

1 **1: Amplification of full length LcMYB2**

2

3 **A: 5'-RACE for *LcMYB2***

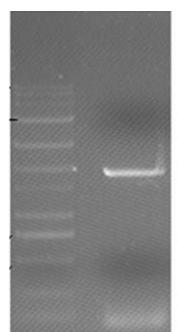
4 The first-strand cDNA was synthesized with SMARTer™ RACE cDNA Amplification Kit (Clontech).A
5 gene specific primer and a universal primer was used for 5'-end amplification.

6 Primers:

7 5'GSP: 5'- GACAGGCATATAAGGGTATTTC -3'

8 UPM: 5'- CTAATACGACTCACTATAAGGGCAAGCAGTGGTATCACACGCAGAGT -3'

9 5'- CTAATACGACTCACTATAAGGGC -3'



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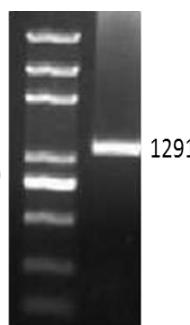
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12 **B: Full length amplification of *LcMYB2***

13 A putative full-length sequence of *LcMYB2* was amplified using the specific pair of primers:

14 LcMYB2-F: 5'-ATGACGAGGCGGTGCTCGCAC-3'

15 LcMYB2-R: 5'-GACAGGCATATAAGGGTATTTC-3'



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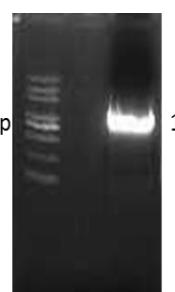
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18 **C: ORF region amplification**

19 Primers:

20 LcMYB2-ORF-F: 5'-ATGACGAGGCGGTGCTCGCAC-3'

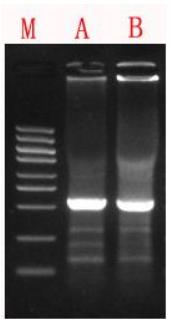
21 LcMYB2-ORF-R: 5'-TCAAACCTGCATGGATTGGGCTG-3'



2: Amplification of LcDREB2 promoter sequence

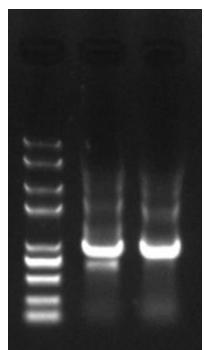
Previous studies revealed that *Leymus chinensis* has closer relationship to *Hordeum vulgare* and *Triticum aestivum* [1], therefore, we search the whole-genome shotgun contigs (WGS) of *Hordeum vulgare* and *Triticum aestivum* with *LcDREB2* (JF754585) and find that partial sequence locating at 5'-end of *LcDREB2* is aligned to gi_413832468 (*Hordeum vulgare*) and gi_439956959 (*Triticum aestivum*) (Fig. 1). Two primers (P1, P4) were designed according to the conserved sequence of *Hordeum vulgare* and *Triticum aestivum* and were used to amplify the putative promoter of *LcDREB2* using total genomic DNA of sheepgrass as template. A fragment about 500bp was amplified and cloned into pMD18-T vector for sequencing (Fig. 2). Three independent clones were sequenced and used to assemble with *LcDREB2* (Fig. 5). According to the assembling sequence (AS1), two primers (P190, P268) were designed to amplify the upstream unknown sequence based the Self-Formed Adaptor PCR [2]. A fragment of about 1000bp was obtained (Fig. 3), ligated into pMD18-T vector, sequenced and assembled with AS1 to get the new sequence (AS2) (Fig. 5). Based on the same method, we acquired another fragment using primers P561 and P784 (Fig. 4). At last, all the sequence were assembled together to form a putative complete *LcDREB2* sequence (Fig. 5). At this time, a primer (F7) picked out in the putative promoter region and another primer (R1821) picked from the coding sequence of *LcDREB2* were used to confirm our previous assembly result. We successfully amplified a 1500bp fragment and sequence analysis revealed that about 200bp of this fragment is the same as the 5'-end of *LcDREB2* (JF754585) (Fig. 6, 7).

Fig. 1 Sequence alignment of *Leymus chinensis* (*LcDREB2*), *Hordeum vulgare* (gi_413832468) and *Triticum aestivum* (gi_439956959)



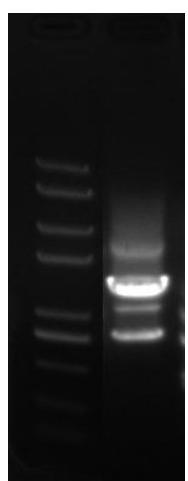
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Fig. 2 The single main band on 1% agarose gel showing the products amplified with P1 and P4
M: BMD5000+1.5k ladder, the bands indicate 5, 3, 2, 1.5, 1, 0.75, 0.5, 0.25, 0.1 (kbp) from up to down



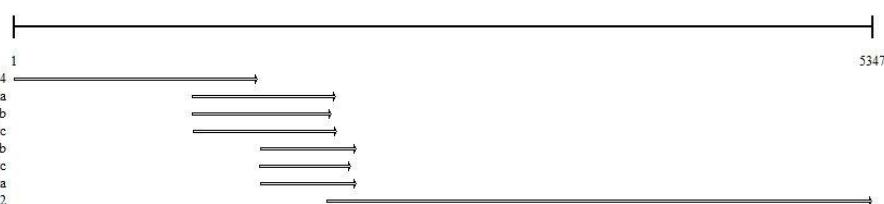
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Fig. 3 The single main band on 1% agarose gel showing the products amplified with P190 and P268



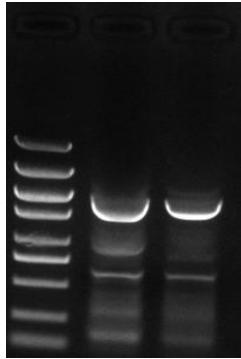
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Fig. 4 The single main band on 1% agarose gel showing the products amplified with P561 and P784



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Fig. 5 The assembly result of putative promoter sequence and transcriptional region of *LcDREB2*



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64 Fig. 6 The single main band on 1% agarose gel showing the products amplified with F7 and R1821
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Leymus chinensis dehydration-responsive element binding protein 2 (DREB2) gene, complete cds, alternatively spliced
Sequence ID: [gbJF754585.1](#) Length: 3396 Number of Matches: 1

Range 1: 1 to 192 GenBank Graphics					▼ Next Match	▲ Previous Match
Score	Expect	Identities	Gaps	Strand		
355 bits(192)	9e-94	192/192(100%)	0/192(0%)	Plus/Plus		
Query 1590	TTTCCGAGTTTCTTtctctccatccacgccttccccctctc	ACGAAGTCGAC	1649			
Sbjct 1	TTTCCGAGTTTCTTCTCTCCACGCCCTCCCCCTCTCAGAAGTCGAC		60			
Query 1650	GCAGCGAAGAAAACAGGCACAAAGATAGCGAACCTAGATACTCTGGACCCGATCCGGATC		1709			
Sbjct 61	GCAGCGAAGAAAACAGGCACAAAGATAGCGAACCTAGATACTCTGGACCCGATCCGGATC		120			
Query 1710	GGGTGGCCATGACGGTAGATCGGAAGGATGCGCGGGGCCCTCTGCGCCCTTCGAG		1769			
Sbjct 121	GGGTGGCCATGACGGTAGATCGGAAGGATGCGCGGGGCCCTCTGCGCCCTTCGAG		180			
Query 1770	ATCCCAGCGCTC	1781				
Sbjct 181	ATCCCAGCGCTC	192				

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67 Fig. 7 Result of blastn in NCBI using the sequence amplified with F7 and R1821
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70 Primers used in this experiment

71 P1: 5-CCTTCCGATCTACCGTCAT-3

72 P4: 5-TCTCACAGTCCACCGTGTC-3

73 P190: 5-AGGCGTGGAGGAGGAGAGAAAGA-3

74 P268: GATACTTCGTGCTCCTGCNNNNNNNNNTATC

75 P561: ATCAGTGGTGGTAAGCGTGGAGTC

76 P784: TTGCGGAGTTGGAGAAGCNNNNNNNNCCAGC

77 F7: TCTCCAGCTGACGCAACCGCG

78 R1821: GAGGCCGGGATCTCGAAGG

80 Promoter sequence and partial CDS amplified with F7 and R1821 primers

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TCTCCAGCTGACGCAACCGCGTGCCTGGCGCGTCAACTGATGGTCAGAAAACCGTCAGATCCC
TCGGTAGGGGTCTGGCGTAGCTGGAAGTCTCAGAACGGAGGTCGACGAGGATTTCACCCAG
GTTCGGGCCTCCGGAGAGTAATACCCTACATCCTGCTCGTGTGTTATTCATAGTAGAGGGATAC
CTCGTGCAGGGGTTACAATGGTGTATGAGATGACTACCGAGAGTTGTTCTACCGTAGTAG
ATGGGAATGAGAGTTCCCTGGCCTCCCTTATATACTCGAGGAGGCTAGGGTTTACTGAGAGAG
GAAACCGATCTAGGTTGCCACCGCACCAACTGGAGGTCAAGTTGCCGCATGGGTTTATCC
TTATCTCGGGCTCCCTTGTCACTGGGCTTGCAGCAGGCTTCTGCGGGGCTC
CCCTCGATGAGCCACCCCCGGTGAACATCAAATTGTCACCCTACTCGTCGCGAATCCATTCCAT
CATAGTGCACAACCTATTGTCATTAACAAAGGAGATCGAAAATTACAGTCATGGCCGGACTA
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90 GAGCGCTCTGCTCTGCGCTGGAGCATCAGGGCTTGGGGCACGCTGCTGGGTGGAACCCGGA
91 GGAGAGGAGGCCGGCGGGCTAGTTGCGGTGGCGCCACATCGCAGCTACGTGCTAACGTGGGG
92 TGGCAGTGGCGCGTAACCCTAGCGCTCGGGCTGGAGAAAGAACGAGAGGAAGAAGTCATGC
93 GTGACCAGAGATGGCAGCCCCTGTTCTCGCGGTGGCATGCTTAAAATTAGCGAAGTAC
94 CAAACCGTGCATTTTCACTTGGCGGTGGTAGGGGGTGAATTGTGTGGACTCCATGAAAATG
95 GAGAATTCTGTGGCGCTGGGAATACCCAGCTTCTCCAACCTCCGCCAAAAAATGCACTGAGGCCA
96 ATACAGCTTCTCGAATTAGCTCGCAGAGCTCAGGCCTCGGCCAGCTCACACCGGGAGT
97 TCGCGAAGTGCAGAGCTGGAGAAGGTTCAGAACACAAGACCCTGGGGACGGATTGTCACGAA
98 TGTAGATGCTCTAAGGAAAAGGAAAAGGTGTGGAGTTGCTCTCGGGACCGGAGAGTGGACTC
99 CACGCTTGCACCACGTATCCATGCAACAAAGATGTCTCACAATCCATCTTGTCACTCGACTGCA
100 CACGTGTCGCCTCCATATCGACCCAGCCCCAGCAATTGCCATACGTGGCCTACACGTGGA
101 ACCCACCCAGAGGAGCACGTCCAATCATAACAAAAAAAGGAAGTATAACAAATACTGGAAAGC
102 GTAAAGGAGAGGCCGAGTTCTCGCACCAAGTCTCCGGAAGTTCCCCGCACCACGGCACT
103 ACTTACGTCACCGCGCCCGAGTCTCACCTCCCACGCACCGCGCCACGTGGACCGTGTCACTTA
104 CGACTGGGCCAACCGGCCAACGTCCGGACCGAGTGACCTAGATAATTCCGGGGCAGGAG
105 CACGAAGTATCCGACCCGGGTATAAATAGGCACCGCCGCGTGGCCCTGGGTTCCGAGT
106 TTTCTTCTCTCCCTCCACGCCCTCCCCCTCTCTCACGAAGTCGACGCAGCGAAGAAAAC
107 AGGCAGACAAGATAGCGAACTCTAGATATCTGGACCCGATCCGGATCGGGTCGCCATGACGGTA
108 GATCGGAAGGATGCGCGGGCCCTCTGCCCTCGAGATCCGGCGCTC

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