Cloning and Characterization of a Molecular Marker Associrance from Soybean Cultivarsated with Salt Tolerance

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Introduction

Much progress has been made towards elucidating the underlying mechanisms controlling salt tolerance in plant (M. R. Foolad et al., 1997; H. Pakniyat et al., 1997). These studies led to the analysis and isolation of genes that encode proteins related to salt tolerance. In previous study, we applied DAF (DNA Amplification Fingerprinting) to screen two salt-tolerant soybean cultivars (Morgan and Wenfeng No.7) and two salt-sensitive ones (Hark and Jackson) and found three polymorphic markers (8.6f/350bp, 8-27/240bp and 8-15/215bp) which only appeared in salt-tolerant cultivars (Zhong et al., 1997). In the present work, we describe the cloning and characterization of the specific marker 8-27/240bp in order to further study whether it plays an important role in salt tolerance of soybean.

Materials and methods

Cloning

The cloning process was carried out according to the methods mentioned in Molecular Cloning (Sambrook et al., 1992). The specific marker 8-27/240bp DNA fragment was reamplified after cut from the polyacrylamide gels. After reamplification, the resulting bands were gel-purified on a 6% polyacrylamide gel. The above steps were repeated several times until a distinct band

appeared. The distinct band was purified by using the Wizard PCR preps DNA purification system and then ligated into the plasmid vector pGEM-T (Promega Inc.). E. coli DH5a competent cells were transformed with the ligation materials and grew on LB plates with color selection. The plasmid DNA was isolated from white colonies and digested with restriction enzyme BglI.

DNA sequencing

The DNA sequencing was carried out using Perkin Elmer ABI Prism

377 sequencer (version 2.1.1) in the Kun-ming Institute of Zoology, Chinese Academy of Sciences. It was sequenced through Prism Ready Reaction Dye-Deoxy Terminator Cycle Sequencing Kit according to the dideoxy-nucleotide chain termination method (Sanger, F. et al., 1977) and sequencing reactions were resolved on 4% polyacrylamide gels.

Computer analysis of sequences

The DNA sequence was analyzed using BLASTn program from the GCG package and compared with the Gene Bank and EMBL nucleic acid database.

Results and discussion

Sequencing results

The sequence of the target DNA fragment was obtained by sequencing (figure 1 and table1).

DNA sequence analysis

A search of the available gene banks using the BLASTN program indicated that the sequence had homology to a number of DNA sequences. It showed it had high similarities to about 250 genes that are from plants, animals or microbes producing high-scoring segment pairs, particularly the sequences from plants such as Oryza Sativa MADS-box protein, Lycopersicon esculentum polygal, Lactuca sativa T-DNA insertions etc. Homology searches of the sequence databases indicate that it had significant homology

to Oryza Sativa MADS-box protein (MADS3) mRNA (length=1361bp). The region made of 15 " TA" in the queried sequence shows 100% identity to the region made of 23 "TA" near the 3'-end in the MADS3 genes. Although the function of the 23 "TA" is not known, we know that Oryza Sativa MADS-box genes that encode regulatory proteins play important roles in flower morphogenesis (Hong-Gyu Kang et al., 1995). They have been highly conserved during evolution and mostly play significant roles in the regulation of these genes (Hong-Gyu Kang et al., 1995). From the above results we deduced that perhaps the queried sequence is not a gene, but a part of a regulatory factor. It maybe plays an important role in regulating the transcription and expression of salt-tolerant genes. Further computer analysis shows that the nucleotide composition of the sequence is very A+T rich, about 65%. This also indicated that it is not a gene but a regulatory region.

Further study required using the specific sequence as a probe to screen the cDNA library and gain its related sequences. Insight into the function of the specific sequence might be gained by transferring it into the salt-sensitive soybean cultivars and observing changes in salt-stress and cell physiology.

References

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(AGRICOLA)
Table 1: The sequence of the target DNA segment

1
CCTCG TGGGG GATGA TATGT ATGTG TGTGT GTGAT GTGTG CTATA

46
TATAT ATATA TATAT ATATA TATAT AATCT ACTAA CTGAT CAATT

91
GGNCC CTCAC AAAAT CTCCG TCTGA TACTG AATTA CTTAT AGNGA

136
TAAAC ANGGT TTTCT GATAG AAAAA CCNGA TTAAT AAACA

176
TAAAT GTATG ATTAC TNTCA GGGTT GTGGG TCTNA AAGGT GTNCA

221
GCCAC GAGGA ATCAC TAGTG CGGNC