

Insilico & Insitu Analysis of Drought Resistant DREB and LEA Genes Isolated from Different Varieties of Rice (Oryza L.)

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Received: 12th March 2017, Accepted: 17th March 2017, Published: 1st May 2017

Abstract

Rapid growth in human population through the world is boosting demand for corresponding increase in grain yield and there is a need to increase production 50% more by 2025. Classical approaches for plant improvement including Plant breeding have been greatly supported by novel techniques of genome sequencing, gene tagging, gene cloning, bioinformatics etc., The present study was undertaken to study the selected genes of rice responsible for imparting the drought resistance to the plant. The genes selected for the study are LEA and DREB both of which are known for their ability to withstand the drought conditions. The gene sequences are collected from the NCBI Database and their complete characterization was performed. The Lab work included the collection of 4 drought resistant varieties from Rain Fed Paddy Research Station, Tuljapur, Maharashtra and 5 susceptible varieties from Directorate of Rice Research Centre Hyderabad. The seeds were sowed in small trays and cultivated in lab conditions for about two weeks. The leaf material was used for the extraction of DNA and the samples were used for sequencing. The complete insilico characterization of the two genes LEA and DREB was performed. Primers were designed for these genes which were used to amplify the genes from the Genomic DNA extracted from the above 9 samples. Based on the presence or absence of the gene in the genomes the amplification can be either positive or negative. These results would enable to classify the plant as drought resistant or sensitive. A complete characterization of the two genes was included in the study.

al., 2011). In Asia, severe and minor drought occurs in East and Northeast India, East

China, Central Myanmar, Thailand. In the eastern states of India viz., Jharkhand, Orissa and Chhattisgarh, 40 per cent of the total rice production is lost in severe droughts valued at \$650 million. (Pandey et al., 2005) and poorest rice farmers are most affected in these areas.

The genes DREB and LEA have been reported to be responsible for drought resistance in a number of plant species including rice. In this study rice varieties susceptible and resistant to drought were selected. The sequence analysis of DREB and LEA genes was conducted and primers were designed for selective amplification of these genes. Using these primers the amplified regions were sequenced and based on these *in silico* and *in vitro* studies efforts have been made to understand the effect of these genes on drought stress.

Materials and Methods

1. Sequence retrieval of DREB and LEA genes from NCBI

NCBI database available at www.ncbi.org was used to retrieve DREB and LEA gene sequences. After entering the name of the gene (DREB and LEA) and the species name (*Oryza sativa*) at the database search box on the webpage www.ncbi.org the available sequence details were retrieved. NCBI is a public database and a premier website available free for all the research applications.

2. Primer Designing

In order to amplify the gene sequences obtained from the samples, primers have to be designed. Primer designing was done using primer 3 software which is a free online tool useful for primer standardization and selection. Each search provides a choice of 5 best primer pairs. All of which differ in the region and size of the amplicons and their properties.

3. Gene analysis:

A. CODON plot:

Codon plot is a software available on (http://www.bioinformatics.org/sms2/codon_plot.html) which was used to identify the exact codon pattern in the gene. Codon plot is useful to find portions of

Introduction

Rice, (*Oryza sativa* L.) is the most important food crop for nearly half of the world population (Sellamuthu et al., 2011). Worldwide, rice is cultivated on an area of 154 million hectares with an annual production of 700 million tones. Globally, India ranks first in cultivable area under rice and second in production, producing 131 million tones. Rice has also become a model organism for genome analysis, having a diploid chromosome number of 24 and the smallest genome size (430 Mb) of all major crops. (Arumuganathan and Earle, 1995).

Drought is an abiotic stress that limits productivity in both rainfed and upland ecosystems (Bimpong et

DNA sequence that may be poorly expressed or to view a graphic representation of a codon usage table.

B. Translation of Gene using EXPASY server:

Translation of gene sequences to corresponding amino acid sequences forming the protein structure can be identified and studied using Translate of EXPASY server available on <http://web.expasy.org/translate>. Translate is a tool which allows the translation of a nucleotide (DNA/RNA) sequence to a protein sequence.

C.SMART:

Conserved domain sequences were identified by using SMART tool (<http://smart.embl-heidelberg>). It is used full in the prediction of probable placements of amino acids in tertiary structures of homologues that overlay in three dimensions. Alignments held by SMART are mostly based on published observations and further updated and edited manually.

4. Obtaining rice plantlets from seeds of different varieties:

Seeds of different rice varieties were sown in separate plastic trays containing soil. They were grown for 15 to 20 days under uniform conditions. The leaves were cut into small pieces and used immediately for DNA extraction.

5. DNA Isolation:

- 1) 1gm of leaf sample was taken and ground thoroughly in CTAB extraction buffer. The slurry was taken in a tube and incubated at 60°C for 1 hour.
- 2) After cooling it to room temperature, 5ml of chloroform: isoamyl alcohol (24:1) was added and mixed thoroughly.
- 3) The tube was centrifuged at 10000rpm for 10 minutes. Supernatant was taken in another tube and above step was repeated.
- 4) The supernatant was taken in a new tube and 5M NaCl was added along with equal volume of chilled isopropanol.
- 5) The tube was incubated at 4°C for 15 minutes and centrifuged at 12,000 rpm for 10 min.
- 6) The pellet was dried and dissolved in TE buffer and kept in refrigerator.

6. DNA quantification:

1. 1 ul DNA sample of each variety was taken in test tubes. In each tube, 2ml of distilled water and 5ml of DPA was added.
2. Tubes were kept in boiling water bath for 15 minutes. After which the volume was made up to 10ml with distilled water.
3. OD reading was taken at 595 nm using a colorimeter.

7. Agarose gel electrophoresis

1. 0.8% agarose was dissolved in 100ml of 1x TAE buffer.
2. The gel was heated in microwave oven and ethidium bromide was added after cooling down to 50°C. The melted gel was immediately poured in the casting tray with the combs. After solidification the gel was used for electrophoresis.
3. 5 µl DNA was mixed with 2µl 1x gel loading dye and loaded on the gel.
4. After electrophoresis results were seen using UV transilluminator and photograph was taken for quality analysis.

8. PCR:

The DNA samples from different rice varieties were subjected to PCR amplification with gene specific primers. Stock DNA was appropriately diluted to obtain working DNA samples of different varieties for conducting PCR amplification. The total volume of PCR reaction mixture was 25 µl having following contents, (Table)

Table 1 Reaction mixture used for conducting PCR

S. No	Reagent Name	Concentration
1	Template DNA	2 µl
2	Primers: Forward Primer Reverse Primer	1 µl 1 µl
3	Composition of amplicon 2X master mix a. Tris HCl(PH8.5) b. 3 Mm Mgcl ₂ c. 0.2% Tween 20 d. 0.4 Mm e. 0.2 units/ µl Taq DNA Polymerase f. Inert red dye and stabilizer	12.5 µl
7	Nuclease free water	8.5 µl
8	Total	25 µl

Table 2: PCR conditions used for amplification of DNA

S.No	Step	Temperature	Time
1	Initial denaturation	94°C	5minutes
2	Denaturation	94°C	30seconds
3	Annealing	55°C	30seconds
4	Extension	72°C	30seconds
5	Final extension	72°C	07minutes

Repeat
ed for
35
cycles

9. Agarose gel electrophoresis of PCR products:

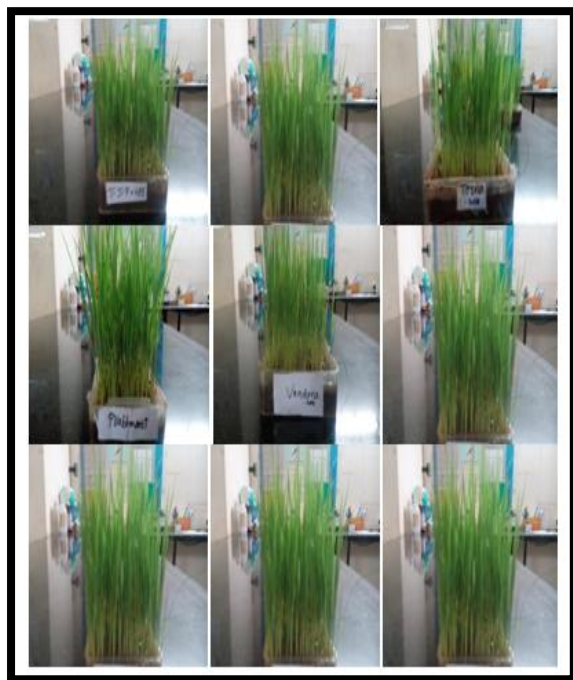
1. 1.2% agarose was dissolved in 100ml of 1x TAE buffer.
2. The gel was heated in microwave oven and ethidium bromide was added after cooling down to 50°C.
3. 5 µl PCR product was mixed with 2µl 1x gel loading dye and loaded on gel.
4. Electrophoresis was carried out at 50 V for 45 minutes using 1x TAE buffer.
5. Results were recorded by using gel documentation unit.

10. Sequencing the amplicons:

The DNA samples were subjected to sequencing using ABI 7700 automated sequencer available at Bio Serve™, Hyderabad.

Results and Discussion:

Fig 1: The invitro Cultivation of the 9 varieties of *Oryza*



Gene Sequence Retrieval:

NCBI database available on (www.ncbi.org) was used to retrieve DREB and LEA gene sequences. After entering the name of the gene (DREB and LEA) and the species name (*Oryza sativa*) at the database search box on the webpage (www.ncbi.org) the available sequence details were retrieved. The results were as follows:

LOC4336585 ethylene-responsive transcription factor ERF038 [*Oryza sativa Japonica Group* (Japanese rice)]

Gene description: ethylene-responsive transcription factor ERF038

Location: chromosome: 4

EXON: 1

GENE LOCATION: 27517168 TO 27518353

>gi|996703429:27517168-27518353 *Oryzasativa Japonica Group* cultivar Nipponbare chromosome 4, IRGSP-1.0

```
CTATTTATAGCCAGAGGCACTCACTCGCTCACTCGCCG
CAGACCGCACTTCCATCGATCTCTCTCCTCGCCACGGG
CCACGGCCTTCTCCTAACGGGAGCCATGGAAAGCAGAC
GCGAGCCATACACCCACCCTCTCTCTCCGTCTCCGT
CTCTTCTCTCTCTCTCGTCTGCTGCTCACTTCTCTCCAC
CTCTCTCTCTGTCGACAATGGCGCGCAAGACCGGCCCA
AGAGTCCAAAGCCCAAACACGCCGCCCAAGAAGCGCAA
GAGAGCGGCCGCGGAGGAACCTGCCAATGCCGCCAC
GGCGCAGGGGAGGATACCAGCAGCTGCAGCACCGGACG
ACAACGCGGCGGCGAGCGGCAAGGCGCAGGCGGGCG
GCGGCGGCGGCGGCGGCTCGACAGCAGCAGCACTGTCAC
CGCCGCTCGGCGCCGAGGAGCGGCTTCAAGCACCCG
TCGTACCGCGGCGTGCGCCGCCGAGCTGGGGGAAGT
GGGTGTGCGGAGATCCGGGAGCCCCGCAAGAAGTCCGG
CATCTGGCTGGGTACCTTCCCAACCGCGGAGATGGCGG
CGCGCGCCACGACGTGGCCGCGCTCGCCATCAAGGG
CCGGAACGCGCACCTCAACTTCCCGGACAGCGCCAC
GAGTGCCCCCGCCGGAGTCCACCTCCCGGCGAGACA
TCCAGGCCGCCGCCCAAGGCTGCGCGGAGGTGCG
GTGCGAGGAGGAGTCTGTCGCGCTGCTCGTCCGCCACC
GCCGAGCAACCCGAGGAGGAAGCCGCTGCCCTGACA
CGGTGCACGCCGACGGCGGCCAGGACAATGCTCTCTT
CGACCTACCCGACCTTCTTCTCGACCTACGGGACGGGC
TCTGGTGGTTCGCCGGTGTGGCCGGCGGCATGGCGGC
CGAGGAGTACGACGGCGGCGACGCCGCTGCTCAAT
GAGCCACTCTTGTGGGCGAGTAGCCATTTACCTTTC
TGGGCTCAATGAATAGGCTACAACGACAATC
TCAAGAAAGAAAGAAAAAAGGGAAAAA
TGTTATTACCGCTATATTTAGTAGTTACTGC
TATTGACAAAGTTTGTCTATGTTGTGCTTG
ATTAGGCCATGAAGCCTGTGTGCTTTGTGT
ACCAACAATCAAAAAGATCTCTCTACTAAT
AGAAGTTAATTAATTCGAAGCAGATTTTTT
TTCA
```

LEA gene sequence

LOC4339480 late embryogenesis abundant protein, group 3 [*Oryzasativa Japonica Group* (Japanese rice)]

Location: chromosome: 5

Gene description: late embryogenesis abundant protein group 3

known as:

LEA; LEA3; LEA-3; OJ1288_A07.1; OJ1362_G11.19

Exon count: 3

FASTA Sequence:

gi|996703428:26927590-26928883 *Oryza sativa Japonica Group* cultivar Nipponbare chromosomes 5, IRGSP-1.0

```
ATTCCAATCCCATCGCCACAGCCAGTGCAAAATCTGATC
GATCAAGATAATCGAGCAAAATCCATCAGAGTTCTAA
CATTCGCGCGTGAATTTTCGAGGTTAATTTTTGAAGCTT
AGGATCAATGGCTTCCACAGGACCAGGCTAGCTAC
CGCGCCGCGGAGACCAAGGCCACACCGAGGTCAGCG
TCAGCCACCACCGGCCGCGCATCTTCTCCAGCTCCGCG
GAGCTTCCAGTTAATTTGTTGATCACTTGATTGTTCTTG
GTGTGACGAGGAAGGCGGGGCGAGGTGATGGGGGCGA
GCAAGGACAAGGCGAGCGAGGCGAAGGACAGGGCGT
CGGAGGCGGCGGGGCGACGCGCGCGGCAAGGGGCGAGG
ATACCAAGGAGGCGACGAAGGAGAAGGCGCAGGCGG
CGAAGGAGAGGGCGTCCGAGACGGCGCAGGCGGCGA
AGGACAAGACCTCCAGCACGTCCGAGGCGGCGAGGGA
```

CAAAGCCGCCGAGAGCAAGGACCAGACCGCGGCTTC
CTCGGCGAGAAGACCGAGCAGGCCAAGCAGAAGGCC
GCCGAGACCGCTGGCGCCGCCAAGCAGAAGACCGCCG
AGACGGCGCAGTACACCAAGGACTCTGCCATCGCCGG
CAAGGACAAGACCGGCAGCGTCTCCAACAGGTACAC
ACAGGATTAAGCAATTCTACCTCCTTTTTTTTTTTT
TTGCGGGGAGCAGAGGTACCGTGAGATAAAAAAATAC
GGTACCAGTTATTAAATTACAATAGAAAAAGTAGTA
CCTTCTTAAGCACTGTAAATTTGCTCTTTGATTAGTTTC
TCCTCGTCGAACACCATTTGTTGTGGATCTTGAGGAGAA
TTGTTTGTGTTTCAGGCGAGTGAGCAGGTGAAGAGCAC
GGTGGTCGGCGCCAAGGACGCGGTGATGAGCAGCTG
GGGATGACCGAAGACGAGGCGGCCACCGACGACGGC
GCCAACAAGGACACCTCTGCCACCGCCGCCGCCACGG
AGACGACGGCGAGGGATCACTAGACGCCGTGAATGAT
TTCCCTTTGGGTCTATTATGTATGTTTTCACCTCAAAAT
TCGGTGCAAGTTTGAATTTGTTTTGTGTCGTTTTGAGT
CTGTATCGATGCTGTATGAAGTGGTGGTCTGCGCAGGG
GAGGATTTCTGACGGGTGTGGGTGATGTGACTGATGA
TGTTACAGTTGTTTTCGTCAGAGTTTCTCGTCTGTGTTCT
GTTTATTATGGCGTAACAATAATAAAGTTGGGCCTA
AAGCCCGCATTTGTGGTTTATCA

Primer designing for the two genes selected:

Primer 3 tool has been used for designing the primers for both DREB and LEA. The primer obtained can be further synthesized and used for PCR amplification and sequencing. The results are as follows:

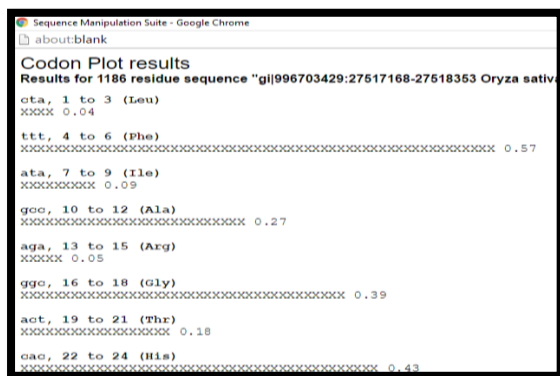
DREB Primer:

Left Primer: GGGCCGAGTAGCCATTTTAC
Right Primer: CACAGGCTTCATGGCCTAAT
Product length: 167

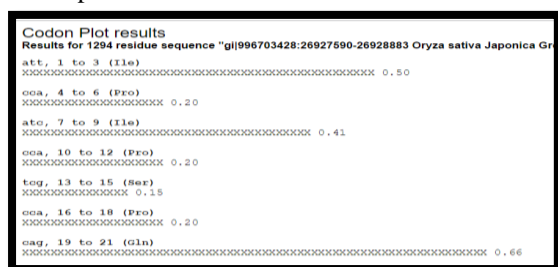
Left Primer: TTGTGTTTCAGGCGAGTGAG
Right primer: TTGCACCGAATTTGAAGTGA
Product Length: 229.

Codon Plot for the two genes:

Codon plot for DREB:



Codon plot for LEA:



From the above two results it can be inferred that the two genes DREB, LEA have their starting codon to be GTA (Leucine) and ILE (Isoleucine). This plot can be used to identify the exact protein coding pattern.

Translation of the gene to Protein using EXPASY Translate:

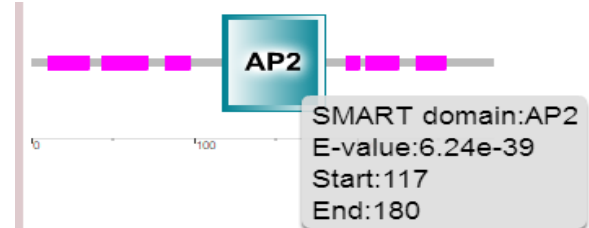
In order to obtain the protein product of the gene sequence Translate tool has been used from the Expasy server. The protein product obtained for the gene sequence is as follows:

XXXXXXXXXXXXXXXXXXXXXYSQRHSLAHSPQTALPSISL¹LATGH
GLLLTGA¹MetEADASHPTPTSSSVSVSFSSSLSTSSSTSS¹SLVDNGA
QDRPKSSKPKHAAKKRKRAAAEEEPANAAHGAGEDTSSCSTD¹
NAAASGKAQAGGGGGVDSSTCTAAASAPRSGFKHPSYRGVRR¹
SWGKVVSEIREPRKKSRIWLGTFPTAEMetAARAHDAALAIK¹
GRNAHLNFPDSEHLPPESTSPADIQAAAAKAAAEVRCEEES¹
PSSSPTAEQPEEEAACPDVTVHADGGQDNALFDLPDLLDLRDL¹
WWSPPVWPAALAAEEYDGGDAVVLNEPLLWAE¹StopPFFYLSG
L¹MetNRLQRQLSRKKKKKGNVITAIFSSCY¹StopQSLVYVVL
D¹StopA¹MetKPVCFVYQSKRSLY¹StopStopKLINSKQIFF¹

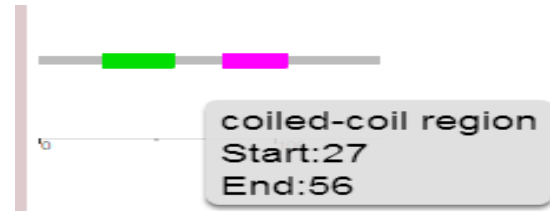
Domain Analysis using SMART:

The conserved domains were identified in the sequences obtained above using SMART tool available on web page.(<http://smart.embl-heidelberg.de/>).

DREB domains:



LEA domains:



The above results shows that there is a prominent AP2 domain in DREB protein whereas no such specific domain is present in LEA protein. These domains indicate localized functional regions.

Amplification of the DNA samples using PCR

Designed primers were synthesized and used for studying amplification of DREB and LEA genes in different rice varieties. The results of PCR were as follows.

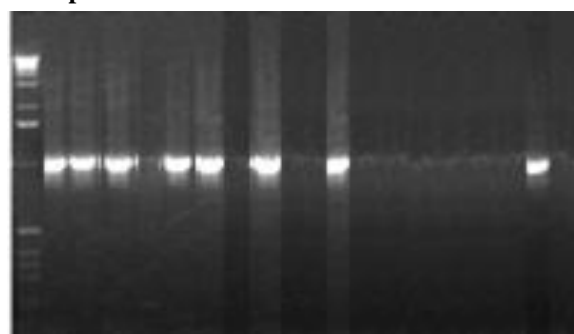
Table 4.1: Results of Amplification

S.No	Name of the Variety	DREB	LEA
1	Vandana	Amplified	Amplified
2	T.J.P	Amplified	Not Amplified
3	Terna	Amplified	Amplified
4	Anjali	Not amplified	Amplified
5	MTU1010	Not Amplified	Not Amplified
6	NDR 359	Amplified	Not Amplified
7	Swarna	Not Amplified	Not Amplified
8	BPT 5204	Not Amplified	Not Amplified
9	Prabavathi	Amplified	Not Amplified

Fig.1 Results of qualitative analysis of DNA isolated from different rice varieties on 0.8% agarose gel.



Fig.2 Amplification of DNA by using DREB and LEA primers



The above picture shows the PCR amplicons which were obtained after amplification for the specific genes.

Gene sequencing:

The distinct bands were cut and DNA was separated and purified. It was sequenced in Bioseve using

AB17700 automated sequencer. The results of gene sequencing were as follows

Results of sequencing:

>Vandana (LEA)

```
TTGTGTTTCAGGCGAGTGAGCAGGTGAAGAGCACGGT
GGTCGGCGCCAAGGACGCGGTGATGAGCACGCTGGGG
ATGACCGAAGACGAGGCCGGCACCACGACGCGGCCA
ACAAGGACACCTCTGCCACCGCCGCCACGGAGAC
GACGGCGAGGGATCACTAGACGCCGTGAATGATTTC
CTTTGGGTCTATTTATGTATGTTTCACTTCAAATT
```

>Vandana (dreb)

```
GGGCCGAGTAGCCATTTTACCTTTCTGGGCTCATGAAT
AGGCTACAACGACAACCTCTCAAGAAAGAAAGAAAAA
AAAGGGAAAAATGTTATTACCGCTATATTTAGTAGTTA
CTGCTATTGACAAAGTTTAGTCTATGTTGTGCTTGATT
AGGCCATGAAGCCTGTGCGGTGCAA
```

>TJP (DREB)

```
GGGCCGAGTAGCCATTTTACCTTTCTGGGCTCATGAAT
AGGCTACAACGACAACCTCTCAAGAAAGAAAGAAAAA
AAAGGGAAAAATGTTATTACCGCTATATTTAGTAGTTA
CTGCTATTGACAAAGTTTAGTCTATGTTGTGCTTGATT
AGGCCATGAAGCCTGTGCGGTGCAA
```

>TJP (LEA)

```
.....
.....NO SEQUENCE AMPLIFIED.....
```

>TERNA (DREB)

```
GGGCCGAGTAGCCATTTTACCTTTCTGGGCTCATGAAT
AGGCTACAACGACAACCTCTCAAGAAAGAAAGAAAAA
AAAGGGAAAAATGTTATTACCGCTATATTTAGTAGTTA
CTGCTATTGACAAAGTTTAGTCTATGTTGTGCTTGATT
AGGCCATGAAGCCTGTGCGGTGCAA
```

>TERNA (LEA)

```
TTGTGTTTCAGGCGAGTGAGCAGGTGAAGAGCACGGT
GGTCGGCGCCAAGGACGCGGTGATGAGCACGCTGGGG
ATGACCGAAGACGAGGCCGGCACCACGACGCGGCCA
ACAAGGACACCTCTGCCACCGCCGCCACGGAGAC
GACGGCGAGGGATCACTAGACGCCGTGAATGATTTC
CTTTGGGTCTATTTATGTATGTTTCACTTCAAATT
```

>Anjali (LEA)

```
TTGTGTTTCAGGCGAGTGAGCAGGTGAAGAGCACGGT
GGTCGGCGCCAAGGACGCGGTGATGAGCACGCTGGGG
ATGACCGAAGACGAGGCCGGCACCACGACGCGGCCA
ACAAGGACACCTCTGCCACCGCCGCCACGGAGAC
GACGGCGAGGGATCACTAGACGCCGTGAATGATTTC
CTTTGGGTCTATTTATGTATGTTTCACTTCAAATT
```

>Anjali (Dreb)

```
.....
.....No sequence obtained.....
```

>MTU1010 (lea)

```
.....
.....No sequence obtained.....
```

>MTU1010 (dreb)

```
.....
.....No sequence obtained.....
```

>NDR 359 (DREB)

```
GGGCCGAGTAGCCATTTTACCTTTCTGGGCTCATGAAT
AGGCTACAACGACAACCTCTCAAGAAAGAAAGAAAAA
AAAGGGAAAAATGTTATTACCGCTATATTTAGTAGTTA
CTGCTATTGACAAAGTTTAGTCTATGTTGTGCTTGATT
AGGCCATGAAGCCTGTGCGGTGCAA
```

>NDR 359 (LEA)

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.....
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.....No sequence obtained.....

>swarna (lea)

.....No sequence obtained.....

>swarna (dreb)

.....No sequence obtained.....

>BPT 5204 (lea)

.....No sequence obtained.....

>BPT 5204 (dreb)

.....No sequence obtained.....

>PRABHAVATHI (DREB)

GGGCCGAGTAGCCATTTTACCTTTCTGGGCTCATGAAT
AGGCTACAACGACAACCTCTCAAGAAAGAAAGAAAAA
AAAGGGAAAAATGTTATTACCGCTATATTAGTAGTTA
CTGCTATTGACAAAGTTAGTCTATGTTGTGCTTGATT
AGGCCATGAAGCCTGTGCGGTGCAA

>PRABHAVATHI (LEA)

GGGCCGAGTAGCCATTTTACCTTTCTGGGCTCATGAAT
AGGCTACAACGACAACCTCTCAAGAAAGAAAGAAAAA
AAGGGAAAAATGTTATTACCGCTATATTAGTAGTTA
TGCTATTGACAAAGTTAGTCTATGTTGTGCTTGATTA
GGCCATGAAGCCTGTGCGGTGCAA

From sequence analysis following results are obtained:

1.Both DREB and LEA genes show variation
interms of amplification in all varieties .

Drought Resistant:

- 1.vandana : DREB AND LEA AMPLIFIED
2. T.J.P: ONLY DREB AMPLIFIED
3. Terna: DREB AND LEA AMPLIFIED
4. Anjali: LEA ONLY AMPLIFIED

Susceptible:

1. MTU1010: BOTH NOT AMPLIFIED:
2. NDR 359: DREB AMPLIFIED
3. Swarna: Not amplified
4. BPT 5204: not amplified
5. Prabavathi. DREB amplified

Following conclusions can be made as per the results if sequencing

1. In vandana DREB and LEA genes were amplified because the samples had the ability to resist complete drought condition.
2. TJP only DREB is amplified
3. Terna DREB and LEA both are amplified. It show complete drought resistance.
4. Anjali LEA only amplified.
5. MTU1010 no amplification
6. In NDR 359 dreb is amplified
7. In swarna not amplified
8. In BPT5204 not amplified
9. Prabhavati DREB is amplified

Conclusion

Deciphering sequences for a particular trait followed by primer designing to amplify an important conserved region of the gene is a useful strategy to find the functional and molecular characteristics of DREB and LEA genes and to use them in plant improvement programme. The work results showed a direct relation with the presence of these selected genes and the ability of the plant against drought conditions. From the results it can be concluded that the transgenic plants possessing these genes can be a beneficial approach for the improvement in the production of essential crops.

References:

- 1) Agarwal P.K., Agarwal P., Reddy.M.K.,Sopory S.K. (2006). Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. *Plant Cell Rep* 25: 1263–1274.
- 2) Agarwal P.K., Jha B. (2010). Transcription factors in plants and ABA dependent and independent abiotic stress signaling. *Biol Plant* 54: 201–212.
- 3) Agarwal, P.K., P. Agarwal, M.K. Reddy and S.K. Sopory, (2006). Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. *Plant Cell Rep.*, **21**: 1263-1274.
- 4) A.R.Srividya, RachanaKatta and V.J.Vishnuvarthan (2014) *Int.J.Curr.Microbiol.App.Sci.*, 3(4): 694-708.
- 5) Bartels D, Sunkar R (2005). Drought and salt tolerance in plants.*Crit Rev Plant Sci***24**: 23–58.
- 6) Bimpong, I. K., R. Serraj, J. H. Chin, J. Ramos, E. M. T. Mendoza, J. E. Hernandez, M. S. Mendioro and D. S. Brar. (2011). Identification of QTLs for Drought-Related Traits in Alien Introgression Lines Derived from Crosses of Rice (*Oryza sativa* cv. IR64) × *O. glaberrima* under Lowland Moisture Stress. *J. of Plant Biol.*, **54**: 237-250.
- 7) Borba, C., D. Oliveria, R. P. V. Brondani, F. Breseghello, A. S. G. Coelho, J. A. Mendonca, P. H. N. Rangel and C. Brondani. (2010). Association mapping for yield and grain quality traits in rice (*Oryza sativa* L.).*Genet. Mol. Biol.*, **33**: 515-524.