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RESEARCH ARTICLE

In silico identification and development of conserved primers from DREB gene in rice

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Abstract

Drought responsive element binding (DREB) proteins plays a key role as transcription factors that bind to the promoter of dehydration responsive genes such as *rd*29A, thereby inducing the expression of downstream genes in response to dehydration due to drought, salt and cold. We have validated hypothesis cum assumption by using bio-informatic tools in rice. Total of 19 sequences of DREB genes were downloaded from Gramene database and 14 non redundant/unigene sequences were identified. Total of 6 conserved primers were identified from the DREB gene sequences. For identification of these conserved primers, whole genome sequence of *Oryza sativa* (IRGCP build 5) was used as reference genome. We had used a high threshold for identification of these conserved primers. When *in silico* PCR studies were conducted out of 6 primers, 4 primers have yielded a product of suitable size (>300 bp amplicon). These 14 sequences were blast searched to identify the putative functions against the NCBI protein data base. Most of the sequences fall under the "fibre protein fb2" category (12 sequences) whereas, the putative function of one sequence is not available which is supposed to be unique for rice. Sequence Os12t0617000-00 had identified with a putative function of drought and salt tolerance. We had studied the localization of the proteins and found maximum of these DREB proteins are localized at intracellular regions followed by plastids, nucleus and mitochondria, which will help to understand the mechanism of drought response in rice. With these *in silico* studies we have validated that DREB genes are not only responsible for drought stress but also have function under cold stress. This study highlights the importance of investigating the genes which interact with markers and suggests following the behavior of marker-related genes at protein level. Identification of genes underlying drought stress will facilitate understanding of molecular mechanisms and will lead to genetic improvement of rice through marker-assisted selection (MAS). We recommend that wet lab study to validate the results obtained through present investigation

Key words: Rice, DREB, Drought, *in silico*, conserved primers

Introduction

Now-a-days the threat of global climate change with its associated fluctuations in patterns of biotic as well as abiotic stresses pose a major challenge in our quest for sustainable food production as it reduces the potential yields in crop plants. Apart from this the burgeoning world population also makes the challenge in this new era for humankind makes the finding of solutions to improving food, feed, fuel and fiber is more urgent than at any time in our history. Although traditional plant breeding has produced impressive gains in world food production and safety, it is unlikely that unassisted breeding will be up to the challenges at hand. The availability of high-quality whole-genome sequences for major crops such as rice, maize etc., creates a paradigm shifting change in how we can approach crop improvement (Scott *et al.*, 2011). The Genomics is useful in identification of the genes in a plant and then to begin to understand the genetic properties and networks that contribute to the development of a superior plant; however, even with these tools, breeding a better variety is still a complicated process. But genomics tools and technological advances will continue to increase the rate of gain from breeding and the precision by which superior genotypes are chosen, and will be a major player in the production of enough food for a growing world population (Evenson and Gollin, 2003).

Rice (*Oryza sativa* L.) is the most important primary cereal crop of the developing world especially Asian continent (Bennetzen, 2007; Devos, 2010) because more than 90% of the rice is produced in the world is consumed in Asia where 60% of the total population live (Khush and Virk, 2000). In spite of technological development, growth rate of global rice production is not keeping pace with the rate of the growing consumers. Also it faces even greater challenges due to various abiotic stresses such as drought, salinity, high radiation and extreme temperatures in changing climatic conditions. These stresses, has greater impact for rice productivity as rice is a highly stress sensitive crop. Therefore, it is important to develop stress tolerant varieties of rice in order to increase rice productivity in the stress areas. Development of stress tolerant varieties is one of the important challenges of traditional rice breeding-programs in the recent past. The main problem in the traditional breeding approach is lack of understanding of genetic mechanisms for stress tolerance. Therefore, it is important to study the stress tolerance mechanism at molecular level and identify novel genes based on its expression under stress condition for the development stress tolerance in rice varieties. In this line several researchers were worked and identified the candidate genes responsible for the stress tolerance in the rice. Among the several genes the DREB transcription factor genes are more important gene for the stress tolerance. DREB transcription factors play an important role in stress tolerance by controlling the expression of many stress related genes. DREB transcription factors (TFs) are a class of transcription factor belonging to the family of AP2/ERF transcription factor, specifically interact with the dehydration-responsive element/Crepeat (DRE/CRT) cis-acting element (core motif: G/ACCGAC) and control the expression of many stress inducible genes in Arabidopsis and other crop plants (Lata and Prasad 2011). In rice, five cDNAs for DREB homologs were identified: *OsDREB1A*, *OsDREB1B*, *OsDREB1C*, *OsDREB1D*, and *OsDREB2A*. Expression of *OsDREB1A* and *OsDREB1B* was induced by cold, whereas expression of *OsDREB2A* was induced by dehydration and high-salt stresses (Lata and Prasad 2011). These genes will provide the means of improving/incorporating stress tolerance in rice through marker assisted selection approaches or direct introduction of genes by genetic engineering seems a more attractive and quick solution for improving stress tolerance. However being exploited these genes in MAS breeding programmes we need to have the molecular markers related to these genes. In the present study an attempt has been made to identify and development of conserved primers from DREB gene in rice using *in silico* methods. The finding of the study will be useful in development of stress tolerance varieties of rice and other similar crops.

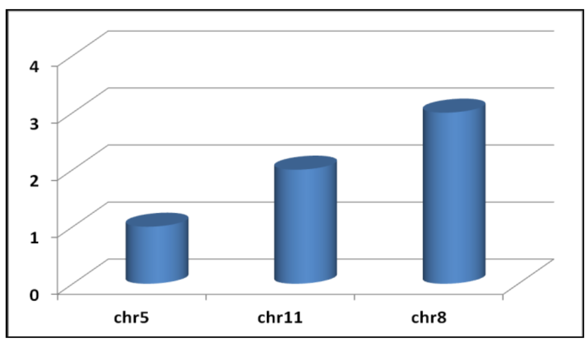
Material and methods

Total of 19 sequences of DREB genes were downloaded from Gramene database (http://archive.gramene.org/rice) as on March, 2012. CAP3 assembler was used to identify Unigenes as suggested by (Huang and Madan, 1999). Identification of conserved primers was carried out by Conserved Primers 2.0: an online software high-throughput pipeline for comparative genome referenced intron-flanking PCR primer design (Frank *et al.,* 2009). We had used a high threshold for identification of these conserved primers. *In silico* PCR studies were conducted aiming the product of size (>300 bp amplicon). These primers were expected to span the predicted rice intron regions in a PCR. Percentage of the identified primers was calculated as total number of primers identified to total number of sequence used for the identification of conserved primers expressed in percentage. We tested the designed primers by electronic PCR (e-PCR). Further, we have validated our results by using online PCR (http://biocompute.bmi. ac.cn/lab/MFEprimer-2.0/) genomic sequences for Arabidopsis and rice, respectively (Qu *et al.,* 2009). The sequences were blast searched to identify the putative functions against the NCBI protein data base for each query sequence with the high threshold.

Results and discussion

In current study, Conserved-PCR primers from Rice DREB genes were designed by using online software ConservedPrimers 2.0. Drought responsive element binding (DREB) proteins plays a key role as transcription factors that bind to the promoter of dehydration responsive genes such as *rd29A*, thereby inducing the expression of downstream genes in response to dehydration due to drought, salt and cold. We have validated hypothesis cum assumption by using bio-informatic tools in rice. Total of 19 sequences of DREB genes were downloaded from Gramene database and 14 non redundant/unigene sequences were identified using CAP3 assembler. DREB Gene sequences used and conserved primers identified along with E-value and results of e PCR are presented in table 1.Total of 6 conserved primers were identified from the 14 DREB gene sequences. We had used a high threshold for identification of these conserved primers. When *in silico* PCR studies were conducted out of 6 primers, 4 primers have yielded a product of suitable size (>300 bp amplicon). The sequence ID's are Viz., CA753127, CA753127_1, CB966387, CB966387_1, CK738403. The conserved primer online software had unique characteristics of *In silico* mapping of the identified markers. It means the software localized the 6 identified conserved markers on rice chromosome and the results are presented in Fig.1.

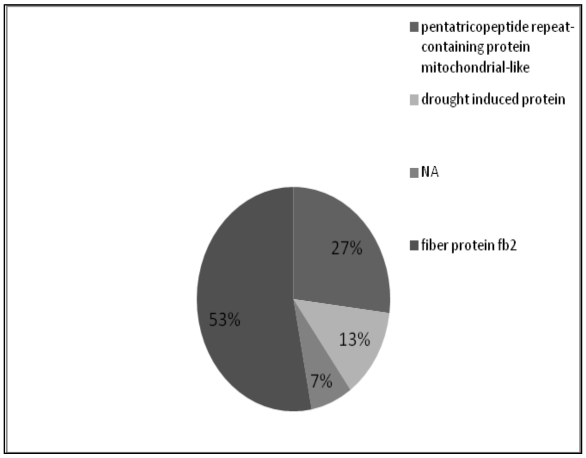
Fig: 1 Localization of 6 conserved primers on rice chromosome



Out of 6 conserved primers/markers identified 3 are localized on chromosome number 8 which are highest in number followed by chromosome

11 with two primers/markers. Only one conserved marker was localized on chromosome 5 of rice genome. Similar results for the localization of ILP markers study was conducted by Dudhe *et al.,* 2012 in safflower and Kumar *et al.,* 2012 in *Artemisia annua*. These 14 unigene DREB sequences were blast searched to identify the putative functions against the NCBI protein data base Fig 2.

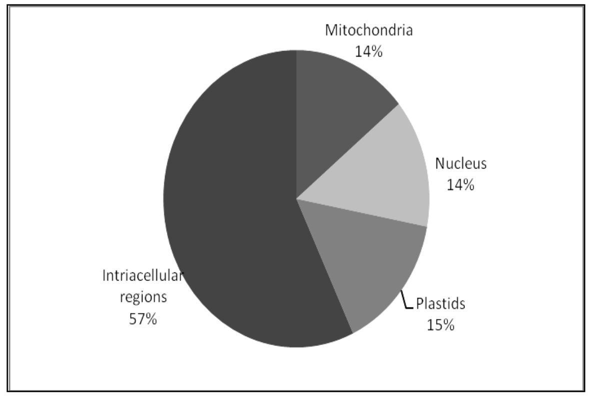
Fig:2 Functional annotation of 14 DREB sequences functions against the NCBI protein database



Most of the sequences fall under the "fibre protein *fb*2**"** category (12 sequences) whereas, the putative function of one sequence is not available which is supposed to be unique for rice. Sequence *Os12t0617000-00* had identified with a putative function of drought and salt tolerance. We had studied the localization of the proteins and found maximum of these DREB proteins are localized at intracellular regions (57%) followed by plastids (15 %), nucleus and mitochondria (14%), which will help to understand the mechanism of drought response in rice (Fig.3).

With these *in silico* studies we have validated the hypothesis that DREB genes are not only responsible for drought stress but also have function under cold stress. This study highlights the importance of investigating the genes which interact with markers and suggests following the

Fig3: Cellular localization of 14 DREB sequences



behavior of marker-related genes at protein level. Identification of genes underlying drought stress will facilitate understanding of molecular mechanisms and will lead to genetic improvement of rice through marker-assisted selection (MAS). We developed the conserved primers which help for breeders in developing the stress tolerance varieties in rice and other related crops using Marker assisted selection.

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References

1. Bennetzen, J.L. 2007. Patterns in grass genome evolution. Curr. Opin. Plant. Biol., 10: 176-81.
2. 2. Devos, K.M. 2010. Grass genome organization and evolution. Curr. Opin. Plant Biol., 13: 139-45.
3. Dudhe, M.Y., Sudhakarbabu, O., and Sudhakar, C. 2014. Development of intron-flanking EST-specific primers from drought responsive expressed sequence tags (ESTs) in safflower. SABRAO J. Breeding Genet., 46 (1): 56-66.

4. Evenson,R.E. and Gollin, D. 2003. Assessing the impact of the green revolution 1960 to 2000. Sci.,300: 758-762.
5. Frank, M. You., Naxin Huo., Yong, Q. Gu., Gerard, R. Lazo., Jan, Dvorak and Olin, D. Anderson.2009.Conserved Primers 2.0: A high-throughput pipeline for comparative genome referenced intron-flanking PCR primer design and its application in wheat SNP discovery. BMC Bioinform., 10:331.
6. Huang, X., Madan, A., CAP3 1999. A DNA sequence assembly program. Genome Res., 9: 868–877.
7. Khush, G. S. and Virk, P.S. 200. Rice breeding: achievements and future strategies. Crop Improv., 27: 115-144.
8. Kumar, J., Bajaj, P., Singh, H., Mishra, G. P., Srivastava, R. B. and Naik, P.K. 2012. Utilization of intron - flanking EST-specific markers in the genetic characterization of Artemisia annuna genotypes from the trans-Himalayan region of Ladakh. India. J. Enivorn. Biol. 33:991-997.
9. Lata, C. and Prasad, M. 2011. Role of DREBs in regulation of abiotic stress responses in plants. J. Exp. Bot., 62: 4731-4748.
10. Qu, W., Shen, Z., Zhao, D., Yang, Y., Zhang, C., 2009. MFEprimer: multiple factor evaluation of the specificity of PCR primers. Bioinform., 25: 276-278.

Table1: Unigene DREB sequences used and conserved primers identified by using *in silico* PCR

Sr. No.	DREB Gene Seq. ID	Primer sequences identified	E-value	Amplicon size (BP)
1	CK738403	**F** AGGTTTCGATCCTGCAAATG	1e-89/1e-70/0.0	682
		R TCCGACAAGATCAACACTGC		
2	CK738241	**F** GTTCGCCTCTTCTTCCTCCT	e-159/9e-62	1009
		R CTGCCTTCCAAACAGACCAT		
3	CA753127	**F** GTCAGACGCGTGTTTGAGAA	7e-84/2e-44	205
		R TCTTGTGCTCCTCCTTCACC		
4	CA753127_1	**F** TGAAGGAGGAGCACAAGACC	2e-35/1e-17	252
		R GTGGTTGTTGCCCTTGTTG		
5	CB966387	**F** TTACCGCACAGAACTGCAAG	8e-62/7e-84/1e-63	639
		R GCATCAGTAGCAGCATCAGC		
6	CB966387_1	**F** CTTACCGCACAGAACTGCAA	8e-62/7e-84/1e-63	641
		R GCCGTAGCAATGTGAGGAAT		