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Plant Science Journal

Print ISSN: 2227-5614 Online ISSN: 2227-5630

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE **PLANT SCIENCE JOURNAL**, 2025 14(1):32-35. **OPEN ACCESS**

In silico Analysis of *NRT2* Gene Family in Chickpea

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DOI: https://doi.org/10.5281/zenodo.17691258
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NRT2 is a High affinity nitrate transporter family which has been found to play an important role in nitrate transportation in *Arabidopsis thaliana*. The family has seven well characterized members in *Arabidopsis thaliana*. Five of the seven Arabidopsis proteins (*AtNRT2.1, AtNRT2.2, AtNRT2.3, AtNRT2.4, andAtNRT2.6*) share a common structure, composed of five identical domains. *AtNRT2.5* is structurally closely related to a yeast transporter, whereas *AtNRT2.7* is similar to fungal and algal proteins. NRT2 proteins of monocotyledons (wheat, rice, barley) are phylogenetically close to each other. Homologs of these proteins in chickpea (*Cicer arietinum*) genome need to be characterized. So, current study includes an *in silico* identification and characterization of *NRT2* genes in *Cicer arietinum* genome. In this study a comprehensive genome wide analysis of *NRT2* genes/proteins, including promoter analysis, phylogenetic analysis, and conserved domains/motifs analysis is done. Moreover, comparative analysis for *NRT2* genes of six plant species (mono- and dicots) has also been performed. The plant species used in the analysis include *Oryzasativa, Cicerarietinum Solanum lycopersicum, Hordeum vulgare, Triticum aestivum*and and *Arabidopsis thaliana*. This study will be important to predict the functions of *NRT2* genes in chickpea.

Keywords: ***NRT2* gene family, Phylogeny, Motif Analysis, Promoter Analysis, *Cicer arietinum*.**

INTRODUCTION

Nitrogen is an essential element for plant growth. It is necessary for achieving high yield potential in crops. NRT2 is a high affinity nitrate transporter family which has been found to play an important role in nitrate transportation in *Arabidopsis thaliana* [1]. The family has seven well characterized members in *Arabidopsis thaliana* [2]. Chickpea (*C. arietinum*) is the third most widely grown grain legume in the world after Beans and Soybean. A better understanding of structural, functional and regulatory aspects of N transport genes could have a positive effect on growth indices and yield components of chickpea as well as better Nitrogen use efficiency of crop [3]. So, current study includes an *in silico* identification and characterization of *NRT2* genes in newly sequenced *Cicer arietinum* genome. In this study a comprehensive genome wide analysis of *NRT2* genes/proteins, including promoter analysis, phylogenetic analysis, and conserved domains analysis is done. *Hordeum vulgare* and *Oryzasativa*, are also used to analyze evolutionary background of chickpea *NRT2* genes.

MATERIALS AND METHODS

Sequence retrieval and identification of NRT2 genes

Gene sequences of NRT2 genes present in Arabidopsis thaliana were extracted from NCBI database (http://www.ncbi.nlm.nih.gov) and used for BLAST on NCBI against chickpea genome.

Multiple sequence alignment and identification of conserved domains

Multiple Sequence alignment was done using CLUSTALW (http://www.genome.jp/tools-bin/clustalw) which is an online available tool. 7 genes of *arabodopsis thaliana* and two genes of chickpea were included in multiple sequence alignment. Aligned sequences were searched for conserved domains.

Phylogenetic Analysis of NRT2 genes in different plant species

Amino acid sequences of all the identified non-redundant chickpea and Arabidopsis NRT2 were aligned using Clustal W [4] program of Molecular Evolutionary Genetics Analysis (MEGA version 6.0) software suite.

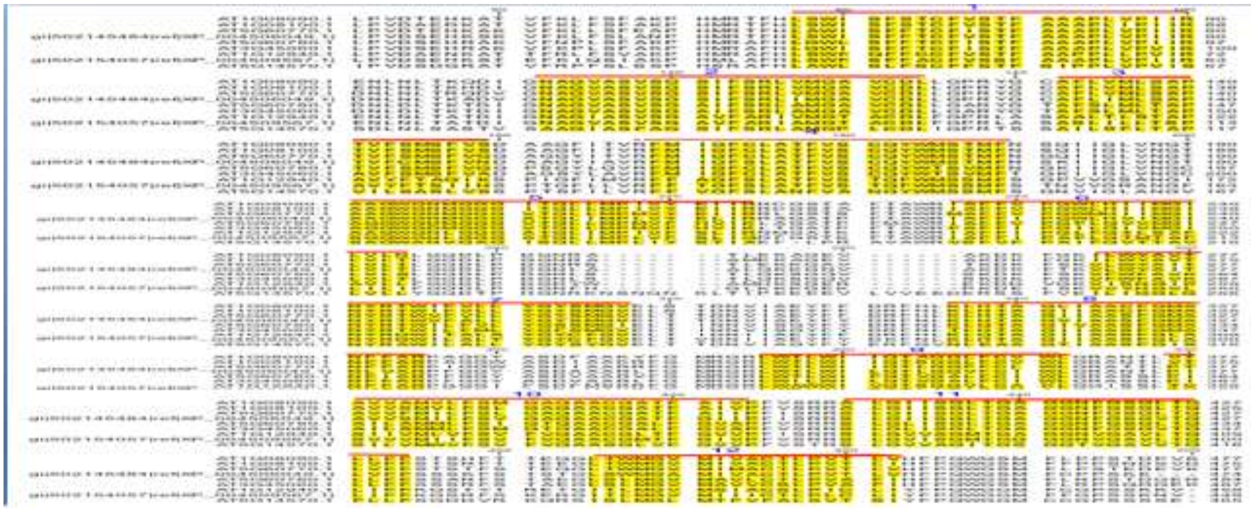

Figure 1: Conserved trans-membrane domains in *Arabidopsis thaliana* and chickpea are highlighted

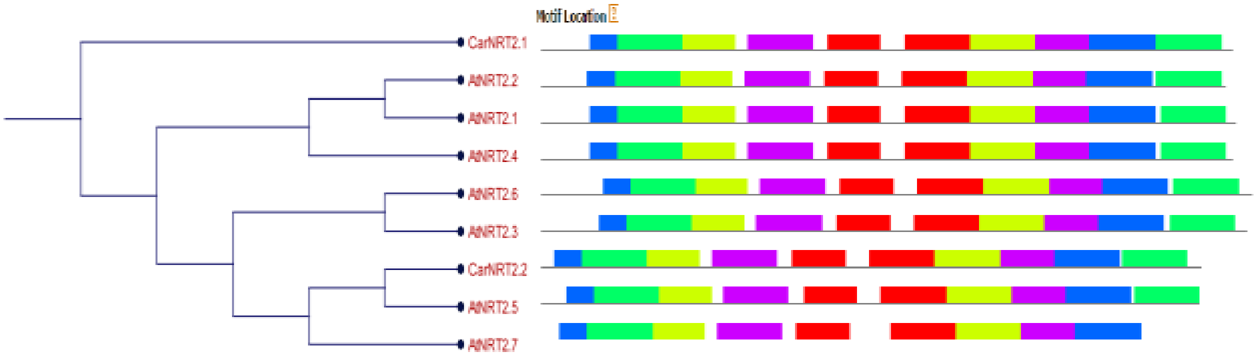

Figure 2: Comparative phylogenetic analysis and conserved domain analysis of NRT2 genes of chickpea and Arabidopsis. Various conserved domains (colored boxes) identified in Car and At NRT2 genes are shown.

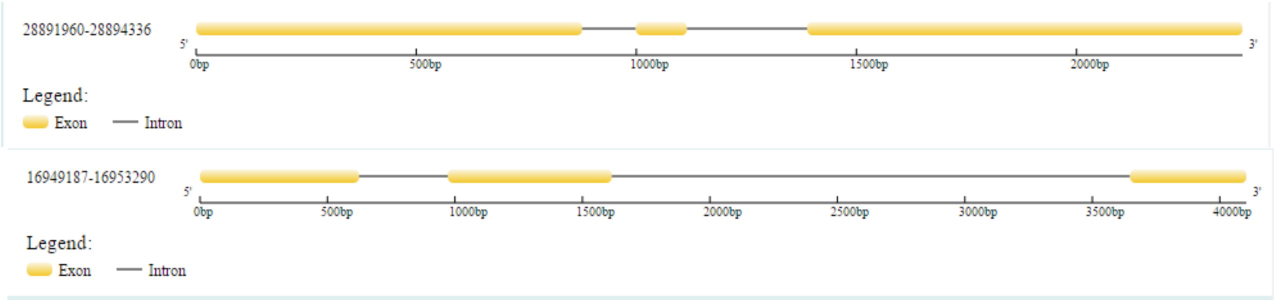

Figure 3: Gene structure analysis of chickpea *NRT2* genes shows the positions of exons and interons in the genes.

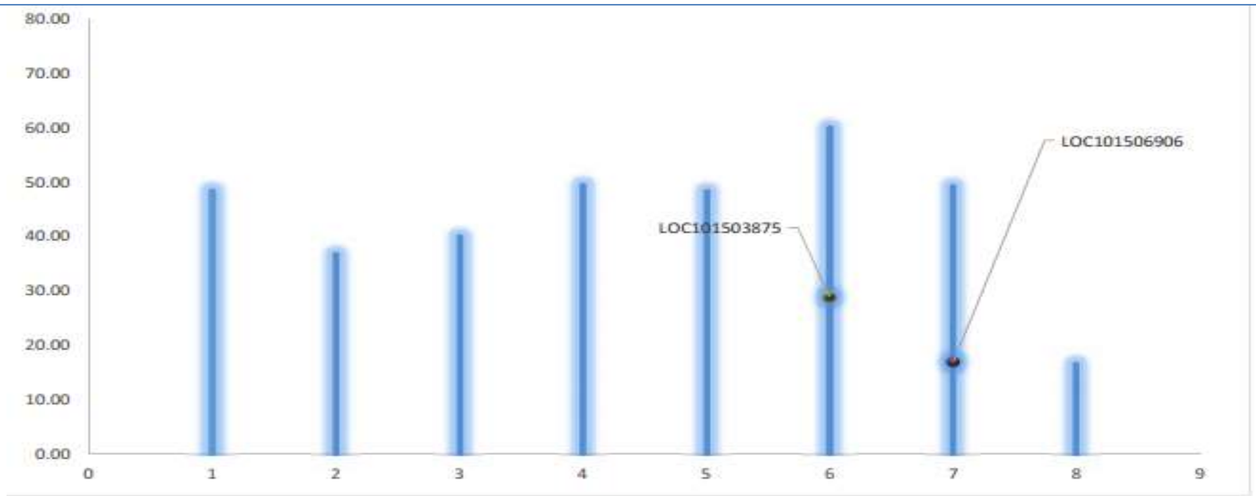

Figure 4: Distribution of Chickpea NRT2 genes on the chromosomes. The scale bar represents Mega bases (Mb).

Discovery of conserved motifs from chickpea and Arabidopsis NRT2 genes

The conserved motifs within chickpea and Arabidopsis NRT2 proteins were determined by using the MEME online server [5]. The parameters were set as follows: maximum numbers of different motifs, 19; minimum motif width, 12; maximum motif width, 52; other parameters retained their default settings

Gene structure analysis of NRT2 gene family of chickpea

Gene structure including introns and exons of chickpea and Arabidopsis NRT2 genes was investigated by using the online Gene Structure Display Server (http://gsds.cbi.pku.edu.cn/) based on genomic and coding sequences [6].

Chromosomal location of NRT2 gene family

The identified non-redundant chickpea NRT2 genes were mapped on all the eight chickpea chromosomes on the basis of the information obtained from NCBI using MapDraw software [7]. After locating all the genes on different chromosomes, they were assigned new names on the basis of their location on the chromosomes i.e, the gene which was located on the chromosome number 6 was assigned the name as CarNRT2.1 and the gene present on the chromosome 7 was assigned as CarNRT2.2.

RESULTS

Identification and prediction of Chickpea NRT2 gene family

A total of 7 genes were found as putative NRT2 genes of chickpea. 5 of them were dropped on the basis of homology. 2 genes named CarNRT2.1 and CarNRT2.2 were selected for further analyses.

Sequence Alignment and Conserved Domains of NRT2 genes

After aligning the sequences of NRT2 proteins with CLUSTALW, they were searched for conserved domains. 12 domains were identified (Fig. 1) as highly conserved in almost all the members of family among both the species. This shows the functional similarity among all the members in different species.

Phylogenetic Analysis of NRT2 genes in different plant species

In order to analyze the evolutionary relationship among chickpea and Arabidopsis thaliana NRT2 proteins, a phylogenetic analysis was done to make a combined phylogenetic tree. The 2 non-redundant chickpea NRT2 protein sequences along with 7 Arabidopsis NRT2 protein sequences were used to make combined phylogenetic tree (Fig. 2). Comparative phylogenetic analysis of chickpea and Arabidopsis NRT2 revealed a close relationship among both species. The combined phylogenetic analysis *Oryz asativa, Cicer arietinum Solanum lycopersicum, Hordeum vulgare, Triticum aestivum* and *Arabidopsis thaliana* showed a majour group of NRT2 genes which shows great similarity in all above species [8, 9].

Discovery of conserved motifs from chickpea and Arabidopsis NRT2 genes

We have identified 5 conserved motifs from 9 NRT2 sequences of chickpea (2) and Arabidopsis (7). All the sequences showed equal number of motifs. The number of conserved motifs is 10 in each chickpea gene (Fig. 2) while 8 conserved sites have been previously identified in *Brassicca napus* [10].

Gene structure analysis of NRT2 gene family of chickpea

Gene structure analysis has shown that NRT2 genes of chickpea contain two introns each (Fig. 3). In recent years, the studies on the role of introns have gain considerable success. Studies in fungi, nematodes, insects, mammals and plants suggest that introns not only play role in regulation of gene expression, but also involved in gene evolution [11].

Chromosomal Location of NRT2 genes

The chromosomal locations of the 2 CarNRT2 genes were investigated according to genome sequencing data of chickpea. It was revealed that, 1 of chickpea NRT2 genes was present on chromosome 6 and other on chromosome 7.

CONCLUSION

In this study, 2 non-redundant NRT2 genes were identified from the sequenced chickpea genome. These chickpea NRT2 genes/proteins contain 10 conserved motifs. Phylogenetic analysis shows the close relationship among NRT2 members present in different plant species. Gene structure analysis revealed that NRT2 genes predominantly contain two introns. This study may provide new insights for the functional characterization of chickpea NRT2 genes.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

DATA AVAILABILITY

All data are included within the article and on the hands of the first author if you needed

ACKNOWLEDGEMENT

Not applicable.

AUTHOR CONTRIBUTION

The author has revised and approved the final version of this manuscript.

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