In-silico promoter analysis and cloning of *OsBBX6* gene towards the development of multi-stress tolerant rice varieties

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Abstract

Rice growth and productivity are seriously limited by several abiotic stresses; temperature, UV-B radiation, drought, salinity and oxidative stress. It has been reported that *Arabidopsis* B-box proteins (BBX) play a key role in light and abscisic acid insensitive abiotic stress regulation pathway indicating that BBX protein could be an ideal candidate to develop multi-stress tolerant crops. However, only a few rice BBX genes have been functionally characterized. *In-silico* designing and simulating cloning protocols in genetic engineering can enhance the accuracy of procedures. The main aims of this study were to identify the abiotic stress regulatory cis-acting element on *OsBBX6* promoter and simulate *in-silico* cloning of *OsBBX6* coding sequence (CDS). *Oryza sativa indica* upstream sequence (1.0 kb) of *OsBBX6* (GenBank: CM000129.1) was retrieved from the NCBI database. PlantCARE and New PLACE tools were used for screening of cis-acting elements. Abiotic stress responsive elements namely salinity stress (GT1GMSCAM4, MYBCORE), dehydration (MYB2CONSENSUSAT, CBFHV, MYBCORE), light (G-Box, GT1-motif, I-box, Sp1, Box 4) and hormone (ABRE, DPBFCOREDCDC3, CGTCA-motif, TGACG-motif) were identified on the *OsBBX6* promoter region. Occurrence of cis-elements related to dehydration and salinity stress, dehydration and abscisic acid in *OsBBX6* promoter are two to five. Other abiotic stress responsive elements occur once in the *OsBBX6* promoter. PCR simulated by SnapGene using forward primer (5'CCCATGGCGATGAAGGTGCAGTGCGACGTG3') and reverse primer **(**5'CGGTAACCTCACCAGTAGGAGTAGGAAGAAG3') amplified the 830 bp *OsBBX6* CDS. Restriction cloning of CDS into pCAMBIA1303 at *Nco*I-*BstE*II restriction sites were carried out by using SnapGene. This simulation showed that the *OsBBX6* CDS in the recombinant plasmid (pCAMBIA1303-OsBBX6) is in frame and therefore, 35S promoter can successfully over-express *OsBBX6* CDS after *Agrobacterium* mediated transformation into rice. Furthermore, this simulation shows that *Nco*I and *BstE*II cleavage sites are not blocked by Dam methylase and Dcm methyltransferases after cloning. Therefore, *E. coli* DH5α can be used for transformation of the pCAMBIA1303-OsBBX6 recombinant plasmid. Taking together, *in-silico* analysis revealed that *OsBBX6* have a putative role in drought and salinity stress regulation and could be a possible candidate to develop multi-stress tolerant rice.

Keywords: Cloning, BBX genes, Simulation, *In-silico*, Promoter

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