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Identification of DREB homologous genes in bread wheat via CODEHOP PCR primer design

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Abstract

In this study, we exploit the useful described CODEHOP primer design and RT-PCR strategy for targeted isolation of homologues in large gene families. The method was tested with two different objectives. The first was to apply CODEHOP strategy for design degenerate oligonucleotide primers in a broad range of plant species. The second was to isolate an orthologus of the transcription factor of dehydration-responsive element binding protein (DREB) and to determine the complexity of gene family in bread wheat. We used a new primer design strategy for PCR amplification of unknown targets that are related to multiply-aligned protein sequences. Each primer consists of a short ٣′ degenerate core region and a longer ٥′ consensus clamp region. Only ٣-٤ highly conserved amino acid residues are necessary for design of the core, which is stabilized by the clamp annealing to templates molecules. This provides the possibility of isolating numerous additional DREB genes by Polymerase Chain Reaction (PCR) with degenerate oligonucleotide primers. The relationship of the amplified products to DREB genes was evaluated by several sequence and genetic criteria. Present data show that expression of DREB and its homologues, is induced by low temperature stress. Towards this step, it found that the expression of DRE-regulated genes increased freezing tolerance in plants. © ٢٠٠٨ Asian Network for Scientific Information.

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Author keywords

CODEHOP strategy; Cold stress; DREB; Expression pattern; Triticum aestivum L.

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EMTREE drug terms: primer DNA; transcription factor; vegetable protein

EMTREE medical terms: amino acid sequence; article; chemistry; genetics; methodology; molecular genetics; nucleotide sequence; polymerase chain reaction; sequence alignment; sequence homology; wheat

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