Rapid detection of 1B, 1BL/1RS heterozygotes in the development of homozygous 1BL/1RS translocation stocks of *Triticum turgidum* (2*n* = 4*x* = 28)

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A biochemical marker was utilized to facilitate detection of chromosome 1B, 1BL/1RS translocation heterozygote plants in segregating backcross progenies during the development of 1BL/1RS homozygous lines in several *Triticum turgidum* L. cultivars (2*n* = 4*x* = 28; AABB). Isoelectric focussing of glucose phosphate isomerase (GPI) on either pH 3.5–9.5 or 5.5–8.5 polyacrylamide gels facilitated the detection of 1B, 1BL/1RS translocation heterozygotes from the homozygous 1B or 1BL/1RS derivatives during each backcross of the heterozygote to the respective recurrent parent. The biochemical diagnostic procedure complements the more time consuming and cumbersome chromosome banding technique. This GPI diagnostic in durum 1BL/1RS development is also swifter than a similar stocks development in *T. aestivum* where both GPI and acid PAGE are essential.

Key words: *Triticum turgidum*, glucose phosphate isomerase, 1BL/1RS translocation, isoelectric focusing.

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Un marqueur biochimique a été utilisé pour faciliter la détection de plantes hétérozygotes via la translocation des chromosomes 1B, 1BL/1RS chez les descendants rétrocroisés en ségrégation, au cours du développement de lignées homozygotes 1BL/1RS de plusieurs cultivars de *Triticum turgidum* L (2*n* = 4*x* = 2; AABB). La focalisation isoélectrique de la glucose phosphate isomérase (GPI) sur gels de polyacrylamide à des pH, soit de 3,5–9,5 ou de 5,5–8,5, a favorisé la séparation des hétérozygotes de translocation 1B, 1BL/1RS des dérivés homozygotes 1B ou 1BL/1RS, lors de chacun des rétrocroisements des hétérozygotes à leurs parents récurrents respectifs. La méthode biochimique de diagnostic constitue un complément à la méthode plus longue et encombrante de la révélation des bandes chromosomiques. Ce diagnostic par la GPI dans le développement de blé durum 1BL/1RS est aussi plus rapide que le développement de souches similaires chez le *T. aestivum*, où la GPI ainsi que l'acide PAGE sont tous deux essentiels.

Mots clés : *Triticum turgidum*, glucose phosphate isomérase, translocation 1BL/1RS, focalisation isoélectrique.

[Traduit par la rédaction]

Introduction

Advanced bread wheat lines, *Triticum aestivum* L. (2*n* = 6*x* = 42; AABBDD), possessing the 1BL/1RS translocation have been attributed with a high yield potential, stability, and wide adaptability (Rajaram et al. 1983). In addition, several important biotic resistances such as *Puccinia recondita* (leaf rust), *P. striiformis* (yellow rust), *P. graminis* (stem rust), and *Erysiphe graminis* (downy mildew) have been associated with the short arm of the rye chromosome 1R involved in the 1BL/1RS translocation. These genes are designated as *Lr26*, *Yr9*, *Sr31*, and *Pm8*, respectively (McIntosh 1983).

In general, the 1BL/1RS wheat cultivars are known to possess inferior dough quality (Dhaliwal et al. 1987) that Peña et al. (1990) consider as not being an exclusive 1RS function. Currently a substantial percentage of CIMMYT advanced bread wheat lines possess the 1BL/1RS translocation (Villareal et al. 1991). No commercial *T. turgidum* L. cultivar possesses the 1BL/1RS translocation but a BC_1 F_2 homozygous 1BL/1RS durum derivative was produced (Friebe et al. 1987) and eventually registered as durum wheat germplasm KS91WGRC14. Because of the desirable agronomic traits associated with the short arm of chromosome 1R and the concept that it may contribute to a yield advantage, a program is underway at CIMMYT to develop in bread wheat (*T. aestivum*) substitution lines to partition the 1RS effects. Simultaneously incorporating 1BL/1RS into elite durum wheat cultivars is also in progress. Such germplasm should unequivocally resolve the 1RS contributions for both desirable and undesirable traits.

The procedure for development of a homozygous 1B line from a 1BL/1RS homozygous *T. aestivum* cultivar essentially requires eight backcrosses (BC_8) of the 1BL/1RS, 1B F_1 heterozygote to the recurrent 1BL/1RS homozygous parent, a selfing of the BC_8 heterozygote derivative and selecting the 1B homozygous derivatives. Both glucose phosphate isomerase (GPI) and acid polyacrylamide gel electrophoresis (A-PAGE) of gliadins are essential as diagnostics for tracking the 1BL/1RS, 1B heterozygote at each backcross generation in *T. aestivum* (William et al. 1992). In durum wheats, however, tracking the 1B, 1BL/1RS heterozygotes at each BC generation only requires the GPI diagnostic methodology and is thus a swifter procedure than chromosome C-banding (Ter-Kuile et al. 1990) or the GPI plus A-PAGE combination used for *T. aestivum* (William et al. 1992). Its application to the development of 1B/1R lines in durum wheats has not been described previously and is the subject of this report.

Materials and methods

A total of eight *Triticum turgidum* (2*n* = 4*x* = 28, AABB) cultivars and their heterozygote F_1 progenies were evaluated. The cultivars Aconchi, Chen/Altar, 86D 21328/DZR, B1A "S", Crox "S"//Chic/Sapi "S"//Yau, BHA (PD10),

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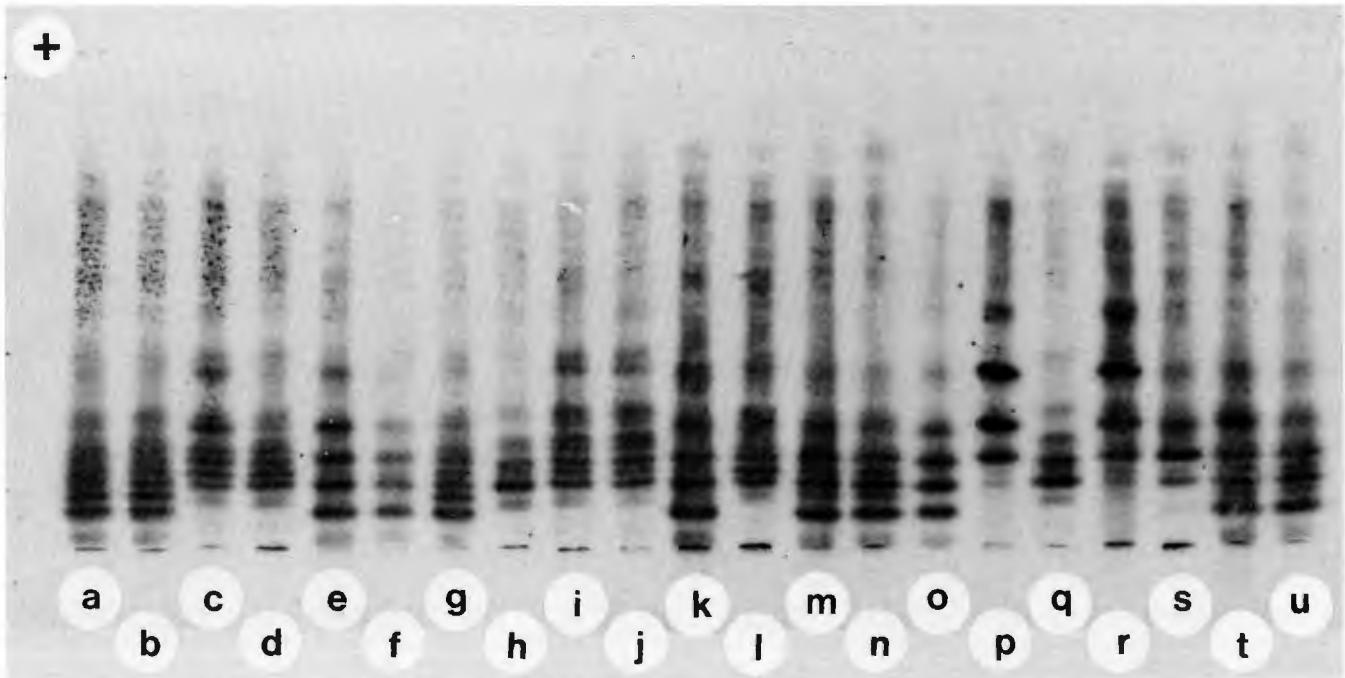

FIG. 1. GPI banding patterns of seed extracts on IEF (pH 5.5–8.5) polyacrylamide gels. Lane a, NIATIB; lane b, NIATID; lane c, NIBTIA; lane d, NIBTID; lane e, NIDTIA; lane f, NIDTIB; lane g, *Triticum aestivum* cv. Chinese Spring; lane h, Imperial rye; lane i, *T. aestivum* cv. Seri 82; lane j, *T. aestivum* cv. Seri 82; lane k, *T. turgidum* cv. Aconchi; lane l, *T. turgidum* cv. Cndo/Veery; lane m, Aconchi//Cndo/Veery F_1; lane n, Aconchi//Cndo/Veery F_1; lane o, *Aegilops speltoides*; lane p, *T. urartu*; lane q, *Ae. squarrosa*; lane r, *T. monococcum*; lane s, *T. boeoticum*; lane t, Aconchi//Cndo/Veery; lane u, Aconchi//Cndo/Veery.

Immer/Altar, and Duergand were homozygous for chromosome 1B. Two 1BL/1RS, 1BL/1RS sources, cv. Cndo/Veery (Friebe et al. 1987) and a BC_8 F_1 1BL/1RS, 1BL/1RS derivative of *T. turgidum* cv. Altar-84 (Ter-Kuile et al. 1990) were crossed onto each of the above homozygous 1B durum cultivars to initiate the homozygous 1B/1R line development. Five parental seeds and their derived F_1's were evaluated. To study the GPI isozyme banding patterns, nullitetrasomic series for homoeologous group 1 chromosomes (source: late Dr. E.R. Sears), *T. monococcum* ($2n = 2x = 14$; AA), *T. boeoticum* ($2n = 2x = 14$; AA), *Aegilops speltoides* ($2n = 2x = 14$; SS), *T. urartu* ($2n = 2x = 14$; AA), *Secale cereale* cv. Imperial ($2n = 2x = 14$; RR), *T. aestivum* cvs. Seri-82 ($2n = 6x = 42$; 1BL/1RS, 1BL/1RS) and Chinese Spring (CS; $2n = 6x = 42$; 1B, 1B), were also included.

Five seeds each of the durum cultivars, F_1 and BC_1 derivatives were cut in half and endosperms directly utilized for GPI analyses. The corresponding embryo halves of each F_1 and BC_1 seeds were saved for planting to verify the heterozygous GPI results by C-banding (Jahan et al. 1990) and to produce BC_2 derivatives by pollinating the verified 1B, 1BL/1RS BC_1 heterozygote plants with their recurrent durum cultivars. For the remaining material evaluated, endosperm halves were ground in 100 μL of 0.5 M Tris–HCl (pH 7.5) buffer, and left at room temperature for 1 h with occasional shaking. After centrifuging (× 10 000 *g*) the samples at room temperature for 5 min, 20 μL of the supernatant was used for isoelectric focusing (IEF) analysis.

Isoelectric focusing

Precast ampholine PAG plates (pH 3.5–9.5) were used for IEF. The anode and cathode buffers were 1 M H_3PO_4 and 1 M NaOH, respectively. Gels were prefocused for 15 min at a constant power of 15 W on an LKB Multiphor flat bed electrophoresis unit. The temperature was maintained at 5°C using the LKB multitemperature 11 thermostatic circulator. Following prefocusing, the seed extracts were applied on paper wicks or the cut endosperm halves were placed directly over the gel 1 cm from the cathode. Isoelectric focusing was continued with the same power setting, with the maximum voltage set at 1300 V for a total running time of 7 h. The paper wicks containing the samples or the cut endosperms were removed after 1 h.

Staining

Staining procedure of Chojecki and Gale (1982) was slightly modified. Staining solution made up of 100 mL of 0.1 M Tris–HCl (pH 7.5), 1 mL 1 M $MgCl_2$, 10 mg fructose-6-phosphate, 7.5 mg NADP, 10 mg dimethylthiazolyl-diphenyltetrazolium bromide (MTT), 2 mg phenazine methosulphate (PMS), and 15 units of glucose-6-phosphate dehydrogenase. Gels were stained in the dark for 3 h at 37°C and then fixed in 10% acetic acid.

Results and discussion

The banding profiles of the homoeologous group 1 nullisomic–tetrasomic series indicate that the most cathodal GPI band results from genes located on chromosome 1B (Fig. 1). Genes for GPI were located within the short arms of homoeologous group 1 wheat chromosomes (Hart 1979). The A genome species *T. monococcum*, *T. urartu*, *T. boeoticum*, and the D genome donor (*Ae. squarrosa*) do not show this most cathodal GPI band (Fig. 1). The debated B genome donor species *Ae. speltoides*, however, possessed the most cathodal GPI band. Chojecki and Gale (1982) established that each *Gpi-1* locus controls the production of at least two enzyme subunits.

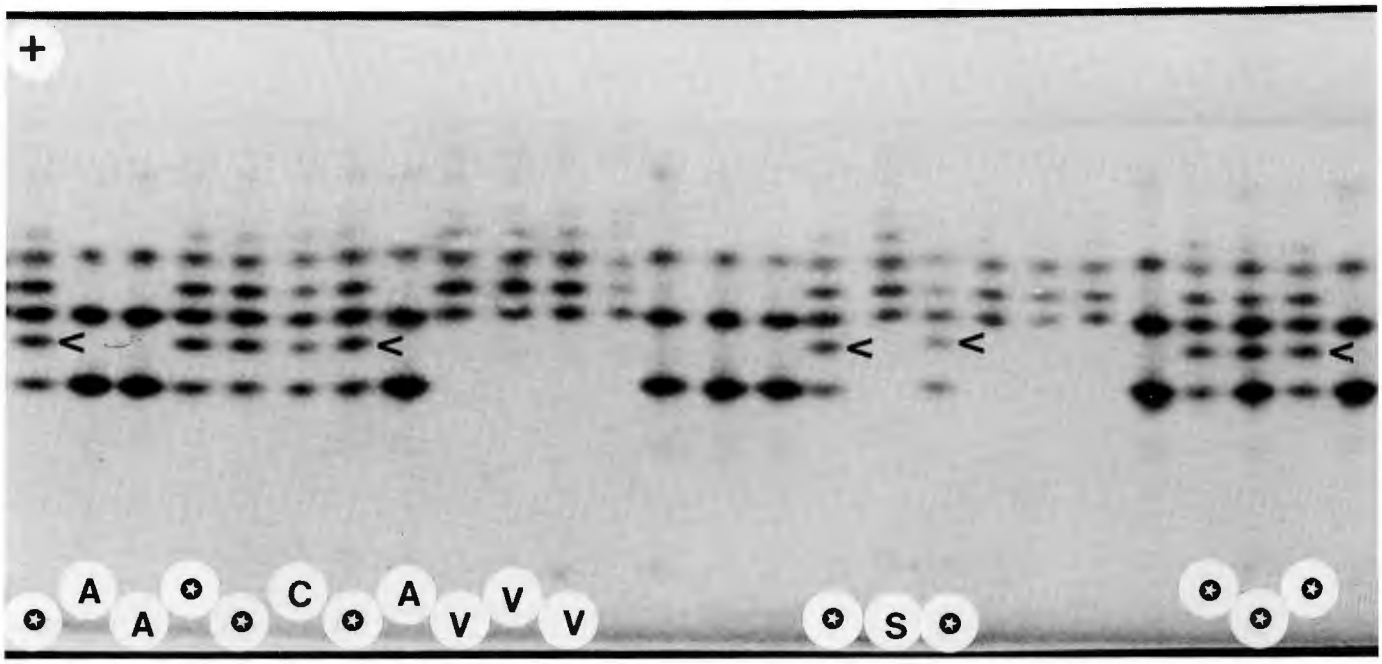

FIG. 2. GPI banding patterns of endosperm halves on IEF (pH 3.5–9.5) polyacrylamide gels. *, F_1 (1B, 1BL/1RS); A, *Triticum turgidum* cv. Aconchi (1B, 1B); C, *T. aestivum* cv. Chinese Spring (1B, 1B); S, *T. aestivum* cv. Seri 82 (1BL/1RS, 1BL/1RS); V, *T. turgidum* cv. Cndo/Veery ($2n = 4x = 28$; 1BL/1RS, 1BL/1RS). The arrow indicates the marker band that identifies the 1B, 1BL/1RS heterozygote.

TABLE 1. Chromosome 1B homozygous or 1B, 1BL/1RS heterozygous status of five BC_1 derivatives from eight different F_1/durum cultivar combinations as determined by GPI analyses

	BC_1 plants analyzed				
BC_1 pedigrees	1	2	3	4	5
F_1/Aconchi	1B, 1B	1B, 1BL/1RS	1B, 1BL/1RS	1B, 1BL/1RS	1B, 1BL/1RS
F_1/Chen/Altar	1B, 1B	1B, 1B	1B, 1B	1B, 1B	1B, 1BL/1RS
F_1/86D 21328/DZR	1B, 1BL/1RS	1B, 1B	1B, 1B	1B, 1BL/1RS	1B, 1B
F_1/B1A "S"	1B, 1BL/1RS	1B, 1BL/1RS	1B, 1B	1B, 1B	1B, 1BL/1RS
F_1/Crex"S"//Chin/Sapi"S"//Yav	1B, 1BL/1RS	1B, 1BL/1RS	1B, 1B	1B, 1BL/1RS	1B, 1B
F_1/BHA (PD10)	1B, 1BL/1RS	1B, 1B	1B, 1BL/1RS	1B, 1B	1B, 1B
F_1/Immer/Altar	1B, 1BL/1RS	1B, 1B	1B, 1B	1B, 1B	1B, 1BL/1RS
F_1/Duergand	1B, 1BL/1RS	1B, 1B	1B, 1B	1B, 1B	1B, 1BL/1RS

Triticum turgidum cv. Aconchi had five bands, with the four cathodal bands more distinct (Fig. 1). The CS banding pattern had 9–10 bands, with the additional bands falling within the *T. turgidum* banding profile. This was possible because of the interaction of the GPI subunits of chromosome 1A and (or) 1B gene products with those of chromosome 1D. The *S. cereale* cv. Imperial banding profile was similar to that of *Ae. squarrosa* (Fig. 1). The location of *Gpi-R1* on chromosome 1R was established by Chojecki and Gale (1982) using a Chinese Spring line with a null-GPI-D1 CS phenotype (designated as CS′).

Triticum turgidum cv. Cndo/Veery and *T. aestivum* cv. Seri-82, both 1BL/1RS homozygous, exhibited eight bands, whereas the F_1 Aconchi//Cndo/Veery 1B, 1BL/1RS heterozygote showed 9–10 bands similar to *T. aestivum* cv. Chinese Spring (Fig. 1). This was expected since the *Gpi-A1* and *Gpi-B1* gene products exhibited a banding pattern with five bands specific to durum wheats that are 1B homozygous. However, with the introduction of GPI-R1 rye subunits, the F_1 heterozygote possesses GPI-A1, GPI-B1, and GPI-R1 subunits. The GPI-R1 subunits behave similar to GPI-D1 subunits (Chojecki and Gale 1982). Upon recombining GPI-R1 subunits with that of GPI-A1 and GPI-B1, the 1B, 1BL/1RS heterozygous durums exhibit a GPI banding pattern essentially similar to cv. Chinese Spring, even when the 1BL/1RS heterozygous durums do not possess GPI-D1 subunits. Comparison of GPI banding patterns among *T. turgidum* cv. Aconchi (1B homozygous), Cndo/Veery (1B/1R homozygous), the F_1 Aconchi//Cndo/Veery 1B, 1BL/1RS heterozygote, and *T. aestivum* cv. Chinese Spring reconfirmed that GPI-R1 subunits behave and interact with GPI-A1 and GPI-B1 subunits in a manner similar to GPI-D1 subunits. This further confirms the specific location of GPI-R1 genes in the short arm of chromosome 1R. Analysis of endosperm halves placed directly on the gel gave similar banding patterns and the translocation heterozygotes (1B, 1BL/1RS) could be detected from the homozygous 1B or 1BL/1RS types (Fig. 2).

GPI analyses of each of the five seeds from eight BC_1 combinations identified homozygous 1B and heterozygous 1B, 1BL/1RS plants (Table 1). All F_1 plants were heterozygous as anticipated. The embryo halves of each F_1 and BC_1 heterozygote plants were germinated for conducting C-banding verification of the heterozygote GPI results on these plants.

When validated, the heterozygous BC_1 plants were utilized for BC_2 production.

The C-banding results of F_1 and BC_1 plants were in total accordance with the GPI observations, confirming the 1B, 1BL/1RS status of all F_1 plants and homozygous 1B, 1B, or 1B, 1BL/1RS heterozygous status of the segregating BC_1 derivatives (Table 1). Several procedures exist for detecting the 1BL/1RS wheats. These have utilized monoclonal antibodies (Howes et al. 1989), SDS–PAGE (Koebner and Shepherd 1986), HPLC (Lookhart et al. 1991), A-PAGE (Berzonsky et al. 1991), and a rye-specific probe (May and Wray 1991). All these methods have facilitated the detection of the 1BL/1RS translocation in bread wheat cultivars only. In durum wheats, Friebe et al. (1989) used PAGE (prolamin) analysis to ascertain the homozygous 1BL/1RS translocation in BC_1F_4 derivatives. They identified the translocation heterozygotes by cytology.

Our results establish for the first time that the IEF banding pattern of GPI can be effectively used to detect the 1B, 1BL/1RS heterozygotes in the development of 1BL/1RS substitution lines of *T. turgidum*. The method we employed enables rapid screening of the germplasm, is swifter than C-banding, and equally accurate. It is also unique in that the GPI diagnostic alone identifies the heterozygote in durums, whereas GPI and A-PAGE are both necessary for *T. aestivum*. The 1BL/1RS detection efficiency using some diagnostic methods has been reported (Javornik et al. 1991; Lookhart et al. 1991); their potential in identifying heterozygotes in durum wheats, however, has yet to be explored.

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