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1B/1R TRANSLOCATION WHEAT CULTIVARS DETECTED BY A-PAGE ELECTROPHORESIS AND C-BANDING IN THE 1990 NATIONAL UNIFORM WHEAT YIELD TRIAL IN PAKSITAN

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Abstract

Bread wheat (*Triticum aestivum* L.) yield trial nurseries comprised of a large proportion of lines with the 1B/1R translocation that has associated with it several positive agronomic and pathological attributes. Thirty eight entries were evaluated in three categories (i.e. normal, short and rainfed) in the 1989-90 National Uniform Wheat Yield Trials (NUWYT) of Pakistan. "Pak-81", "Faisalabad-83" and "Lyallpur-73" served as checks for each category. There was an overall predominance of lines with the 1B/1R translocation with a cumulative frequency of slightly over 60%. The 1B/1R entries were 40%, 61.5% and 84.6% for the normal, short, and rainfed durations, respectively. Acid-polyacrylamide gel electrophoresis (A-PAGE) and Giemsa C-banding identification of the 1B/1R or 1B homozygous lines was perfectly correlated and either procedure could be used for analysis based upon convenience and facilities. Seed purification necessity was identified for a few entries.

Introduction

The 1B/1R translocation *Triticum aestivum* L. (2n=6x=42;AABBDD) germplasm (McIntosh, 1983) possesses resistances to leaf rust (*Lr26*), stem rust (*Sr31*), stripe rust (*Yr9*) as well as powdery mildew (*Pm8*). The 1B/1R lines may also possess resistance to *Septoria tritici* blotch, moderate aluminum toxicity tolerance, with high yield, stability and adaptability (Rajaram *et al.*, 1983). This germplasm has been successfully incorporated globally into many hexaploid wheat cultivars (Rajaram *et al.*, 1990) and to a limited extent in durum wheat; *T. turgidum* L. 2n=4x=28, AABB (Friebe *et al.*, 1987, 1989). In Pakistan, the 1988-89 national uniform wheat yield trial (NUWYT) comprised of 38 entries including three checks. The cytological analysis led to identification of 25 entries that were homozygous for the 1B/1R translocation (Jahan *et al.*, 1990). Some concerns do exist with respect to adverse bread baking quality and disease vulnerability of such wheats, possibly associated with the 1RS segment that in all cases has a common origin; *Secale cereale* L. cv. Petkus (Jahan *et al.*, 1990). Thus regular analyses are warranted to enable the breeders to make appropriate selective decisions as to presence or absence of 1B/1R chromosomes and swifter biochemical analytical techniques complementary to chromosome band

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Table 1. The "normal" duration *Triticum aestivum* L., lines from the 1989-90 NUWYT (National Uniform Wheat Yield Trial) analyzed for their 1B/1R status (+ or -) by A-PAGE and C-banding analysis of partial samples.

Serial Number	*T. aestivum* Cultivar/line	Pedigree	A-PAGE and C-banding Analysis				
			Seed (Plant) Number				
			1	2	3	4	5
1	Pak-81	Check	⊕ *	⊕	+	+	+
2	PR-34	SNI-TR²xBB	- -	- ⊖	⊕	⊖ -	⊖ -
3	PR-35	SA-75-K7185	-	⊖	-	-	-
4	SS-5-1	(Lu 230xA24)23584	-	-	⊖	-	-
5	S-230	N.A.	+	+	⊕	+	+
6	S-232	N.A.	⊕	+	+	⊕	+
7	V-1	N.A	-	-	-	⊖	-
8	V-5005	MRL'S'/Buc 'S' CM 61949-3M-4Y-1M-3Y-1M-0Y	-	-	-	-	⊖
9	V-6300	WE//CNO/NO66/3/ALDON'S' CM 59161-3B-2B-0B	+	+	⊕	⊕	+
10	V-6751	Veery'S' CM 33027-F-12M-1Y-1M-1Y-1M-1M-0Y	-	-	⊖	-	-
11	V-85060-1	WL-711/CROW'S' Pb.19545-9a-0a	-	-	⊖	-	-
12	V-85054	WL-711/3/KAL/BB//ALD Pb.19282-1a-1a-0a	+	⊕	+	⊕	+
13	V-83035-2	CROW'S'/BAYA'S' CM 67475-2a-0a	-	-	⊖	-	-
14	V-86038	LU 26/HD 2179 Pb.19738-14a-2a-0a	-	⊖	-	-	-
15	V-86369	INIA 66/A.DIST//INIA 66/3/ GEN 81 W.5898-1	+	⊕	+	+	+
16	V-86371	INIA 66/A.DIST//INIA 66/3/GEN81 W.8461-3	+	+	⊕	+	+

* = Circled + or - are indicative of plants that were giemsa C-banded and identified as 1B/1R (+) or 1B (-)

ing would be preferred. The last NUWYT trial (1989-90) was consequently analyzed using acid-polyacrylamide gel electrophoresis (A-PAGE) for 1B/1R detection together with cytological (chromosome banding) confirmation of some samples. This methodology as applied to the current NUWYT trial is presented in this paper.

Materials and Methods

Germplasm for this study was provided by the National Wheat Co-Ordinator's office at the National Agricultural Research Centre (NARC) in Islamabad, Pakistan. The material was categorized under (i) Normal duration (16 entries with Pak-81 check), (ii) Short duration (14 entries with Faisalabad-83 check), (iii) Rainfed (15 entries with LYP-73 check). The pedigrees of these lines are listed in Tables 1, 2 and 3.

***Sample Preparation*:** Ten seeds of each entry in the NUWYT were individually numbered (1 to 10) and each seed was cut with a sharp scalpel. This allowed the embryo portion to be germinated for selective banding analysis and the endosperm portion for A-PAGE analysis. Only 5 seeds were analyzed but where A-PAGE banding profiles were variable in an entry then the remaining five were also utilized. Chromosome C-banding was performed on a limited number of plants with at least one from each entry and more if A-PAGE results indicated line variation.

***Cytology*:** Upon seed germination root tips were collected from specifically identified plants of each entry (1 to 5 or 6 to 10) and their respective plants grown to maturity. The root tips were pre-treated for 3 hours 30 minutes in a 8-hydroxyquinoline + colchicine + dimethylsulfoxide solution (Mujeeb-Kazi & Miranda, 1985), fixed in 0.1% aceto-carmine for 48 h, squashed in 45% acetic acid and processed on dry ice for making permanent slides. The Giemsa C-banding procedure applied was similar to that described by Bennett *et al.*, (1977) with staining and diagnostic aspects identical to those reported by Jahan *et al.*, (1990).

***Acid-polyacrylamide gel electrophoresis* (A-PAGE):** Endosperm seed halves were ground and extracted with five times its weight of 70% ehtanol. Samples were left at room temperature for two hours with occasional vortex mixing. The contents were then centrifuged at 10,000 Xg for 5 minutes at room temperature on a micro-centrifuge. The supernatant was mixed with an equal volume of buffer solution (sodium lactate pH 3.1) containing 40% w/v sucrose and 0.5% methyl green. This diluted supernatant was used for A-PAGE. The A-PAGE procedure of Bushuk & Zillman (1978) employed was slightly modified by the use of a Hoeffer Scientific Vertical electrophoresis system. The gels contained 0.05% sodium lactate buffer (pH 3.1), with 6% w/v acrylamide, 0.3% w/v N,N-methylane -bis-acrylamide (bis), 0.1% w/v L-ascorbic acid and 0.0015% w/v ferrous sulfate heptahydrate. Hydrogen peroxide (30 µl of 3% w/v) was used as a catalyst. The gel dimensions were 140mm x 160mm x 15mm. Each gel had 15 slots and 25 µl of the diluted supernatant was loaded per slot. The electrophoretic run was at 600 V and at a constant temperature of 10C. The run was stopped 1 hour 15 minutes after the methyl green marker dye reached the bottom of the gel (which took approximately 2 h). Gels were then stained overnight in a solution containing 50 ml of 0.4% Coomassie Brilliant Blue (R-250) dissolved in ethanol + 950 ml of aqueous 10% trichloroacetic acid, destained in water and photographed using basal fluorescent transmitted light.

Table 2. The "short" duration *Triticum aestivum* L., lines from the 1989-90 NUWYT (National Uniform Wheat Yield Trial) analyzed for their 1B/1R status (+ or -) by A-PAGE and C-banding analysis of partial samples.

Serial Number	*T. aestivum* Cultivar/line	Pedigree	A-PAGE and C-banding Analysis: Seed (Plant) Number 1	2	3	4	5
1	Fbd-83	Check	-	-	⊖ *	-	-
2	PR-31	A-I FUNG#DOVE	+	+	⊕	⊕	+
3	PR-32	KVZ/3/TOP/CTN/BB/4/BLD/5/ V4/6/BOW'S'	+	⊕	⊕	+	⊕
4	V-5002	LIRA'S'	⊕ +	⊖ +	+ ⊕	+ +	+ ⊕
5	V-6566	742(H.2123-1)PIMA/CD//TORIM 73 Pb.18406-1B-0B	-	⊖	-	⊖	-
6	V-6632	MAYA 74'S'/MONCHO'S'//BB BR.186-3B-0B	+	⊕	+	+	+
7	V-7061	AU/UP301//GLL/Sx/3/PEW"S"/4/ MAI"S"/MAYA "S"//PEW"S" CM 67245-C-2M-0Y	+	+	⊕	+	+
8	V-8203-S-1	[(CNO-8156xTOB-CNO(NO.66/1230x LR-8156)]TRF'S'	-	-	-	⊖	-
9	V-82274-4	INIA/Pb.81xBOW'S' Pb.17928-2a-0a-4SHP	-	-	⊖	-	-
10	V-84133-8	SA-75[Ti-71(KAL-SKAxSNO'S'-INIA'S'/CN-CHR)] AU.ERECTION Pb.18260-4a-2a-0a	+	+	+	⊕	+
11	V-85110	HD 2204-JUN'S' Pb.18843-7a-6a-0a	-	-	-	⊖	-
12	V-85205	Lu28/KVZ-JB216-87xSIS'S' Pb.17229-9a-0k-1a-0a-4a-0a	+	⊕	+	+	+
13	V-85276-2	CHB-70[(K.N83xCHB-70-ALD)CHB-70 (INIAxCNO-CAL/LR-SON)] Pb.17528-A-1N-6N-2N-0N	-	⊖	-	-	-
14	V-86299	[Pb.78(H.6494-71AxHORK'S'/JUP)] Pb.76-KLTxPb.78-TTR'S' Pb.19874-2a-1a-0a	+	+	+	⊕	+

* = Circled + or - are indicative of plants that were giemsa C-banded and identified as 1B/1R (+) or 1B (-)

Table 3. The "rainfed" *Triticum aestivum* L., lines from the 1989-90 NUWYT (National Uniform Wheat Yield Trial) analyzed for their 1B/1R status (+ or -) by A-PAGE and C-banding analysis of partial samples.

Serial Number	*T. aestivum* Cultivar/line	Pedigree	A-PAGE and C-banding Analysis				
			Seed 1	(Plant) 2	3	Number 4	5
1	LYP-73	Check	+ −	⊖ * +	⊕ ⊕	⊖ +	+ −
2	NR-14	BAGULA'S' CM 59123-3M-1Y-2M-1Y-2M-1Y-0M	+	⊕	+	+	+
3	PR-33	KVZ-CNO-CHRxONE 375-125-35 FR 2208-7F-1F-0F	+	⊕	+	+	+
4	PR-36	VL/PVN//TAN'S'	−	⊖	+	−	⊖
5	RF-9	TTR'S'-JUN'S' CM59123-4M-2Y-2M-2Y-2M-1Y-0M-9q	+	+	⊕	+	+
6	SA-1982	N.A.	+	+	⊕	+	+
7	V-6594	KVZ//CNO 67/PJ 62/3/JAN'S' CM 74220-3B-0B	+	⊕	+	+	+
8	V-6602	PJN'S'/GOLAN 81 CM 74478-2B-0B	+ −	− −	⊖ −	− +	⊖ +
9	V-8557	Vee'S'/FLN-ACCxANA CM 64576-9Y-1M-3Y-2M-0Y	+	⊕	+	+	⊕
10	V-8561	(TAS 58/KAL-BBxALD'S')OLN-TRM 76-ALD'S'	+	+	⊕	+	+
11	V-86007	HD 2204/HORK 'S' CM 39808-58M-2Y-4M-1Y-1M-1Y-0B	−	⊖	−	−	−
12	V-86175	BAR.70/SNB'S' Pb.19805-24a-2a-0a	+	+	⊕	+	+
13	V-86247	ALDAN"S"//AS.58 CM 53574-10M-1Y-4M-1Y-0M	+	⊕	+	+	+
14	V-86371	INIA 66/A.DIST//INIA 66/3/GEN 81 W.8461-3	+	⊕	+	+	+
15	V-8701	JUPATECO/HD 1944xCNO'S'-GALLO RP 645-5R-1R-0R	⊕	+	+	+	⊕

* = Circled + or - are indicative of plants that were giemsa C-banded and identified as 1B/1R (+) or 1B (-)

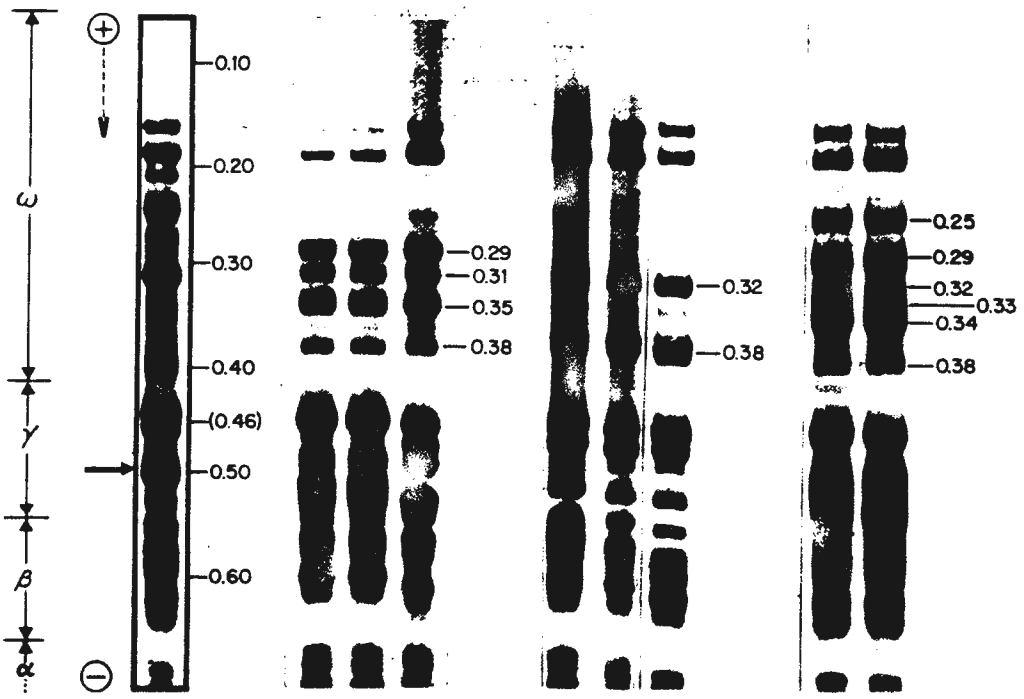

Fig.1. Gliadin separations on A-PAGE of some Pakistan NUWYT wheat cultivars from normal (N), short (S) and rainfed (R) environments as compared to Marquis wheat. From left to right: (a) Marquis, standard; (b) 1B/1R wheats S-230 (N), V-84133-8 (S), NR-14 (R); (c) 1B wheats V-5005 (N), V-6566 (S), V-86007 (R); (d) 1B/1R wheats V-7061 (S), SH-1982 (R).

Results and Discussion

Marquis was used as a standard for A-PAGE gliadin analysis with band 0.50 (Fig.1a) being the standard reference for the band mobility rate for all *Triticum aestivum* wheats (Bushuk & Zillman, 1978). This standard enabled identification of the homozygous IB/IR lines with the omega (ω) region being of analytical significance.

The secalin-1 locus located on the short arm of *Secale cereale* chromosome 1R (Lawrence & Shepherd, 1981; Shewry *et al.*, 1984) is characterized by two distinct gliadin marker bands (in a zone of four); dark (0.29), light (0.31), dark (0.35), light (0.38), (Fig. 1b). These two bands are associated with the 1RS arm and are designated as "a" and "b" (Friebe *et al.*, 1989). The presence of chromosome 1B/1R was observed in 59.5% of the 42 NUWYT wheat entries analyzed in this study (3 checks not included in the percentage of which "Pak-81" was 1B/1R homozygous). In general, all wheats homozygous for chromosome 1B/1R had the consistently repetitive 1RS "a" and "b" secalin associated bands. The 1B homozygous wheats did not possess these characteristic band marker sites.

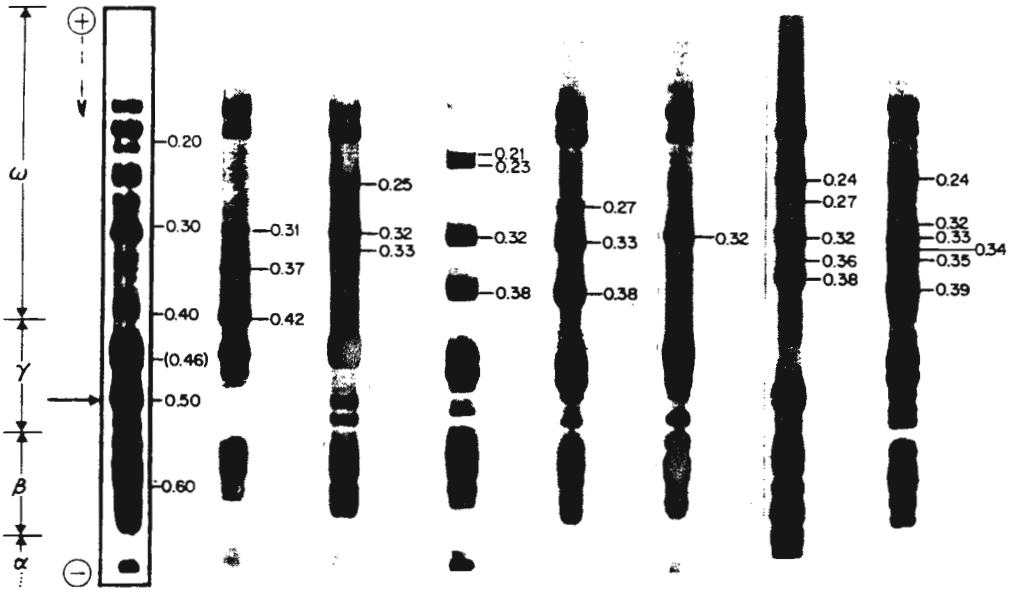

Fig.2. Gliadin separations on A-PAGE of some 1B,1B Pakistan NUWYT wheat cultivars from normal (N), short (S) and rainfed (R) environments as compared to Marquis wheat. From left to right: (a) Marquis, standard; (b) PR-35 (N); (c) SS-5-1 (N); (d) V-1 (N); (e) V-8203-S-1 (S); (f) V-85110 (S); (g) V-85276-2 (S); (h) PR-36 (R).

Polymorphisms of the gliadin banding profiles were evident in some wheat cultivars. Three varieties; "V5005", "V6566", "V86007"; representative of each of the three environments (normal, short, rainfed) had the same banding pattern in the omega region (0.32 and 0.38, Fig. 1c). These varieties however, when compared with two others ("V 7061", "SA 1982") from "short" and "rainfed" environments had the identical 0.32 plus 0.38 bands but additionally possessed four other bands (0.25, 0.29, 0.33, 0.34, Fig. 1d). As demonstrated for these two cases (Figs. 1c., 1d), several other wheat cultivars also exhibited similar gliadin band polymorphisms in the " w " region.

There were many 1B homozygous wheat cultivars which had totally distinct banding patterns within the same or across different environmental groupings. Some of these polymorphisms are represented in Fig.2, with band 0.50 of Marquis being the wheat standard (Fig. 2a). The bands 0.32 and 0.38 seen in Normal "V-1" (Fig. 2d) are very similar to bands seen for the three cultivars presented in Fig. 1c, except that for cultivar Normal "V-1" (Fig. 2d) two extra bands (0.21 and 0.23) were also resolved. Similarly, the banding pattern of Short "V85276-2" and Rainfed "PR36" (Figs. 2g, 2h) were quite similar to the two entries of Fig. 1d, allowance being made for extremely minor banding variations. Additional examples are further shown in Fig.2.

Although a precise standard cannot be formed for the homozygous 1B types, we can logically infer that A-PAGE gliadin analysis can be used to assess and characterize the amount of variability in " ω " gliadins encoded by sequences in the small arm

Fig.3. C-banded 1B and 1B/1R chromosomes in which the 1BL arms of both have a terminal banding site. The short arm (1BS) and (1RS) show banding discriminations (left to right) for: (a) A C-banded 1B chromosome with the banding sites on the 1BS arm, and (b) A C-banded 1B/1R chromosome showing the band variation on the 1RS arm.

of chromosome 1B (1BS). Whether these variations are associated with homozygosity and whether they will constitute towards diagnostic assistance for varietal detection/tracking in crossing is at this stage premature to conclude.

Gliadin analysis using A-PAGE to discriminate homozygous 1B/1R from 1B wheat cultivars is a simplistic, non-destructive and rapid technique because the secalins encoded by the secalin-1 locus are always observed in 1B/1R translocation wheats and are absent in the homozygous 1B cultivars. Chromosome C-banding analysis showed characteristic band positive sites as reported earlier (Jahan *et al.*, 1990; Ter-Kuile *et al.*, 1990). The 1B/1R chromosome had a terminal and interstitial band positive site on the 1R short arm with a terminal site on the 1B long arm with diffuse banding sites located midway on 1BL and the centromeric region (Fig. 3b). The 1RS C-banding pattern was distinctly different from that of the 1BS arm (Fig. 3a). Thus, the translocated chromosome could always be unequivocally identified.

A-PAGE analysis conclusively identified 1B/1R cultivars to comprise of 40% of the 15 normal duration entries (Table 1), 61.5% of the 13 short duration entries (Table 2) and 84.6% of the 13 in the rainfed category (Table 3) with "V-6602" not included because of seed impurities (Table 3). Some 1B/1R and 1B seed impurities

were also observed in "PR-34" (Normal) and "V-5002" (short). The rainfed category check (LYP-73) warrants more stringent purification since 6 of the 10 seeds were 1B/1R band positive (A-PAGE) although the pedigree does not include any 1B/1R parent. The entry "V-6602" also needs purification. The restricted C-banding analysis perfectly corroborated the above A-PAGE data. Either analytical test may be adopted based upon laboratory facilities or researcher convenience.

As compared to the previous NUWYT varietal trials (1988-89), the percentages of the 1B/1R wheat cultivars in each category has somewhat decreased (40.0 : 42.8 for normal, 61.5:72.7 for short, 84.6 : 90.0 for rainfed). It is still remarkably high. This status theoretically remains prone to the dogma of genetic vulnerability as a consequence of the narrow genetic base contributed by the 1RS chromosome of Petkus rye. It is unlikely that a breakdown of the rust and mildew resistances located on the 1RS arm will curtail the future diversification of 1B/1R wheat cultivation since its yield advantages and agronomic attributes continue to demonstrate considerable merit (Rajaram *et al.*, 1990; Villareal *et al.*, 1991). The inferior bread-baking quality of 1B/1R wheats are being reconsidered (Pena *et al.*, 1990) to be complemented by the development of 1B/1R or 1B isogenic lines (Ter-Kuile *et al.*, 1990). These isoline contributions may further enhance the future spread of 1B/1R wheats. This discussion however, has attempted to express a certain degree of caution associated with restricted genetic diversity. The surprising pre-ponderance (61.5%) of 1B/1R wheats in the short duration category (a second year) despite the well recognized "lateness" associated with such wheats is encouraging. The high frequency of 1B/1R wheats in rainfed areas is also seen as a bonus for enhancing cultivation of these wheats.

We are cognizant of the fact that our current observations only validate the documentation of 1B/1R wheats in national yield trials. The yield impacts of the various entries need to be addressed to in the future. This shall hopefully provide interesting data on yield advantages as a consequence of the 1B/1R translocation in wheats. Lacking will be the critical isoline test; for which the germplasm is keenly awaited (Ter-Kuile *et al.*, 1990).

References

Bennett, M.D., J.P. Gustafson and J.B. Smith. 1977. Variation in nuclear DNA in the genus *Secale*. *Chromosoma*, 61: 149-176.

Bushuk, W. and R.R. Zillman. 1978. Wheat cultivar identification by gliadin electrophoregrams. I. Apparatus, method and nomenclature. *Can. J. Pl. Sci.*, 58:505-515.

Friebe, B., F.J. Zeller and R. Kunzmann. 1987. Transfer of the 1BL-1RS wheat-rye translocation from hexaploid bread wheat to tetraploid durum wheat. *Theor. Appl. Genet.*, 74:423-425.

Friebe, B., M. Heun and W. Bushuk. 1989. Cytological characterization, powdery mildew resistance and storage protein composition of tetraploid and hexaploid 1BL/1RS wheat-rye translocation lines. *Theor. Appl. Genet.*, 78: 425-432.

Jahan, Q., N. Ter-Kuile, N. Hashmi, M. Aslam, A.A. Vahidy and A. Mujeeb-Kazi. 1990. The status of the 1B/1R translocation chromosome in some recent varietal releases and in the 1989 national uniform wheat yield trials (NUWYT) in Pakistan. *Pak. J. Bot.*, 22: 1-10.

Lawrence, G.J. and K.W. Shepherd. 1981. Chromosomal locations of genes controlling seed proteins in species related to wheat. *Theor. Appl. Genet.*, 59: 25-31.

McIntosh, R.W. 1983. A catalogue of gene symbols. In: Proc. 6th IWGS, Kyoto, Japan, pp. 1197-1255.

Mujeeb-Kazi, A. and J.L. Miranda. 1985. Enhanced resolution of somatic chromosome constrictions as an aid to identifying intergeneric hybrids among some Triticeae. *Cytologia*. 50: 701-709.

Pena, R.J., A. Amaya, S. Rajaram and A. Mujeeb-Kazi. 1990. Variation in quality characteristics associated with some spring 1B/1R translocation wheats. *J. Cereal Sci.*, 12:105-112.

Rajaram, S., Ch. E. Mann, G. Ortiz-Ferrara and A. Mujeeb-Kazi. 1983. Adaptation, stability and high yield potential of certain 1B/1R CIMMYT wheat *In*: Proc. 6th. IWGS, Kyoto, Japan, pp. 613-621.

Rajaram, S., R. Villareal and A. Mujeeb-Kazi. 1990. The global impact of 1B/1R spring wheats. *Agr. Abst. (ASA Annual Meeting)* pp. 105.

Shewry, R.P., D. Bradberry, J. Franklin and R.P. White. 1984. The chromosomal loca- tions and linkage relationships of the structural genes for the prolamine storage proteins (secalins) of rye. *Theor. Apll. Genet.*, 69: 63-69.

Ter-Kuile, N., Q. Jahan, V. Rosas, A.A. Vahidy and A. Mujeeb-Kazi. 1990. Development of 1B/1R based genetic stocks associated with *Triticum aestivum* and *T. turgidum*. *Agr. Abs. (ASA Annual Meeting)* pp. 112.

Villareal, R.L., S. Rajaram, A. Mujeeb-Kazi and E. Del Toro. 1991. The effect of chromo-some 1B/1R translocation on the yield potential of certain spring wheats (*Triticum aestivum* L.). *Pl. Breeding* 106: 77-81.