Nitrogen assimilation in cassava: implications for carbon metabolism and biomass synthesis

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Abstract. The nitrogen assimilation pathway in cassava was reconstructed by comparative genomics approach to understand the underlying metabolism as well as the interaction between carbon and nitrogen assimilation towards the synthesis of metabolic phenotype. First, the proteins of cassava were annotated via sequence similarity search against genes of 11 template plants obtained from KEGG and PMN databases, employing reciprocal BLASTp(E-value ≤ 1x10⁻10, identity percentage ≥ 60, and coverage percentage ≥ 80). The template plants comprised well-known plant, starchy crops, nitrogen-fixing crops and crops that are evolutionarily related to cassava and included *Arabidopsis thaliana*, *Oryza sativa*, *Zea mays*, *Ricinus communis*, *Solanum tuberosum*, *Brassica rapa*, *Cicer arietinum*, *Jatropha curcas*, *Medicago truncatula*, *Phaseolus vulgaris* and *Glycine max*. The pathway was then curated with reactions obtained from the CassavaCyc database to ensure full pathway connectivity. It was subsequently validated with cloned sequence of cassava from the GenBank and cassava transcriptome data from literature. The resulting N-assimilation pathway, covering the conversion of nitrate to amino acids (glutamine and glutamate), consists of 14 biochemical reactions corresponding to 59 genes, 73 proteins and 2 transport reactions. At least 92 percent of the identified proteins in the pathway were supported by the transcriptome data. In addition, the proposed N-assimilation pathway contains four additional enzymes, including glutamate synthase, nitrilase, formamidase and carbamoyl phosphate synthase compared to the existing N-assimilation pathway in CassavaCyc database. Taken together, the N-assimilation pathway herein proposed identified reactions involved in N-assimilation and represents a forward step...
towards understanding metabolic basis for cassava yield as well as its phenotypic plasticity and adaptation to stress.

1. Introduction
Cassava is an important tropical crop grown primarily for its carbohydrate-rich storage roots, which contain approximately 70-85% starch, on dry weight basis [1]. It is the highest supplier of carbohydrates among staple crops [2] and is widely utilized as food, feed and raw material for the biofuel, cosmetic and pharmaceutical industries [3]. Cassava is relatively tolerant to drought and poor soil conditions [3], and over 800 million people in the tropics rely on it for their calorie needs. Hence, ensuring adequate supply of cassava is crucial for achieving food security in a world threatened by rapid population growth and climate change, resulting in resource scarcity (e.g. arable land, water, etc.). Enhancing cassava yield will require a comprehensive understanding of the genetic basis for its phenotypic plasticity and ways to improve resource (e.g. land, fertilizer, etc.) use efficiency through innovative breeding, for which adequate knowledge and quantification of the underlying metabolism is critical.

Metabolism refers to the complex network of interdependent chemical reactions required for regulating cellular processes and for synthesizing organic molecules in organisms [4]. It determines the genetic potential of an organism, such as the capacity for growth, development and yield. Metabolic study has been enabled by the advent of high-throughput sequencing technology and availability of complete genomic data of species. These resources have facilitated system level reconstruction of metabolic pathways in a broad range of organisms, prokaryotes and eukaryotes alike, and the modeling of metabolic phenotype and adaptation of plants to environmental stressors. The biochemical reactions are often inferred from publicly available databases such as KEGG (Kyoto Encyclopedia of Genes and Genomes) [5], PMN (Plant Metabolic Network) [6], MetaCyc [7] and BioCyc [7].

Research has sought to understand the impact of climate change on cassava production and the metabolic advantage of cassava over other crops in relation to starch synthesis. The starch biosynthesis and related pathways of cassava which are carbon assimilation pathways have been proposed [8], but research on nitrogen metabolism in cassava comparatively is lagging behind. Nitrogen and carbon assimilation are essential for the growth, development and yield of crops [9], and the interaction and dynamics of these metabolic pathways are likewise crucial. Carbon assimilation generates energy and provides the C-skeleton for nitrogen assimilation. Nitrogen uptake and assimilation in plants are regulated by sugars produced during photosynthesis [10]. Inorganic nitrogen (nitrate or ammonium) is assimilated into amino acids (glutamine and glutamate) required for the synthesis of nitrogen containing compounds such as nucleic acids, proteins and chlorophyll [11] [12], and the latter is a vital pigment in chloroplast, used in photosynthesis for light capture [13]. Increases in total soluble saccharides, non-reducing saccharides and inorganic phosphate have been reported in leaves of cassava exposed to nitrogen fertilizer [14].

Considering the obvious link between the carbon and nitrogen assimilation pathways, and the far-reaching role of amino acids, this research aimed to reconstruct the N-assimilation pathway of cassava based on comparative genomics approach with a view to gain a better understanding of how both pathways affect biomass synthesis in cassava. The reconstructed pathway was used to investigate the metabolic conversion of nitrogen source (nitrate) from soil to amino acids (glutamine and glutamate) in plant cells. The association of N- and C-assimilation pathways was investigated through the reactions connecting both pathways, believed to be a channel of communication between nitrogen and carbon to modulate growth and biomass yield of plants.
2. Methodology
The N-assimilation pathway was reconstructed following the genome-based approach proposed by [8], the research was divided into four parts: 1) pathway reconstruction 2) calculation of confidence scores 3) pathway validation and 4) pathway analysis.

2.1. Pathway reconstruction
Cassava proteins were annotated via sequence similarity search against 11 template plants (Arabidopsis thaliana, Oryza sativa, Zea mays, Ricinus communis, Solanum tuberosum, Brassica rapa, Cicer arietinum, Jatropha curcas, Medicago truncatula, Phaseolus vulgaris and Glycine max) through reciprocal BLASTp (E-value ≤ 1x10^{-10}, identity percentage ≥ 60, and coverage percentage ≥ 80). The template plants comprised well-known plants, starchy crops, N-fixing plants and plants that are evolutionarily related to cassava. The functions of proteins in the metabolism were annotated according to their orthologues in the template plants. First, the protein sequences of the 11 plant templates involved in N-assimilation were obtained from the KEGG (Kyoto Encyclopedia of Genes and Genomes) [5] and PMN (Plant Metabolic Network) [6] databases. The sequences were aligned (BLASTp-1) against the cassava genome (version 4.1) and subsequently, the matched cassava protein sequences that passed the criteria in BLASTp-1 were in turn aligned back (BLASTp-2) against each template plant genome. Next, the N-assimilation pathway was reconstructed based on the annotated cassava proteins. The resulting pathway was curated with reactions obtained from the CassavaCyc database to ensure full pathway connectivity and was visualized using SmartDraw tool, following the approach of [8].

2.2. Calculation of confidence scores
The reliability of the proteins annotation was determined by computing confidence scores, match score (MS) and conservation score (CS), as proposed in [8]. The MS score indicates similarity between the protein sequence of cassava and its orthologues in template plant species (equation 1), whereas the CS score represents the conservation of the annotated protein sequence across the species under study (equation 2). Both MS and CS scores range from 0 (low) to 1 (high).

\[ MS = \frac{\sum_i^N m(H_i \times F_i)}{N_m} \]  \hspace{1cm} (1)

\[ CS = \frac{\sum_i^N p(H_i \times F_i)}{N_t} \]  \hspace{1cm} (2)

where  
- \( H_i \) = match quality of the aligned protein sequence of cassava and template plant species \( i \)
- \( F_i \) = clarity of the orthologous gene function in template plant species \( i \)
- \( N_m \) = number of template plants from which an orthologous gene of cassava was annotated
- \( N_t \) = total number of template plant species

2.3. Pathway validation
The annotated cassava proteins were verified by two ways. First, the existence of these proteins was confirmed with cloned sequences of cassava available in the GenBank database. Second, gene expression was determined using six transcriptome datasets on leaf, stem, and root development [15] [16], responses to fungal infection [17], cold stress [18] and drought stress [19]. Sequence comparison was performed through BLAST with the following criteria: E-value ≤ 1x 10^{-10}, and identity percentage ≥ 95.

2.4. Pathway analysis
The N-assimilation pathway was analysed to explore its association to the C-assimilation pathway via identifying the common reactions and metabolite of the N- and C-assimilation pathways and infer their potential roles in biomass synthesis based on scientific evidence in literature.
3. Results and Discussion

3.1. N-assimilation pathway of cassava

The reconstruction of the N-assimilation pathway in cassava followed the approach of [8], employing enzymatic proteins involved in N-assimilation in 11 template plant species available in KEGG [5] and PMN [6] databases (table A1). The N-assimilation pathway of the template plants consists of 8-16 enzymes (EC numbers) that are highly conserved, and 17 non-redundant enzymes (EC numbers) were found in the entire set of proteins obtained. In total, 71 orthologous proteins (57 genes) of cassava were identified (Table 1), and 30 percent (21/71) of the cassava proteins were annotated from their orthologues in a single template species. The 24 percent (17/71) of the orthologous proteins are highly conserved among cassava and the 11 plant species (figure 1).

Overall, 71 cassava proteins (57 genes) related to 13 enzymes (EC numbers) that catalyse 15 reactions (12 enzymatic reactions, one spontaneous reaction, and two transport reactions) were annotated to be involved in N-assimilation pathway.

Table 1. The number of protein sequences related to the N-assimilation pathway in cassava, and the template protein sequence retrieved from KEGG and PMN databases.

| Plant templates         | Template protein sequences | Cassava genome |
|-------------------------|---------------------------|----------------|
|                         |                           | Protein sequences | Genes |
| *Arabidopsis thaliana*  | 47                        | 39              | 29    |
| *Oryza sativa*         | 34                        | 28              | 23    |
| *Zea mays*             | 66                        | 30              | 25    |
| *Ricinus communis*     | 32                        | 46              | 35    |
| *Solanum tuberosum*    | 37                        | 38              | 26    |
| *Brassica rapa*        | 75                        | 40              | 28    |
| *Cicer arietinum*      | 30                        | 34              | 27    |
| *Jatropha curcas*      | 31                        | 44              | 33    |
| *Medicago truncatula*  | 21                        | 28              | 20    |
| *Phaseolus vulgaris*   | 37                        | 32              | 27    |
| *Glycine max*          | 73                        | 59              | 47    |
| Total                   | -                         | 71              | 57    |

Figure 1. The number of template plants possessing cassava orthologous proteins involved in the N-assimilation pathway.
The reconstructed N-assimilation pathway was compared with the CassavaCyc database (figure 2). Nine of 13 enzymes in our reconstructed pathway overlapped with CassavaCyc pathway (table B1), indicating that these enzymes are truly functional in the N-pathway. Four additional enzymes, namely glutamate synthase, nitriase, formamidase and carbamoyl phosphate synthase were identified (table 2) particularly in our reconstruction. Nitrate reductase (EC 1.7.1.1) found in the CassavaCyc pathway was included in our pathway despite not meeting the set criteria for similarity of protein sequence alignment, to ensure full pathway connectivity. Nitrate reductase catalyses the conversion of nitrate to nitrite.

![Figure 2](image)

**Figure 2.** A comparison of enzymes (EC numbers) in the reconstructed N-assimilation pathway and CassavaCyc database.

**Table 2.** The four enzymes (EC numbers) annotated to the nitrogen assimilation pathway in cassava not found in the CassavaCyc pathway.

| EC numbers | Enzyme functions            | Reactions                                      |
|------------|-----------------------------|------------------------------------------------|
| 1.4.1.13   | Glutamate synthase          | 2L-Glutamate + NADP+ <=> L-Glutamine + 2-Oxoglutarate + NADPH + H+ |
| 3.5.5.1    | Nitrilase                   | Nitrile + 2 H2O <=> Carboxylate + NH3          |
| 3.5.1.49   | Formamidase                 | Formamide + H2O <=> Formate + NH3             |
| 6.3.4.16   | Carbamoyl phosphate synthase| NH3 + CO2 + 2 ATP + H2O -> carbamoyl-phosphate + 2 ADP + phosphate + 3 H+ |

The four enzymes newly reconstructed to the N-assimilation in cassava were as follows. Glutamate synthase (EC 1.4.1.13), a complex iron–sulphur flavoprotein, plays an important role in the ammonia assimilation pathway in plants [20], and its activity along with those of enzymes such as nitrate reductase, glutamine synthetase and glutamate dehydrogenase depend on nitrate supply; low activities of these enzymes have been reported in cassava under low nitrate availability [21]. Nitrilases (EC 3.5.5.1) are involved in nitrile degradation. They are produced naturally and xenobiatically [22] and play critical roles in plant–microbe interactions for defence, detoxification, nitrogen utilization and plant hormone synthesis [23]. Formamidase (EC 3.5.1.49) catalyses the conversion of formamide into formate and ammonia [24], and the reaction may be a channel of connection between the N-assimilation pathway and N-derivative pathways, e.g., the cyanogenic acid pathway (cyanide assimilation). Cyanogenic glycosides have β-linkage with D-glucose and are substrates for the production of cyanide, which reportedly increases during drought stress in cassava [25]. Carbamoyl phosphate synthase enzyme (EC 6.3.4.16) is involved in the synthesis of arginine, an amino acid with a high nitrogen to carbon ratio, used for the storage and transport of nitrogen in plants and may be involved in the development and defence against stress [26].

After the curation of the reconstructed N-assimilation pathway, 14 enzymes (EC numbers), 16 reactions (13 enzymatic reactions, one spontaneous reaction and two transport reactions), 59 genes and 73 proteins (table C1) were obtained. The pathway was visualized employing the SmartDraw platform. The annotated proteins were denoted as 12-digits IDs, consisting of gene and protein IDs, based upon Phytozome database. The confidence scores (CS and MS) of the proteins are provided, and the
annotated proteins that were validated using the expression datasets are underlined (Figure 3). The N-assimilation pathway can be accessed at http://bml.sbi.kmutt.ac.th/.

Figure 3. The reconstructed nitrogen assimilation pathway in cassava, visualized using the SmartDraw platform.

3.2. Confidence of cassava protein annotation and pathway reconstruction

The CS and MS scores of the protein annotation ranged from 0 (low) to 1 (high), as shown in Table C1 and Figure 4, and can be divided into three groups. The proteins in group-one have both low CS and low MS scores, indicating low conservation with template plants and low sequence alignment confidence, and represent 21 percent (15/71) of all annotated proteins. The group-two proteins, consisting of 38 percent (27/71) of all annotated proteins, have low CS and high MS scores, indicating low conservation with template plants and high sequence alignment confidence. Group-three represents proteins annotated with high conservation with template plants and extremely high sequence alignment confidence (high CS and high MS) and constitutes 41 percent (29/71) of all annotated proteins.

Figure 4. The MS-CS plot of all annotated cassava proteins. The groups represent the confidence level of annotation and include: group1: low confidence (low CS and low MS), group2: high confidence (low CS and high MS) and group3: extremely high confidence (high CS and high MS).
3.3. Validation of the reconstructed N-assimilation pathway by expression data
The cloned sequences of cassava (3,867) obtained from the GenBank database were employed to
determine the existence of the annotated proteins in cassava, but the annotated proteins did not match
the cloned sequences probably due to limited cassava data as only about 10% of genes in the genome
were available in the database. Moreover, almost all the entries (cloned sequences) are related to
starch biosynthesis. Next, the annotated proteins were verified based on the six transcriptome
datasets on leaf, stem, and root development [15] [16] and responses to cold stress [20], drought stress
[21] and fungal infection [22]. The results showed that up to 92 percent (67/73) of proteins in the
pathway were validated by at least one transcriptome data (figure 5).

![Figure 5. Expression evidence of proteins in the cassava N-assimilation pathway, validated based on six transcriptome datasets on leaf, stem, and root development [15] [16], and responses to cold [20] and drought [17]–[19] stresses and fungal infection [22].]

3.4. Relationship between the N- and C-assimilation pathways
Nitrogen and carbon are important skeletons of metabolites in metabolism of plants. These metabolites
are used for energy production, growth, development and stress responses [27]. The nitrogen
assimilation pathway, in the case of nitrate-the most commonly used nitrogen compound, starts with
the uptake of nitrate and its subsequent reduction to nitrite (by nitrate reductase) and then to
ammonium (by nitrite reductase). The latter is then fixed into glutamine and glutamate, which serve as
substrates for transamination reactions for the production of other amino acids [28] [29]. Glutamine,
 glutamate and 2-oxoglutarate link both the N- and C-assimilation pathway together (figure 6). Besides
the induction of the nitrate-assimilation enzymes, nitrate affects carbohydrate metabolism and the
relation between starch synthesis and sucrose synthesis. Carbon fixed during photosynthesis is
converted to carbon compounds, such as sucrose and glucose, utilized in glycolysis and TCA cycle for
producing energy and other intermediate compounds for metabolism, e.g. UDP-D-glucose utilized in
the starch biosynthesis pathway [30] and 2-oxoglutarate utilized in the N-assimilation pathway [27].
Moreover, nitrogen uptake and assimilation are activated by photosynthesis and regulated by circadian
[10]. The uptake of nitrate and ammonium as well as the activities of nitrate reductase (NR) and nitrite
reductase (NiR) are regulated by sugars produced during photosynthesis [21]. Meanwhile,
photosynthesis requires chlorophyll, a nitrogen based compound [27]. Thus, understanding N- and C-
assimilation as well as the interaction and dynamics between both pathways is essential for identifying
engineering targets for crop improvement.
4. Conclusions
The N-assimilation pathway of cassava was reconstructed by comparative genomics approach [8] based on 11 template plant species. The pathway covers the conversion of nitrate to amino acids. The cassava proteins were annotated to the pathway according to their orthologues in the template species, and confidence scores (CS and MS scores), were calculated for the annotations. The reconstructed pathway was then curated reactions obtained from the CassavaCyc database to eliminate metabolic gaps and ensure full pathway connectivity. The reconstructed N-assimilation pathway of cassava thus contains 73 proteins (59 genes) related to 14 enzymes (EC numbers) and 16 reactions (13 enzymatic reactions, one spontaneous reaction and two transport reactions). At least 92 percent of proteins in the pathway were validated using the six transcriptome data. The reconstructed pathway provides a comprehensive description of the N-assimilation pathway and offers a blueprint to investigate the association the N- and C-assimilation pathways and implications for the growth, development and biomass (e.g. starch, protein, etc.) biosynthesis.

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Table A1: A comparison of the protein IDs and enzymes (EC numbers) of the 11 template plants retrieved from KEGG and PMN databases.

| Plant templates            | Protein IDs | EC numbers |
|----------------------------|-------------|------------|
| *Arabidopsis thaliana*     | KEGG = 42   | KEGG = 14  |
| (Arabidopsis)              | PMN = 25    | PMN = 12   |
|                            | Total = 47  | Total = 16 |
| *Oryza sativa*             | KEGG = 27   | KEGG = 14  |
| (Rice)                     | PMN = 21    | PMN = 9    |
|                            | Total = 34  | Total = 15 |
| *Zea mays*                 | KEGG = 52   | KEGG = 14  |
| (Maize)                    | PMN = 29    | PMN = 11   |
|                            | Total = 66  | Total = 15 |
| *Ricinus communis*         | KEGG = 32   | KEGG = 14  |
| (Castor bean)              | PMN = 0     | PMN = 0    |
|                            | Total = 32  | Total = 14 |
| *Solanum tuberosum*        | KEGG = 30   | KEGG = 13  |
| (Potato)                   | PMN = 21    | PMN = 8    |
|                            | Total = 37  | Total = 14 |
| *Brassica rapa*            | KEGG = 66   | KEGG = 13  |
| (Turnip)                   | PMN = 36    | PMN = 11   |
|                            | Total = 75  | Total = 15 |
| *Cicer arietinum*          | KEGG = 30   | KEGG = 13  |
| (Chickpea)                 | PMN = 0     | PMN = 0    |
|                            | Total = 30  | Total = 13 |
| *Jatropha curcas*          | KEGG = 31   | KEGG = 13  |
| (Physic nut)               | PMN = 0     | PMN = 0    |
|                            | Total = 31  | Total = 13 |
| *Medicago truncatula*      | KEGG = 21   | KEGG = 8   |
| (Barrel medic)             | PMN = 0     | PMN = 0    |
|                            | Total = 21  | Total = 8  |
| *Phaseolus vulgaris*       | KEGG = 37   | KEGG = 13  |
| (Common bean)              | PMN = 0     | PMN = 0    |
|                            | Total = 37  | Total = 13 |
| *Glycine max*              | KEGG = 62   | KEGG = 13  |
| (Soy bean)                 | PMN = 11    | PMN = 9    |
|                            | Total = 73  | Total = 14 |
### Table B1
The nine enzymes (EC numbers) found in both the reconstructed N-assimilation pathway and N-assimilation pathway in CassavaCyc database, and their catalytic reactions.

| EC numbers | Enzyme functions | Reactions |
|------------|------------------|-----------|
| 1.4.1.14   | Glutamate synthase | 2L-Glutamate + NAD+ <=> L-Glutamine + 2-Oxoglutarate + NADH + H+ |
| 1.4.1.2    | Glutamate dehydrogenase | L-Glutamate + NAD+ + H2O <=> 2-Oxoglutarate + NH3 + NADH + H+ |
| 1.4.1.3    | Glutamate dehydrogenase | L-Glutamate + NAD+ + H2O <=> 2-Oxoglutarate + NH3 + NADH + H+ |
| 1.4.1.4    | Glutamate dehydrogenase | L-Glutamate + NADP+ + H2O <=> 2-Oxoglutarate + NH3 + NADPH + H+ |
| 1.4.7.1    | Glutamate synthase | 2 L-Glutamate + 2 Oxidized ferredoxin <=> L-Glutamine + 2-Oxoglutarate + 2 Reduced ferredoxin + 2 H+ |
| 1.7.7.1    | Ferredoxin-nitrite reductase | NH3 + 2 H2O + 6 Oxidized ferredoxin <=> Nitrite + 6 Reduced ferredoxin + 7 H+ |
| 4.2.1.1    | Carbonic anhydrase | CO2 + H2O -> HCO3 + H+ |
| 4.2.1.104  | Cyanase | Cyanate + H+ + HCO3- <=> CO2 + Carbamate |
| 6.3.1.2    | Glutamine synthetase | NH3 + L-glutamate + ATP -> L-glutamine + ADP + phosphate + H+ |

### Table C1
Characteristics of the reconstructed N-assimilation pathway.

| EC numbers | Enzyme functions | Reactio n IDs | Reactions | Protein IDs | CS | MS |
|------------|------------------|---------------|-----------|-------------|----|----|
| 1.4.1.13   | Glutamate synthase (NADPH) | R00114 | 2L-Glutamate + NADP<=> L-Glutamine + 2-Oxoglutarate + NADPH+H+ | 000038_000038 | 0.5 | 0.5 |
|            |                   |               |           | 000038_000039 | 0.9 | 1  |
|            |                   |               |           | 000038_000061 | 0.5 | 0.5 |
|            |                   |               |           | 000040_000040 | 0.5 | 0.5 |
| 1.4.1.14   | Glutamate synthase | R00093 | 2L-Glutamate + NAD<=> L-Glutamine + 2-Oxoglutarate + NADH + H+ | 000038_000038 | 0.5 | 0.5 |
|            |                   |               |           | 000038_000039 | 0.9 | 1  |
|            |                   |               |           | 000038_000061 | 0.5 | 0.5 |
|            |                   |               |           | 000040_000040 | 0.5 | 0.5 |
|            |                   |               |           | 003306_003306 | 0.1 | 0.5 |
|            |                   |               |           | 003327_003327 | 0.02 | 0.3 |
| 1.4.1.2    | Glutamate dehydrogenase | R00243 | L-Glutamate + NAD+ + H2O <=> 2-Oxoglutarate + NH3 + NADH + H+ | 008693_008693 | 0.8 | 0.8 |
|            |                   |               |           | 008701_008701 | 0.5 | 0.5 |
|            |                   |               |           | 008713_008713 | 0.5 | 0.5 |
|            |                   |               |           | 001205_001205 | 0.02 | 0.3 |
|            |                   |               |           | 001233_001233 | 0.05 | 0.5 |
|            |                   |               |           | 001244_001244 | 0.02 | 0.3 |
| 1.4.1.3    | Glutamate dehydrogenase | R00243 | L-Glutamate + NAD+ + H2O <=> 2-Oxoglutarate + NH3 + NADH + H+ | 008690_008690 | 0.8 | 0.8 |
|            |                   |               |           | 008690_009673 | 0.5 | 0.5 |
|            |                   |               |           | 008693_008693 | 0.8 | 0.8 |
|            |                   |               |           | 008701_008701 | 0.5 | 0.5 |
|            |                   |               |           | 008713_008713 | 0.5 | 0.5 |
|            |                   |               |           | 010581_010581 | 0.8 | 0.8 |
|            |                   |               |           | 001205_001205 | 0.02 | 0.3 |
|            |                   |               |           | 001244_001244 | 0.02 | 0.3 |
| EC numbers | Enzyme functions | Reaction IDs | Reactions                                                                 | Protein IDs               | CS | MS |
|------------|------------------|--------------|---------------------------------------------------------------------------|---------------------------|----|----|
| 1.4.1.4    | Glutamate dehydrogenase | R00248      | L-Glutamate + NADP$^+$ + H$_2$O $\rightleftharpoons$ 2- Oxoglutarate + NH$_3$ + NADPH + H$^+$ | 003494_003494 001205_001205 001244_001244 001503_001503 001621_001621 001624_001624 001637_001637 001652_001652 001733_001733 001205_001205 001244_001244 001503_001503 001621_001621 001624_001624 001637_001637 001652_001652 001733_001733 001205_001205 001244_001244 001503_001503 001621_001621 001624_001624 001637_001637 001652_001652 001733_001733 | 0.9 | 1   |
| 1.4.7.1    | Glutamate synthase (ferredoxin) | R00021      | 2 L-Glutamate + 2 Oxidized ferredoxin$\rightleftharpoons$ L-Glutamine + 2- Oxoglutarate + 2 Reduced ferredoxin + 2 H$^+$ | 002214_002214 004233_004233 005383_005383 007321_007321 | 0.8 | 0.8 |
| 1.7.7.1    | Ferredoxin-nitrite reductase | R00790      | NH$_3$ + 2 H$_2$O + 6 Oxidized ferredoxin$\rightleftharpoons$ Nitrite + 6 Reduced ferredoxin + 7 H$^+$ | 004233_004233 005383_005383 007321_007321 | 0.9 | 0.9 |
| 3.5.1.49   | Formamidase      | R00524      | Formamide + H$_2$O $\rightleftharpoons$ Formate + NH$_3$ | 008899_008899 | 0.7 | 0.9 |
| 3.5.5.1    | Nitrilase        | R00540      | Nitrile + 2 H$_2$O $\rightleftharpoons$ Carboxylate + NH$_3$ | 008964_008964 009445_009445 010906_010906 010906_012664 | 0.1 | 1   |
| 4.2.1.1    | Carbonic anhydrase | R00132      | CO$_2$ + H$_2$O $\rightarrow$ HCO$_3^-$ + H$^+$ | 030179_030179 011104_011104 011104_011560 011104_014338 011432_011432 011432_011843 011432_011848 011432_013910 011432_015976 012242_012242 012277_012277 013547_013547 013547_014390 013547_014416 014374_014374 014842_014842 023722_023722 024791_024791 | 0.1 | 1   |
| EC numbers | Enzyme functions | Reaction IDs | Reactions | Protein IDs | CS | MS |
|------------|------------------|--------------|-----------|-------------|----|----|
|            |                  | R03546       | Cyanate + H⁺ + HCO₃⁻ ⇌ CO₂ + Carbamate | 027820_027820 | 0.2 | 1  |
|            |                  | R03546       | Carbamate <=> NH₃ + CO₂ | 017479_017479 | 0.9 | 1  |
| 4.2.1.104  | Cyanase (first step) spontaneous | R07316 |            | 000819_008013 | 0.9 | 0.9 |
| 4.2.1.104  | Cyanase (second step) spontaneous | R07316 |            | 008019_008019 | 0.5 | 0.5 |
| 6.3.1.2    | Glutamine synthetase | R00253 | NH₃ + L-glutamate + ATP -> L-glutamine + ADP + phosphate + H⁺ | 000808_008086 | 0.5 | 0.5 |
| 6.3.1.2    | Glutamine synthetase | R00253 | NH₃ + L-glutamate + ATP -> L-glutamine + ADP + phosphate + H⁺ | 010581_010581 | 0.8 | 0.8 |
| 6.3.1.2    | Glutamine synthetase | R00253 | NH₃ + L-glutamate + ATP -> L-glutamine + ADP + phosphate + H⁺ | 010597_010597 | 0.6 | 0.6 |
| 6.3.4.16   | Carbamoyl phosphate synthase | R00149 | NH₃ + CO₂ + 2 ATP + H₂O -> carbamoyl-phosphate + 2 ADP + phosphate + 3 H⁺ | 000597_000597 | 0.1 | 1  |
| 1.7.1.1    | Nitrate reductase | R00794 | Nitrite + NAD⁺ + H₂O ⇌ Nitrate + NADH + H⁺ | 007225_007225 | -  | -  |

**Nitrate Transporter**

| Protein IDs | CS | MS |
|-------------|----|----|
| 007354_007354 | 0.5 | 0.9 |
| 021575_021575 | 0.9 | 0.9 |
| 024356_024356 | 0.5 | 0.5 |
| 030050_030050 | 0.7 | 0.7 |
| 031095_031095 | 0.6 | 0.6 |
| 031702_031702 | 0.6 | 0.6 |
| 031702_031702 | 0.6 | 0.6 |

**Nitrite Transporter**

| Protein IDs | CS | MS |
|-------------|----|----|
| 007354_007354 | 0.5 | 0.9 |
| 021575_021575 | 0.9 | 0.9 |
| 024356_024356 | 0.5 | 0.5 |
| 030050_030050 | 0.7 | 0.7 |
| 031095_031095 | 0.6 | 0.6 |
| 031702_031702 | 0.6 | 0.6 |
| 031702_031702 | 0.6 | 0.6 |

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