

In vitro Studies of DREB, LEA and OsDHODH1 Genes in *Oryza sativa* Cultivars

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Abstract

Paddy has been the staple food in India since generations. India being one of the densely populated countries has an ever ending demand for the food requirement. The only way to achieve its needs is to increase the production of the staple crop or develop the quality of the current crops in terms of disease and drought resistance. The study includes recording the occurrence of three different drought resistance genes among 19 varieties of *Oryza sativa* grown in south India. All the 19 varieties were sown under lab conditions and the leaf material was collected for DNA extractions. The target specific primers were designed and band pattern was recorded upon amplification. The successfully amplified samples were sequenced to confirm the presence of these genes

Keywords

Cultivars, Drought Tolerance, Abiotic Stress, OsDHODH1, DREB, LEA, Amplification, RFLP

Introduction

Rice, (*Oryza sativa* L.) is the most important food crop for nearly half of the world's population (Sellamuthu et al., 2011). Worldwide, rice is cultivated on an area of 154 million hectares with an annual production of 700 million tonnes (FAO, 2011). Globally, India ranks first in cultivable area under rice and second in production, producing 131 million tonnes. Water is the critical and most important factor in rice production. The 70% of the world's food-growing areas turn increasingly parched (IRRI, 2009), so that main challenge for rice producers is to develop a strategy to raise yields of the water-intensive crop. Drought is a major abiotic stress that limits rice productivity in rainfed and upland ecosystems (Bimpong et al., 2011) and worldwide, drought affects approximately 27 million ha of rainfed (IRRI, 2011) crop fields. In India, area under rice cultivation remained stagnant and even declined in the recent years due to water unavailability. Drought reduces yield by 15–50% depending on the stress intensity and crop growth period at which the stress occurs in rice (Srividhya et al., 2011). Eastern India, comprising Jharkhand, Orissa, and Chhattisgarh alone accounts loss of about 40% of the total rice production due to severe drought (Pandey and Bhandari, 2009). Developing high yielding and drought resistant varieties for rainfed area is priority for improving rainfed rice

production. Plants' response to drought stress is one of the most complex biological processes, and it involves numerous changes at the physiological, cellular, and molecular levels. Many genes have been identified to be involved in the response of drought stress in plants (Zhang et al., 2012). The effect of drought on rice plants considerably varies with genotypes, developmental stages, and degree and duration of drought stress (Wang et al., 2011). A better understanding of the complex physiological mechanisms underlying drought response is important to improve rice yields under water-limited environments. Roots, the hidden half of a plant, are important for numerous functions including water and nutrient uptake that make it difficult to overlook their importance to plant productivity (MacMillan et al., 2006).

DREB, LEA and OsDHODH1 genes are known for their association with Drought resistance in plants. DREB (Dehydration responsive element binding factor) play key roles in plant stress signalling transduction pathway, they can specifically bind to DRE/CRT element (G/ACCGAC) and activate the expression of many stress inducible genes. LEA proteins are late embryonic proteins abundant in higher plant embryos. It has been found that LEA genes are a gene family and play important roles in the protection of water stress. In a study conducted by Liu *et al* the overexpression of the OsDHODH1 gene in rice increased the DHODH activity and enhanced plant tolerance to salt and drought stresses as compared with wild type and OsDHODH1-antisense transgenic plants. Their findings revealed that the cytosolic dihydroorotate dehydrogenase is involved in plant stress response and that OsDHODH1 could be used in engineering crop plants with enhanced tolerance to salt and drought.

Materials and methods

Collection of Paddy seeds for Seedling development:

In the current study 19 varieties of Paddy were randomly collected from various government and private research laboratories viz Jaiprabha Gram Centre and KVK Centre. They were sown in different pots under normal laboratory conditions and incubated for a period of 3 weeks for the complete plantlet to generate. During the incubation care was taken for providing proper sunlight, air and water uniformly to all the samples. No specific drought condition was provided to any sample so

that the natural and native gene expression of all the samples can take place without any alteration by modified environmental condition.

DNA extraction from plant varieties and Band pattern identification

The plant material from all the 19 varieties is collected and Genomic DNA was extracted using CTAB Method for obtaining pure DNA. For this 0.2gm of leaf sample was grinded in 2ml of extraction buffer [3% CTAB (Cetyl Trimethyl Ammonium Bromide), 100mM Tris HCl, 20mM EDTA, 1.4M NaCl, 0.2% Beta mercapta ethanol, 0.2% PVP (Polyvinyl Pyrrolidone)] and incubated at 65°C for 40 mins. It was then centrifuged at 10000 rpm for 10 mins. To the supernatant 2ml of Chloroform: Isoamyl alcohol in the ratio 24:1 was added and again centrifuged at 10000rpm for 10mts. To the uppermost aqueous layer of the supernatant equal volume of Isopropanol /ethanol was added. The middle layer of organic matter of the leaf and the down most 3rd layer of Chloroform should not be taken for DNA precipitation. It was incubated at 4°C overnight and centrifuged at 12000rpm for 10 mins. The obtained pellet was further purified using ethanol wash, dried and preserved in TE buffer for analysis.

The genomic DNA was run on Agarose Gel to obtain their Bands which indicates the level of purity of the sample. A single sharp band indicates pure DNA.

Selection of Genes and their sequence retrieval from NCBI Database

The three known drought related genes of *Oryza sativa* DREB, LEA and OsDHODH1 were selected for the study. These three genes are known to have their vital role in Drought resistance under stress conditions. The presence of these genes with some SNP/Mutation may lead to a failure in their function in imparting drought resistance to the plants. The sequences of these genes were retrieved from NCBI to further design the specific primers for them.

Primer Designing and PCR

Three set of primers were designed for the target regions. The tool used for primer designing was Primer 3. All the 19 samples were amplified on these three target regions using these novel synthesized primer sets. The PCR Conditions were common to all these primers as described below

Pre-denaturation: at 94°C for 5 mins

Denaturation: at 94°C 30 sec

Annealing: at 55°C for 30 sec

Extension: at 72°C for 45 sec

Final Extension: at 72°C for 10 mins

The steps of Denaturation, Annealing and Extension were repeated for 30 cycles each.

The reaction mixture of 50µl consisted of 20 ng of genomic DNA, 2.5 U of Taq DNA polymerase, 5 µl of 10 X Taq buffer (100 mM Tris-HCl, , 500 mM KCl pH-8.3) , 200µM dNTP, 10 pmoles each primer (forward primer and reverse primer) and 2.0 mM MgCl₂ was used.

The amplicons were run on 1% agarose gel electrophoresis along with 1kb DNA marker as a qualitative check and the successful ones were purified by washing with 1M Sodium acetate and 70% of ethanol and subjected for sequencing on ABI3500 sequencer.

Results and Discussion

Fig 1: Seedlings germinated in laboratory conditions



Fig 2: Bands of Genomic DNA Extracted from leaves



Sequence Retrieval and Primer Designing:

Sequence Retrieval of DREB, LEA and OsDHODH1 has been done from NCBI taking *Oryza sativa* as the source. The sequences were further used for the Primer Designing using Primer

3 tool so as to use the same for the amplification of the respective genes. The fig 3a, 3b and 3c shows the Primer 3 results for all the three genes respectively.

Fig 3a: Showing the Primer 3 result for DREB gene from *Oryza sativa*

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PRIMER PICKING RESULTS FOR gi|996703428:c16300377-16297058 Oryza sativa Japonica Group cultivar Nipponbare chromosome 5, IRGSP-1.0

No mispriming library specified
Using 1-based sequence positions
OLIGO      start len  tm    gc%  any  3' seq
LEFT PRIMER 2467  20   60.02 55.00 6.00 2.00 TCAGCATGCGAGTCCACTAC
RIGHT PRIMER 2703  20   59.96 55.00 4.00 0.00 AAAGACCTCCTCGCCTCTTC
SEQUENCE SIZE: 3320
INCLUDED REGION SIZE: 3320

PRODUCT SIZE: 237, PAIR ANY COMPL: 5.00, PAIR 3' COMPL: 0.00
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Fig 3b: Showing the Primer 3 result for LEA gene from *Oryza sativa*

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PRIMER PICKING RESULTS FOR gi|996703428:26927590-26928883 Oryza sativa Japonica Group cultivar Nipponbare chromosome 5, IRGSP-1.0

No mispriming library specified
Using 1-based sequence positions
OLIGO      start len  tm    gc%  any  3' seq
LEFT PRIMER 860   20   60.02 50.00 4.00 2.00 TTGTGTTTCAGGCGAGTGAG
RIGHT PRIMER 1088  20   60.23 40.00 4.00 1.00 TTGCACCGAATTGAAGTGA
SEQUENCE SIZE: 1294
INCLUDED REGION SIZE: 1294

PRODUCT SIZE: 229, PAIR ANY COMPL: 4.00, PAIR 3' COMPL: 0.00
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Fig 3c: Showing the Primer 3 result for OsDHODH1 gene from *Oryza sativa*

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Using 1-based sequence positions
OLIGO      start len  tm    gc%  any  3' seq
LEFT PRIMER 635   20   60.07 50.00 2.00 0.00 GAGGTCTGCGGTTGGATAAA
RIGHT PRIMER 827   20   60.00 55.00 4.00 3.00 CCTCCACACAAGGTTCTGGT
SEQUENCE SIZE: 1446
INCLUDED REGION SIZE: 1446

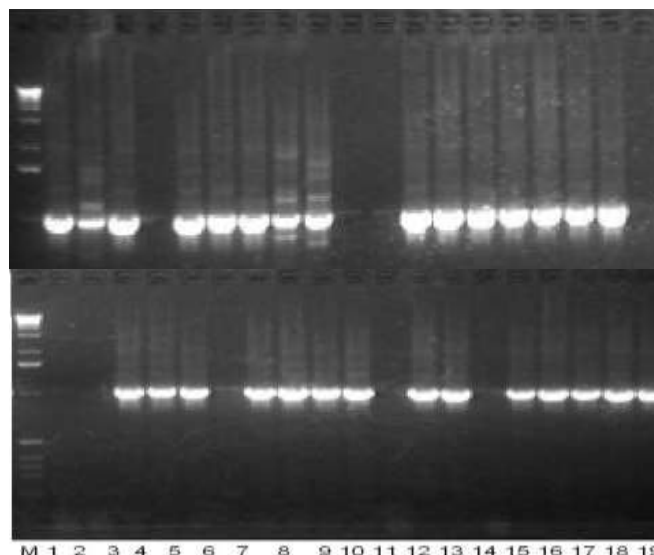
PRODUCT SIZE: 193, PAIR ANY COMPL: 4.00, PAIR 3' COMPL: 1.00
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PCR Amplification of the genome using above three Primers

Upon amplification with the three sets of primers clear sharp bands were obtained in most of the samples employing successful amplification. However in a few samples the amplification could not be successful and hence no bands were obtained at the desired position.

Fig 4: The amplicons' bands of DREB, LEA and OsDHODH1 respectively





Sequencing Result Analysis

The sequences for all the successfully amplified products were obtained. The results obtained for sequencing are as follows:

Table 1: showing the result of sequencing (Wherein A: DREB gene, B: LEA gene and C: OsDHODH1 gene)

S.NO	SAMPLE	FORWARD PRIMER	REVERSE PRIMER	SEQUENCIG RESULT
1	1A	TCAGCATGCGAGTCCACTAC	AAAGACCTCCTCGCCTCTTC	POSITIVE
2	1B	TTGTGTTTCAGGCGAGTGAG	TTGCACCGAATTTGAAGTGA	POSITIVE
3	1C	GAGGTCTGCGGTTGGATAAA	CCTCCACACAAGGTTCTGGT	NEGATIVE
4	2A	TCAGCATGCGAGTCCACTAC	AAAGACCTCCTCGCCTCTTC	NEGATIVE
5	2B	TTGTGTTTCAGGCGAGTGAG	TTGCACCGAATTTGAAGTGA	POSITIVE
6	2C	GAGGTCTGCGGTTGGATAAA	CCTCCACACAAGGTTCTGGT	NEGATIVE
7	3A	TCAGCATGCGAGTCCACTAC	AAAGACCTCCTCGCCTCTTC	POSITIVE
8	3B	TTGTGTTTCAGGCGAGTGAG	TTGCACCGAATTTGAAGTGA	POSITIVE
9	3C	GAGGTCTGCGGTTGGATAAA	CCTCCACACAAGGTTCTGGT	POSITIVE
10	4A	TCAGCATGCGAGTCCACTAC	AAAGACCTCCTCGCCTCTTC	POSITIVE
11	4B	TTGTGTTTCAGGCGAGTGAG	TTGCACCGAATTTGAAGTGA	NEGATIVE
12	4C	GAGGTCTGCGGTTGGATAAA	CCTCCACACAAGGTTCTGGT	POSITIVE
13	5A	TCAGCATGCGAGTCCACTAC	AAAGACCTCCTCGCCTCTTC	POSITIVE
14	5B	TTGTGTTTCAGGCGAGTGAG	TTGCACCGAATTTGAAGTGA	POSITIVE
15	5C	GAGGTCTGCGGTTGGATAAA	CCTCCACACAAGGTTCTGGT	POSITIVE
16	6A	TCAGCATGCGAGTCCACTAC	AAAGACCTCCTCGCCTCTTC	POSITIVE
17	6B	TTGTGTTTCAGGCGAGTGAG	TTGCACCGAATTTGAAGTGA	POSITIVE
18	6C	GAGGTCTGCGGTTGGATAAA	CCTCCACACAAGGTTCTGGT	NEGATIVE
19	7A	TCAGCATGCGAGTCCACTAC	AAAGACCTCCTCGCCTCTTC	POSITIVE
20	7B	TTGTGTTTCAGGCGAGTGAG	TTGCACCGAATTTGAAGTGA	POSITIVE
21	7C	GAGGTCTGCGGTTGGATAAA	CCTCCACACAAGGTTCTGGT	POSITIVE
22	8A	TCAGCATGCGAGTCCACTAC	AAAGACCTCCTCGCCTCTTC	POSITIVE
23	8B	TTGTGTTTCAGGCGAGTGAG	TTGCACCGAATTTGAAGTGA	POSITIVE
24	8C	GAGGTCTGCGGTTGGATAAA	CCTCCACACAAGGTTCTGGT	POSITIVE
25	9A	TCAGCATGCGAGTCCACTAC	AAAGACCTCCTCGCCTCTTC	POSITIVE
26	9B	TTGTGTTTCAGGCGAGTGAG	TTGCACCGAATTTGAAGTGA	POSITIVE
27	9C	GAGGTCTGCGGTTGGATAAA	CCTCCACACAAGGTTCTGGT	POSITIVE
28	10A	TCAGCATGCGAGTCCACTAC	AAAGACCTCCTCGCCTCTTC	POSITIVE
29	10B	TTGTGTTTCAGGCGAGTGAG	TTGCACCGAATTTGAAGTGA	NEGATIVE
30	10C	GAGGTCTGCGGTTGGATAAA	CCTCCACACAAGGTTCTGGT	POSITIVE
31	11A	TCAGCATGCGAGTCCACTAC	AAAGACCTCCTCGCCTCTTC	POSITIVE
32	11B	TTGTGTTTCAGGCGAGTGAG	TTGCACCGAATTTGAAGTGA	NEGATIVE

33	11C	GAGGTCTGCGGTTGGATAAA	CCTCCACACAAGGTTCTGGT	NEGATIVE
34	12A	TCAGCATGCGAGTCCACTAC	AAAGACCTCCTCGCCTCTTC	POSITIVE
35	12B	TTGTGTTTCAGGCGAGTGAG	TTGCACCGAATTTGAAGTGA	POSITIVE
36	12C	GAGGTCTGCGGTTGGATAAA	CCTCCACACAAGGTTCTGGT	POSITIVE
37	13A	TCAGCATGCGAGTCCACTAC	AAAGACCTCCTCGCCTCTTC	NEGATIVE
38	13B	TTGTGTTTCAGGCGAGTGAG	TTGCACCGAATTTGAAGTGA	POSITIVE
39	13C	GAGGTCTGCGGTTGGATAAA	CCTCCACACAAGGTTCTGGT	POSITIVE
40	14A	TCAGCATGCGAGTCCACTAC	AAAGACCTCCTCGCCTCTTC	NEGATIVE
41	14B	TTGTGTTTCAGGCGAGTGAG	TTGCACCGAATTTGAAGTGA	POSITIVE
42	14C	GAGGTCTGCGGTTGGATAAA	CCTCCACACAAGGTTCTGGT	NEGATIVE
43	15A	TCAGCATGCGAGTCCACTAC	AAAGACCTCCTCGCCTCTTC	POSITIVE
44	15B	TTGTGTTTCAGGCGAGTGAG	TTGCACCGAATTTGAAGTGA	POSITIVE
45	15C	GAGGTCTGCGGTTGGATAAA	CCTCCACACAAGGTTCTGGT	POSITIVE
46	16A	TCAGCATGCGAGTCCACTAC	AAAGACCTCCTCGCCTCTTC	POSITIVE
47	16B	TTGTGTTTCAGGCGAGTGAG	TTGCACCGAATTTGAAGTGA	POSITIVE
48	16C	GAGGTCTGCGGTTGGATAAA	CCTCCACACAAGGTTCTGGT	POSITIVE
49	17A	TCAGCATGCGAGTCCACTAC	AAAGACCTCCTCGCCTCTTC	POSITIVE
50	17B	TTGTGTTTCAGGCGAGTGAG	TTGCACCGAATTTGAAGTGA	POSITIVE
51	17C	GAGGTCTGCGGTTGGATAAA	CCTCCACACAAGGTTCTGGT	POSITIVE
52	18A	TCAGCATGCGAGTCCACTAC	AAAGACCTCCTCGCCTCTTC	POSITIVE
53	18B	TTGTGTTTCAGGCGAGTGAG	TTGCACCGAATTTGAAGTGA	POSITIVE
54	18C	GAGGTCTGCGGTTGGATAAA	CCTCCACACAAGGTTCTGGT	POSITIVE
55	19A	TCAGCATGCGAGTCCACTAC	AAAGACCTCCTCGCCTCTTC	NEGATIVE
56	19B	TTGTGTTTCAGGCGAGTGAG	TTGCACCGAATTTGAAGTGA	NEGATIVE
57	19C	GAGGTCTGCGGTTGGATAAA	CCTCCACACAAGGTTCTGGT	POSITIVE

Table 2: showing the genes present in each sample

SAMPLE No.	SAMPLE NAME	DREB	LEA	OsDHODH1
1	Pusa-1612	✓	✓	
2	Rajshree		✓	
3	Pratikshaa	✓	✓	✓
4	Malviya Dhan-2	✓		✓
5	Rajendra Mehsuri	✓	✓	✓
6	Annada	✓	✓	
7	Komal-1	✓	✓	✓
8	NDR-2064	✓	✓	✓
9	MTU-Swarna / 7029	✓	✓	✓
10	PNR-301	✓		✓
11	Lalat	✓		
12	Prabhat	✓	✓	✓
13	Kalanamak-3		✓	✓
14	Gotra Bidhan		✓	
15	Pusa Sugandha-5	✓	✓	✓
16	Malviya Dhan-2	✓	✓	✓
17	Pusa-1612	✓	✓	✓
18	Sarju-52	✓	✓	✓
19	NDR-97			✓

Conclusion

The current research was undertaken to detect/identify the presence of three of the drought resistant genes DREB, LEA and OsDHODH1 among 19 varieties of paddy. The results of amplification showed that DREB was present in all the varieties except Rajshree, Kala namak-3, Gotra Bidhan and NDR-97 while LEA could not be detected in Malviya Dhan-2, PNR-301, Lalat and

NDR-97. OsDHODH1 was comparatively much rare than these two genes and was not identified in Pusa-1612, Rajshree, Annada, Lalat and Gotra Bidhan. The samples in which all these three genes were identified were Pratikshaa, Rajendra Mehsuri, Komal-1, NDR-2064, MTU-Swarna/7029, Prabhat, Pusa sugandha-5 and Sarju-52.

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