Cloning of DREB Transcription Factor from Buffalo Grass

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Key words : *Buchloe dactyloides* , DREB , gene cloning , drought resistance , stress

Introduction Buffalo grass (*Buchloe dactyloides*) is a fine turf grass which needs less intensive management and has strong resistance against adverse environment . Addressing the molecular mechanism that buffalo grass resists the drought is very important to take full advantage of its adversity-resistance . Separation and clone of genes related to drought stress is an important step to clarify the mechanism .

Materials and methods Total RNA was extracted from fresh Buffalo grass induced by 20% PEG for 10 days . A pairs of degenerated primers was designed based on the multiply alignment of DREB amino acid sequence from seven other species to amplify the middle fragment . Up special primer was design for 3 race and down special primer was designed for 5 race based on the middle sequence . SMARTTM technology was used to amplify 3 and 5 ends of DREB gene .

Results and discussions A 209bp middle fragment was acquired by using the degenerated primer pairs . It was confirmed as the DREB fragment after Blast . Based on the sequence of the middle segment , primer designed for 3 RACE and a 653bp segment was acquired ; another primer designed for 5 RACE and a 426bp cDNA segment was acquired . The full-length cDNA gained by overlapping sequences and the analysis of the sequence indicated that it contained an open reading frame encoding a protein of 254 amino acid with a predicted molecular mass of 38 .75 kDa and isoelectric point of 5 .85 . Compared Blast with DREB of other plants , the amino acid sequence has the best similarity to Cynodon dactylon(AAS46285)(with 89%) . The result indicated the gene cloned from Buffalo grass was a DREB gene named as BdDREB2 . The GenBank accession number was EF512460 .

Conclusions Gene clone is a prerequisite for subsequent analysis , such as protein function analysis . There were several methods to amplify 3 and 5 cDNA ends . Since the utilization of SMART RACE technology , RACE became simpler , especially 5 RACE . DREB gene coding Dehydration Responsive Element Binding protein is induced by drought and salt stress . Cloning of DREB transcription factor from drought stressed Buffalo grass was useful to uncover the molecular mechanism of Buffalo grass drought resistance and to improve the drought tolerance of other species .

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