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SHORT COMMUNICATION

ISOLATION OF DIFFERENTIALLY REGULATED PARTIAL cDNA WITH RESPECT TO WATER STRESS FROM RICE

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In the present study a partial cDNA sequence of 308 bp showing induction under water stress was isolated from rice cv. Nagina-22 (N22) and sequenced. The sequence has 150 bp 5' untranslated region and the 156 bp coding sequence. Coding sequence has ATG initiation codon and encodes 52 amino acids. On homology search analysis, it was observed that the coding sequence had 100 % homology with protein phosphatase 2C gene of *Arabidopsis thaliana* and corresponds to N terminal region of the gene which has very important role in ABA signaling. Protein phosphatase 2C negatively regulates ABA response under water deficit stress.

Key words: **ABA, water stress**

Plants are constantly exposed to a variety of abiotic stresses that adversely affect plant growth and limit the crop yield. Among the abiotic factors that are involved in plant growth and development, water availability is most important factor, which in broadest sense encompasses both drought and salt stresses (Bartels and Sunkar 2005, Kar *et al*. 2008). Cellular and molecular responses of plants to environmental stresses have been studied extensively (Umezawa *et al.* 2006, Shao *et. al.* 2008). To survive these challenges, plants have developed elaborate mechanisms to perceive external signals and to manifest adaptive responses (Bohnert *et al*. 1995, Shinozaki *et al*. 2003). Rice is the most important staple food crop in the world and is grown under a broad range of environmental conditions (Boyer 1982, Munns and Tester 2008). Drought is one of the main factors causing severe rice yield loss in most rice growing areas. Water stress is becoming increasingly important as the climate change scenarios suggest an increase in aridity in many parts of globe.

The physiological and biochemical processes underlying plant responses to water deficit and oxidative stresses include expression of specific genes and large number of stress proteins that have putative role in stress adaptation and plant defense. Hundreds of genes that are induced under water deficit stress have been identified and isolated and are being used to study their specific functions and role in plant acclimatization(Nguyen *et al*. 2004). Biochemical and biotechnological approaches may help us to find out stress induced genes to understand the mechanism of plant responses to water deficit stress. In view of this in the present study, a differentially regulated gene sequence with respect to moisture stress was isolated from an early maturing deep-rooted drought tolerant rice cultivar Nagina-22 (N-22).

Drought tolerant rice cultivar (N22) was grown in growth chamber under controlled conditions of 30^0C/ 25^0C (day/night) under the light intensity of 600 mmol

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m^{-2} s^{-1} PPFD (Photosynthetic photon flux density). Forty five days old seedlings were gradually subjected to severe water stress by withholding the water supply. Relative water content (RWC) of leaf tissues was determined according to the method of Weatherly (Weatherly 1950). Control plants were watered daily and their RWC was found to be 96-98 %. Rice plants having attained 65-85% RWC after withholding water supply were used as water stressed samples.

For RNA isolation, leaf samples were collected on the day of corresponding RWC and immediately frozen in liquid nitrogen and stored till RNA isolation. Total RNA was isolated by using guanidinium isothiocyanate (GTC) method (Sambrook *et al*. 1998) and was subjected to electrophoresis on 1.2% agarose gel containing 20mM GTC. mRNA was isolated from ~250 mg of total RNA from stressed leaf tissues using oligotex mRNA spin column following the protocol supplied with Qiagen poly A^+ RNA isolation kit (Qiagen Inc., USA)

cDNA was synthesized from 1 mg of total mRNA using Smart™ cDNA library construction kit from BD Biosciences Clontech,USA. A 5 µl sample of single stranded cDNA product was checked on 1.1 % agarose gel. The cDNA was cloned into PCR-Script™ vector using the protocol provided with PCR-Script™ Amp cloning Kit procured from Stratagene, USA. The ligated products were then transformed in *E.coli* strain DH5a and grown on LBA (Luria Bertani agar) plates containing 100mg/ml ampicillin, 40 mg/ml X-gal and 0.1M isopropyl thiogalactoside (IPTG). The recombinants were selected by blue/white screening and transferred to fresh LBA plates containing ampicillin 100mg/ml. The clones were grown O/N at 37^0C and stored at 4^0C for further analysis. Randomly selected clones were analyzed for the presence of insert by PCR amplification using T_3 and T_7 universal primers. Amplified products were checked on 1.2% agarose gel. Amplified products were labeled with a ^{32}P dATP using Hexalabel™ DNA labeling kit of MBI Fermentas Inc., USA following manufacturer's protocol. Northern analysis was done using 10 mg of total RNA of control plants and plants grown under water deficit stress and standard protocol was followed for hybridization (Sambrook *et al*. 1998). Automated sequencing of the putative clones was got done from DNA sequencing facility, South Campus, Delhi University, New Delhi.

Cloned plant genes and transgenic plants have become a standard tool in plant stress biology. These techniques have greatly enlarged the knowledge of mechanisms of tolerance to various abiotic stresses. Total RNA was isolated from the control plant (RWC 98%) as well as stressed leaf tissue (RWC 65-85 %) of rice (Fig.1). Further cDNA was synthesized from 1 mg mRNA purified from stressed leaf tissue. The cDNA was cloned into PCR-Script™ vector (SK^+). The

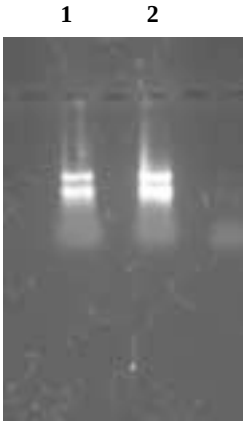

Fig. 1. Agarose gel (1.2%) showing total RNA isolated from leaves of *Oryza sativa* cv. N 22 grown under control (lane 1) and water deficit stress conditions (lane 2)

ligated product was then transformed in *E.coli* strain DH5a . Randomly selected five of the recombinant clones were analyzed for the presence of insert using PCR with T_3 and T_7 primers and the amplified products were checked on 1.2 % agarose gel (Fig.2). Clone no. 1 and 2 showed the presence of ~300 bp bands while clone no. 3-5 showed ~500-600 bp inserts. These amplified products were used as probes for Northern analysis. Northern analysis of the RNA isolated from leaf tissues of control and stressed samples revealed that clone no. 2 showed differential expression with respect to water deficit stress (Fig.3). Sequencing of the Clone 2 revealed presence of a 308 bp insert. Sequence was submitted to Genbank (Acc. N. DQ269475). Sequence analysis of the sequence using BLAST N search for similarity showed 99% homology with water deficit stress specific cDNA clone (Accession No.GB672963).

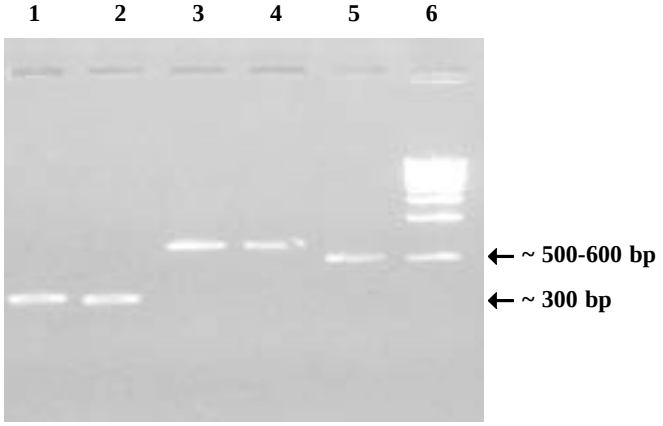

Fig. 2. Agarose gel(0.8 %) showing PCR amplified inserts using T3 and T7 primers from random clones (lane 1-5) and lambda *Hin*d III marker (lane 6)

Fig. 3. Autoradiograph of Northern hybridization of fig. 1.

Analysis of the cloned sequence showed that it had 150 bp 5' untranslated region and partial coding sequence. It has ATG initiation codon and encodes 52 amino acids (Fig.4). It was observed that the coding sequence had 100 % homology with protein phosphatase 2C gene of *Arabidopsis thaliana* (Altachul *et al*. 1990). Homologous sequence was found to correspond to N terminal region of the gene.

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GCGTGCGAGACGGTGCTTCGTTTCGCCTCACGTTTTCCGAGGGCTACGGCGACTTGGTGGGA
GCGCGGAGAAAATCTAGCGAGCCGTCGGAGTTGGGTGGCGCGCGGCCGGCTGTGCTTCTG
AAGGAGGAGTAATTGGAGTAGTTGCTTGCCATGGCCGAGATCTGCTGCGAGGAGGCCATG
                                  M  A  E  I  C  C  E  E  A  M
TCGCCGCCGGCCACTGCCACGGCTGCTGTCGCGGCAGCGGTCTCCGCCTCGGCCGCCGCA
 S  P  P  A  T  A  T  A  A  V  A  A  A  A  V  S  A  S  A  A  A
GCCGTCTCCTCGGCGATAGACAGGCGGCGCCGCAGGATGGAGATGAGGCGCATCCGCAC
  A  V  S  S  A  I  D  R  R  R  R  R  M  E  M  R  R  I  R  I
GCAAGT
 A  S
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Fig. 4. Nucleotide and amino acid sequence encoded by water deficit stress responsive cloned insert (Acc. No. DQ269475)

Protein phosphatase 2C has an important role in ABA signaling pathway. It is a negative regulator of ABA response under water deficit stress conditions. ABA plays an important role in plant growth and response to environment stresses such as drought, salinity and low temperature. Normally this protein is not expressed in vegetative parts but gets induced under water deficit stress. Reversible protein phosphorylation is involved in the early event of ABA signal transduction (Sheen 1998). Specific protein kinases are activated in response to ABA and have been proposed to play a positive role in ABA signaling. Coordinated activities of protein kinases and protein phosphatases regulate the gene expression by reversibly modulating transcription factor function and thereby gene expression in response to signaling stimuli . Biochemical and genetic studies have cofirmed that protein phosphatase 2C acts as negative modulator of stress in animals yeast and plants (Mayada 1994).

The random moisture-stress specific cDNA clone isolated in present study encodes for putative phosphatase 2C, which has a role in ABA signaling, The N terminal region of PP2C obtained in study has been proposed to mediate the interaction with cellular substrates, regulatory proteins and secondary messenger. Hence it is important to isolate the complete gene sequence of this gene by using this cDNA sequence as probe to define its role under water deficit stress and manipulation of cloned genes to alter the function of the gene product in transgenic plants provide novel opportunities to guess their biological role in stress response.

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