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Identification and isolation of a salt inducible gene from chickpea (*Cicer arietinum L.*)

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Abstract

Chickpea (*Cicer arietinum* L.) is the second largest produced grain legume in the world contributing in supplying high quality dietary protein and fixing atmospheric nitrogen biologically. Mostly grown in arid and semi-arid areas, India is the largest producer of chickpea in the world with an estimated production of 7.17 Mt from an area of 8.6 Mha (FAOSTAT, 2015) and accounts for over 67.4 % of the total world production. Chickpea is a salt-sensitive crop species and soil salinity impedes chickpea production in many parts of the world, including large areas of farming land in India. Salinity is a major abiotic stress limiting plant survival and having adverse effects on growth and productivity. Genes that are induced in response to salt stress in plants are thought to play important role in conferring salt tolerance. In the present study a putative salt responsive gene was identified, isolated and gene expression studies were carried out through both semi quantitative RT-PCR and real-time qRT PCR. The present study was an attempt to successfully study the salt responsive gene expression in chickpea gene and to isolate a gene.

Key Words: *Abiotic stress, Salt inducible, Chickpea, Gene expression analysis*.

1. Introduction

Chickpea (*Cicer arietinum*) is the most important pulse crop in the Indian subcontinent. Chickpea productivity is adversely affected by a large number of stresses like biotic and abiotic. MicroRNAs (miRNAs) have been implicated in the regulation of plant responses to several biotic and abiotic stresses. Many miRNAs responded in a similar fashion under both biotic and abiotic stresses, indicating the existence of cross talk between the pathways that are involved in regulating these stresses. The potential target genes for the conserved and novel miRNAs were predicted based on sequence homologies. miR166 targets a HD-ZIPIII transcription factor and was validated by 5′ RLM-RACE. This study has identified several conserved and novel miRNAs in the chickpea that are associated with gene regulation following exposure to wilt and salt stress (Kolhi *et al.*, 2014). Chickpea (*C. arietinum* L.) ranks third in food legume crop production in the world. However, drought poses a serious threat to chickpea production, and development of drought-resistant varieties is a necessity. Unfortunately, cultivated chickpea has a high morphological but narrow genetic diversity, and understanding the genetic processes of this plant is hindered by the fact that the chickpea genome has not yet been sequenced and its EST resources are limited

(Jain and Chattopadhyay, 2010). In this study RT-PCR and qRT-PCR (Quantitative real-time PCR) were used to validate novel salt inducible genes.

2. Materials and methods:

For the isolation of salt inducible chickpea gene the primer designing for both semi qRT, real time qRT was done using freely available software IDT. Seeds of the chickpea genotypes tolerant to salinity (JG64, JG11) and sensitive to salinity (ICCV2, DCP-92-3) were procured from the Genetics, Indian Agricultural Research Institute, New Delhi. Both salt tolerant and salt sensitive chickpea genotypes were grown for 14 days and 80Mm salt (NaCl) stress was given for 6h and 24h. At least three biological replicates of each tissue sample were harvested. Total RNA was isolated from both control and salt stressed salt tolerant and salt sensitive chickpea genotypes using Trizol method and checked the quality and quantity. cDNA was made and checked the quality by using housekeeping gene GAPDH. Semi qRT PCR to validate expression pattern of selected putative salt responsive gene was performed and an up-regulation was observed. Real time qRT PCR to validate expression pattern of selected putative salt responsive gene was performed and an up-regulation was observed. The gene of interest was PCR amplified using gene specific primers using cDNA of salt stressed plant as template. The amplified product was checked on agarose gel followed by gel elution and cloning of gene sequence (~1.15 kb) into a suitable sequencing vector pGEM-T (3.0kb). The cloned product was screened on LA plates containing ampicillin and checked for the presence of insert after plasmid isolation using colony PCR.

3. Results and conclusion:

We have identified and cloned a putative salt inducible gene in chickpea. Gene expression analysis by semiquantitative RT-PCR and real-time q RT PCR revealed that the gene is significantly upregulated under salt stress with a possible role in salt tolerance. In future, to further investigate the functional role of the isolated gene from chickpea, it will be overexpressed in model plant *Arabidopsis thaliana* under the control of strong constitutive promoter, CaMV35S using a binary vector and the transformed *Arabidopsis* will be screened for salt tolerance. Such genes will be useful in generating salt tolerant plants.

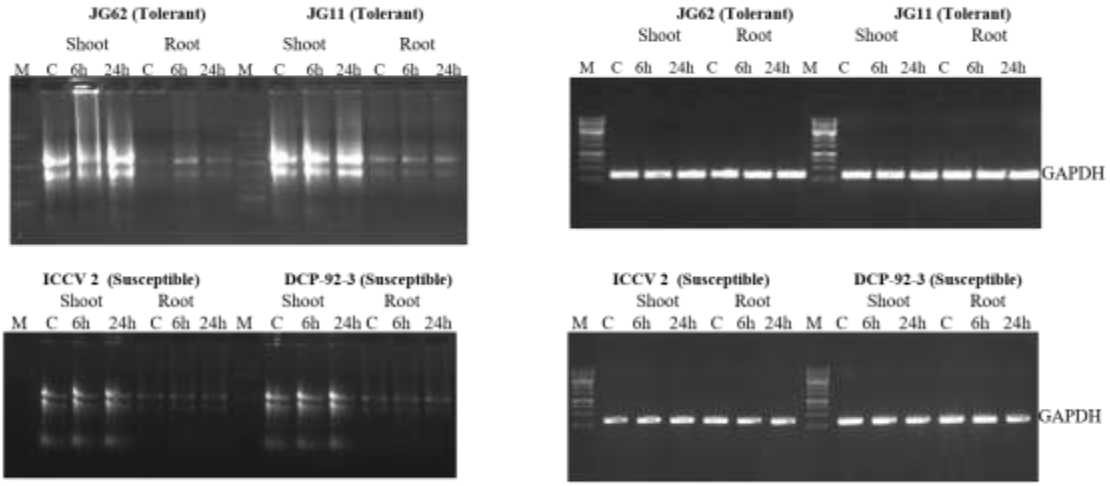

Fig. 1. Isolated total RNA and cDNA check with housekeeping gene GAPDH from both shoots and roots separately from both Tolerant and susceptible genotypes of chickpea treated with 80 mM NaCl stress and sampled at 6 and 24 hour post treatment (hpt).

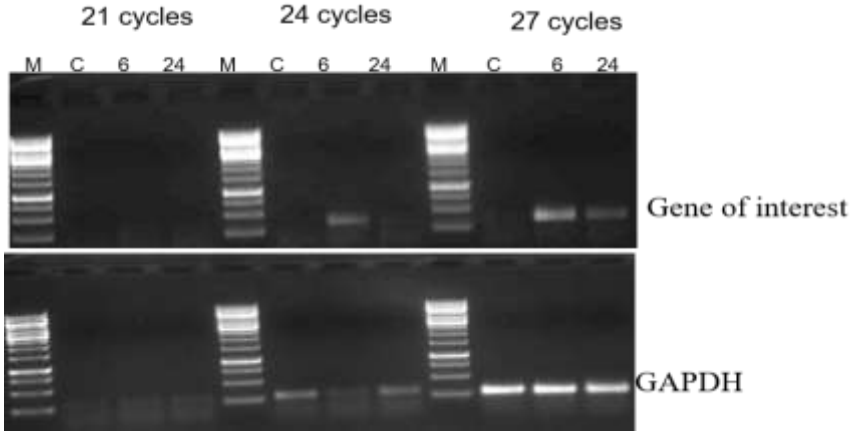

Fig.2. Semi qRT PCR showing up-regulated gene in response to salt stress in 6h and 24h corresponding to a house-keeping gene.

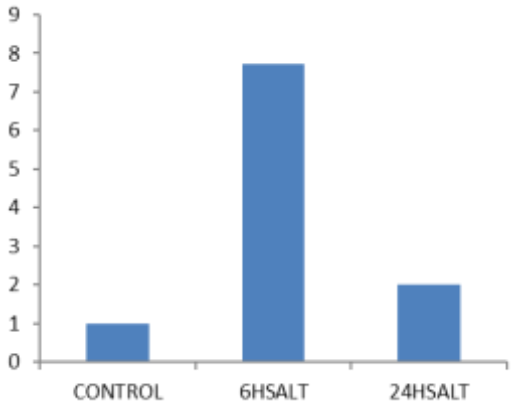

Fig 3. Quantitative RT-PCR analysis of a putative salt responsive gene of chickpea in response to salt stress of 80mM NaCl at 6 and 24h time interval. GADPH gene was used for normalization. The expression level of genes in control sample was used as calibrator to calculate fold change.

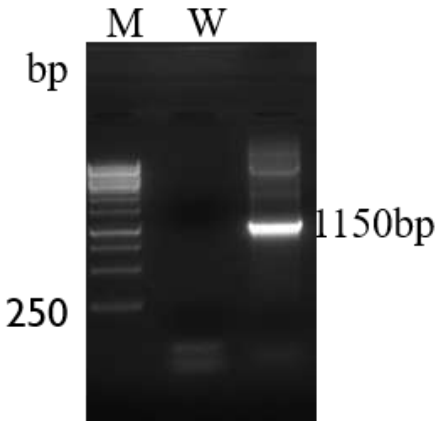

Fig. 4 PCR amplified gene of interest (~1150bp)

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