Isolation of stress responsive Psb A gene from rice (Oryza sativa L.) using differential display

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Differential display (DD) experiments were performed on drought-tolerant rice (Oryza sativa L.) genotype N22 to identify both upregulated and downregulated partial cDNAs with respect to moisture stress. DNA polymorphism was detected between drought-stressed and control leaf tissues on the DD gels. A partial cDNA showing differential expression, with respect to moisture stress was isolated from the gel. Northern blotting analysis was performed using this cDNA as a probe and it was observed that mRNA corresponding to this transcript was accumulated to high level in rice leaves under water deficit stress. At the DNA sequence level, the partial cDNA showed homology with psb A gene encoding for D1 protein.

Keywords: Gene, Drought stress, Rice, Oryza sativa L.

The productivity of plants is greatly affected by environmental stress and the genetic improvement of stress tolerance is an urgent need for future agriculture. Drought stress is one of the most important abiotic factors that affects crop yield. Plants respond and adapt to water stress by means of physiological, developmental and biochemical changes including the synthesis of a number of proteins and induction of gene expression. The response of plants to osmotic stress has been an important subject of physiological as well as molecular and transgenic studies. A number of genes have been reported to be induced by drought, high salinity and low temperature stresses and their products are thought to function in stress tolerance and response. It is important to analyze the function of differentially expressed genes for further understanding of molecular mechanism of stress tolerance and responses of higher plants. Identification of novel genes, determination of their expression pattern in response to stresses and an improved understanding of their functions in stress adaptation would provide us the basis for improving stress tolerance of crops by gene manipulation.

New tools are now available to further our understanding of the genetics of abiotic stress tolerance, allowing us to address the complexity of stress responses on a larger scale through genome wide expression profiling. Differential display (DD) is one of the most powerful techniques to study the expression of differentially expressed genes. Earlier, using this approach several drought responsive cDNAs have been reported. In a previous study, we reported qualitative and quantitative differences in protein profile of N22 genotype under control and water stress conditions using 2-D gels. In the present study, an early maturing deep-rooted, drought-tolerant rice cultivar Nagina-22 (N22), adapted to upland conditions was used for studying novel drought responsive genes using DD technique.

Materials and Methods

The rice cultivar namely N22 (drought-tolerant) was procured from Genetics Division, I.A.R.I, New Delhi. Fifteen-days old seedlings were planted in pots and grown under controlled conditions (25 ± 5°C), 14 hr D/N photoperiod. The plants were uniformly watered once a day. Water stress treatment was imposed at vegetative stage (40 days after transplanting) by withholding water. Water status was monitored by measuring relative water content (RWC), which was computed using 1 cm long leaf pieces excised from 3rd most recent fully-expanded leaf. Leaf tissue was collected at different RWC varying from 60 to 85% and frozen in liquid N₂.

Total RNA was extracted from 2 g of leaf tissue from control and water-stressed leaf samples as per method. Total stressed RNA was pooled from different RWC varying from 60 to 85%. Control RNA was isolated from tissue having approx. 95% RWC. RNA isolated from control and stressed leaf tissues were used for differential display as well as Northern blot hybridization.

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Differential display (DD) was carried out as described previously. The polymorphic cDNA bands specific to water stress were carefully cut out, soaked in 100 µl of dH2O for 10 min, boiled for 15 min and subjected to brief spin (2 min). The supernatant was transferred to fresh tube and precipitated in the presence of glycogen and 3 M sodium acetate. It was again pelleted, air-dried and redissolved in sterilized distilled water and used for reamplification. The reamplified products were checked on 1.5% agarose gel. The reamplified cDNA fragments were cloned in PCR trap vector (Gene Hunter Corp. MA) following manufacturer’s instructions. Northern blot hybridization and other routine procedures were carried out essentially as per protocol.

The cDNA with induced expression was sequenced using automated DNA sequencer at Delhi Univ. and sequence analysis was carried out using BLAST sequence search homology program.

Results and Discussion

Differentially expressed cDNAs from control and water-stressed samples were visualized on 6% denaturing polyacrylamide-sequencing gels. Each RNA sample was studied with 8 random primers (HAP1-8) and 3 single nucleotide anchored poly A tail-specific primers (H-T11N). Polymorphism was detected between control and stressed leaf tissues on DD gels (Fig. 1). cDNA fragments specific to stressed leaf tissue were eluted out from the middle one third of sequencing gel, as proper resolution was not observed in upper one third of sequencing gel and fragments in the lower one third of gel, were too short. The cDNA fragments obtained were relatively short (200 to 600 bp) and correspond theoretically to the 3’ end of mRNA which contains non-coding sequences of variable length.

Some cDNAs were found to have induced expression with respect to stress (upregulated) or control (downregulated) genes, but, some were specific to stress only i.e., they were not observed in control and vice versa. Polymorphic cDNA bands having induced expression with respect to stress were eluted out from the gel, amplified and cloned. Inserts from these clones were obtained by restricting with Hind III enzyme (Fig. 2) or by amplifying with vector-specific primers. Inserts of clones namely 2, 3, 4 and 5 were radiolabelled and used for Northern hybridization by hybridizing with RNA form control and stressed leaf tissues. Induced expression was found with one of the clones corresponding to lane 2 of Fig. 2 under stress conditions (Fig. 3), while other clones showed no such expression.

The cDNA with induced expression was sequenced and found to have an insert of 218 bp (Fig. 4). The sequence was submitted to Genbank (accession #811435). Blast N result showed 100% homology with psb A gene of Oryza sativa. The fragment of cDNA corresponds to 1160 to 1348 bp of coding sequence of the psb A (accession # M36191). The 218 bp differentially expressed cDNA sequence was found to

Fig. 1—Autoradiogram of differential display pattern showing differentially expressed cDNAs in water stressed (S) and control (C) leaf tissue [Arrows indicate differentially expressed cDNAs specific to water stress. The figure is just a part of DD gel showing polymorphism between water-stressed (S) and control (C) leaf tissue of drought-tolerant N22 genotype]

Fig. 2—Restriction pattern of cloned fragments with Hind III [Reamplified fragments eluted out from DD gels were cloned in PCR Trap vector. M, λ Hind III marker; lane 1, cDNA clone was not restrictable; lanes, 2, 3, 4, 5 depict different cDNA clones; Upper band and lower bands depict vector DNA and cloned insert respectively]
have 30 bp non-coding sequence corresponding to 3′ untraslated region of psb A gene sequence. Psb A gene codes for chloroplast quinone-binding Q(B) protein, a photosystem II (PSII) protein D1 precursor. Drought is one of the most important environmental factor limiting photosynthetic CO₂ assimilation. PSII plays a key role in response of leaf photosynthesis to environmental perturbations and D1 metabolism is very important for stress adaptations of plants. Induced expression of mRNA corresponding to psb A, as observed in present study, should reflect in enhanced synthesis of D1 during water deficit stress, yet decreased level of D1 is possibly due to enhanced degradation. Thus, D1 degradation, rather than D1 synthesis caused a decreased steady-state level of D1 in water-stressed plants. The higher rate of synthesis may reflect preferential translation of psb A message in response to greater depletion of the protein as seen in senescing leaves. Large accumulation of Psb A mRNA is reported in Cyanobacterium syneccoyctis during photo-inhibition of photosynthesis by visible light. Thus, it is suggested that the biochemical response at the level of D1 turnover could act as general adaptation signal for the plant in response to environmental stress.

To conclude, a 218 bp EST corresponding to psb A having induced expression in water-deficit stress is reported for the first time in Oryza sativa using differential display approach.

References

Fig. 3—Autoradiogram showing Northern hybridization of total RNA of rice using psb A cDNA probe [10 µg of RNA from control (C) and water-stressed leaf tissues (S) was run on 1.2% agarose gel containing 25 mM GTC. The blots were hybridized with α32P d ATP- labelled psb A cDNA probe]

Fig. 4—218 bp sequence of psb A (accession # 811435) gene sequence showing induced expression with respect to water stress