Sprime: A database on promoters of abiotic stress inducible genes in rice

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Submitted: 3 Apr. 2008; Accepted: 17 May 2008

Abstract
Promoter is an essential constituent of the gene transfer cassette, and the expression of transgene(s) is under its direct control. However, we are limited by the availability of appropriate abiotic stress inducible promoters for routine use in rice transformation programs. This led to the development of a database exclusive on drought and salt stress inducible promoters in rice. Sprime: Stress inducible promoter database comprise of the upstream sequences from experimentally reported drought/salinity stress inducible genes in rice. A 3 kbp sequence upstream to the known stress inducible gene was demarcated, curated and developed into a promoter database using MS SQL and ASP.NET.

Keywords: Rice, Drought, Salt, Inducible promoter, Database.
URL: http://www.btistnau.org/Default1.aspx

INTRODUCTION
Abiotic stress, particularly drought and salinity affects the crop productivity worldwide. Developing transgenic crop plants tolerant to drought and/or salt stress is a felt objective in agricultural biotechnology. Development of transgenic rice plants against abiotic stress using constitutive promoters (e.g. CaMV 35S promoter, maize ubiquitin promoter and rice actin promoter) has been the most commonly followed approach. However, transgenic plants expressing transgenes in a regulated manner (only at times of stress) adds greater strength to the transgenic technology. The use of drought and/or salt stress inducible promoters to drive transgenes alleviates the problems arising out of constitutive over expression of transgenes (Kasuga et al., 1999; Garg et al., 2002).

The present investigation is aimed towards developing a database on abiotic stress inducible promoters, with the input data selectively sourced out from the transcript information available on rice under drought and salinity stress conditions. Rice with its relatively small genome has been adopted as a monocotyledonous model plant for dissecting genetic networks of biotic and abiotic stress responses. A detailed genome wide expression analysis in rice was carried out by Zhou et al. (2007), in which a 70mer oligomer microarray covering 36,926 unique genes or gene models were used to profile transcript level changes in rice shoot, flag leaf and panicle under drought and high salinity stress conditions. Of which, 322, 415 and 174 genes were found to be upregulated in each of the above organs. It was also shown that most of these genes exhibited high levels of expression as well as strong inducibility under drought and salinity stress conditions. In an earlier study, Rabbani et al. (2003), reported a list of drought and salinity stress responsive genes in rice. Based on the above two investigations, we aim at developing a promoter database on abiotic stress inducible genes in rice. This will directly be of use to the transformation scientists, and throws open a...
wider choice of drought/salinity stress inducible promoters for their transgenic research programs.

**MATERIALS AND METHODS**

**Dataset**

We primarily targeted the genes that are commonly expressed in rice shoot, flag leaf and panicle by comparing the genes induced in each of the three organs under drought and salinity stress conditions. Besides, we also included the genes that are induced under drought and salinity conditions in rice seedlings. Next, the NCBI Map Viewer was used to retrieve a 3 kbp nucleotide sequence upstream to the translation start site (ATG) of the drought/salt stress inducible genes. Each of the resulting sequence was subsequently curated so as to exclude any 3'-Exon and/or 3'-UTR region of the preceding gene that happen to fall within the demarcated 3 kbp region. FGENESH, a gene prediction tool based on Hidden Markov Model (HMM) was used to predict the presence of plausible Exon/UTR in the putative promoter region (http://www.softberry.com/berry.phtml). The curated promoter sequences were submitted to the Plant database of cis-acting Regulatory DNA Elements: PLACE (Higo et al., 1999; www.dna.affrc.go.jp/PLACE) for finding the presence/absence of characteristic stress response elements such as ABRE, DRE, MYB and MYC.

**Database architecture**

The **Sprom**e database is an object model database (Fig. 1). The information in the **Sprom**e database is of two types (1) information which is descriptive in nature like: Home, About database, Linkouts, Help desk and Reference. (2) and information which are tabulated. The descriptive information is linked to buttons which are nestled in the left, top and bottom frames. In the case of tabulated information, the table is accessed by its name (eg, Rice) via a drop-down list. The original table(s) is in the backend of the database, user can retrieve data from the selected table with the help of a search engine using suitable key words. The user interface of the database was enabled using Microsoft ASP.NET a standard generalized and most advanced web development application. The overall layout of the database was implemented using Microsoft Structure Query Language (MS SQL), and consist of six modules: **Sprom**e Id, Chromosome No, Gene Id, Gene description, Kind of stress (drought/salinity), Expressed tissue, Sequence and PLACE results.

**RESULTS AND DISCUSSION**

The **Sprom**e database is a compilation of putative promoters on drought and salt stress inducible genes based primarily on the research data of Zhou et al. (2007) and Rabbani et al. (2003). **Sprom**e stands for Stress inducible **promoter** database. A total of 49 genes were enlisted to be induced in different tissues of rice which includes, shoot, flag leaf, panicles and in seedlings under drought and/or salinity stress conditions. Of which, 34 genes were drought and salinity inducible, 5 genes were induced under drought and 10 genes under salinity stress (Table 1 [Supplementary data]). A 3 kbp nucleotide sequence upstream to the translational start site of the drought/salt stress inducible genes formed...
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the very basis of Srome. The demarcated 3 kbp upstream region was curated using a gene finding tool FGENESH, to avoid inclusion of any coding region in the promoter sequence. Finally, the above promoter sequences were made into a database using MS SQL as the backend. Search and retrieval of data is one of the most basic and important feature of a database. The web interface of Srome allows the user to access the data through list browsing and by key word search. The search function adopted in the database comprise of two basic layers. The first layer is the navigation layer which is used to select the database table (Rice/others) in which the search is to be performed and the next layer does the actual searching. Based on the match with the key word and the data content in the table the search tool picks up the entire row and display in an easily understandable way (Fig. 2).

Further, we analyzed the promoter sequences of the Srome database for the presence of drought and/or salt stress responsive sequence motifs. PLACE a database on plant cis-acting regulatory DNA elements is used for analyzing the promoter sequence. The PLACE result revealed that the promoters have one or more of the stress responsive motifs in them, this includes: Dehydration Responsive Element: DRE (Yamaguchi-Shinozaki and Shinozaki, 1994), ABA Responsive Element: ABRE (Marcotte Jr et al., 1989; Mundy et al., 1990), Myeloblastosis oncogene: MYB and MYC (Urao et al., 1993; Abe et al., 1997). The DRE fall under the ABA-independent pathway, whereas ABRE, MYB and MYC function in an ABA-dependent manner. Based on the analysis of 49 promoters, the core DRE sequence (CCGAC/YCGAC) is found in 42 of the 49 promoter sequences and the ABRE motif (ACGTG) is found in 42 promoters. Further, the binding sites for MYB (TAACTG/CNGTTR) and MYC (CACATG/CANNTG) are present in 47 and 49 sequences, respectively. The study put forth a total of 37 promoters which is governed by both ABA-independent (DRE) and ABA-dependent (ABRE) mechanism for drought and salinity stress tolerance (Table 1 [Supplementary data]; Fig. 3). However, more than one ABA-dependent mechanism operates in most of the promoters as has been evidenced by the presence of MYB/MYC binding sites, besides ABRE (Table 1). Of which, 41 promoters has all the three (ABRE, MYB and MYC) motifs in common. In a related comparison, DRE, MYB and MYC binding sites are found to be common in 40 promoter sequences (Table 1). Thus an appreciable cross-talk exists between the ABA-dependant and ABA-independent pathways, as well as among the ABA-dependant pathway. The results of the motif analysis indicate that the promoters constituting the Srome are doubly armoured with multiple stress response mechanism, accomplishing strong inducibility under drought and salinity stress conditions.

Figure 3: Venn diagram showing the interacting cis-elements (ABRE and DRE) in the promoter sequences

CONCLUSION

The Srome database facilitates experimental characterization of one or more of the enlisted...
stress inducible promoters for both basic and applied research. Some of the promising promoters can be used for engineering rice and other cereals for fighting against abiotic stresses. Presently, the database has been developed for rice but it is also extendable to other crops in the future.

Acknowledgement
This work was carried out at the Bioinformatics facility developed with the financial support of the Department of Biotechnology, Government of India under the Biotechnology Information System (BTIS) program.

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