# D7 2BS/2RL 전좌 계통 선발을 위한 AFLP marker 및 STS marker 개발

장철성, 김동섭, 김진백, 부소영, 서용원†
고려대학교 식량자원학과

# Development of AFLP markers and STS markers to select gemplasms carrying 2RL

Jang, C. S., D. S. Kim, J. B. Kim, S. Y. Bu, and Y. W. Seo†
Department of Agronomy Korea University

## Objectives

Objectives of this study were to identify 2RL specific polymorphism by AFLP analysis using near isogenic lines which carried resistant gene on biotype L of Hessian fly and to develop sequence-specific PCR markers which were easy used to select 2BS/2RL germplasm by wheat breeders.

## Materials and Methods

Plant materials : Coker 797, Near isogenic line carrying 2RL, and Hamlet.

Methods : AFLP analysis was conducted using AFLP Analysis System (Life Technologies). Sixty-four primer combinations were used for selective amplification. Polymorphic DNA fragments were ligated into pGEM-T Easy Vector Systems (Promega). Insert DNA was amplified with T3 promoter primer or SP6 promoter primer (Promega) using the BigDye terminator cycle sequencing ready reaction kit (Perkin Elmer). In order to develop STS marker systems oligo primers were synthesized by sequence obtained from the *Eco*R1 adapter at one end and the *Mse*1 adapter at other end (Table 1).

## Results and discussion

1. As expected to derive from 2RL, twelve of reproducible polymorphic fragments were generated by nine primer combinations between Coker 797 and near isogenic line carrying 2RL (Fig. 1).

2. Sequences of small fragment out of two DNA fragments generated from the primer combinations of E+AAC and M+CTA showed high significant homology with two sequences. One is rye specific repeated sequence known as R173-1. Other is Wis-2-1A retrotransposon like element of wheat.

3. As result of PCR amplification for developing STS markers, only SJ07 and SJ09 out of twelve STS primer combinations were represented to polymorphism between Coker 797 and near isogenic line. As result of amplification with SJ07 primer combination, the PCR products were generated in two rye cultivars, tiriticale, 1RS & 2RL translocation, near isogenic line, and Hamlet, but not in Coker 797, Geumgangmil, 6RL translocation, and 1RS translocation (Fig. 2).

† Tel : 02-3290-3005, E-mail : seoag@kuccnx.koera.ac.kr

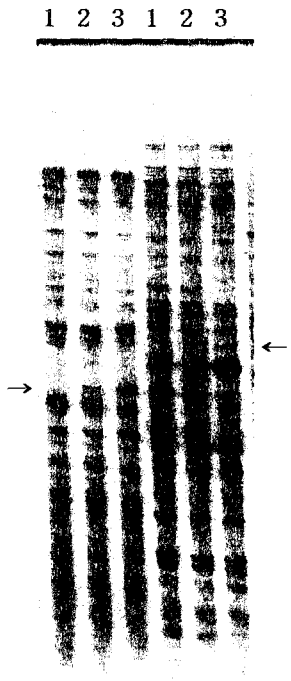


Fig. 1. Variation in AFLP patterns between Coker797, near isogenic line, and Hamlet. Lane 1: Coker797, 2: NIL, 3: Hamelt.

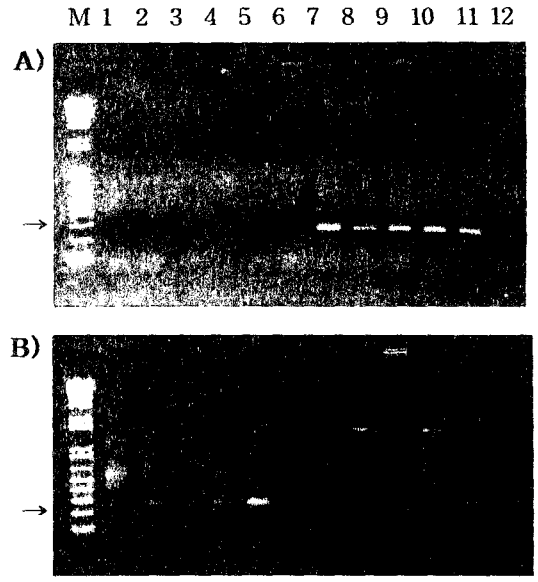


Fig. 2. PCR products amplified with SJ07(A) and SJ09(B) primer combinations. Lane 1: Coker797, 2: Geumgangmil, 3~4: 6RL, 5~6: 1RS, 7~8: rye 9: triticale, 10: 1RS&2RL, 11: near isogenic line, 12: Hamlet. M: 1 Kb ladder.

Table 1. Profile of primer combinations which were detected to polymorphism between coker and nearisogenic line possessing 2BS/2RL through AFLP analysis and sequences of STS primers derived from polymorphic DNA fragments.

| AFLP primer† | Size (bp)‡ | STS primer§ | AFLP primer | Size (bp) | STS primer |
|---|---|---|---|---|---|
| EACT+MCTC (SJ01) | 223 | GAATTCACTCCCTACTTGGC TTAACTCAACAGATGTTGCC | EACC+MCTC (SJ07) | 266 | GAATTCACCGACCAGTTG TTAACTCCAAAGAACCATGT |
| EACT+MCTC (SJ02) | 222 | ACTCTTCAGAACTCATTCGTGC CTCGGCAGAGTCATATTGGTTGCA | EACC+MCTC (SJ08) | 253 | ACCGCATTGGTTCTCGACCCAATC CTCCTTGCTTCGGAAGTTATCCGG |
| EAAC+MCTA (SJ03) | 207 | GAATTCAACTGGCCGGTG TTAACTATGCAGCCAGGC | EACG+MCAA (SJ09) | 264 | GAATTCACGATCATCTTTCC TTAACAAGGAGGGGGGGCAA |
| EAAC+MCTA (SJ04) | 200 | GAATTCACAGGTGTAAGCT TTAACTAGGTCGTGTCCGATCA | EACG+MCAT (SJ10) | 239 | GAATTCACGGTGCTTTCT TTAACATGTCGCGGCAAT |
| EAAG+MCTA (SJ05) | 226 | GAATTCAAGAACTCCAAT TTAACTACTTCGGATTGC | EACG+MCTC (SJ11) | 269 | GAATTCACGTGCGTGTTGG TTAACTCAAAGGTCAAAACT |
| EAAG+MCTT (SJ06) | 409 | GAATTCAAGCATAACATCCG TTAACTTGCCGGCATGTTTG | EAGG+MCTA (SJ12) | 269 | GAATTCAGGCGGTGGTGGGT TTAACTAACATTAGGATCCC |

† Primer combinations which were detected polymorphism, ‡ Size of DNA fragment represented polymorphism, § Sequences of STS primer derived from polymorphic DNA fragment.