D6 1AL/1RS 밀-호밀 전좌 계통의 PCR 표지인자를 통한 검정

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Identification of wheat-rye translocation stocks (1AL/1RS) by sequence specific PCR marker

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1. Purpose

The purpose of this research was to develop sequence specific marker system for the identification of wheat-rye chromosomal translocations presently encounted in wheat breed population.

2. Materials and Methods

—Materials

- K-14 (1AL/1RS translocation line) and "Geumgangmil"
- Forty-two $F_{2:3}$ plants from cross between cultivar K-14 (1AL/1RS) and "Geumgangmil"

—Primer sequence

- RAPD primer : Sixty-eight of 10-mer primer (UBC)
- SCAR primer : Oligo-primer sequences derived from primer 336(5'-GCCACGGAGA-3')
  5'-GCCACGGAGACGAACCGAGT-3'
  5'-GCCACGGAGAGATGTGGAGA-3'

3. Results and Discussion

Ⅰ. One out of 68 UBC primers gave a polymorphic product for 1RS translocations.

Ⅱ. The polymorphic band of 300 bp was cloned and sequenced. Based on the sequence data, the sequence specific 20-mer primers were synthesized.

Ⅲ. To verify 1RS as the origin of the PCR marker, cosegregation of the amplified product with sequence specific primers and secalin subunits was examined in 42 F_2 plants from cross between K-14 and "Geumgangmil" The PCR product showed complete cosegregation with secalin subunits which were encoded by the genes located on 1RS. Therefore, RAPD and SCAR marker should be useful to plant breeders as an alternative method for identifying 1AL/1RS wheat-rye translocation stocks.

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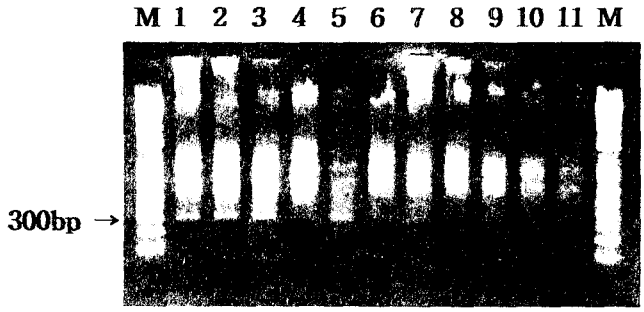

Fig 1. Amplification of the 300 bp polymorphic DNA fragment by UBC primer-336 (5'-GCCACGGAGA-3'). Lane 1 ; K-14, lanes 2 and 3 ; K-14 homo, lanes 4~7, K-14 & "Geumgangmil" hetero, lanes 8~10 ; "Geumgangmil" homo, lanell ; " Geumgangmil", M ; 1kb DNA molecular weight marker (GIBCO BRL).

GGCCGCGAATTCACTAGTGATTGCCACGGAGACGAACCGAGTGTAGTCGAACAAATCCTCACGATCGCAA
CGAAACAGGAACTAACGAGAAGAAGCAAACAACATGGTAAACACACCCCACATAAACAAGGCATGATGCT
CAACCAAGTATGATGCATGACANGGCTACATGATTCAAAACATGGCAAGAGATGAAGCTCACAAGATCAA
CACACAAAGCAAGTACAACCCATTTTCTAGGGGAAATGGCGACTCCCACCTCTCCACCGCCACCTTAACC
CTAGCCACCACCACCACCGGCAATCTCCACATCTCTCCGTGGCAATCGAATTCCCGCGGCCGCCA

Fig 2. Sequence of the amplified products with primer 336 (UBC). Dark region showed the sequences of pimer 336, and underline indicated sequence specific primer sites.

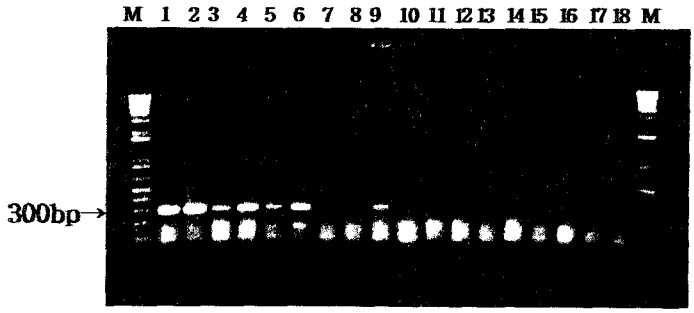

Fig 3. Amplification of the 300 bp polymorphic DNA fragment by SCAR primer. Lane 1 ; K-14, lanes 2 and 6 ; K-14 homo, lanes 7~12 ; K-14 & "Geumgangmil" hetero ; lanes 13~17 ; "Geumgangmil" homo, lane 18 ; "Geumgangmil", M ; 1kb DNA molecular weight marker (GIBCO BRL).