3994182
0.292268	-0.468419	-0.120381	0.4108025	0.0627645
0.538618	0.5195436	0.2826143	-0.397405	-0.160476

0.409867	0.0828489	0.7384058	0.2650252	-0.390532
0.054172	0.1654964	0.3957375	-0.18693	-0.417171
-0.07483	-0.649045	0.1231594	0.6177381	-0.154466
0.070952	-0.064237	0.116397	0.3404595	0.1598256
0.335155	-0.436796	0.0836721	0.0308378	-0.48963
0.246547	0.0940575	0.1475921	-0.049403	-0.102937
0.975794	-0.363643	-0.983756	2.5182024	3.1383155
-0.54358	-	-	-	-
0.08072	0.089052	0.2715425	-0.27957	-0.46206
-0.03214	0.0492138	0.409599	0.0565866	-0.303799
3.063257	-0.793631	0.0745428	-0.611081	-1.479254
-0.34947	-0.396826	-0.158859	0.3545894	0.116623
0.323178	0.0408769	0.230128	-0.047387	-0.236638

Supplement figures

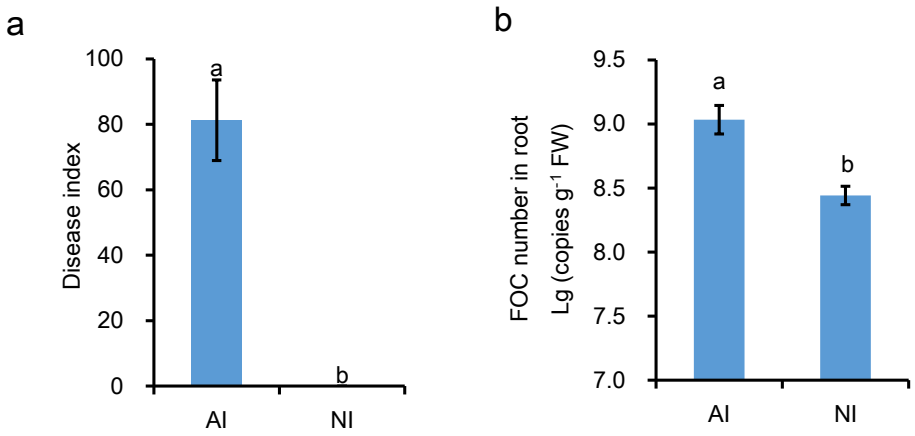


Figure S1 Effects of different nitrogen forms on disease index of Fusarium wilt and root Fusarium oxysporum f. sp. cucumerinum (FOC) colonization. Cucumber plants were supplied with ammonium or nitrate, and inoculated with FOC for eight days. FOC-inoculated ammonium-grown plants are labelled AI; FOC-inoculated nitrate-grown plants are labelled NI. The results represent the means ± SD of five replicates.

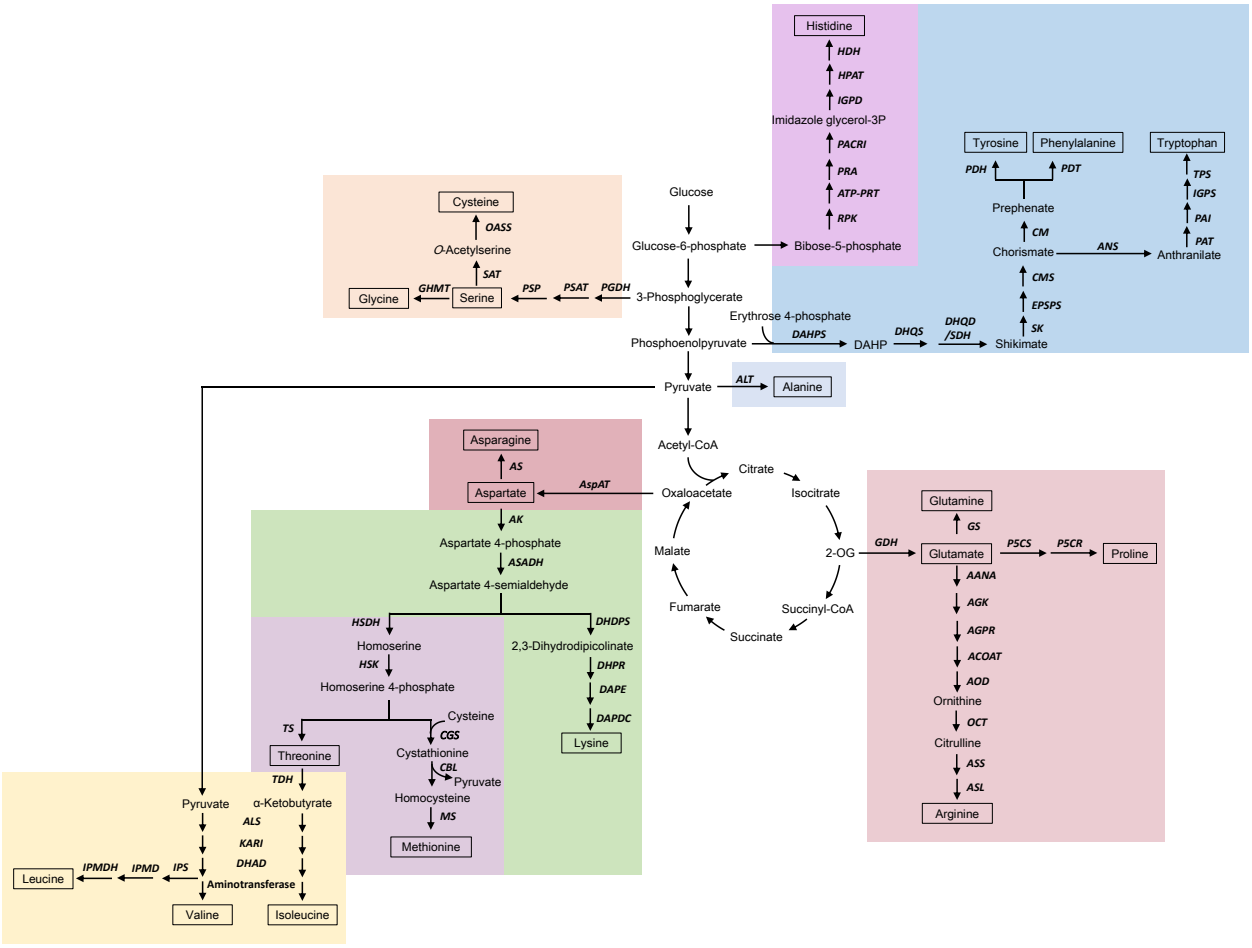
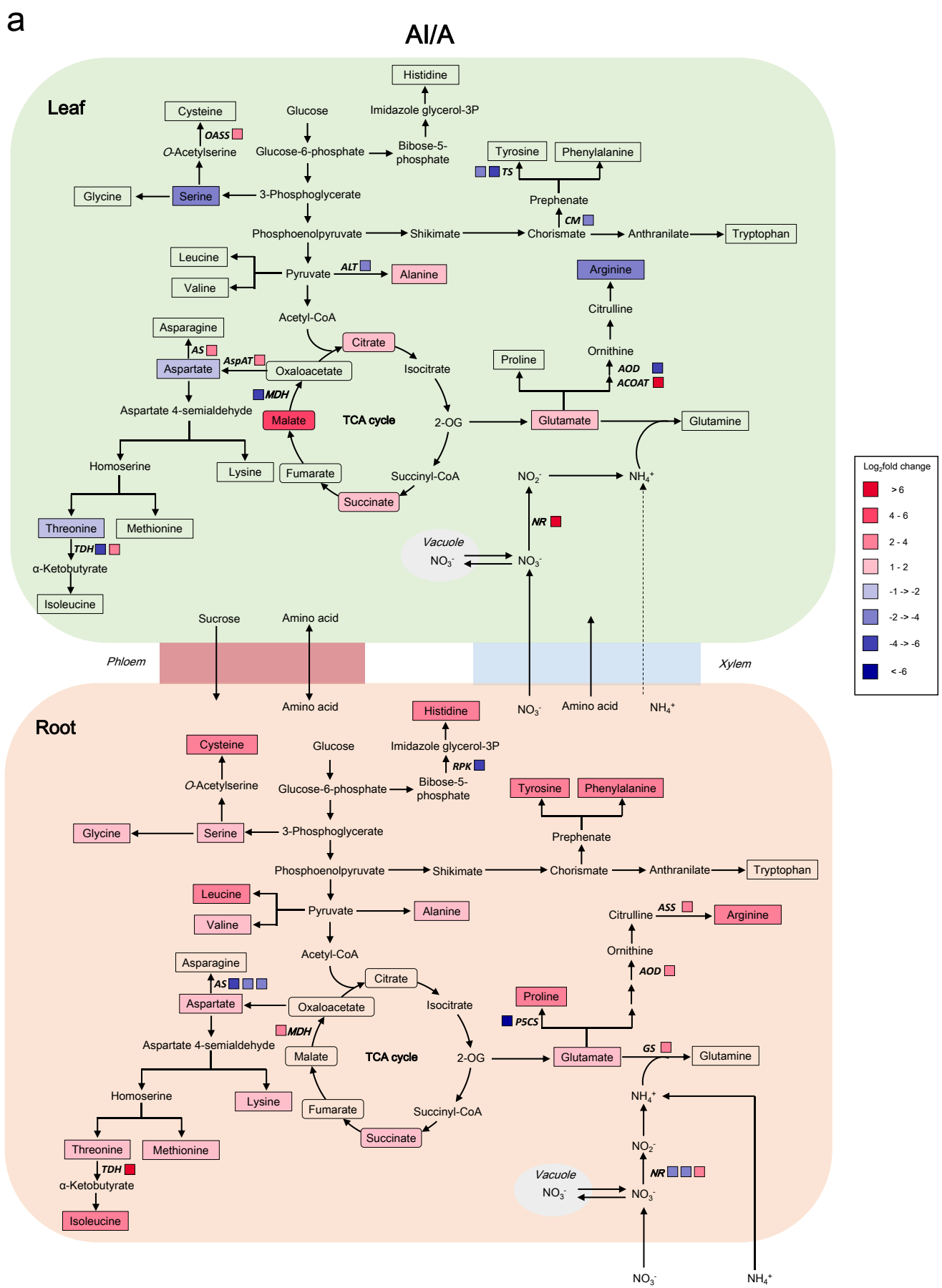


Figure S2 Overview of amino acid biosynthesis in higher plants (Modified from Buchanan et al., 2000).

a


AI/A
Leaf
Histidine
Imidazole glycerol-3P
Cysteine
Glucose
OASS
O-Acetylserine
Glucose-6-phosphate
Bibose-5-phosphate
Tyrosine
Phenylalanine
TS
Glycine
Serine
3-Phosphoglycerate
Prephenate
CM
Phosphoenolpyruvate
Shikimate
Chorismate
Anthranilate
Tryptophan
Leucine
Valine
Pyruvate
ALT
Alanine
Arginine
Citrulline
Acetyl-CoA
Asparagine
AS
Citrate
Ornithine
Proline
AOD
ACOAT
Aspartate
AspAT
Oxaloacetate
Isocitrate
MDH
Aspartate 4-semialdehyde
Malate
TCA cycle
2-OG
Glutamate
Glutamine
Homoserine
Lysine
Fumarate
Succinyl-CoA
NO2-
NH4+
Succinate
NR
Threonine
Methionine
Vacuole
NO3-
TDH
α-Ketobutyrate
Isoleucine
Sucrose
Amino acid
Phloem
Xylem
Log2fold change
> 6
4 - 6
2 - 4
1 - 2
-1 -> -2
-2 -> -4
-4 -> -6
< -6
Root
Histidine
Imidazole glycerol-3P
RPK
Cysteine
Glucose
O-Acetylserine
Glucose-6-phosphate
Bibose-5-phosphate
Tyrosine
Phenylalanine
Glycine
Serine
3-Phosphoglycerate
Prephenate
Phosphoenolpyruvate
Shikimate
Chorismate
Anthranilate
Tryptophan
Leucine
Valine
Pyruvate
Alanine
Citrulline
ASS
Arginine
Acetyl-CoA
Ornithine
Asparagine
AS
Citrate
AOD
Proline
Aspartate
Oxaloacetate
Isocitrate
P5CS
MDH
Aspartate 4-semialdehyde
Malate
TCA cycle
2-OG
Glutamate
GS
Glutamine
Homoserine
Lysine
Fumarate
Succinyl-CoA
NH4+
NO2-
Succinate
Threonine
Methionine
NR
Vacuole
NO3-
TDH
α-Ketobutyrate
Isoleucine

b

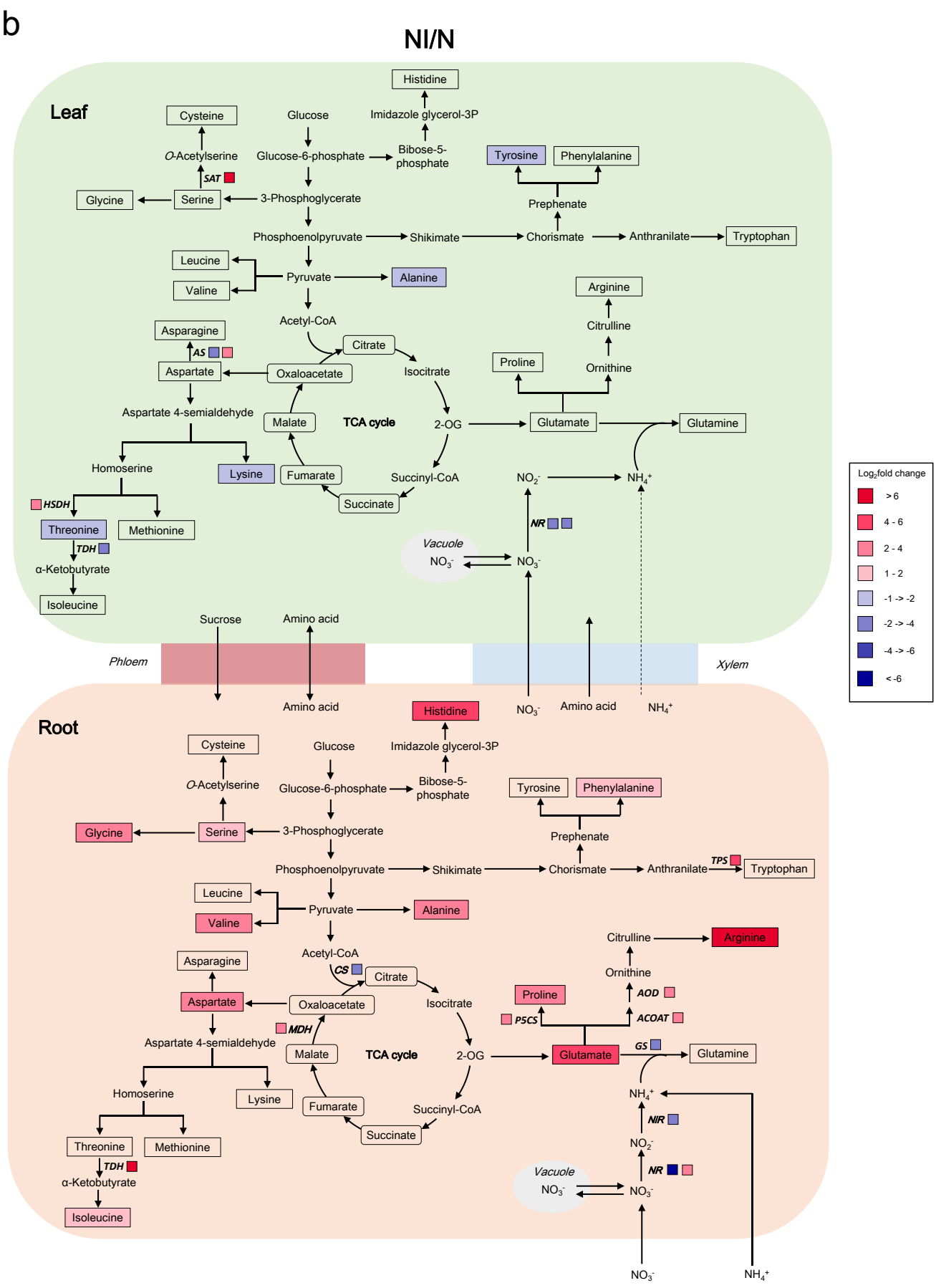

NI/N
Leaf
Histidine
Cysteine
Glucose
Imidazole glycerol-3P
O-Acetylserine
Glucose-6-phosphate
Bibose-5-phosphate
Tyrosine
Phenylalanine
SAT
Glycine
Serine
3-Phosphoglycerate
Prephenate
Phosphoenolpyruvate
Shikimate
Chorismate
Anthranilate
Tryptophan
Leucine
Valine
Pyruvate
Alanine
Arginine
Acetyl-CoA
Citrulline
Asparagine
AS
Citrate
Proline
Aspartate
Oxaloacetate
Isocitrate
Ornithine
Aspartate 4-semialdehyde
Malate
TCA cycle
2-OG
Glutamate
Glutamine
Homoserine
Lysine
Fumarate
Succinyl-CoA
NO2-
NH4+
HSDH
Succinate
NR
Threonine
Methionine
Vacuole
NO3-
TDH
α-Ketobutyrate
Isoleucine
Sucrose
Amino acid
Phloem
Xylem
Root
Histidine
Cysteine
Glucose
Imidazole glycerol-3P
O-Acetylserine
Glucose-6-phosphate
Bibose-5-phosphate
Tyrosine
Phenylalanine
Glycine
Serine
3-Phosphoglycerate
Prephenate
Phosphoenolpyruvate
Shikimate
Chorismate
Anthranilate
TPS
Tryptophan
Leucine
Valine
Pyruvate
Alanine
Citrulline
Arginine
Acetyl-CoA
CS
Ornithine
Asparagine
Citrate
Proline
AOD
Aspartate
Oxaloacetate
Isocitrate
P5CS
ACOAT
MDH
Aspartate 4-semialdehyde
Malate
TCA cycle
2-OG
Glutamate
GS
Glutamine
Homoserine
Lysine
Fumarate
Succinyl-CoA
NH4+
NIR
Succinate
NO2-
Threonine
Methionine
NR
Vacuole
NO3-
TDH
α-Ketobutyrate
Isoleucine
Log2fold change
> 6
4 - 6
2 - 4
1 - 2
-1 -> -2
-2 -> -4
-4 -> -6
< -6

Figure S3 Changes in nitrogen and carbon metabolism in response to Fusarium oxysporum infection in cucumber seedlings under different forms of nitrogen nutrition. The amino acid levels, organic acid levels and related genes expression were compared between different nitrogen forms and FOC infection. Panels a and b present the ratio of AI to A (AI/A) and NI to N (NI/N), respectively. The coloured bar limits show 6-fold up- or down-regulation (blue indicates low content or expression; red indicates high content or expression).