

# 1BL.1RS translocation in some Indian bread wheat genotypes and strategies for its use in future wheat breeding

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**Abstract** - In the present study 17 bread wheat genotypes including Chinese Spring were screened to identify 1BL.1RS translocation (T1BL.1RS). Both molecular approaches and karyotype analysis were used. In 8 of the 17 bread wheat genotypes, the translocation 1BL.1RS was identified using GISH (genomic *in situ* hybridization) involving total rye genomic DNA as a probe, which allowed identification of a rye chromosome arm carrying a relatively small satellite that is characteristic of 1RS. The microsatellite (SSR) markers specific for 1BS successfully amplified only in 9 genotypes including Chinese Spring, and gave no PCR product in the remaining 8 genotypes, thus confirming the loss of 1BS arm in these 8 genotypes. The 1BL specific microsatellite (SSR) markers, on the other hand, amplified successfully in all the 17 genotypes. Further, in all the above 8 genotypes, instead of normal four prominent satellites, only two prominent satellites were observed at somatic metaphase confirming the replacement of 1BS with 1RS, resulting in T1BL.1RS in these genotypes. The 8 genotypes that carried T1BL.1RS included the following: PBW373, PBW343, PBW175, UP2338, UP2425, UP2418, UP2382 and CPAN3004. These varieties may be considered as a useful germplasm resource for wheat breeding.

**Key words:** GISH, STMS, translocation, wheat.

## INTRODUCTION

Rye (*Secale cereale*) is an important source of alien genetic variation for improvement of bread wheat (*Triticum aestivum*). Wheat varieties with the short arm of rye chromosome 1R (1RS) translocated to long arm of wheat chromosome 1B (1BL) are being extensively grown and cover over five million hectares of cultivated area (VILLAREAL *et al.* 1998; RABINOVICH 1998). The chromosome arm 1RS has genes conferring resistance to leaf rust (*Puccinia recondita* Rob. et. Desm. f. sp. *tritici*, Lr26), stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Erikss. et Henn., Sr31), stripe rust (*Puccinia striiformis* Westend f. sp. *tritici*) and powdery mildew (*Blumeria graminis* (DC.) EO Speer f. sp. *tritici* Em. Marchal Pm8) (HUEN

and FISCHBECK 1987; SINGH *et al.* 1990; MCINTOSH *et al.* 1993). It also has genes for resistance against insects (MARTIN *et al.* 1976; MARAIS *et al.* 1994). Besides these resistance genes, 1RS has genetic factors for wide adaptation and tolerance to abiotic stresses (RAJARAM *et al.* 1983; VILLAREAL *et al.* 1994) and also contributes towards higher grain yield (SCHLEGEL and MEINEL 1994; CAVER and RAYBURN 1994; MORENO-SEVILLA *et al.* 1995; VILLAREAL *et al.* 1991, 1997, 1998).

Biochemical, cytogenetic, and molecular approaches have been used for characterization of 1RS in wheat background (for a review, see BERZONSKY and FRANCKI 1999). Using some of these approaches, presence of T1BL.1RS has been confirmed in a number of wheat varieties from U.S.A., Europe, Mexico and Pakistan, etc. (RAJARAM *et al.* 1983; ZELLER and HSAM 1984; BENNETT 1984; LUCASZEWSKI 1990; JAHAN *et al.* 1990; MCKENDRY *et al.* 1996a,b; MUZEEB-KAZI

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*et al.* 1996). However, no precise information regarding the presence of T1BL.1RS is available in bread wheat genotypes developed in India. The identification of T1BL.1RS in these wheat genotypes will prove helpful in selection of material for use in wheat breeding programmes. In the present study, using genomic *in situ* hybridization (GISH) we identified the presence of a wheat-rye translocation (presumed to be T1BL.1RS) in eight out of 17 bread wheat varieties/genotypes examined, and in these eight genotypes we later also confirmed the absence of 1BS by SSR markers for this arm and by the presence of one instead of two pairs of prominent satellites (1BS, 6BS) generally observed in bread wheat. The presence of 1BL was also confirmed through the use of SSR markers for this arm.

## MATERIAL AND METHODS

### Plant material

In the present study 16 bread wheat (*Triticum aestivum*) genotypes from Indian bread wheat germplasm, including 12 released varieties and four other genotypes were used. An “unknown” strain of diploid ( $2n=2x=14$ ) rye (*Secale cereale*) and Chinese Spring were utilized as controls (Table 1). A nullisomic 1B-tetrasomic 1A was also used as an addition-

al control. The seed material for bread wheat genotypes was obtained partly from our own germplasm collection and partly from collections available at G. B. Pant University of Agriculture and Technology, Pantnagar, India and Punjab Agricultural University, Ludhiana, India. The seed of the rye genotype was procured from I.A.R.I., New Delhi, India.

### DNA sample

The DNA sample of nullisomic1B-tetrasomic1A line of Chinese Spring was obtained from M. Roder, I.P.K., Gatersleben, Germany.

### Slide preparation

For identification of SAT-chromosomes, somatic metaphase spreads from root tip cells were prepared following standard aceto-carmin procedure.

For genomic *in situ* hybridization (GISH), fixed root tips were treated with a mixture of 2% cellulase Onozuka R10 and 20% pectinase (solution in 40% glycerol, Sigma) for 40 min and squashed in 45% acetic acid to obtain good metaphase spreads. The slides were subsequently frozen in liquid nitrogen and the cover slips were flicked off. The slides were then air dried, dehydrated in ethanol series (70%, 90%, 100%) and stored in 100% glycerol at 4° C for subsequent use in GISH experiments.

### Genomic *in situ* hybridization (GISH)

Sheared total rye (*Secale cereale*) genomic DNA labeled with biotin-14-dCTP was used as a probe for

Table 1 – A summary of results of somatic chromosome analysis for satellites, GISH and STMS analysis in 17 bread wheat genotypes, a single rye genotype and nullisomic 1B-tetrasomic 1A line of Chinese spring.

S. No.	Name of variety/genotype	No. of SAT-chromosome pairs	Presence of short arm of a rye chromosome*	Amplification of microsatellite marker**	
				Specific to 1BS	Specific to 1BL
1	PBW 373	1	+	-	+
2	PBW 233	2	-	+	+
3	PBW 343	1	+	-	+
4	WL 711	2	-	+	+
5	PH 132	2	-	+	+
6	UP 2338	1	+	-	+
7	PBW 396	2	-	+	+
8	CPAN 3004	1	+	-	+
9	HD 2627	2	-	+	+
10	HD 2329	2	-	+	+
11	UP 2418	1	+	-	+
12	PBW 175	1	+	-	+
13	Sonalika	2	-	+	+
14	C 591	2	-	+	+
15	UP 2382	1	+	-	+
16	UP 2425	1	+	-	+
17	Chinese Spring	2	-	+	+
18	Nulli 1B- tetra 1A	1	-	-	-
19	Rye (unknown)	1	-	-	-

\* + = present, - = absent

\*\* + = amplification of DNA, - = no amplification of DNA

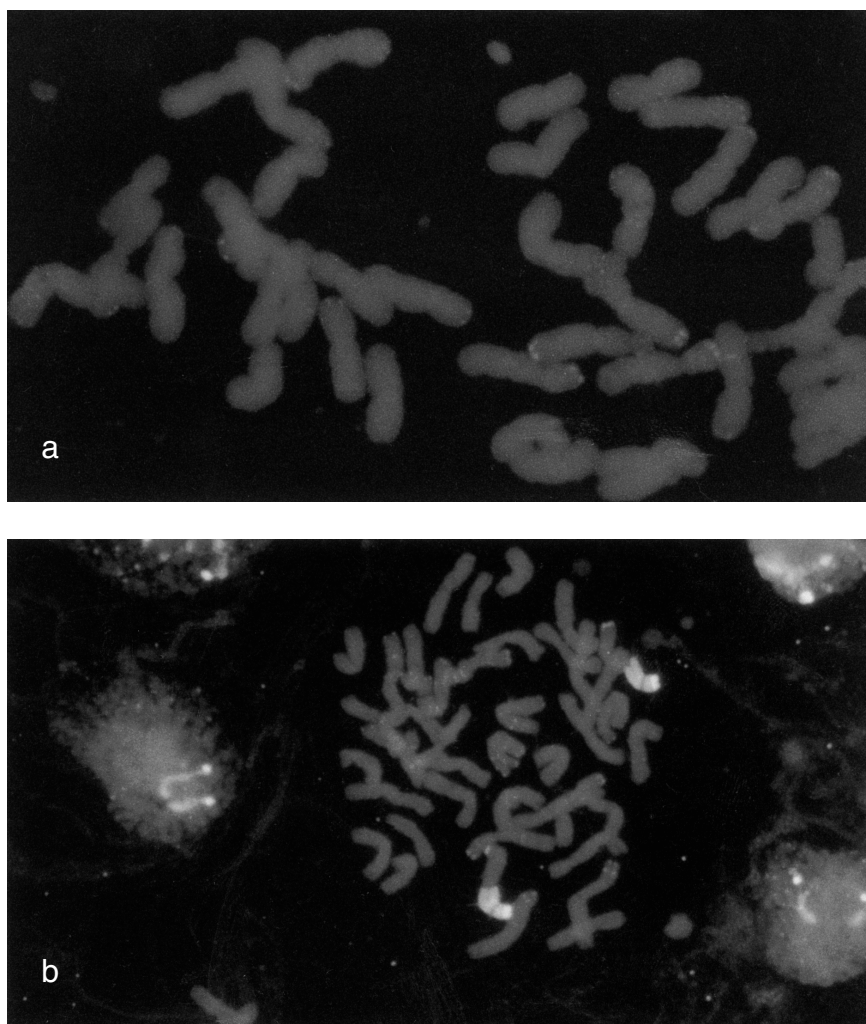


Fig. 1 – GISH on somatic metaphase chromosomes of bread wheat ( $2n=6x=42$ ) using total rye genomic DNA as probe. (a) Chinese Spring, without 1BL/1RS translocation. (b) UP 2338 with 1BL/1RS translocation showing painting of 1RS (short arm of rye chromosome 1).

GISH on wheat somatic metaphase cells. The hybridization and signal amplification were achieved following LEITCH *et al.* (1994). For the amplification of hybridization signals two different antibodies (avidin-FITC and biotinylated-anti-avidin) were used. The slides were counterstained with DAPI (4', 6-diamidino-2-phenylindole). The *in situ* signals were visualized under a fluorescence microscope (Leica) using high-performance universal objectives and by using suitable epifluorescence filter set. The photomicrographs were taken using Kodak 400 film.

#### STMS analysis

For STMS analysis, microsatellite primer pairs for four 1B chromosome specific gwm loci two each mapped on short (gwm11 and gwm18) and long (gwm124 and gwm153) arms of the 1B chromosome of bread wheat (RODER *et al.* 1998) were selected. Each PCR reaction was performed in 25 $\mu$ l volume

containing 250 nM of each STMS primer, 2mM of each deoxynucleotide, 1.5 mM MgCl<sub>2</sub>, 1U *Taq* polymerase, and 50-100 ng of genomic DNA of bread wheat. PCR amplifications of DNA were carried out in Eppendorf Mastercycler using following PCR profile: initial denaturation at 94° C for 3 min, 45 cycles at 94° C for 1 min, 50 or 60° C for 2 min, 72° C for 2 min; a final extension at 72° C for 10 min. The amplified products were resolved on 10% polyacrylamide denaturing gels (PAGE) followed by silver staining (Tegelstrom 1992). The presence and absence of products of expected size were recorded.

#### Field evaluation of genotypes for grain yield and disease resistance

Thirteen of the 16 bread wheat genotypes studied during the present study were evaluated earlier in replicated trials for grain yield and field resistance to leaf rust, stem rust and powdery mildew at six loca-

tions in North Western Plain Zones in India by the Directorate of Wheat Research, Karnal, India. The data from these trials was used to calculate the average grain yield (as % of check cultivars) and the field resistance against the above diseases.

## RESULTS

### *Genomic in situ hybridization (GISH)*

Using total rye genomic DNA as probe, GISH on somatic metaphase chromosomes was conducted. Painting of short arms of a solitary pair of chromosomes was observed in eight of the 16 genotypes suggesting the presence of an arm from rye genome (Table 1, Fig. 1a). In the remaining nine genotypes including Chinese Spring, none of the 42 available chromosome arms was painted. Small hybridization signals on telomeric and intercalary regions were also detected in several chromosomes in each of the genotypes examined (Fig. 1b).

### *STMS analysis*

The results of amplification of four STMS markers, two of them specific for the short arm (1BS) and the other two specific for the long arm (1BL) of chromosome 1B of bread wheat, are summarized in Table 1 and Figure 2. All the above four STMS markers were amplified not only in Chinese Spring but also in eight of the 16 other bread wheat genotypes. These eight genotypes were the same in which none of the chromosome arms was painted following GISH. In

the remaining 8 bread wheat genotypes, the two markers specific to 1BL gave a PCR product while the two markers specific to 1BS did not give any PCR product. Obviously, these eight genotypes were the same in which one arm was painted during GISH with rye genomic DNA. As expected, none of the four markers belonging to chromosome 1B of wheat showed amplification in either the rye genotype used or in the nullisomic 1B-tetrasomic-1A line of Chinese Spring.

### *Analysis of satellited chromosomes*

The root tip somatic metaphase preparations in each of the 17 genotypes including Chinese Spring were also studied and the prominent satellites were examined in each case. The somatic chromosome number in each of the 17 wheat genotypes was  $2n=6x=42$ . In eight of these 17 genotypes one pair of prominent satellites in the short arms of a single pair of chromosomes was observed, while in the remaining nine genotypes two pairs of prominent satellites in the short arms of two different pairs of chromosomes were observed (Table 1 and Fig. 3). The eight genotypes showing only one pair of prominent satellited chromosomes, presumably lacked 1BS and contained 1RS.

### *Grain yield and disease resistance*

The data on mean grain yield (as % of checks) and field resistance to leaf rust, stem rust and powdery mildew was available for 13 of the 16 Indian bread wheat genotypes and the data is summarized in Table 2.

Table 2 – Mean grain yield and score for field resistance for three diseases in 13 bread wheat varieties/genotypes evaluated at six locations in North Western Plain Zones, India.

S. No.	Variety/genotype	1BL/1RS	Grain yield as % of check cultivar	Disease score		
				Stem rust	Leaf rust	Powdery mildew
1	HD2687	Absent	102.55	-	5S	4
2	PBW343	Present	106.75	-	tMS	6
3	PBW175	Present	107.44	-	20S	0
4	CPAN3004	Present	100.24	-	10S	3
5	WL711	Absent	104.08	-	90S	7
6	Sonalika	Absent	88.50	20S	80S	0
7	UP2418	Present	93.95	tS	20S	-
8	PBW396	Absent	117.41	-	15S	3
9	PDW233	Absent	96.89	-	5S	3
10	UP2338	Present	102.89	0	5MS	4
11	PBW373	Present	110.03	-	5S	9
12	UP2425	Present	103.24	-	20S	6
13	HD2329	Absent	95.90	-	60S	6

S= S=susceptible, MS= moderately susceptible, t= traces, 0= no disease, 9= highly susceptible

## DISCUSSION

Significant improvement in many wheat cultivars worldwide is attributed to the presence of T1BL.1RS so that the characterization of this translocation in bread wheat cultivars has been undertaken in a number of studies using a variety of approaches. These approaches involved biochemical, cytological and molecular techniques (for a review see BERZONSKY and FRANCKI 1999). However, the present study is the first of its kind where a set of Indian bread wheat cultivars was subjected to such screening. Three different approaches used in the present study complemented each other for identification of eight genotypes that carried T1BL.1RS. While the karyotypic analysis established the absence of 1BS due to the presence of one pair instead of two pairs of chromosomes with prominent satellites (when 1R is placed in wheat genetic background, the satellite in its short arm is not expressed; MERKER 1982), GISH facilitated the detection of short arm belonging to rye genome in a pair of translocated chromosomes. The absence of 1BS and presence of 1BL in these eight cultivars was also confirmed by using SSR markers specific for both these arms.

In GISH experiments, small hybridization signals in telomeric and intercalary regions were also observed that were common to all the bread wheat genotypes. The observation suggested distribution of repetitive sequences that are found

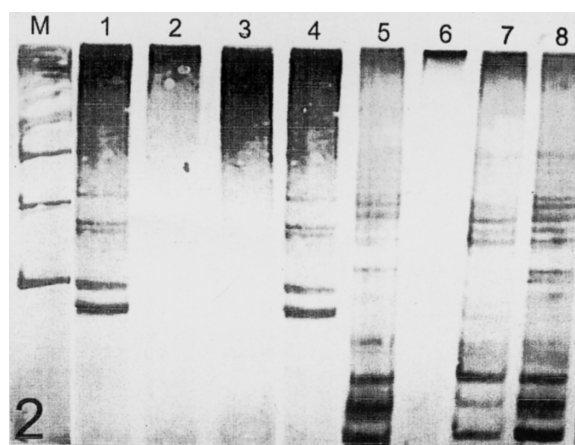


Fig. 2 – DNA amplification patterns in eight bread wheat genotypes using two STMS primer pairs each specific to the short and long arms of chromosome 1B of bread wheat. M=100 bp ladder marker; 1-4 (short arm specific STMS primer gwm 18): 1=Chinese Spring, 2=Rye, 3=UP 2338, 4=WL 711; 5-8 (long arm specific STMS primer gwm 153): 5=Chinese Spring, 6=Rye, 7=UP 2338, 8=WL 711.

in all Triticeae members and therefore are common between wheat and rye. In an earlier study, using multicolour fluorescence *in situ* hybridization (Mc-FISH), repeated DNA sequences from rye were also shown to be distributed on many wheat chromosomes (MUKAI 1996).

The eight genotypes detected to carry T1BL.1RS included seven cultivars, and each of them were found to be relatively high yielding, possessing high level of field tolerance against three important diseases including leaf

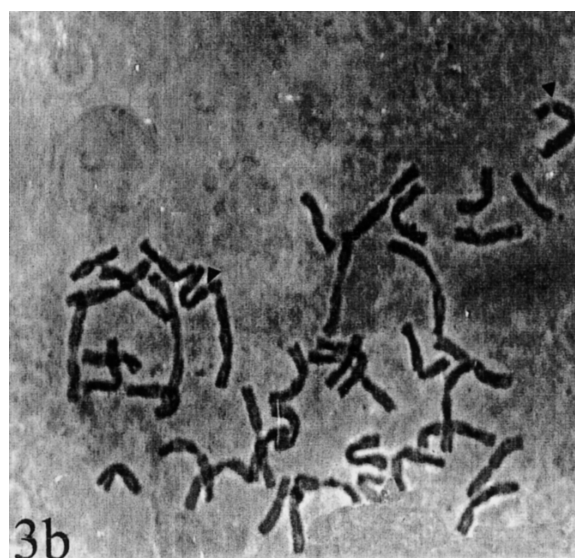
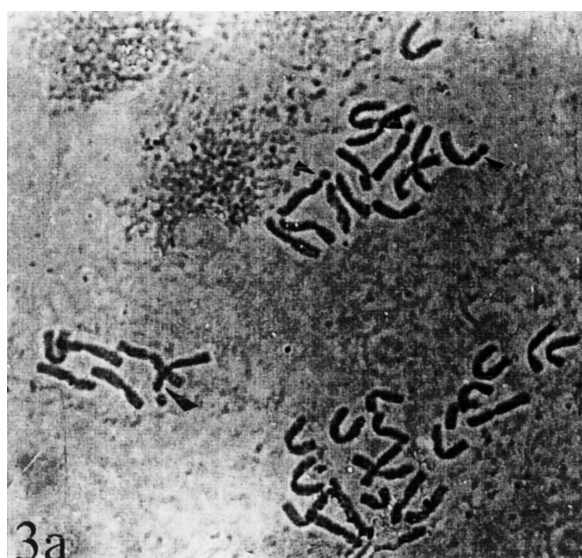


Fig. 3 – Somatic metaphase of bread wheat ( $2n=6x=42$ ) genotypes (a) Chinese Spring, showing two pairs of satellited chromosomes, and (b) UP 2338, showing one pair of satellited chromosomes.

rust, stem rust and powdery mildew (Table 2). In earlier studies on bread wheat these useful traits have often been attributed to T1BL.1RS (SINGH *et al.* 1990; MCINTOSH *et al.* 1993; CARVER and RAYBURN 1994; MORENO-SEVILLA *et al.* 1995; VILLAREAL *et al.* 1998). The T1BL.1RS is also known to have imparted adaptation and stress tolerance (RAJARAM *et al.* 1983; VILLAREAL *et al.* 1994). These useful attributes in 1RS are, however, associated with poor bread making quality (DHALIWAL *et al.* 1987; GRAYBOSCH *et al.* 1993; FENN *et al.* 1994). In wheat genotypes with T1BL.1RS, available evidence also indicates breakdown of resistance to leaf rust due to *Lr26* and that of powdery mildew due to *Pm8* in Europe and Mexico (ZELLER and HSAM 1984; BENNETT 1984; LUTZ *et al.* 1992; see VILLAREAL *et al.* 1998). Breakdown of resistance imparted by 1RS to several diseases may also be witnessed in other parts of the world including India, where bread wheat varieties containing T1BL.1RS are currently grown on a large scale. This is often attributed to narrow genetic base contributed by the 1RS chromosome arm from rye cv. Petkus in all wheat genotypes (VILLAREAL *et al.* 1998). Therefore, in wheat breeding programmes, it is necessary to take immediate measures to ensure long-term resistance to diseases and to offset the negative effects of 1RS on grain quality. Following measures may be used to achieve this objective. (i) The sources of 1RS having different genes for disease resistance need to be used to diversify resistance genes; (ii) 1RS may be translocated to either 1A or 1D instead of 1B. Some efforts in this direction have already been made. For example, recently KO *et al.* (2002) produced T1BL.1RS using Korean varieties of wheat and rye with a view to diversify the source of 1RS in wheat breeding programmes. However, the agronomic value of the genes located on 1RS from this new source of Korean rye still remains to be examined. Similarly, T1AL.1RS in wheat cv. Amigo, where 1RS has been contributed by rye cv. Insave, has powdery mildew resistance gene *Pm17* instead of *Pm8* located on 1RS derived from rye cv. Petkus (HEUN *et al.* 1990). The T1AL.1RS is also less detrimental to bread making quality than T1BL.1RS translocation (GRAYBOSCH *et al.* 1993). Therefore, efforts need to be made to exploit these sources in future wheat breeding programmes to exploit the beneficial effects of 1RS.

## CONCLUSIONS

Eight Indian bread wheat varieties/genotypes containing T1BL.1RS, identified during the present study, may be used as genetic resource in wheat breeding programmes. However, resistance to some diseases provided by genes on 1RS has broken down in some wheat growing areas and this may also breakdown in Indian varieties carrying T1BL.1RS. The absence of 1BS in these varieties/genotypes also led to poor grain quality, so that efforts need to be made to use 1RS from diverse sources, and translocated to 1A or 1D chromosomes instead of 1B to ensure long term resistance provided by 1RS and to develop high yielding wheat varieties, which possess not only tolerance to stress but also better grain quality.

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