Functional Analysis of Drought-Induced *Os*LEA3 Promoter Isolated from Batutegi Rice Cultivar

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Abstract

Drought is the most significant environmental stress in agriculture and many efforts have been made to improve crop productivity under water-limiting conditions. Improving yield under drought is a major goal of plant breeding. Late embryogenesis abundant (LEA) proteins have been implicated in many stress responses of plants. It has been found that LEA genes are a gene family and play important roles in the protection of water stress. Expression of *Os*LEA3 is induced by abiotic stresses, including drought and high salinity. The promoter of *Os*LEA3 was cloned from Batutegi, Rojolele and Nipponbare cultivars and its function was analyzed in transgenic Nipponbare plant by a GUS reporter gene. Transient assay was conducted in order to investigate GUS expression driven by *Os*LEA3 promoter. *Os*LEA3 promoter was expressed in embryogenic callus indicated by the appearance of blue spots. Differences in color produced in embryogenic callus showed different expression level.

Key words: *rice, drought, Batutegi, Rojolele, Nipponbare, GUS, OsLEA3 promoter*

Introduction

Rice (*Oryza sativa* L.) is one of the major staple foods. As the population growth goes up, the rice production must be increased. However, the field for rice cultivation has decreased because of the change of paddy field into housing and industry. Moreover, drought influences the rice production especially in North China and South Asian. Thus, rice becomes one of the target source of gene and genome sequence analysis project among cereal plants (Goff *et al.* 2002).

LEA proteins constitute a large group of proteins that specifically expressed during seed development. LEA proteins are located in seeds and pollen. Many vegetative organs can also express some LEA proteins under conditions of dehydration, osmotic stress, cold or exogenous ABA. First LEA proteins were identified during the desiccation phases of seed development. These proteins were reported to protect specific cellular structures or ameliorate the effect of drought stress by sequestering ions and maintaining minimum cellular water requirements (Hu, 2008).

Plant has physiological and molecular response mechanisms to tolerate drought. Those mechanisms are osmotic accumulation, scavenging ROS, and osmoprotectant protein production such as late embryogenesis abundant (LEA) and heat shock protein. LEA gene is identified as an expresed gene in the late phase of seed growth. Research on LEA genes has showed that LEA 3 gene is responsible for drought tolerance. A major problem under severe dehydration is that the loss of water leads to crystallization of cellular components during the course of dehydration and dormancy, which damages cell structures (Spelundm *et al* 1996). Some of the LEA proteins could essentially be considered compatible solutes that likely play the role similar to sugars in maintaining the structure of the cytoplasm in the absence of water (Hu 2008). The goal of this research is to study the function of LEA3 promoter isolated from rice cv. Batutegi.

Materials and Methods

Promoter OsLEA3

Promoter sequence primers were designed based on sequence of genes LEA3 HVA-like gene promoter from rice cultivar IRAT 109 (GenBank Acc. DQ837728) and has been adapted to the BAC sequence of chromosome No. 5 rice cultivar Nipponbare (GenBank Acc. AC104713). Primer sequences are also designed to include the start codon (ATG) of mRNA sequences LEA3 gene (GenBank Acc. DQ789359) and the addition sequence EcoRI (G↓ AATTC) and BglII (A↓GATCT) in order to add *EcoR*I and *Bgl*II restriction sites in the primers. The predicted promoter was amplified and isolated by PCR. The amplified fragments were sequenced to ensure fidelity.

Isolation of rice genomic DNA

Isolation of genomic DNA of rice cv. Batutegi was conducted by CTAB method (Saghai-Maroof *et al*., 1984) that has been optimized. Rice leaf samples were taken from 1-month-old plants. A total of 2 g leaf samples were prepared using aluminum foil wrapping. Leaf powder was put into polypropylene tubes containing 10 ml of buffer solution which has been has been preheated in the incubator at 65^{o}C for 30 minutes.

PCR analysis

All PCR reactions were done using 20 ng DNA template. PCR reaction mix contained 6.5 uL nuclease free water, 12.5 uL Green Go Taq 2x Master, 2.5 uL for each LEA F primer (0.1 µM) and LEA R (0.1 µM) promoter LEA3, and 1 µl template DNA. PCR conditions were: initial denaturation 95^{o}C for 3 minutes, followed by 35 cycles of denaturation at of 95^{o}C for 1 minute, annealing at 60°C for 1 minute, and initial extention at 72^{o}C for 1 minute. The cycle was terminated by final extention at 72^{o}C for 10 minutes.

Vector Construction

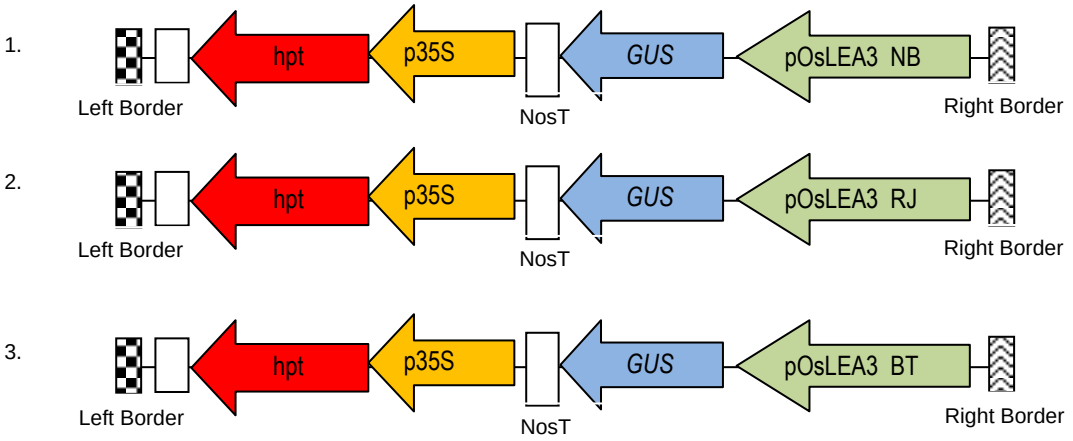

Figure 1. Vector Construction of OsLEA3 promoter::GUS.

Agrobacterium mediated transformation

Agrobacterium tumefaciens LBA4404 strain was transformed using recombinant plasmid pC1305 containing hygromycin phosphotransferase gene (hpt) controlled by CAMV 35S promoter, and GUS gene controlled by OsLEA3 promoter.

Histochemichal GUS assay

Water deficit treated and non treated plant tissues were vacuum infiltrated for 1 h in the GUS reaction mixture containing 1 mM 5-bromo-4-chloro-3-indolyl- b-D-glucuronide (X-gluc) and 50 mM sodium phosphate buffer and incubated at 37^{o}C overnight. The reaction was stopped by adding 75% ethanol, and the pigments and chlorophylls were removed by repeated ethanol treatment.

Results and Discussion

Amplification of OsLEA3 promoter

Isolation of 1.291 bp OsLEA3 gene promoter was conducted by amplification of DNA genome using PCR with specific primers and visualized with 0.8% agarose gel (Figure 2). The PCR fragment was then cloned into pGEM-T Easy and then used to construct pC1305 based recombinant plasmid.

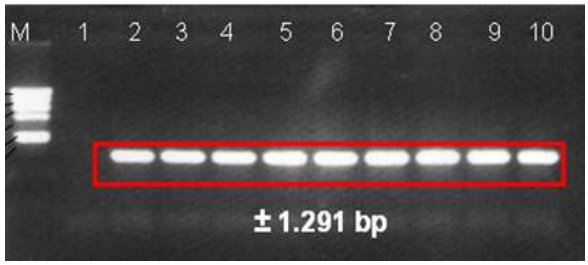

Figure 2. Amplification of OsLEA3 promoter.

Verification of the recombinant pGEM-T plasmid was conducted using *EcoR*I restriction analysis . The result showed 3 fragments, which were fragment with size 3.001 bp (pGEM-T Easy vector plasmid), 1.291 bp (gene OsLEA3 promoter), and 12 bp (nucleotides between EcoRI site of OsLEA promotor gene and EcoRI site of pGEM-T Easy) fragments (Figure 3).

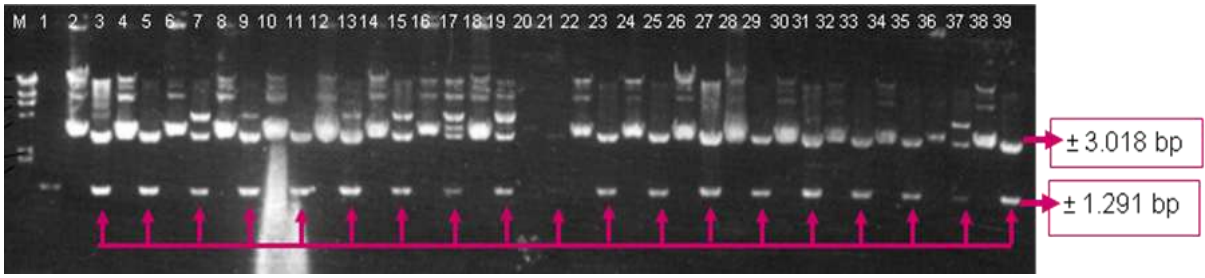

Figure 3. Electrophoresis of recombinant vector digested with *Eco*RI.

Rice Transformation using *Agrobacterium*

Recombinant vector containing OsLEA3promoter was successfully constructed and trasformed to rice cv. Nipponbare. Selection of Transformed plants was selected on hygromisin contained medium.

Figure 4. Successfull transformed plant in the selection medium.

Some transformed calli survived in medium containing hygroimisin. Hygromisin resistant celli were subcultured to regeneration medium. Calli with green spot grew into planlets. Table 1 showed low transformation efficiency. It needs to be increased by optimizing conditions of the culture. Factors that influence the low efficieny of transfomation are method of tranformation, type of explants and condition of culture.

Table 1. Genetic transformation of *Os*LEA3 ::GUS to rice cv. Nipponbare

No	Recombinant vectors	Rice Varieties	∑ infected calli	GUS assay	Number of Planlets
1.	*Os*LEA3 NB:: GUS	Nipponbare	600	Positive	32
2.	*OsLEA3* Rj:: GUS	Nipponbare	600	Positive	19
3.	*OsLEA3* BT:: GUS	Nipponbare	600	Positive	7

Histochemical Assay

Transient expression assaysof OsLEA3::GUS promoter of rice cv. Nipponbare, Rojolele and Batutegi were successfully conducted. GUS gene expression was identified with blue spot on the transformed calli .Figure 5 showed the difference intensity of blue spot in three cultivars. It means that the expression level of GUS gene differ among different OsLEA3 promoters. Real Time PCR will be conducted for further qualitative analysis.

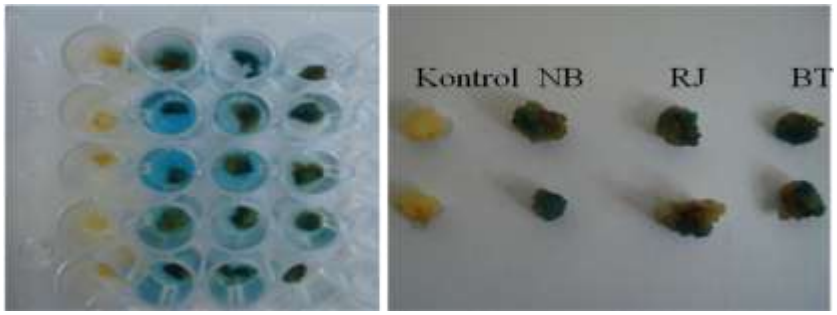

Figure 5. Histochemical assay of transformed calli, NB= Niopponbare, RJ= Rojolele, BT= Batutegi.

Conclusion

1. Recombinant plasmid pCambia1305 of OsLEA3::GUS of Batutegi, Rojolele and Nipponbare cultivars was succesfully constructed.
2. Promoters OsLEA3 of Batutegi, Rojolele and Nipponbare cultivars controlled GUS expression differently among those cultivars

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