2BS/2RL 밀 계통의 cDNA library 구축과 발현 유전자의 염기서열 동정

장 철성*, 서 용원, 홍 병희, 박 용학, 김 진백
고려대학교 식량자원학과

cDNA Library Construction and Expressed Genes Sequencing of the Wheat(*Triticum aestivum* L.) Germplasm Possessing 2BS/2RL

Jang C.S.*, Y.W. Seo, B.H. Hong, Y.H. Park, J.B. Kim,
Department of Agronomy Korea University

1. Objectives

The purpose of this study is to construct cDNA library and to identify genes expressed of the wheat(*Triticum aestivum* L.), 2BS/2RL, which is resistant to Hessian fly, biotype L. and possesses favorite agronomic characteristics.

2. Materials and Methods

The wheat germplasm used for cDNA library construction was obtained by backcross introgression $BC_3F_{3:4}$('Coker'797*4/'Hamlet'). Total RNA was extracted from 4 weeks old leaves using Trizol reagent(GibcoBRL). Poly(A)$^+$ RNA was separated from total RNA using PolyATract mRNA isolation system(Promega). cDNA library of 2BS/2RL was constructed with ZAP-cDNA GigapackⅢ cloning kit(Stratagen). Inserted DNA was amplified by PCR with two primers(T3 and T7 promoter primers) and separated in 1% agarose gel. Expressed genes were prepared by BigDye terminator cycle sequencing ready reaction kit(Perkin Elmer Applied biosystems) for sequencing.

3. Results and Discussion

1. The recombinant efficiency of cDNA library was more than 90%. Plaque forming unit(pfu) of primary library was 0.9×10^6. After primary stock was amplified, pfu was 3×10^9/㎖ in SM buffer(total buffer volume; 120㎖).

2. Inserted DNA sizes of randomly selected clones were 0.4Kb to 2.0Kb(ave. 0.98Kb) with 21 clones being larger than 0.9Kb.

3. The expressed genes were identified 17 clones that had a significant homology(score > 80) to a known amino acid sequence, and 2 clones to a known nucleotide sequence in the NCBI database. Eleven of clones were found that they were not homology to both amino acids and nucleotide sequences.

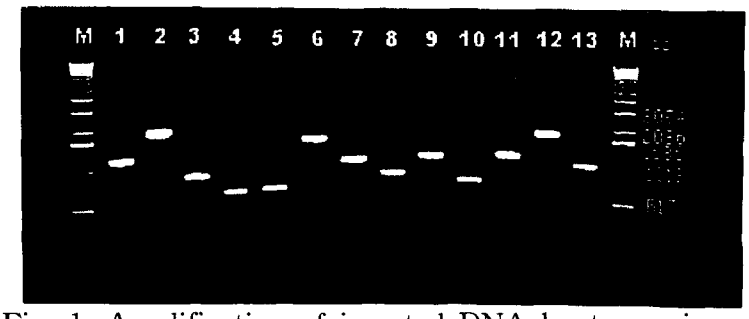

Fig. 1. Amplification of inserted DNA by two primers (T3 and T7 promoter primers). Lane 1 through 13; clone 1, 5, 14, 16, 18, 19, 20, 22, 23,26, 27, 28, and 30. M; Molecular size marker.

Table 1. Putative identification of randomly selected clones by single pass sequencing.

No.	Size	Putative identification	score†	Species¶	No.	Size	Putative identification	score	Species
1	1.0†	Phenylalanine tRNA synthetase	175	H.S.	16	0.5	Unknown		
2	0.6	Unknown			17	0.5	Putative calcium-dependent ser/thr protein kinase	116	A.T.
3	1.6	Unknown			18	0.5	LHC I	533§	H.V.
4	1.3	Chlorophyll a/b binding protein	195	T.A.	19	1.3	Unknown		
5	1.8	Rubisco activase	177	H.V.	20	1.0	Predicted protein	193	A.T.
6	1.5	Unknown			21	0.4	Unknown		
7	1.0	Rubisco activase	168	H.V.	22	0.8	Glyceraldehyde 3-phosphate dehydrogenase	238	H.V.
8	0.9	Unknown			23	1.0	Unknown protein	110	A.T.
9	1.0	Chlorophyll a/b binding protein	152	T.A.	24	0.9	Unknown		
10	0.9	Putative protein	98	A.T.	25	1.2	Putative dTDP-glucose 4-6-dehydratase	240	A.T.
11	0.9	Pollen specific protein C13 precursor	101	Z.M.	26	0.8	PS I reaction centre subunit precursor	186	H.V.
12	0.8	PS II 10KD polypeptide precursor	138	H.V.	27	1.1	LHC II type I protein	138	H.V.
13	0.4	Thioredoxin peroxidase	331§	S.C.	28	1.8	Pyruvate kinase, chloroplast isozyme A precursor	200	R.C.
14	0.9	Unknown			29	1.0	Chlorophyll a/b binding protein	195	T.A.
15	0.5	Unknown			30	1.4	Unknown		

†cDNA insert size(kbp), ‡Amino acid homology score, §Nucleotide homology score, ¶Species abbreviations: H.S. *Homo sapiens*, H.V. *Horedeum vulgare*, T.A. *Triticum aestivum*, A.T. *Arabidopsis thaliana*, Z.M. *Zea mays*, S.C. *Secale cereale*, R.C. *Ricinus communis*.