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A combination of protein electrophoretic techniques for the detection of 1B, 1B/1R heterozygotes in *Triticum aestivum* L.

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ABSTRACT

In the development of a 1B, 1B isoline of a 1B/1R, 1B/1R *Triticum aestivum* L. ($2n = 6 x = 42$, AABBDD) cultivar, an F_1 heterozygote must be generated. This F_1 is advanced by backcrossing to the 1B/1R parent and the heterozygote identified in each of the subsequent eight backcross generations. Selfing after the last backcross allows for extraction of the 1B, 1B isoline. The C and N banding techniques used for heterozygote identification are time consuming and cumbersome as opposed to biochemical procedures. A combination of two protein separation techniques, namely isoelectric focusing (IEF) of glucose phosphate isomerase (GPI) isozymes and polyacrylamide gel electrophoresis on an acid medium (Acid-PAGE) of the gliadins, was used to unequivocally identify the 1B, 1B/1R heterozygotes without destroying the embryo. The speed and accuracy of these techniques enhance the efficient development of several near isogenic, homozygous lines with chromosomes 1B or 1B/1R. These near isogenic lines will serve as the basis of evaluating yield components, adaptability, stability and quality characteristics of homozygous 1B/1R wheat cultivars.

Key words: Isozyme analysis, Protein separation, *Triticum aestivum*, 1B/1R translocation, 1RS secalins.

INTRODUCTION

Spring x winter wheat crossing has led to the development of the highly successful spring wheat Veery "S" lines (RAJARAM *et al.*, 1983) through the use of the winter cultivar Kavkaz. This cultivar possesses the 1RS chromosome arm from *Secale cereale* cv. Petkus in the form of a 1BL/1RS translocation (METTIN *et al.*, 1973; ZELLER, 1973). Associated with this chromosome are several important disease resistance traits such as: *Puccinia recondita* Roberge ex Desmaz. (leaf rust), *P. graminis* Pers. (stem rust), *P. striiformis* Westend. (yellow rust) and *Erysiphe graminis* DC. ex Merat (powdery mildew). These important genes are designated *Lr26, Sr31, Yr9* and *Pm8*, respectively (MCINTOSH, 1983). It has been contended that in addition to these desirable agronomic features, the 1B/1R wheat cultivars generally produce a flour with inferior dough quality (DHALIWAL *et al.*, 1987). This adverse association, however, is disputed by the recent findings of PEÑA *et al.* (1990). To better understand the effect of the 1B/1R translocation versus a normal 1B chromosome on these attributes, it is imperative that adequate germplasm controls be developed, which in this case means the production of isolines.

Developing a 1B, 1B isoline of a 1B/1R, 1B/1R wheat cultivar involves crossing the 1B/1R wheat with a 1B donor thus generating the F_1 1B, 1B/1R heterozygote. The F_1 is advanced by backcrossing (BC) to the 1B/1R, 1B/1R female parent for eight generations (BCVIII). The heterozygote BCVIII derivatives are selfed and from this selfed progeny the homozygous 1B and 1B/1R isolines are selected.

In each BC generation, it is necessary to identify the 1B, 1B/1R heterozygotes for subsequent crossing to advance them to the next generation and eventually to BCVIII. The heterozygote identification at each backcross is possible by applying the diagnostic C and/or N banding techniques (TER-KUILE *et al.*, 1990) that are time consuming and cumbersome. Accordingly this study

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was conducted to develop more efficient means of tracking the 1B, 1B/1R heterozygotes in segregating backcross populations by using protein separation techniques. A combination of isoelectric focusing (IEF) of glucose phosphate isomerase isozymes (GPI, D-glucose 6-phosphate ketol-isomerase, E.C. 5.3.1.9) and polyacrylamide gel electrophoresis in acid medium (Acid-PAGE) of the gliadin storage proteins was used. Genes for GPI are located on the short arms of homoeologous group 1 chromosomes in wheat (HART, 1979). Genes for ω-gliadins are specifically located on the short arms of homoelogous group 1 wheat chromosomes (PAYNE *et al.*, 1982) and on the short arm of chromosome 1R of rye (LAWRENCE and SHEPHERD, 1981; SHEWRY *et al.*, 1984).

MATERIALS AND METHODS

Germplasm. A total of eight 1B or 1B/1R homozygous *Triticum aestivum* L. ($2n$ = 6 x = 42, AABBDD) cultivars and their heterozygote (1B, 1B/1R) F_1 progenies were evaluated. The 1B or 1B/1R homozygous status of these parents was confirmed by giemsa C-banding (MUJEEB-KAZI, unpublished data). The cultivars Ocoroni, Opata, Papago"S" and Yaco"S" (all homozygous for 1B) were crossed with Glennson 81 (homozygous for 1B/1R). The other four cultivars, Bau, Fink, Kauz and Spinebill (all homozygous for 1B/1R), were crossed with Pavon "S" (homozygous for 1B). Five randomly chosen seeds from each parent (homozygous for chromosome 1B or 1B/1R) and their F_1 hybrids (1B, 1B/1R heterozygotes) were analyzed for GPI and gliadin electrophoretic patterns. *T. aestivum* cv. Chinese Spring (CS) ($2n$ = 6 x = 42), chromosome 1R disomic Imperial rye addition in the CS background ($2n$ = 6 x = 42 + 2) and *Secale cereale* cv. Imperial ($2n$ = 2 x = 14, RR) were also included in the two analyses as standard checks. Seeds of the checks were generously supplied by the late Dr. E.R. SEARS, University of Missouri, Columbia, MO, USA.

Isoelectric focusing (IEF). Precast ampholine PAG plates of pH 5.5-8.5 were used for GPI isoelectric focusing. The anode and cathode buffers were 0.5M hydroxyethyl-piperazine ethylsulphonic acid (HEPES) and 0.5M sodium hydroxide, respectively. Gels were prefocused for 30 min at a constant power of 15 watts on an LKB Multiphor flat bed electrophoresis apparatus with the temperature maintained at 5°C using the LKB multitemperature II thermostatic circulator. Following prefocusing, the cut endosperms from each seed were laid directly over the gel and IEF continued at the same power setting with maximum voltage set at 1700 volts for 6.0 h. The cut endosperm samples were removed after 1.5 h while the IEF run was in progress. Staining was similar to that reported by CHOJECKI and GALE (1982).

Acid-PAGE (A-PAGE). The same endosperm seed halves that were first used for GPI analyses were also utilized for gliadin extraction. The endosperms were ground and extracted with 70% ethanol using a 1:2 w/v ratio (e.g., 25 mg of ground material extracted with 50 µl of ethanol). The samples were vortex mixed and held at room temperature (18 to 20°C) for 2 h. The contents were diluted with an equal volume of 10% sucrose with 5% methyl green. The samples were vortex mixed briefly, centrifuged for 5 min. at 10,000 g at room temperature (18-20°C) and the supernatants used for A-PAGE analysis. The protocols used for A-PAGE were similar to those of NG *et al.*, 1988. The buffer solution used was 0.25% w/v aluminium lactate with pH adjusted to 3.1 with lactic acid. The gel contained the same buffer solution with 6% (w/v) acrylamide and 0.3% (w/v) N,N-methylene-bis-acrylamide, 0.1% (w/v) L-ascorbic acid and 0.0015% (w/v) ferrous sulfate. The catalyst solution was 3% (w/v) hydrogen peroxide of which 116 µl was used for a 70 ml gel solution. Gels were polymerized for at least 2 h. Gel dimensions were 22 x 20 x 0.15 cm. A constant current of 60 mA was maintained until the purple dye front had migrated to the end of the gel. Temperature was maintained at 5°C by circulating cold water through the gel apparatus. A Bio-Rad Protean II apparatus was used for electrophoresis. Gels were stained overnight in a solution of 12% trichloroacetic-acid (TCA) containing 0.4% Coomassie G and destained with a 12% TCA solution.

RESULTS AND DISCUSSION

The F_1 cross combinations heterozygous for the 1B, 1B/1R chromosomal constitution can be readily differentiated from their homozygous (1B or 1B/1R) parents by using a combination of GPI IEF and gliadin A-PAGE. Genes for GPI are located in the short arms of homoeologous group 1 wheat chromosomes (HART, 1979). The most cathodal band of GPI zymograms of wheat seed extracts is the product of the gene located on the short arm of chromosome 1B (CHOJECKI and GALE, 1982). Eight bands were clearly distinguished in the GPI banding patterns of wheats that were 1B, 1B (homozygous) or 1B, 1B/1R (heterozygous). Wheat lines homozygous for 1B/1R express either six or, in some cases, seven bands, with the most cathodal bands being absent (Fig. 1). The most cathodal band can serve as a distinct marker in the differentiation of the homozygous 1B/1R types from the homozygous 1B or the 1B, 1B/1R heterozygote genotypes since this marker band was consistently absent in all homozygous 1B/1R cultivars, i.e., in Bau, Fink, Kauz, Spinebill and Glennson 81 (Table 1). This initial screening has the advantage that 1B/1R homozygotes can be

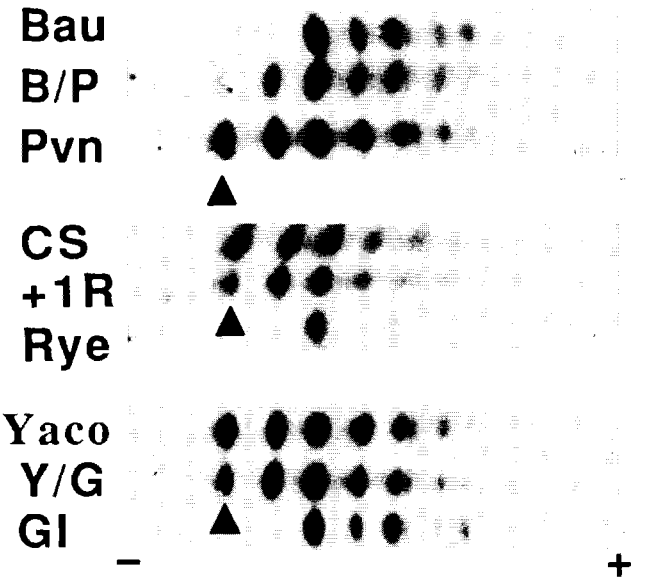

FIGURE 1 - Grain glucose phosphate isomerase banding patterns of two different combinations of parents, their F_1 progeny, Chinese Spring (CS), 1R disomic addition line (+1R) and Imperial rye (Rye). The germplasm brevity is : Bau = Bau "S" B/P = Bau/Pavon, Pvn = Pavon "S", Yaco = Yaco "S", Y/G = Yaco/Glennson 81, and Gl = Glennson 81.
Marker band from the short arm of chromosome 1B indicated by arrows.

TABLE 1

Screening of *Triticum aestivum* cultivars homozygous for the 1B/1R or 1B chromosomes and their heterozygote F_1 hybrids (1B, 1B/1R) using GPI-isoelectric focusing on polyacrylamide gels.

Cultivars: female (F) male (M) and F_1 combinations			1B/1R, 1B/1R	1B, 1B	1B/1R, 1B F_1
F		Yaco		*[b]	*
	F_1	Yaco/Glennson		*	*
M		Glennson 81	+[a]		
F		Opata		*	*
	F_1	Opata/Glennson		*	*
M		Glennson 81	+		
F		Ocoroni		*	*
	F_1	Ocoroni/Glennson		*	*
M		Glennson 81	+		
F		Papago "S"		*	*
	F_1	Papago"S"/Glennson		*	*
M		Glennson 81	+		
F		Kauz	+		
	F_1	Kauz/Pavon		*	*
M		Pavon		*	*
F		Spinebill (Spb)	+		
	F_1	Spb/Pavon		*	*
M		Pavon		*	*
F		Bau	+		
	F_1	Bau/Pavon		*	*
M		Pavon		*	*
F		Fink	+		
	F_1	Fink/Pavon		*	*
M		Pavon		*	*

[a] Indicates genotypes that can be isolated using GPI-IEF.
[b] Indicates genotypes that cannot be separated from each other using GPI.

rapidly screened out, requiring subsequent A-PAGE analysis only on those endosperm halves that exhibit the 1B banding characteristic. A single GPI IEF gel enables rapid screening of at least 48 cut endosperms. In comparison, an A-PAGE gel accomodates only 15 to 20 samples per run, and is more time consuming because seed halves have to be ground and extracted for analysis. The GPI banding pattern of Chinese Spring was essentially similar to that of the 1R disomic addition (Fig. 1), which is probably due to the co-focusing of the Chinese Spring and rye dimers.

The omega-gliadin region (asterisks, Fig. 2) in the banding patterns resolved by A-PAGE electrophoresis is characterized by two distinct gliadin marker bands (in a zone of four) in *T. aestivum* cultivars that are homozygous for the 1B/1R translocation chromosome (TER-KUILE *et al.*, 1991). These two distinct marker bands are specific to the short arm of *Secale cereale* chromosome 1R (FRIEBE *et al.*, 1989), diagnosed as products of the rye *Sec-1* locus (LAWRENCE and SHEPHERD, 1981). These two rye-specific marker bands are present in the CS + 1R disomic addition line as well as in the parents that are homozygous for 1B/1R. (Fig. 2). The 1B homozygous wheats, CS and Yaco, show some bands in the ω-gliadin region which apparently belong to the short arm of 1B. These are distinctly different from the two marker bands that are rye specific (Fig. 2). The 1B, 1B/1R heterozygotes also show these two rye-specific bands in addition to those specific for the 1B, 1B parent. This group of bands resulting from the genes located on 1RS + 1BS unequivocally diagnoses the heterozygotes (Table 2, Fig. 2) and will enhance the efficiency of isoline development in *T. aestivum* 1B, 1B or 1B/1R, 1B/1R cultivars. GPI analysis identifies 1B/1R homozygotes, and A-PAGE

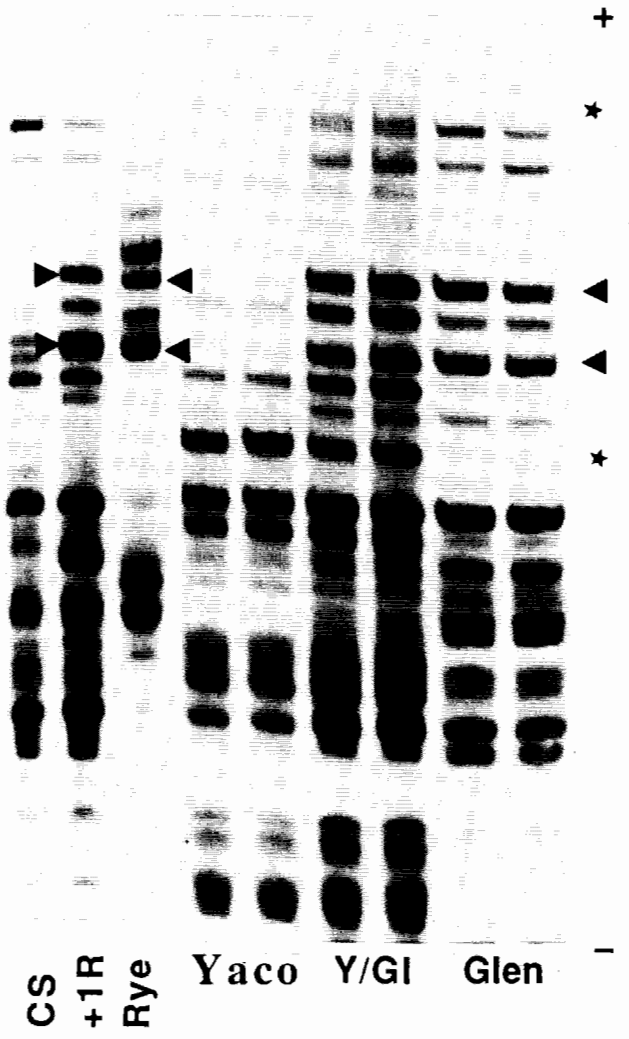

FIGURE 2 - Gliadin A-PAGE patterns of two parents, their F_1 progeny, Chinese Spring (CS), 1R disomic addition line (+1R) and Imperial rye.
Marker bands of rye are indicated by arrows. The omega-gliadin region falls between the asterisks.

analysis categorically distinguishes between the 1B homozygotes and the 1B, 1B/1R heterozygotes that are to be utilized in a backcrossing program for isoline development (TER-KUILE *et al.*, 1990). The resolution of the prominent secalin bands in the 1RS test material was well correlated with their presence in the 1R disomic addition and Imperial rye and their absence in Chinese Spring (Fig. 2).

Both techniques used are nondestructive (i.e., embryos can be germinated after confirmation is made of their heterozygous 1B, 1B/1R status), and they allow for efficient analysis to be conducted on

TABLE 2

Screening of *Triticum aestivum* cultivars and F_1 derivatives homozygous for 1B, 1B or heterozygous for 1B, 1B/1R using Acid-PAGE

Cultivars: female (F) male (M) and F_1 combinations		1B, 1B	1B/1R, 1B, F_1
F	Yaco	+[a]	
F_1	Yaco/Glennson		+
F	Opata	+	
F_1	Opata/Glennson		+
F	Ocoroni	+	
F_1	Ocoroni/Glennson		+
F	Papago "S"	+	
F_1	Papago "S"/Glennson		+
F_1	Kauz/Pavon		+
M	Pavon	+	
F_1	Spb/Pavon		+
M	Pavon	+	
F_1	Bau/Pavon		+
M	Pavon	+	
F_1	Fink/Pavon		+
M	Pavon	+	

[a] Indicates genotypes that can be distinguished using Acid-PAGE.

a large number of samples with high accuracy. As a consequence of these advantages, the 1B or 1B/1R isoline development in *T. aestivum* cultivars and 1B/1R isolines in *T. turgidum* can be extended over a wider range of cultivars than has been undertaken so far (TER-KUILE *et al.*, 1990). This will facilitate determination of the agronomic values of the presence or absence of the 1B/1R translocation in wheats and the effect on quality as well.

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