फसलों में नाइट्रोजन-उपयोग दक्षता का अध्ययन

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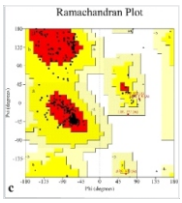
टिकाऊ खाद्य सुरक्षा के लिए नाइट्रोजन उपयोग दक्षता में सुधार के लिए बाजरा में अमोनियम ट्रांसपोर्टर जीन की क्षमता को उजागर करनाः एक इनसिलिको विश्लेषण

तनुश्री सरकार और सुमन बक्शी*
नाभिकीय कृषि एवं जैव प्रौद्योगिकी प्रभाग , भाभा परमाणु अनुसंधान केंद्र, ट्रांबे-४०००८५, भारत



पी. जी. ए. एम. टी. प्रोटीन का रामचंद्रन भूखंड

सारांश

बाजरे (पेनिसेटम ग्लौकम) में अमोनियम ट्रांसपोर्टर (AMT) जीन परिवार के सिलिको विश्लेषण पर केंद्रित है। हम नाइट्रोजन के अवशोषण और उपयोग के आनुवंशिक आधार का पता लगाते हैं, जीन संरचनाओं, कार्यों और विकासवादी संबंधों का पूर्वानुमान लगाने के लिए जैव सूचना विज्ञान का उपयोग करते हैं। बाजरे में AMT जीन के बारे में अध्ययन नाइट्रोजन उपयोग दक्षता (NUE) में सुधार के लिए नई जानकारी के रूप में काम करेगी।

Understanding Nitrogen-use Efficiency in Crops

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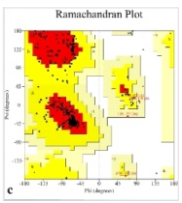
Unlocking the Potential of Ammonium Transporter Genes in Pearl Millet to Improve Nitrogen Use Efficiency for Sustainable Food Security: An *insilico* Analysis

Tanushree Sarkar and Suman Bakshi*
Nuclear Agriculture & Biotechnology Division, Bhabha Atomic Research Centre, Trombay-400085, INDIA



Ramachandran plot of PgAMT protein

ABSTRACT

This study focuses on the *insilico* analysis of the Ammonium Transporter (AMT) gene family in Pearl millet (*Pennisetum glaucum*). We explore the genetic basis of nitrogen uptake and utilization, harnessing bioinformatics to predict gene structures, functions, and evolutionary relationships. Understanding about *AMT* genes in Pearl millet will serve as novel information for improving nitrogen use efficiency (NUE).

KEYWORDS: *In silico analysis, P. glaucum, Ammonium transporter, Nitrogen uptake, NUE*

**Author for Correspondence: Suman Bakshi*
E-mail: sumansud@barc.gov.in

Introduction

Nitrogen (N), a macronutrient for plant growth and development, exists in the form of organic nitrogen, ammonium (NH_4^+), or nitrate (NO_3^-) in soil [1]. Plants have preference for NH_4^+ over NO_3^- due to its direct assimilation into amino acids within plant cells, thus maximizing nutrient uptake efficiency [2]. However, excessive NH_4^+ absorption can be toxic to plants, necessitating precise regulation of NH_4^+ uptake systems to optimize NUE and prevent growth inhibition. Ammonium transporters play a crucial role in NH_4^+ uptake and function as NH_4^+ uniporters, NH_4^+/H^+ symporters, or NH_3/H^+ co-transporters [4]. Pearl millet is a resilient cereal crop and widely grown in arid and semi-arid regions. Understanding and optimizing NUE in this crop is essential for maximizing its productivity and ensuring global food security [6]. Advancements in computational biology have assisted molecular understanding of genes. An *in silico* analysis of the *AMT* gene family was carried out to unravel the genetic basis of ammonium transport in Pearl millet, paving the way for experimental validation and utilization of *AMT* genes for enhancing nitrogen use efficiency.

Materials and Methods

For identification of AMTs, the protein, genomic and coding sequences were downloaded from the IPMGSC (https://cegsb.icrisat.org/ipmgsc/). A Hidden Markov model search was done to obtain ammonium transporter genes. To confirm the candidate gene sequences, belonging to the *AMT* gene family, the NCBI database and HMM-BLAST scores were used. The theoretical molecular weight and isoelectric point of *AMT* proteins were calculated using the ExPASy server [4]. The grand average of hydropathicity (GRAVY) of all the identified proteins was evaluated (www.gravy-calcul-ator.de/). The predictions of the subcellular localization of *AMT* genes were verified using Plant-mPloc online tool [4]. The gene structure of the *AMT*s were determined using the Gene Structure Display Server (https://gsds.gao-lab.org/). Furthermore, the TMHMM server (https://services.healthtech.dtu.dk/services/TMHMM-2.0/) was used for the prediction of transmembrane helices in AMT proteins. Secondary (http://vadar.wishartlab.com/) and tertiary protein (https://swissmodel. expasy.org/) structure was predicted and verified by the PROCHECK test (www.ebi.ac.uk/thorntonsrv/databases/pdbsum/Generate.html), and visualized using Pymol software [4]. The phylogenetic analysis was done by ClustalW and MEGA-XI [4].

Individually, the physical locations of *PgAMT*s were obtained from the database of Pearl millet genome and then, the map of the chromosome location of genes was constructed through the MG2C software (http://mg2c.iask.in/mg2c_v2.1/). Further, *Ks* and *Ka* substitution rates of the paralogs genes were investigated (https://bio.tools/kaks_calculator). Syntenic relationships of the *AMT* genes in *Poaceae* were determined (https://bat.infspire.org/circoletto). To predict cis-regulatory elements on the promoter regions of *PgAMT*s, 2 kb upstream genomic DNA sequences were submitted to the PlantCARE (https://bioinformatics.psb.ugent.be/webtools/plantcare/html/) and visualized using TBtools [4].

Gene	Gene identifier	Strand	Protein (aa)	MW (kDa)	pI	GRAVY	TM	Category
PgAMT2.1	Pgl_GLEAN_10026660	Reverse	492	51.8	7.1	0.555	11	AMT 2
PgAMT3.3	Pgl_GLEAN_10007710	Forward	479	51.85	5.97	0.564	11	AMT 2
PgAMT3.2	Pgl_GLEAN_10030271	Forward	480	51.25	6.29	0.539	11	AMT 2
PgAMT2.3	Pgl_GLEAN_10012021	Forward	445	47.04	7.15	0.513	11	AMT 2
PgAMT2.2	Pgl_GLEAN_10012022	Forward	454	48.56	6.87	0.318	9	AMT 2
PgAMT1.2a	Pgl_GLEAN_10027285	Forward	474	50.24	6.54	0.495	10	AMT 1
PgAMT1.2b	Pgl_GLEAN_10027258	Forward	392	41.34	6.7	0.569	8	AMT 1
PgAMT1.1	Pgl_GLEAN_10009225	Reverse	334	35.55	6.64	0.466	6	AMT 1

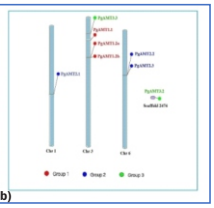


Fig.1: (a) Physicochemical properties of P. glaucum AMT proteins; (b) Chromosomes of P. glaucum showing distribution of PgAMT genes.

Results and Discussion

Among plants, AMT gene was first recognized in Arabidopsis thaliana [2]. In this study, a total of 8 AMT protein sequences were identified and termed as *PgAMT*s. The physiochemical features of AMT proteins include: chromosome location, strand orientation, protein length, molecular weight (MW), isoelectric point (pI), hydrophobicity prediction (GRAVY), subcellular localization, and family categorization were assessed (Fig.1(a)). The length, MW and pI of AMT proteins ranged from 334 (*PgAMT*1.1) to 492 amino acids (*PgAMT*2.1), 35.45 (*PgAMT*1.1) to 51.85kD (*PgAMT*3.3) and 5.97 (*PgAMT*3.3) to 7.15 (*PgAMT*2.3) respectively. Subcellular localization found that of *PgAMT*s were primarily located in the cell membrane. Predictions about the hydrophobic nature of the deduced amino acid sequences indicated that all the AMT proteins exhibit polar characteristics. Chromosomal distribution revealed that *PgAMT*s were located on chromosome 1, 3, 6 and scaffold 2474 (Fig.1(b)). Notably, structural analysis revealed the presence of introns in both the AMT1 and AMT2 subfamilies (Fig.2(a)). The occurrence of introns in the AMT1 subfamily has also been reported in rapeseed [7]. Domain analysis revealed ubiquitous existence of ammonium transporter domain identified in all the queried proteins.

Motif analysis showed that the AMT1 and AMT2 subfamilies had variable motif compositions. Proteins within the same subgroup has identical motif components signifies that differences in the motifs can differentiate subgroups and has evolutionary significance (Fig.2(b)).

Transmembrane domain analysis of *PgAMT*s indicated the presence of 6-11 conserved transmembrane domains (Fig.3(a)). In the secondary structure, *PgAMT*s have shown 56-60% helix, 1-6% beta, 33-40% coil, and 15-17% turn in their structures. The 3D structure of *PgAMT* proteins were predicted based on homology modelling (Fig.3(b)) and found to be analogous to the AMTs analysis in *S. lycopersicum* reported by Filiz and Akbudak (2020). Ramachandran plot was used for validation of the protein 3D structures of *PgAMT* proteins for exploring its biological functions (Fig.3(c)). In the Gene Ontology (GO) study of predicted *PgAMT*s, the AMT1 and AMT2 showed GO terms related to ammonium transportation (Fig.4(a)). The protein-protein interaction study (Fig.4(b-c)) has shown that the proteins associated with *PgAMT* proteins in the network comprise enzymes that participate in nitrogen assimilation and comparable findings were found in the

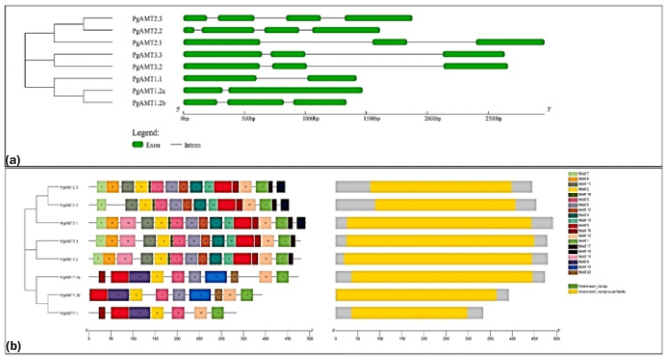


Fig.2: (a) Structure showing exons and introns with the green boxand black lines, respectively; (b) Illustration of conserved protein motifs and conserved domain of PgAMTs.

This paper received the best oral presentation award in the 'International Conference on Millets Cropping in Thar Desert of India and Role of Rural Community in Sustainable Dry Ecology' held at HARSAC, Hisar, Nov. 9-11, 2023.

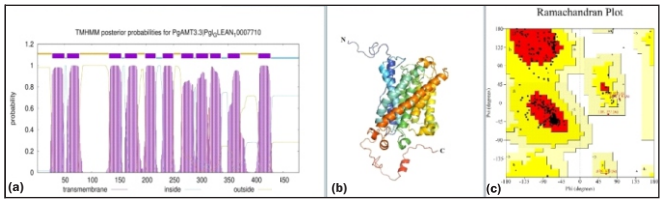


Fig.3: (a) Representative figure of transmembrane structure prediction of AMT proteins; (b) Predicted 3D structure and (c)Ramachandran plot of PgAMT protein.

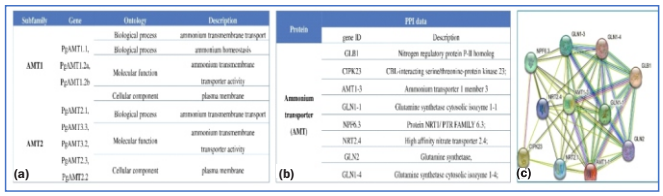


Fig.4: (a) Gene ontology (GO) enrichment analyses of PgAMT genes; (b) List of protein- protein interaction network members of PgAMT proteins; (c)Visualization Protein-Protein Interaction analysis.

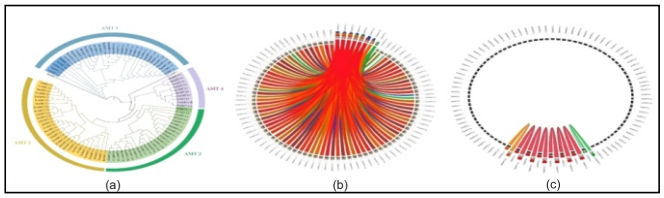


Fig.5: (a) Phylogenetic tree of AMTs of Poaceae; (b) Representation of synteny of PgAMTs; (c)Synteny blocks in best match synteny analysis parameter.

Paralogous Pairs	Chromosomal location	Duplication event	Ka/Ks	Selection
PgAMT1.2b/PgAMT1.2a	Chr 3/Chr 3	Tandem	0.205	Purifying
PgAMT3.2/PgAMT3.3	scaffold 2474/Chr 3	Segmental	0.190	Purifying
PgAMT2.2/PgAMT2.1	Chr 6/Chr 1	Segmental	0.341	Purifying

(a) (b)

Fig.6: (a) Ka/Ks ratios for paralogous PgAMT genes; (b) Chromosomal distribution and inter-chromosomal relationships among AMT genes of P. glaucum.

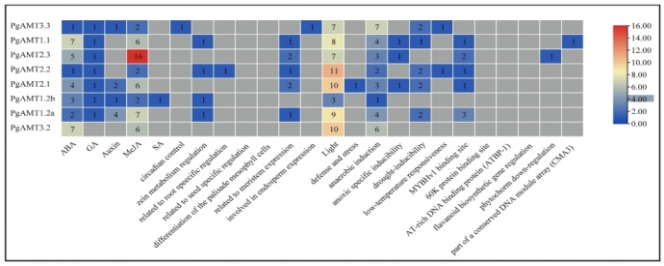


Fig.7: Representation of promoter cis-element analysis of AMT genes in P. glaucum.

analysis of *SlAMT1* of *S. lycopersicum* [5] thus, elucidating an active involvement of AMT proteins in the uptake and assimilation of nitrogen in plants. The phylogenetic analysis categorically separated all plant AMTs to two subfamilies, AMT1 and AMT2 (AMT2/AMT3/AMT4) (Fig.5. a). Phylogenetic grouping showed close association of AMTs in the *Poaceae* family, which includes millets and other cereals. Some similarities exist among AMTs of *Poaceae* with the AMT genes from *A. thaliana*, however, form distinct cladeindicating the divergence of AMTs of monocots from the dicot. The phylogenetic grouping in this study is similar to findings reported in other crops, Apple [1], Soybean [2], Wheat [3] and Tomato [5]. Synteny analysis showed strong conservation among the *PgAMTs* and AMTs of other Poaceae members (Fig.5(b)). The best matched blocks were identified between *PgAMTs* and other members (*S. italica, S. viridis & P. miliaceum*) validating evolutionary similarities (Fig.5(c)).

The cis-elements of *PgAMTs* respond to signals related to plant growth and development (zein metabolism, circadian control), hormones (auxin, gibberellin, and abscisic acid), and environmental stresses (light response, low-temperature, defense, anaerobic conditions, and drought). All the *AMT* gene promoters respond to light, thus, play role in plant growth. Each promoter contains elements for different phytohormones, implying hormone-mediated regulation (Fig.7). In similar studies, Arabidopsis, Tomato and Wheat [3, 4] AMTs display diurnal expression patterns, indicating increased ammonium absorption during daylight hours. Rice AMTs are essential for ammonium uptake under anaerobic conditions. Wheat and rice AMTs are associated with defence response to pathogens [3, 4]. *Lotus japonicus* and *Medicago truncatula* AMTs facilitate ammonium transport in plant-microbe symbiosis [4].

Conclusion

Through in silico analysis, a total of eight *AMT* genes were identified, categorized into AMT1 and AMT2 subfamilies. Investigation of gene sequences, chromosomal locations, and conserved motifs underscores their conservation. These findings elucidate the role of AMTs in efficient ammonium N utilization and stress adaptation paving the way for efficient utilization of AMTs in Pearl millet.

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