

C6. Biochemical and molecular characterization of Amigo-derived 1AL/1RS translocated wheat (*Triticum aestivum* L.) lines

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Objectives

The purpose of this study was to identify Amigo-derived 1AL/1RS among numerous 1RS wheat-rye translocations by employing biochemical and DNA marker systems. The possibilities of application of Amigo specific secalin subunit and AFLP conversion of STS marker in the wheat breeding program were discussed.

Materials and Methods

Plant materials: Numerous wheat-rye translocations and non-translocation lines were used to develop rye 1RS specific markers. The wheat-rye translocations are "TAM107" (1AL/1RS), "TAM200" (1AL/1RS), "TAM202" (1AL/1RS), "Century" (1AL/1RS), "TXGH125885" (1AL/1RS), "GRS1201" (1AL/1RS) and "GRS1201"/"Redland" (1AL/1RS), "Siouxland" (1BL/1RS), "Gabo" (1DL/1RS), "Hamlet" (2BS/2RL), "Chochunhomil" (rye), "Chilbohomil" (rye), "Suwon15" (triticale) and "Karl" (nontranslocation line) which is used as control materials.

Each twenty F₃ kernels derived from forty-two F₂ plants of cross between Amigo-derived 1AL/1RS translocation line and Keumgangmil (nontranslocation line) were analyzed through SDS-PAGE.

1D SDS-PAGE: Secalins were separated on 12% SDS-polyacrylamide gel and silver stained.

AFLP analysis: Primer combinations 64 *EcoRI* / *MseI* (methylation insensitive) were applied to develop AFLP markers specific to Amigo derived 1AL/1RS chromosomal translocation.

Conversion of AFLP markers to STS: Targeted AFLP fragment were excised, sequenced and cloned. Based on the sequences of cloned fragment, 12 new primers internal to the AFLP selective primers were designed.

Results and Discussion

Identification of 1RS sepcific secalins (Fig1)

The presence of 70 Kda secalin subunit : Amigo-derived 1AL/1RS translocation lines such as TAM107, TAM 200, TAM202, Century, TXGH125885, Chochunhomil, and Suwon15(triticale)

The absence of 70Kda secalin subunit : GRS-derived 1AL/1RS translocation lines, other translocation lines such as Siouxland(1BL/1RS), Gabo(1DL/1RS), and Hamlet(2BS/2RL).

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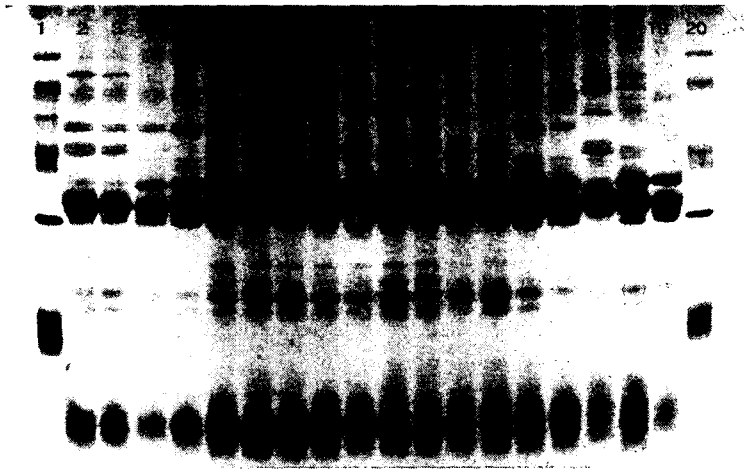


Fig 1. One-dimensional 12% SDS-PAGE separation of 70% ethanol soluble unreduced seed storage protein (prolamins) from various crop species. Lane 1 and 20, Molecular size marker; lane 2, Amigo-derived 1AL/1RS translocation line; lane 3, Amigo homozygous line; lane 4, Keumgangmil; lane 5, Keumgangmil homozygous line; lane 6, TAM 107; lane 7, TAM200; lane 8, TAM202; lane 9, Century; lane 10, TXGH125885; lane 11, GRS1201; lane 12, GRS1201*Redland; lane 13, Siouxland (1BL/1RS); lane 14, Siouxland*N86L177; lane 15, Gabo (1DL/1RS); lane 16, Hamlet (2BS/2RL); lane 17, Chochunhomil (rye); lane 18, Suwon15 (triticale); lane 19, Karl (non-translocated wheat).

AFLP analysis and conversions of AFLP markers to STS: Twenty seven primer combinations produced in the 1AL/1RS specific 42 bands were detected. These polymorphic bands were excised from the AFLP gels, reamplified, cloned and sequenced. Twelve polymorphic bands were converted to sequence specific STS markers. Especially, WRT-04 primer set was amplified only in Amigo-derived 1AL/1RS translocation lines, rye and triticale (Fig 2).



Fig 2. Amigo-derived 1AL/1RS translocation lines specific PCR products amplified with WRT-04 primer set. Lane 1 and 15, Molecular size marker; lane 2, Amigo-derived 1AL/1RS translocation line; lane 3, Amigo homozygous lines; lane 4, Keumgangmil; lane 5, Keumgangmil homozygous lines; lane 6, Coker (nontranslocation line); lane 7, TAM200 (1AL/1RS); lane 8, TAM202 (1AL/1RS); lane 9, Karl (nontranslocation line); lane 10, GRS1201 (1AL/1RS); lane 11, Chochunhomil (rye); lane 12, Chilbohomil (rye); lane 13, Blanco (rye); lane 14, Suwon15 (triticale).

Therefore, both AFLP conversion of PCR-based STS marker and a 70 Kda secalin subunit would be useful as 1RS selectable markers in wheat breeding programs.