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EXPRESSION OF DREB2A FOR DROUGHT STRESS IN SELECTED INDIAN WHEAT CULTIVARS UNDER GREEN HOUSE CONDITIONS

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ABSTRACT

Environmental constraints that include abiotic stress factors such as salt, drought, cold and extreme temperatures severely limit crop productivity. Drought stress is one of the major limitations to crop productivity worldwide due to its multigene nature, making the production of transgenic crops a challenging prospect. Transcription factors DREB1A/CBF3 and DREB2A specifically interact with *cis*-acting dehydration-responsive element/C-repeat (DRE/CRT) involved in cold and drought stress–responsive gene expression in Arabidopsis thaliana. Intact DREB2A expression does not activate downstream genes under normal growth conditions, suggesting that DREB2A requires posttranslational modification for activation, but the activation mechanism has not been clarified. Identification of stress related target genes and regulatory elements are expected to open new avenues for rational biotechnological intervention to achieve dependable drought tolerance in crops. Genetic engineering methods allow identification, isolation and characterization of the desired genes from the organism of choice and transfer it into the desired system in a regulated manner beyond the boundaries of sexual compatibility. 20 germplasm lines were randomly selected out of 175 germplasm lines. Genotypes like NL 897/NL 724//BL 2281, NL 781/NL 724//BL 2017, NL 897/NL 714//BL 2218 and BAW 969/SHATABDI (having DRC 1 or >1) were considered drought tolerant.

Key words : *Triticum aestivum, Dreb, drought, AP2 domain, DRC.*

Rice, Maize and Wheat provide 60% of the energy consumed as food in the world making them the most important food crops. Drought and water shortages threaten the agricultural productivity of many developing countries Improving drought tolerance and productivity is one of the most difficult tasks for plant or wheat breeders because under drought prone conditions, plant themselves adopts diverse strategies to combat drought stress depending on the timing, severity and stage of crop growth. Functional Genomics has become an essential tool to identify and define the function of genes and uncover when and how genes work together to produce traits. Functional genomics goes further to examine the interrelationship and interactions between thousand of genes to determine when and why certain traits are expressed, which sets of genes are specifically responsible for that expression and under what conditions (1). Recent advances in plant genomics have led to the identification of a vast number of potentially beneficial water-stress-related genes, plus technologies for gene over-expression or silencing. It has been seen that the tolerant genotypes in most of the cases, have DREB/CBF genes which is a small family of transcription factors that bind to the drought responsive elements (DRE, 9 bp consensus sequences TACCGACAT) found in the promoters of many drought-responsive genes of Arabidopsis (rd 29/ Iti 78/ cor 78, kin 1, cor 6.6/ kin 2 and cor 47/rd 17) (2, 3), rice (4) and other plants. This DRE is responsible for the regulation of dehydration responsive gene expression. Two DRE binding proteins DREB 1 and DREB 2 of Arabidopsis interact with the DRE sequence in the promoter region of rd 29 in dehydrated and low temperature stress. The induction of the dehydration-responsive Arabidopsis gene, rd29B is mediated mainly by ABA (5), whereas the stress induced gene rd29A is induced through the ABA-independent pathway (2). The Dehydration- Responsive element binding gene 1 (DREB1 and DREB2) are transcription factors that bind to the promoter of the genes such as rd29A, thereby inducing expression in response to drought, salt and cold (4). Because DREB 1 binds to a DRE/CRT (dehydration- responsive element/C-repeat) cis-acting element, it was also termed as DREBI/CBF (DRE-binding protein 1/C-repeat binding factor). It was recently reported that over-expression of DREB 2A CA

gene (an active form of DREB2A gene) induces not only drought and salt-responsive genes but also heat-shock (HS)-related genes (6). The DREB2A up-regulated genes are classified into three groups based on their expression patterns: genes induced by heat stress, genes induced by drought stress, and genes induced by both heat stress and drought stress.

The functional genomic analysis of DREB gene families may reveal the differential expression in different Indian wheat cultivars. The information about presence and differential expression of DREB gene families can play a vital role in the breeding of drought tolerant varieties. In wheat, substantial variability for drought tolerance has not been unexploited so far. Hence, there is possibility of getting a suitable molecular marker for this trait. The enhancement of drought efficiency is expected to be much higher if information is generated about the presence of genetic variation for drought trait in Indian wheat cultivars, molecular analysis of DREB gene families, association of drought traits with a suitable marker and contribution of these genes behind the traits. The relative yield under drought was calculated as the yield of a specific genotype under drought divided by that of the highest yielding genotype in the population.

MATERIALS AND METHODS

A total of six genotypes including drought tolerant and drought susceptible were used which are C 306, NL 897/NL 724//BL 2281, NL 781/NL 724//BL2017, NL 897/NL 714//BL 2218, BAW 969/SHATABDI and HUW 206. C 306 is a known drought tolerant variety and HUW 206 is a known drought susceptible variety. The genotypes were provided by Eastern Gangetic plain nursery. A time course drought stress experiment was carried out in green house *viz.,* 0, 1, 4, 6, 7 and 10 days under water stressed condition where, 0 represent the time when pots were lastly filled with 100 ml of water as shown in Fig. 1. A leaf was collected from each plant of every genotype at each time point and frozen in liquid nitrogen. Total RNA was extracted from frozen leaf samples collected at different time points as given above using Guanidine thiocyanate phenol extraction method. Pellets were dried until the white pellets became clear. Then, 53 ìl H_2O was added and pellet was dissolved by vigorous vortexing several times. Centrifugation was done at 13,500 rpm for 10 min. 50 ìl

Table-1: Expression of DREB2A, correlated with Drought Resistance Coefficient and Relative yield.

S.No.	Germplasm line	Expression of DREB2A	DRC	RY
1.	C 306	High	1.13	0.77
2.	HUW 206	Very low	0.66	0.39
3.	BAW 969/SHATABDI	High	1.04	0.70
4.	NL 897/NL 724//BL 2281	High	1.05	0.86
5.	NL 781/NL 724//BL 2017	High	1.01	0.59
6.	NL 897/NL 714//BL 2218	High	0.89	0.76
7.	CR0C 1/AE.SQUA (205)//2*BCN/BL 1887//BL 2473	Very low	0.64	0.35
8.	BABAX//IRENA/KAUZ/3/HUITES	High	0.93	0.59
9.	URES/JUN//KAUZ/3/K 9211	No expression	0.33	0.28
10.	FRET2/TUKURU//FRET2	High	1.02	0.65
11.	CS/TH.CS//3*PVN/3/MIRLO/BUC/4/MILAN/5/TILHI	Moderate	0.88	0.54
12.	SW90.1057/3/KAUZ*2/YACO//KAUZ*2/YACO//KAUZ	Moderate	1.09	0.68
13.	BAW 923/BAW 1008	Moderate	0.91	0.60
14.	BL 3494=NL 699/NL 352//PWL6/3/CMH84.A.1294/*2KAUZ	High	1.21	0.60
15.	PBW 502	Moderate	1.00	0.73
16.	PBW 343	Moderate	1.29	0.67
17.	GOURAB/RAWAL 87	Moderate	1.18	0.45
18.	GARUDA/3//A6/GLEN	High	0.94	0.43
19.	KAN//IAS 63/ALDAN	Moderate	1.34	0.52
20.	BAW 966/BAW 748	High	1.18	0.47

Fig. 1(a) : Cultivars without stress condition under green house.

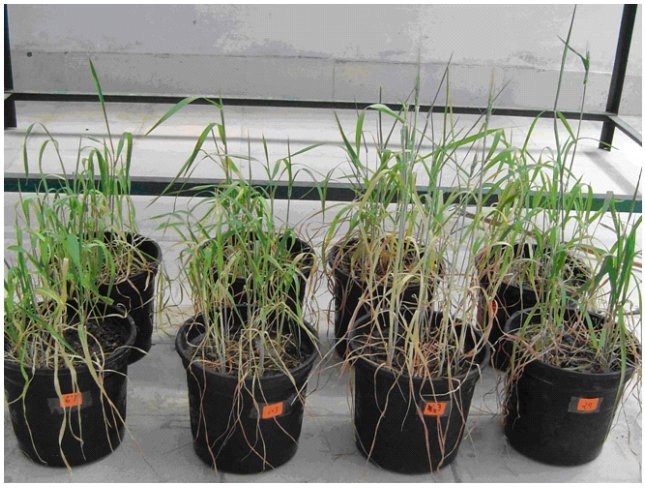

Fig. 1(b) : Cultivars without stress condition under green house.

of the RNA solution was transferred to a new 1.5ml reaction tube (leaving behind polysaccharide slime). Before using the RNA as a template for RT-PCR, a *DNAse I* treatment was performed in order to eliminate the presence of eventual DNA contaminations in the samples. cDNA were synthesized, using total RNA as a template, with gene specific primers. Oligo dT (18 mer) was used to construct cDNA. Reaction was performed in 20µl of volume with high fidelity MMLV reverse transcriptase enzyme**.** Each polymerase chain reaction was performed in 20µl final volume in Eppendorf Thermo-cycler (Germany) comprising 6.5 PCR mix, 1 forward primer, 1 reverse primer, 2 cDNA and 9.5 dd water. PCR profile was initial denaturation at 94°C followed by 45 amplification cycle (94°C for 1 min 1min. 55°C for 1 min. and 72°C for 1 min). The following oligos were selected by the "Gene Fisher" web tool in http://bibiserv.techfak.uni-bielefeld.de/genefisher/- using as template, a sequence from TIGR wheat database that was strictly related to a transcription factor DREB2A drought induced from *Arabidopsis thaliana*: Dreb-for1: 5'CATGACGGTAGATCGGAA3' Dreb-rev1: 5'CTGAAATTCGGGAGCCAA3' Dreb-for2: 5' ATGATCCACAGGGTGCAA3' Dreb-rev2: 5'GCTCCCGAATTTCAGCAA3' Dreb-for3: 5'CTGAAATTCGGGAGCCAA3'. The PCR amplification product were separated on ethidium bromide stained 1% (w/v) SFR agarose gels (Biogene) and visualized with UV light.

Drought resistant coefficient (DRC) was calculated following the suggested formula (7). The relative yield under drought was calculated as the yield of a specific genotype under drought divided by that of the highest yielding genotype in the population.

RESULTS AND DISCUSSION

In larger genomes, target sequences, are often controlled by additional regulators. The evolution of major phenotypic differences across organisms may be the effect of transcription factors, which provide regulatory inputs, rather than changes in genes repertoire itself. No specific results were obtained when oligo pair dreb-for1/rev1, dreb- for2/rev2, dreb-for1/rev2, dreb-for/rev1 were used as primers. However dreb-for2/rev1 oligo pair produced a band of 350bp in case of NL 897/NL 724//BL 2281 while dreb-for3/rev1 amplified a band of 250bp in case of C-306, NL 897/NL 724//BL 2281, NL 781/NL 724//BL2017, NL 897/NL 714//BL 2218 and BAW 969/SHATABDI. After 4 days without water the intensity of bands was higher followed by slight decrease on 6 and 7 days respectively as shown in Fig. 2. Sequencing was done of the PCR products obtained after amplification with Dreb-for3:5'CTGAAATTCGGGAGCCAA3' Dreb-rev1: 5'CTGAAATTCGGGAGCCAA3'primers.

The relative yield was highest for GARUDA/ 3//A6/GLEN (1.00). The relative yield for NL 897/NL 724//BL 2281 is 0.86, for NL 781/NL 724//BL 2017 0.59, NL 897/NL 714//BL 2218 is 0.76, for BAW 969/SHATABDI is 0.70 which is more than that of C 306 i.e. 0.77. The germplasm line CR0C 1/AE.SQUA (205)//2*BCN/BL 1887//BL 2473 has relative yield 0.35 and HUW 206 has 0.39 which are drought susceptible.

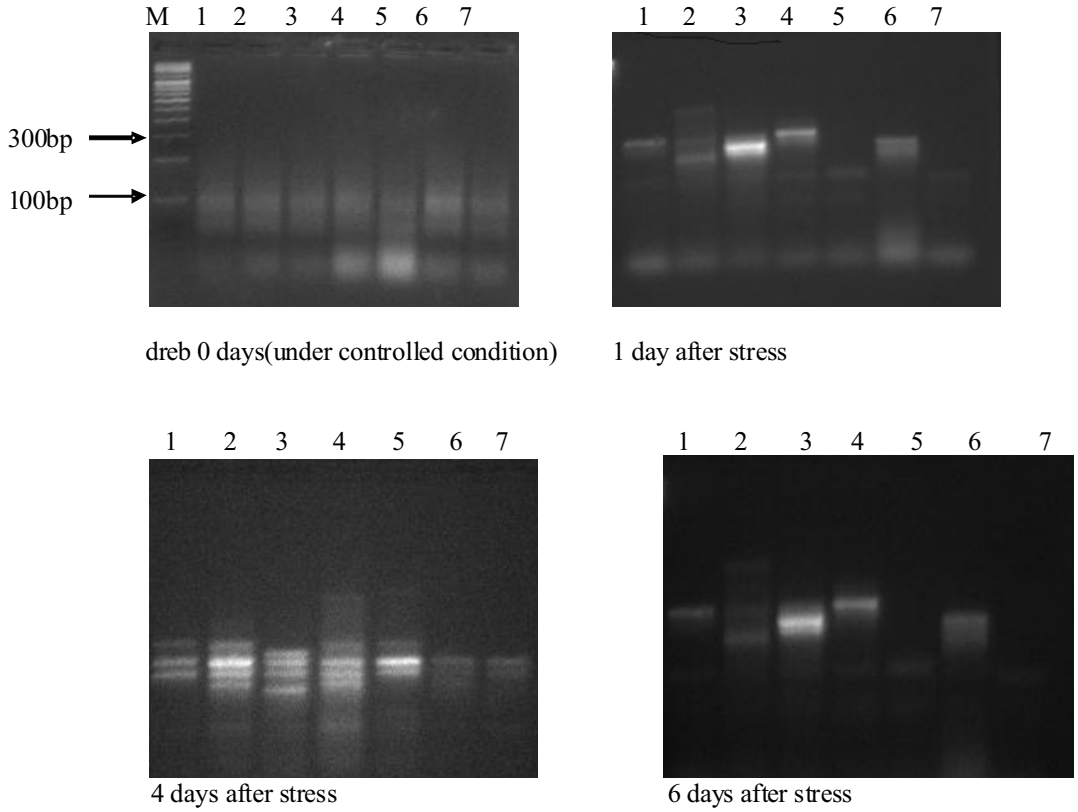

Fig. 2 : Dreb gene expression of different selected varieties at control and at different time points M=100bp ladder

1. C 306 2. NL 897/NL 724//BL 2281 3. NL 781/NL 724//BL2017
4. NL 897/NL 714//BL 2218 5. BAW 969/SHATABDI
6. CR0C 1/AE. SQUA(205) //2* BCN/BL 1887//BL 2473 7. HUW 206

The DRC for NL 897/NL 724//BL 2281 is 1.04, for NL 781/NL 724//BL 2017 is 1.05, NL 897/NL 714//BL 2218 is 1.01, for BAW 969/SHATABDI is 0.89 which is more than that of C 306 i.e. 0.96. These varieties have high relative yield, also showed the presence of DREB gene and expression also at molecular level. The germplasm line CR0C 1/AE.SQUA (205)//2*BCN/BL 1887//BL 2473 has DRC 0.59 and HUW 206 has 0.66 which are drought susceptible as the DRC is low. The varieties showing DRC higher than 1 were also showing the presence of DREB gene and also the expression. The varieties showing DRC higher than one were considered as drought tolerant *viz.* BAW 969/SHATABDI. C-306, NL 897/NL 724//BL 2281 and NL 781/NL 724//BL 2017. The varieties CR0C 1/AE.SQUA (205)//2*BCN/BL 1887//BL 2473 and HUW 206 were drought susceptible and the DRC was comparatively low in comparison to drought tolerant lines. Higher relative yield shows that the genotype performed relatively well under drought. Other researchers (8, 9) found relative yield to be a useful criteria for assessing drought response of wheat genotypes. Genotypes with higher DRC can be considered as drought tolerant because they exhibited smaller yield reductions under water stress compared with well watered conditions than mean of all genotypes

CONCLUSION

Our knowledge of the mechanism of drought tolerance has been enhanced by research programmes targeting specific physiological, genetic or molecular aspects of the drought response. However, in wheat, these approaches have not led to an increase in tolerance over that already achieved by breeders using empirical selection. Although the idea of linking physiology, 'omics and quantitative genetics have already been proposed, only a few research programmes have taken this integrative approach. The great strength of genetic and genomics analysis in wheat has been the ability to generate large populations and well-developed field phenotyping capabilities. Analysis of the response to drought has been further complicated by the absence

of a genome sequence and the generally poor genomics resources have been limiting. New developments in sequencing, marker development, and genome analysis have created the opportunity to revisit the way in which we structure populations for analysis and tackle specific components of drought tolerance.

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