Homozygous 1B and 1BL/1RS Chromosome Substitutions in *Triticum aestivum* and *T. turgidum* cultivars

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Accepted March 6, 1996

The 1BL/1RS homozygous chromosome translocation involves the short arm of *Secale cereale* L. chromosome 1R (1RS) and the long arm of chromosome 1B (1BL) of *Triticum aestivum* L. The 1RS chromosome arm possesses leaf, stem, stripe rust and mildew resistance genes (McIntosh 1983, Zeller and Fuchs 1983, Heun and Fischbeck 1987). Presence of the rye arm has been further associated with high yield, stability and wide adaptability of wheat germplasm, specifically for the cultivar Veery cross (Rajaram *et al.* 1983, Villareal *et al.* 1994). The Veery lines were derived from the 1BL/1RS winter bread wheat variety Kavkaz. The translocation was simultaneously and independently identified in Veery “S” by Merker (1982) and Mujeeb-Kazi (1982). Global wheat varietal releases possessing the 1BL/1RS translocation currently occupy over five million hectares of cultivated area. Approximately 40.0% of our germplasm (Rajaram *et al.* 1990) and up to 85.0% of Pakistan wheat varieties in yield trials (Jahan *et al.* 1990, Ter-Kuile *et al.* 1991) possess the homozygous 1BL/1RS translocation.

Recently it was suggested (Villareal *et al.* 1994) that 1BL/1RS wheat varieties exhibit a yield advantage over 1B wheat varieties. Further (Villareal *et al.* 1995) in a two year yield trial study based upon random F₂ derived F₆ advanced lines from a Nacozari (1B)/Seri 82 (1BL/1RS) cross concluded that there was a 4.3% significant yield advantage for the 1BL/1RS derivatives.

We felt that a more stringent test was necessary and through this report describe the production of germplasm that shall more precisely elucidate the effects of the 1BL/1RS translocation in a bread and durum wheat variety. This was made possible by substituting in *T. aestivum* L. cv. Seri 82 (1BL/1RS, 1BL/1RS) a 1B chromosome, and a 1BL/1RS chromosome in *T. turgidum* L. cv. Altar 84 (1B, 1B) by using the backcross procedure. For critical testing of the 1BL effect on yield components the “extracted” Seri 82 and Altar 84 derivatives after eight backcrosses and a selfing were also selected. These “extracted” derivatives may differ from the original breeders Seri 82 and Altar 84 varieties by recombinational changes on the 1BL chromosome arm, a minor but significant feature not met by random F₂ derived F₆ germplasm also. The diagnostic biochemical, molecular and cytological procedures used in development of the substituted germplasm are also elucidated.

Materials and methods

Production and cytological diagnostics

Triticum aestivum L. cv. Seri 82 (1BL/1RS, 1BL/1RS) as the female parent was pollinated by *T. aestivum* L. cv. Pavon 76 (1B, 1B). The resulting Seri 82 × Pavon 76 F₁ (1BL/1RS, 1B) was backcrossed to Seri 82. BC₁ plants were screened by C- or N-banding techniques (Jahan *et al.* 1990, Jewell and Mujeeb-Kazi 1982) for identifying up to three 1BL/1RS, 1B heterozygote derivative plants which were then each backcrossed to Seri 82 again and similarly

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advanced up to backcross VIII (BC₈); Fig. 1. The 1BL/1RS transfer into *T. turgidum* L. cv. Altar 84 (1B, 1B; Fig. 2a) involved a procedure similar to that described above for Seri 82. Altar 84, as the female parent, was initially crossed by Seri 82 to generate the F₁ pentaploid ($2n=5x=35$, AABBDD; 1BL/1RS, 1B). Backcrossing this F₁ pentaploid onto Altar 84 gave BC derivatives from which $2n=4x=28$, 1BL/1RS, 1B plants were identified (Fig. 2b) by chromosome banding and advanced to BC₈ (Fig. 3). Selfing after the last backcross (BC₈) in both cases (Seri 82 and Altar 84) gave progeny from which the following categories were selected:

- Seri 82 with 1B homozygous,
- Seri 82 with 1BL/1RS homozygous; categorized as 'extracted',
- Altar 84 with 1BL/1RS homozygous, and
- Altar 84 with 1B homozygous; categorized as 'extracted'

Biochemical diagnostics

Endosperm halves of the germplasm used for fluorescent *in situ* hybridization (FISH) were utilized for biochemical based identification of 1BL/1RS homozygotes, 1BL/1RS + 1B heterozy-

Fig. 1. Crossing methodology for the substitution of chromosome 1B for 1BL/1RS in bread wheat Seri 82 from the cultivar Pavon 76.

Fig. 2. N-banded partial mitotic spreads of *Triticum turgidum* cv. Altar 84 showing in (a) homozygosity of chromosome 1B and in (b) heterozygous 1B, 1BL/1RS cell of a plant used in further backcrosses for the substitution of 1BL/1RS in Altar 84.

Fig. 3. Crossing methodology for the substitution of chromosome 1BL/1RS for chromosome 1B from a bread wheat Seri 82 to a durum wheat Altar 84.

gotes and 1B homozygotes in both Seri 82 and Altar 84 backcross or selfed derivatives. Glucose phosphate isomerase (GPI; Chojceki and Gale 1982) and gliadin polyacrylamide gel electrophoresis (A-PAGE; Bushuk and Zillman 1978) facilitated additional validation of the homozygous 1BL/1RS and 1B as well as the 1BL/1RS, 1B heterozygote derivatives.

Molecular cytogenetic diagnostics

The plant material used was Seri 82 (1BL/1RS, 1BL/1RS), Seri 82 (BC₈ 1BL/1RS, 1B), Altar 84 (BC₈ 1BL/1RS, 1B) and Altar 84 (1BL/1RS, 1BL/1RS). The heterozygotes and homozygotes were ascertained on respective endosperm halves by biochemical assays except for the 1BL/1RS, 1BL/1RS Seri 82 where the commercial variety was utilized. Seed germination, root-tip collection and slide preparation procedures were essentially similar to those of Mujeeb-Kazi and Miranda (1985).

The FISH procedure utilized the published protocols of Schwarzacher *et al* (1992), Islam-Faridi and Mujeeb-Kazi (1995). *S. cereale* DNA was biotin labelled by nick translation following instructions accompanying the GIBCO-BRL bionick translation kit. Genomic *T. aestivum* cv. Seri 82 and *T. turgidum* cv. Altar 84 DNA sheared by autoclaving at 10 psi for 5 min was used 15 times in excess of biotin-14-dATP labelled *S. cereale* genomic DNA. Two stringency washes were done at 37°C with 30% formamide in 0.5×SSC, followed twice again with 4×SSC plus Tween-20 (0.2%) at room temperature. Biotin labelled *S. cereale* DNA detection utilized fluorescein-avidin coupled with biotinylated anti-avidin for signal amplification using Vector Labs. Inc. protocols.

Results and discussion

Production and cytological diagnostics

When C-banded, the 1BL/1RS chromosome is characterized by prominent bands in the terminal and sub-terminal regions of the short arm (1RS) as well as the centromeric region and terminal region of the long arm (1BL). In contrast, the short arm of chromosome 1B of *T. aestivum* shows a striking banding site variation (Figs. 4a, b). Occasionally a faint interstitial band is prevalent in the 1BL arm of the 1BL/1RS translocated chromosome. When N-banded, the 1BL/1RS translocated chromosome can be distinguished from 1B because of its unbanded

Fig. 4. Chromosome Giemsa banding patterns elucidating the 1RS rye segment from left to right a to f as follows: a) C-banded 1B wheat chromosome, b) C-banded 1BL/1RS translocation chromosome of wheat, c) C-banded 1R chromosome of *Secale cereale*, d) N-banded 1B wheat chromosome, e) N-banded 1BL wheat telocentric f) N-banded 1BL/1RS translocation chromosome of wheat.

1RS arm and copiously banded 1BL characteristics (Figs. 4d, e).

A C-banded 1BL/1RS chromosome can be confused with the 1R rye chromosome substitution if the cross pedigree of the wheat derivatives is unknown (Figs. 4b, c) and N-banding done to ascertain the rye status. In our case, since the cultivar pedigree of (Seri 82) was identified to possess the 1BL/1RS translocated chromosome, C-banding alone was used for screening. The Seri 82 $\times$ Pavon 76 F₁ derivatives of *T. aestivum* were advanced by backcrossing to Seri 82 for producing BC₁ progeny. The heterozygote BC₁ plants (1BL/1RS, 1B) were further backcrossed by Seri 82. Heterozygotes were identified, backcrossed and advanced similarly to BC₈. This BC₈ progeny was selfed and plants homozygous for 1B and 1BL/1RS selected. Both categories of plants in general resembled Seri 82 in phenotype, grain color, maturity and height.

Biochemical diagnostics

The GPI genes are located on the short arms of the homoeologous group I chromosomes of wheat (Hart 1979), and the gene product of chromosome 1BS is observed as the most cathodal band on GPI zymograms. This band is marked for the homozygous 1B wheat cultivar Pavon (Fig. 5a) and in the heterozygous 1B, 1BL/1RS Seri 82/Pavon F₁ hybrid (Fig. 5b). The band is missing in the homozygous 1BL/1RS cultivar Seri 82 (Fig. 5c). When the 1B, 1BL/1RS heterozygotes are backcrossed by the recurrent 1BL/1RS homozygous parent Seri 82, progeny is produced that is 1BL/1RS, 1BL/1RS or 1B, 1BL/1RS. GPI screening of individual endosperm halves detects the 1BL/1RS homozygous progeny which is eliminated whereas the 1B, 1BL/1RS heterozygotes are retained for further backcrosses with Seri 82 until eight are completed (Fig. 1 schematic).

After eight backcrosses a selfing of the heterozygote plants is required for separating the homozygous 1B substitution, homozygous 1BL/1RS extracted Seri 82, and the critical 1B, 1BL/1RS progeny that is identical to the homozygous 1B Seri 82 banding pattern. These heterozygotes have to be identified and discarded. Endosperm halves are subjected to GPI analyses that separates the 1BL/1RS extracted Seri 82 progeny. The remaining endosperm halves which could represent 1B homozygotes or the 1B, 1BL/1RS are subjected to A-PAGE analyses. Seri 82 progeny homozygous for the 1B substitution is separated since it lacks the rye secalin bands

Fig. 5. • Glucose phosphate isomerase banding pattern of grain from (a) *Triticum aestivum* cv. Pavon "S" (1B, 1B), (b) F₁ of cv. Pavon/Seri 82 (1B, 1BL/1RS), (c) Cultivar Seri 82 (1BL/1RS, 1BL/1RS). Marker band for the 1B short arm indicated by an arrow. • Gliadin A-PAGE patterns of the cultivar Seri 82 (1B, 1B) (d), two tracks of the Seri (1B, 1BL/1RS) F₁ (e) and Seri 82 (1BL/1RS, 1BL/1RS) cultivar (f). The rye (1RS) marker bands are arrowed and are within the omega-gliadin region. • GPI banding patterns on IEF polyacrylamide gels for *T. aestivum* cv. Chinese Spring (1B, 1B) (g), a durum based 1B, 1BL/1RS F₁ (h) *T. turgidum* cv. Altar 84 (1B, 1B) (i) and a durum 1BL/1RS, 1BR/1RS translocation line (j). The arrow identified the 1B, 1BL/1RS heterozygote band.

(Fig. 5d). By GPI Seri 82 homozygous for 1BL/1RS derivatives had already been separated and A-PAGE unequivocally identifies the homozygous 1B derivatives. A-PAGE tracks that show the secalin bands (Fig. 5e) are of heterozygotes and discarded.

Wheat cultivars homozygous for chromosome 1B have a GPI zymogram pattern (Fig. 5g) that is identical to that of a 1B, 1BL/1RS heterozygote durum wheat (Fig. 5h). The arrowed diagnostic band in the heterozygote is contributed by the 1RS arm. This is consistent with the findings of Chojceki and Gale (1982) that suggested the GPI-R₁ subunits behaved similar to the GPI-D₁ units. The 1B, 1BL/1RS $2n=4x=28$ heterozygotes were advanced by eight backcrosses to the durum 1B cultivar Altar 84 recurrent parent. Over this advance GPI analyses enabled identification of the 1B homozygotes that were discarded. After reaching the BC₈ generation the heterozygotes were selfed. The GPI analyses on individual endosperm halves identified 1B homozygous altar 84 derivatives categorized as "extracted" Altar 84. The corresponding embryo halves of these seeds were seed increased. The remaining embryo halves were germinated and root-tips analyzed by Giemsa C-banding to identify plants that were 1BL/1RS homozygotes. The heterozygote seedlings were discarded.

Molecular cytogenetic diagnostics

We utilized the FISH molecular diagnostics to ascertain that the BC₈ selfed derivatives

Fig. 6. Fluorescent *in situ* hybridization micrographs using filter sets 02, 09 and 23 of a Zeiss epifluorescence research microscope associated with identification of the 1BL/1RS translocation chromosome in bread and durum wheat germplasm. Photographs on a Fujicolor super HG400 print film. (a) *T. aestivum* cv. Seri 82 homozygous 1BL/1RS (DAPI stain, filter 02) chromosomes arrowed, (b) A backcross heterozygote where Seri 82 is the recurrent parent. The 1BL/1RS heterozygote arrowed (filter 23), (c) The 1BL/1RS heterozygote of a BC Seri 82 in an interphase mitotic cell (Filter 23), (d) a backcross heterozygote where the durum wheat Altar 84 is the recurrent parent. Cell photographs under filter 23 (left) and 02 (right) show the single 1BL/1RS chromosome in the $2n=4x=28$ complement, (e and f) Selfing of the BC₈ Altar heterozygote (1B, 1BL/1RS) showing the homozygous 1BL/1RS derivative. Mitotic cell photographed under filters 02 and 09 respectively with the two 1BL/1RS chromosomes arrowed.

complemented the inferences obtained from the cytological and biochemical data about chromosomal uniformity of physical structure. There appears to be no visible structural change in the 1RS segment of the 1BL/1RS translocation (Figs. 6a–f). The homozygous 1BL/1RS parents Seri 82 and the substituted derivatives of Altar 84 characterize the centric-break association that produced the 1BL/1RS translocation (Figs. 6a, e, f). The 1B, 1BL/1RS heterozygotes (Figs. 6b, d) also represent the normal 1RS arm location, and this single rye chromosome arm is elaborately illustrated in mitotic interphase (Fig. 6c).

Summary

Wheat (*Triticum aestivum* L.) varieties with the 1BL/1RS chromosome translocation exhibit a major role in agricultural production, partly attributed to their high yield advantage. The evidence as to whether the 1RS segment may have genetically contributed to this yield advantage has not been unequivocally demonstrated due to lack of appropriate testor germplasm. This analysis now appears possible through the testing of a bread wheat (*T. aestivum* L. cv. Seri 82) and a durum wheat variety (*T. turgidum* L. cv. Altar 84) in which their homozygous 1BL/1RS and 1B chromosomes have been substituted by 1B and 1BL/1RS chromosomes respectively. Heterozygote (1BL/1RS, 1B) F_1 and backcross derivatives were advanced by eight backcrosses respectively to Seri 82 or Altar 84 with an ultimate selfing to obtain homozygous 1B Seri 82 and 1BL/1RS Altar 84 derivatives. Diagnostic procedure for tracking the 1BL/1RS chromosomes in heterozygous and homozygote derivatives utilized Giemsa C- or N-banding differential staining, electrophoretic biochemical assays and FISH.

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