IN SILICO IDENTIFICATION OF *MEDICAGO TRUNCATULA* GENES INVOLVED IN PURINE TRANSPORT AND METABOLISM

IDENTIFICAREA *IN SILICO* A GENELOR IMPLICATE IN TRANSPORTUL SI METABOLISMUL PURINELOR IN *MEDICAGO TRUNCATULA*

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Abstract. *Leguminous plants grow on nitrogen-limited soils based on their symbiosis with rhizobial nitrogen-fixing bacteria. This symbiosis provides the majority of biologically available nitrogen for cycling in the biosphere and serves as an important natural fertilizer in agricultural systems by allowing natural enrichment of soils with nitrogen. In consequence, leguminous plants are a crucial part of the human diet and of animal feeds and their optimal growth is of major economical and agricultural importance. Better knowledge on the genetic and metabolic regulation of symbiotic nitrogen fixation, can be implemented for improvements on the efficiency of such systems in the field. For this purpose, a bioinformatics approach was undertaken to study purine transport and metabolism, an aspect not studied in the past. Several Medicago truncatula genes involved in purine transport and metabolism were identified including the nodule-specific purine permease Pup1 and the nodule-induced genes coding for adenine phosphoribosyltransferase-like protein (APT1), adenosine/AMP deaminase (AMPD), inosine-uridine preferring nucleoside hydrolase (IUNH5) and nucleobase-ascorbate transporter-like protein (NAT14).*

Key words: *Medicago truncatula*, symbiotic nitrogen fixation, purine transport, purine metabolism, bioinformatics, gene expression, *M. truncatula* Gene Atlas

Rezumat. *Plantele leguminoase cresc pe soluri cu un conținut limitat de azot, datorită simbiozei acestora cu bacteriile fixatoare de azot. Această simbioză, asigură majoritatea azotului disponibil biologic pentru circuitul acestuia în natură şi serveşte ca important îngrăşământ natural în sistemele agricole, permițând îmbogățirea naturală a solurilor cu azot. In consecință, plantele leguminoase reprezintă o componentă crucială a dietei umane şi a hranei pentru animale, iar creşterea lor optimă are o importantă economică şi agricolă majoră. O mai bună cunoaştere a reglării genetice şi metabolice a procesului de fixare simbiotică a azotului, poate fi aplicată pentru îmbunătățirea eficientei acestor sisteme*

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în domeniul agricol. În acest scop, a fost efectuată o analiză bioinformatică, pentru a studia transportul şi metabolismul purinelor, aspect care, în trecut, nu a mai fost abordat. Au fost identificate câteva gene implicate în transportul şi metabolismul purinelor, în Medicago truncatula, inclusiv, purin permeaza Pup1, specifică nodozităţilor, precum şi genele induse in nodozitati care codifica proteinele adenin fosforiboziltransferaza (APT1), adenozina/AMP deaminaza (AMPD), inozin-uridina cu afinitate pentru nucleozid-hidrolaza (IUNH5) şi nucleobaza/transportor ascorbat (NAT14).

Cuvinte cheie: *Medicago truncatula*, fixarea simbiotică a azotului, transportul purinelor, metabolismul purinelor, bioinformatică, expresie genică, *M. truncatula* Gene Atlas

INTRODUCTION

Molecular Nitrogen (N_2) is extremely abundant, comprising about 79% of the atmosphere (Postgate, 1998). However, plants cannot convert N_2 to useful organic forms and mineral nitrogen is limited in soils, commonly restricting the plant growth (Tamm, 1991; Vitousek and Howarth, 1991; Berendse *et al.*, 1993; Vitousek *et al.*, 1997). One of the biggest challenges of the 21st century is to sustain adequate production of food so as to cover the needs of the continually increasing human population. Symbiotic nitrogen fixation in legumes has strategic significance to long-range improvement and sustainability of modern agriculture. Aiming to gain knowledge on the genetic and metabolic regulation of symbiotic nitrogen fixation systems, which might be implemented for improvements on the efficiency of such systems in the field, this work questions the putative implication of purine transport and metabolism in symbiotic nitrogen fixation since little is known about the precise molecular and biochemical mechanisms governing this process and their physiological role in the establishment and function of symbiotic nitrogen fixation. The model symbiotic system that was used in this work is that of *Medicago truncatula – Sinorhizobium meliloti*. *M. truncatula* represents the primary model organism or reference legume species for genomic and functional genomic research (Barker *et al*., 1990; Young and Udvardi, 2009). A bioinformatics approach was undertaken to identify genes of interest using latest version of *M. truncatula* genome released at http://www.plantgdb.org/MtGDB and the *M. truncatula* Expression Gene Atlas (http://mtgea.noble.org/v2).

MATERIAL AND METHOD

International genome sequencing efforts have yielded genomic sequences for the majority of *M. truncatula* genes and a latest version of *M. truncatula* genome has been released at http://www.plantgdb.org/MtGDB. *In silico* genome searches were carried out in order to identify *M. truncatula* genes involved in purine transport and metabolism. For the identification of such gene homologs, well annotated genes of model organisms were identified in the KEGG database and were used as "bait" sequences for the Blast algorithm. At the same time, manually cured analysis of candidate genes involved size of the gene product, presence of conserved characteristic domains (Pfam and InterPro), Gene Ontology (GO), as well as sequence similarity to proteins in another well-annotated database

(SwissProt and NCBI). Furthermore, detailed gene expression data are provided by the *M. truncatula* Gene Expression Atlas (http://mtgea.noble.org/v2). These recourses were used to identify nodule-specific and/or nodule-induced *M. truncatula* genes involved in purine transport and metabolism.

RESULTS AND DISCUSSIONS

To fulfill the objectives of this study, a bioinformatics approach was undertaken to identify *M. truncatula* genes involved in purine transport and metabolism. For this purpose, *in silico* genome searches were conducted using Blast algorithm on the latest version of *M. truncatula* genome released at http://www.plantgdb.org/MtGDB. For the identification of such gene homologs, well annotated genes of model organisms were identified in the KEGG database and were used as "bait" sequences. *In silico* genome searches resulted in the identification of forty four *M. truncatula* genes involved in purine transport and metabolism including one 5-hydroxyisourate hydrolase (*Urah*), four adenine phosphoribosyltransferase-like proteins (*Apt1-Apt4*), two adenine/guanine permease AZG2-like proteins (*Agp1-Agp2*), one adenosine/AMP deaminase (*Ampd*), one allantoinase (*Aln*), one hypoxanthine-guanine phosphoribosyltransferase (*Hgrt*), eight inosine-uridine preferring nucleoside hydrolases (*Iunh1-Iunh8)*, one purine permease (*Pup1*), seventeen nucleobase-ascorbate transporter-like proteins (*Nat1-Nat17*), one urease (*Ure*), four urease accessory proteins (*UreD*, *UreF1*, *UreF2*, *UreG*), one uricase/urate oxidase/nodulin 35, putative (*Uox*), one xanthine dehydrogenase/oxidase (*Xdh*) and one xanthine/uracil permease family protein, putative (*Xup*) (fig.1). The biochemical pathway of purine transport and metabolism in *M. truncatula* was designed (fig. 2). according to the identified genes. Moreover, the organization on the eight chromosomes (fig. 3) and the structure (fig. 4) of *M. truncatula* genes involved in purine transport and metabolism genes were studied. Furthermore, a thorough and detailed in silico search was performed using *M. truncatula* gene expression Atlas (MtGEA, http://mtgea.noble.org/v2) (Benedito *et al*., 2008), in order to identify nodule-specific and/or nodule-induced *M. truncatula* genes involved in purine transport and metabolism. Detailed gene expression data revealed that purine permease *Pup1* gene is expressed in a nodule specific way which is also in accordance with the results presented by Fedorova *et al*. 2002. In addition, adenine phosphoribosyltransferase-like protein *Apt1*, adenosine/AMP deaminase *Ampd*, inosine-uridine preferring nucleoside hydrolase *Iunh5* and nucleobase-ascorbate transporter-like protein *Nat14* showed strong induction in nodules at all developmental stages in comparison to non-symbiotic organs (fig. 5). Furthermore, the induced expression of the identified genes in the different developmental stages of the nodule of *M. truncatula* will be determined by real-time RT-qPCR. In order to investigate if gene expression is linked directly to the symbiotic nitrogen fixation, the expression levels of these genes will be determined in symbiotic and non-symbiotic organs of *M. truncatula* plants which will be inoculated either with wild-type rhizobia or with *Fix*$^-$ mutant rhizobia namely rhizobia that induce nodule development, but they lack the ability of fixing atmospheric nitrogen. These results will allow us to establish a possible link between purine transport and metabolism and symbiotic nitrogen fixation.

Gene code	Gene symbol	Description
Medtr8g010550.2	*Urah*	5-hydroxyisourate hydrolase
Medtr3g106780.1	*Apt1*	adenine phosphoribosyltransferase-like protein
Medtr3g103010.1	*Apt2*	adenine phosphoribosyltransferase-like protein
Medtr5g012210.1	*Apt3*	adenine phosphoribosyltransferase-like protein
Medtr4g101460.1	*Apt4*	adenine phosphoribosyltransferase-like protein
Medtr3g075120.1	*Agp1*	adenine/guanine permease AZG2-like protein
Medtr3g094320.1	*Agp2*	adenine/guanine permease AZG2-like protein
Medtr2g022780.1	*Ampd*	adenosine/AMP deaminase
Medtr2g013060.6	*Aln*	allantoinase
Medtr1g097460.1	*Hgrt*	hypoxanthine-guanine phosphoribosyltransferase
Medtr1g039400.1	*Iunh1*	inosine-uridine preferring nucleoside hydrolase
Medtr1g039410.2	*Iunh2*	inosine-uridine preferring nucleoside hydrolase
Medtr1g007110.1	*Iunh3*	inosine-uridine preferring nucleoside hydrolase
Medtr2g020750.2	*Iunh4*	inosine-uridine preferring nucleoside hydrolase
Medtr2g020760.1	*Iunh5*	inosine-uridine preferring nucleoside hydrolase
Medtr4g118570.1	*Iunh6*	inosine-uridine preferring nucleoside hydrolase
Medtr4g118590.1	*Iunh7*	inosine-uridine preferring nucleoside hydrolase
Medtr7g104270.1	*Iunh8*	inosine-uridine preferring nucleoside hydrolase
Medtr2g015470.1	*Pup1*	Purine permease
Medtr3g078270.2	*Nat1*	nucleobase-ascorbate transporter-like protein
Medtr4g055290.1	*Nat2*	nucleobase-ascorbate transporter-like protein
Medtr4g106750.3	*Nat3*	nucleobase-ascorbate transporter-like protein
Medtr8g068550.1	*Nat4*	nucleobase-ascorbate transporter-like protein
Medtr8g086520.1	*Nat5*	nucleobase-ascorbate transporter-like protein
Medtr1g021120.1	*Nat6*	nucleobase-ascorbate transporter-like protein
Medtr1g106085.1	*Nat7*	nucleobase-ascorbate transporter-like protein
Medtr2g016460.1	*Nat8*	nucleobase-ascorbate transporter-like protein
Medtr2g084895.1	*Nat9*	nucleobase-ascorbate transporter-like protein
Medtr2g103510.1	*Nat10*	nucleobase-ascorbate transporter-like protein
Medtr3g080390.1	*Nat11*	nucleobase-ascorbate transporter-like protein
Medtr3g103230.1	*Nat12*	nucleobase-ascorbate transporter-like protein
Medtr5g022110.1	*Nat13*	nucleobase-ascorbate transporter-like protein
Medtr5g032020.1	*Nat14*	nucleobase-ascorbate transporter-like protein
Medtr5g035180.1	*Nat15*	nucleobase-ascorbate transporter-like protein
Medtr8g063220.1	*Nat16*	nucleobase-ascorbate transporter-like protein
Medtr8g086535.1	*Nat17*	nucleobase-ascorbate transporter-like protein
Medtr3g085640.1	*Ure*	urease
Medtr1g050428.1	*UreD*	urease accessory protein UreD
Medtr5g088570.1	*UreF1*	urease accessory protein UreF
Medtr5g088615.1	*UrefF2*	urease accessory protein UreF, putative
Medtr4g096770.1	*UreG*	urease accessory protein UreG
Medtr1g048370.1	*Uox*	uricase/urate oxidase/nodulin 35, putative
Medtr2g098030.1	*Xdh*	xanthine dehydrogenase/oxidase
Medtr1g079900.1	*Xup*	xanthine/uracil permease family protein, putative

Fig. 1 *M. truncatula* genes involved in purine transport and metabolism

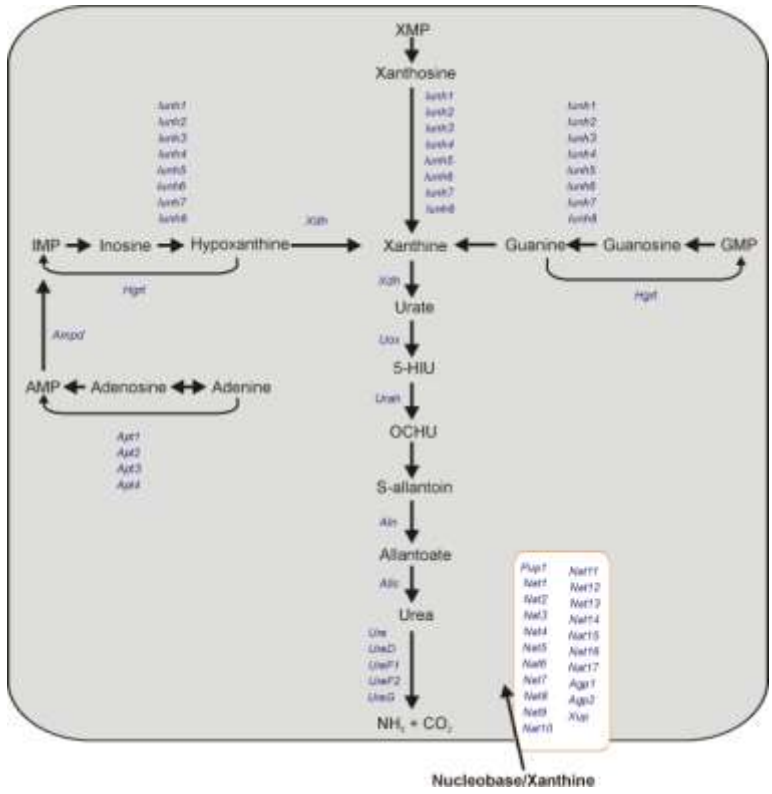

Fig. 2 Schematic representation of the biochemical pathway of purine transport and metabolism in *M. truncatula*

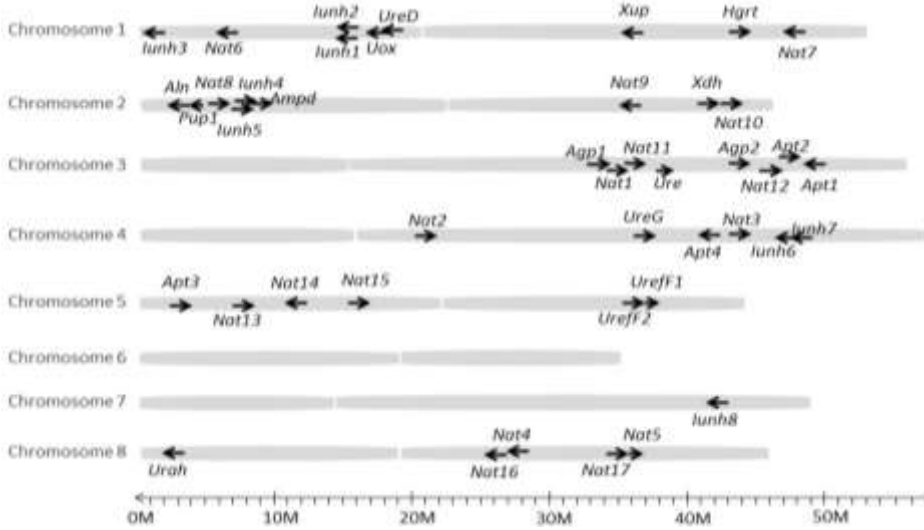

Fig. 3 Schematic representation of the organization of genes involved in purine transport and metabolism on the eight chromosomes of *M. truncatula*.

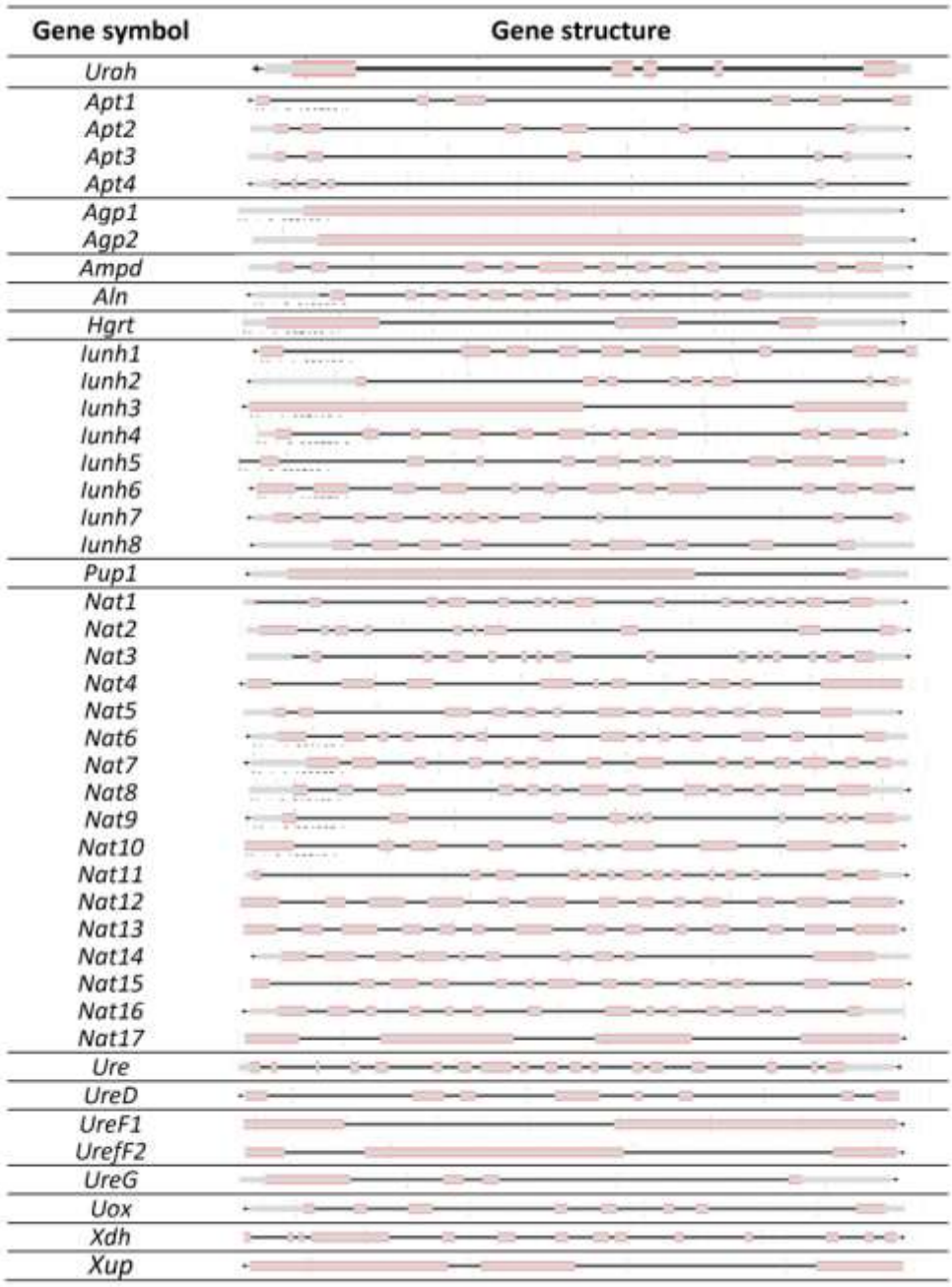

Fig. 4 Schematic representation of the structure of *M. truncatula* genes involved in purine transport and metabolism (: exons, : introns, : untranslated region)

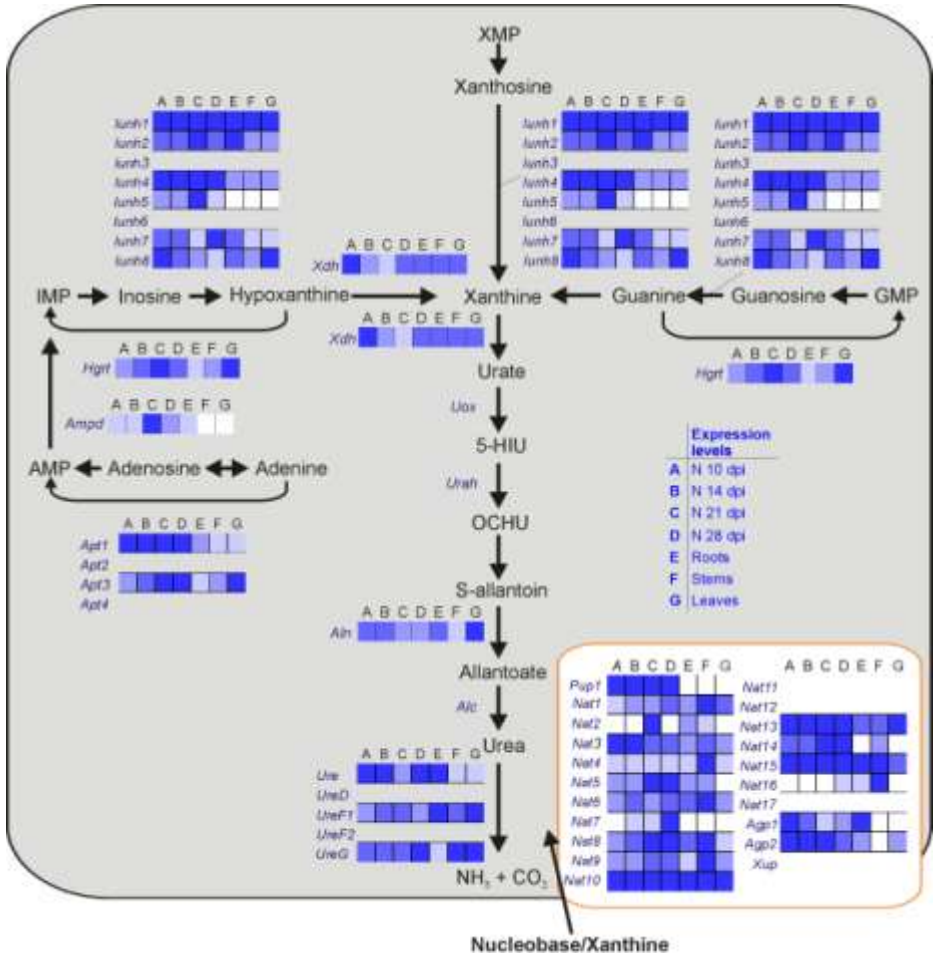

Fig. 5 Heat map of the expression levels of *M. truncatula* genes involved in purine transport and metabolism in non-symbiotic organs of the plant as well as in nodules of different developmental stages, according to data retrieved from the *M. truncatula* Gene Expression Atlas.

CONCLUSIONS

1. A bioinformatics approach including use of Gene databases and gene expression Atlases allows the identification of genes of interest and the study of the related metabolic pathways.

2. Detailed gene expression data provided by the *M. truncatula* Gene Expression Atlas revealed the nodule specific and/or the induced expression of several *M. truncatula* genes involved in purine transport and metabolism.

3. Transcriptional profiling data from symbiotic and non-symbiotic organs of *M. truncatula* plants which will be inoculated either with wild-type rhizobia or with *Fix-* mutant rhizobia namely rhizobia that induce nodule development, but they lack the ability of fixing atmospheric nitrogen using the extremely sensitive and reliable qRT-PCR approach will facilitate a better understanding of the molecular and biochemical mechanisms governing purine transport and metabolism during symbiotic nitrogen fixation.

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