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RESEARCH ARTICLE

Identification of Alien Chromosome/Chromatin Introgressions in Triticale × Wheat Derived Stable Lines Through Molecular Cytogenetic Analysis

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ABSTRACT

The present study identified rye chromosomes/chromatin in 17 BC1F7 and 12 F7 lines. The GISH and FISH methods were both successful in detecting rye chromatin during metaphase. Except for a few lines, all of the lines were linked to foreign chromosomal translocation, addition, or substitution. The majority of the recombinants found in TW 1, TW 2, and TW 6 crosses were found to be associated with important alien chromatin translocations such as 1BL/1RS, 1R (1D), 5R (5D), and a combination of both, i.e. 1BL/1RS + 5R (5D), as well as the presence of more than four rye chromosomes in some cases. The chromosomal composition of the hybrids TW 3 and TW 5 contained 8 to 14 rye chromosomes. As a result, their morphological expression resembled that of triticale. These restored lines will be extremely useful in future wheat breeding efforts.

Keywords: Chromosomes; Introgressions; Wheat; Triticale; FISH; GISH

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INTRODUCTION

Translocation lines are commonly developed using wheat–alien chromosomal addition or replacement lines as a bridge material in breeding programmes (Smith et al., 1968; Graybosch, 2001). After the creation of an interspecific or intergeneric hybrid, the nature of the hybrid must be confirmed, or repeated DNA sequences can be used as probes to ensure that the parental genomes are clearly distinguished. Similarly, Carvalho et al. (1997) employed this technique to measure the level of alien chromatin in translocated chromosomes, and to detect wheat–barley spontaneous translocations (Carvalho et al., 1997). " (Molnar-Lang and colleagues, 2000; Prieto and colleagues, 2001)."

In several wheat breeding initiatives throughout the world, the rye chromosomal arm 1RS is being used (Zhou et al., 2004). In all wheat, Villareal et colleagues (1998) found that a tiny genetic base provided by the 1RS chromosomal arm from the cv. Petkus rye cultivar was responsible for the genetic weakness caused in 1BL.1RS cultivars. The Korean rye cultivar Paldanghomil has produced a number of unique wheat lines with the 1BL.1RS translocation (Zhang and Ren, 2007; Ren et al., 2009). Aiming to bridge triticale and bread wheat, this study aimed to introduce the rye chromatin from triticale.

MATERIALS AND METHODS

Plant Materials

Triticale x wheat recombinants of various generations were created from triticale x wheat hybrids, such as ITSN 105/58 x VL 802 and TL 2908 x HS 396, as well as back crosses, such as (ITSN 105/58 x VL 802) x VL 802, (TL 2908 x VL 802) x VL 802, (TL 2900 x RL-14-1) x RL-14-1 In this study, wheat-like plants from 43 stable triticale x wheat derivatives in BC1F7 and F7 generations were used for molecular cytogenetic analysis using the GISH and FISH approaches (Yamamoto and Mukai, 1989; Mukai et al., 1993) to detect and characterise the introgressed rye chromatin.

Molecular Probes and labelling

To detect and characterise alien introgressions, molecular probes such as the rye genomic probe, ribosomal DNA probe (pTa 71), and repetitive DNA sequence probes (pSc119 and pAs1) were utilised (Table 1). Following the nick translation methodology provided by, the haptens biotin (Vitamin H) and digoxigenin (Steroid) were used to label all of the probes (Maniatis et al., 1975). The fluorophores fluorescein-conjugated streptavidin used to detect the tagged sites.

Table 1. Details of molecular probes and sources

S.No.	Probes	Source
1.	Rye Genomic	Total rye genome DNA from Himalayan collection
2.	pTa 71	45S rDNA from *Triticum aestivum*
3.	pSc 119	*Secale cereale*
4.	pSc 74	-do-
5.	pAs 1	*Aegilops squarrosa*

Preparation of Chromosomes spreads

A Petri dish lined with Whatman filter paper was used to germinate the seeds of 43 fixed lines. We extracted 2-3 cm long roots and placed them in a vial filled with deionized water, which was stored in ice and processed for 18-20 hours at 40 °C. They were placed on Whatman filter paper after 15 minutes in acetocarmine, and then crushed in 45 percent acetic acid. On the prepared slide, chromosomes were inspected using a phase-contrast microscope. With the coverslip facing up, the chromosome-containing slide was placed on dry ice for 15 to 30 minutes. on the slides. As seen under a phase-contrast microscope, we selected slides with a small number of cells and a fair distribution of chromosomes at the somatic metaphase stage. It is intended for future use and should be stored at -20°C.

Denaturing of Chromosomes

The selected slides for the FISH (experiment were placed in coupling jars containing 70% formamide produced in 2x SSC and maintained in a water bath at 69°C for 2 minutes. After two minutes, the slides were transferred to -20°C 70%, 95%, and 100% ethanol for five minutes each to remove water and formamide. The denatured chromosome is unable to rejoin as a result of this treatment. The slides were taken one by one after the ethanol treatment and dried with a blower.

DNA Probe Mixture preparation

50 percent formamide (3 l), 50 percent Dextran sulphate (2 l), 20x SSC (1 l), 5 g Salmon DNA (0.5 l), 0.1 g labelled DNA (2 l), and sterilised doubly distilled water (1.5 l) were mixed in 1.5 ml microtubes for the probe DNA preparation. Microtubes containing the probe mixture were maintained in 100°C boiling water for at least 10 minutes to denature the tagged probe DNA.

The probe combination comprising microtubes was kept in the icebox for at least 5 minutes after the requisite period had passed. The 10-l probe combination was applied to the treated slide, 18 x 18 mm coverslips were placed on top. The humid chamber was removed from the incubator the next day, and placed in a 2x SSC containing a 100 ml beaker for removal of the coverslips. The slides were then placed at RT for 5 minutes. After five minutes, the slides were moved to a slide basket and placed in a coupling jar containing 50 percent formamide, which was held at 40 °C in the water bath for 15 minutes.

Fluorescence detection mixture

The fluorescent mixture includes 4x SSC+1% BSA, Avidin-FITC (Fluorescein Isothiocyanate) 2.6 µl and Rhodamine-conjugated anti-digoxigenin 2.6 µl. The fluorescence mixture was mixed properly, and 65 µl/slide was poured to each slide. The 24 x 32 mm size parafilm was cut and placed on the fluorescence detection mixture. The slides were placed in the humid chamber which was further kept in the incubator for 1 hr at 37 °C.

Washing of the slides

After one hour, the humid chamber was removed from the incubator and the slides were transferred to 4x SSC and 2x SSC solutions for 15 and 5 min, respectively in the dark with gentle shake.

Preparation of antifade solution

The antifade solution was produced with 25 l DABCO and 0.25 l DAPI (per slide) and placed onto the slide. The slides were covered with 24 x 32 mm coverslips and left in the dark for 30 minutes.

RESULTS

TW1-TW3 Lines

The cross (ITSN105/58 x VL802) x VL 802 probes *viz*., Himalayan rye genomic, rDNA, pAs1 and pSc119 were utilized for the identification of rye chromosome introgression (Table 2). *Secale cereale* of Himalayan origin rye was used as a probe for identification of rye chromatin introgression. The clone pTa71 helped identify major NORs in 1R, 1B and 6B and minor NORs in 1A and 5D (Mukai and Gill, 1991).

Lines derived from this cross, namely TW-1-12 (Figure 1a) have substitution as 1R(1D) and line TW-1-35 possessing the 1BL/1RS translocation. Two of the 42 chromosomes in the translocation lines showed an exchange of 1BL with 1RS. The bright fluorescence revealed the 1R chromosome arms presence in the 1BL/1RS translocated line.

Table 2. Molecular cytogenetic analysis of stable lines derived from triticale x wheat

Sr. No.	Line Name	Probe used	Chromosome number	Result obtained
1.	TW-1-12	Bio: Rye genomic Dig: r DNA	42	1R(1D) substitution
2.	TW-1-35	Bio: Himalaya Rye genomic Dig: r DNA Bio: pAs1 Dig: pSc 119	42	IBL/IRS translocation
3.	TW-1-50	Bio: pSc 119 Dig: Rye genomic	42	No rye chromatin
4.	TW-1-280	Bio: Rye genomic Dig: r DNA	42	No rye chromatin
5.	TW-2-7	Bio: Rye Genomic Dig: r DNA	42	IBL/IRS translocation
6.	TW-2-10	Bio: Rye genomic Dig: r DNA (pTa 71) Bio: r DNA Dig: pAsI Bio: pSc 119 Dig: r DNA	42	IBL/IRS translocation
7.	TW-2-27	Bio; Rye Genomic Dig: r DNA	42	IBL/IRS translocation
8.	TW-2-153	Bio; Rye Genomic Dig: r DNA	42	IBL/IRS translocation
9.	TW-2-160	Bio; Rye Genomic Dig: r DNA	42	IBL/IRS translocation
10.	TW-2-181	Bio; Rye Genomic Dig: r DNA	42	IBL/IRS translocation
11.	TW-2-184	Bio; Rye Genomic Dig: r DNA Bio: pSc 119 Dig: pSc 74	42	1R(1D) substitution
12.	TW-2-186	Bio; Rye Genomic Dig: r DNA Bio: pSc 119 Dig: Rye Genomic	42	1 pair rye chromosome
13.	TW-3-5	Bio: Rye genomic Dig: r DNA	42	14 rye chromosome substitution
14.	TW-3-8	Bio: Rye genomic Dig: r DNA	42	-do-
15.	TW-3-11	Bio: Rye genomic Dig: r DNA	42	-do-
16.	TW-3-24	Bio: Rye genomic Dig: r DNA	42	-do-
17	TW-3-26	Bio: Rye genomic Dig: r DNA	42	-do-
18.	TW-3-29	Bio pSc 119 Dig: pSc 74	42	8 rye chromosome substitution
19.	TW-3-41	Bio pSc 119 Dig: pSc 74		-do-
20.	TW-4-1	Bio: Rye genomic	42	IBL/IRS translocation

		Dig: Rye genomic		
		Bio: rye genomic, pAs1		
		Dig: r DNA, pSc 119		
21.	TW-4-2	Bio: Rye genomic, pAs1	42	No translocation
		Dig: r DNA, pSc 119		
22.	TW-4-9	Bio: Rye genomic	42	No translocation
		Dig: Rye genomic		
23.	TW-4-19	Bio: Genomic Rye	42	No translocation
		Dig: r DNA		
24.	TW-4-22	Bio: Rye genomic	42	No translocation
		Dig: r DNA		
25.	TW-5-23	Bio: pAs1	42	No translocation
		Dig: pSc 119		
26.	TW-4-24	Bio: Rye genomic	42	No translocation
		Dig: r DNA		
27.	TW-4-43	Bio: rye genomic	42	No translocation
		Dig: r DNA		
28.	TW-4-83	Bio: rye genomic	42	No translocation
		Dig: r DNA		
29.	TW-4-122	Bio: rye genomic	42	1BL.1RS translocation
		Dig: r DNA		
30.	TW-5-1	Bio: pAs1	42	No translocation
		Dig: pSc 119		
31.	TW-5-4	Bio: Rye genomic, pAs1	42	4 pair of rye chromosome substitution
		Dig: r DNA, pSc 119		
32.	TW-5-6	Bio: pSc 119, rye genomic	42	-do-
		Dig: rye genomic r DNA		
33.	TW-5-18	Bio: Rye genomic, pAs1	42	No rye chromosome
		Dig: r DNA, pSc 119		
34.	TW-5-31	Bio: Rye genomic	42	10 Rye chromosome substitution
		Dig: r DNA		
35.	TW-5-32	Bio: pSc 119	42	-do-
		Dig: rye genomic		
36.	TW-6-4	Bio: rye genomic	42	IBL/IRS translocation
		Dig: pSc 119		
37.	TW-6-7	Bio: pSc 119	42	IBL/IRS translocation
		Dig: r DNA		
38.	TW-6-243	Bio: Rye genomic	42	IBL/IRS translocation
		Dig: DNA		
39.	TW-6-245	Bio: Rye genomic	42	IBL/IRS translocation
		Dig: Rye genomic		
40.	TW-6-250	Bio: pSc 119	42	No translocation
		Dig: r DNA		
41.	TW-6-261	Bio: Rye genomic	42	IBL/IRS translocation
		Dig: r DNA		
42.	TW-6-267	Bio: Rye genomic	42	IBL/IRS translocation
		Dig: r DNA		
43.	TW-6-285	Bio: rye genomic	42	No translocation
		Dig: rDNA		

There were three probes employed in the cross (TL 2908 X VL 802): rDNA from the Rye genome, as well as pSc119 and Psc74 from the Wheat genome. The TW-2-7, TW-2-27, TW-2-153, TW-2-160, TW-2-181,

and TW-2-10 lines generated from this cross were 1BL/1RS translocations in the wheat genome. FISH with a biotin-labeled rDNA probe produced robust signals at metaphase chromosomes in four triticale x wheat-derived lines studied in this experiment. FISH signals from rDNA locations on six bread wheat somatic chromosomes revealed the short arms of the six chromosomes, while six NOR signals were detected. It was determined that lines TW-2-184 and TW-2-186 were substituted with 1R (1D) (Figure 1b & 1c). In the rDNA, you can see the non-coding portions of chromosomes. Fourteen rye chromosomes were also discovered using whole genomic DNA as a probe. The rye chromatin's vivid green hybridization signals and rDNA signals at the terminal ends of the chromosomes are readily apparent.

TW4-TW6 Lines

For the cross (TL 2900 X RL-14-1) x RL-14-1 probes viz., rye genomic and rDNA, pSc119 and pAs1 were used to analyze the alien introgression. But all the lines were not having any translocation, addition and substitution except the lines TW-4-1, and TW-4-122 possessing 1BL/1RS translocation evident from the 1 pair of rye chromosome translocation showing yellow-green fluorescence. The lines derived from TL 2908 X HS 396 were analyzed for translocation and substitution lines. Line TW-5-4, TW-5-6 and TW-5-31 were having 4, 4 and 5 pairs of rye chromosomes, respectively (Figure 2a, 2b & 3). The rye genomic DNA probe introgressed in the rye chromosomes present in the lines showing strong green colour signal. These lines were close to the triticale. Line TW-5-18 was not carrying the rye chromosome.

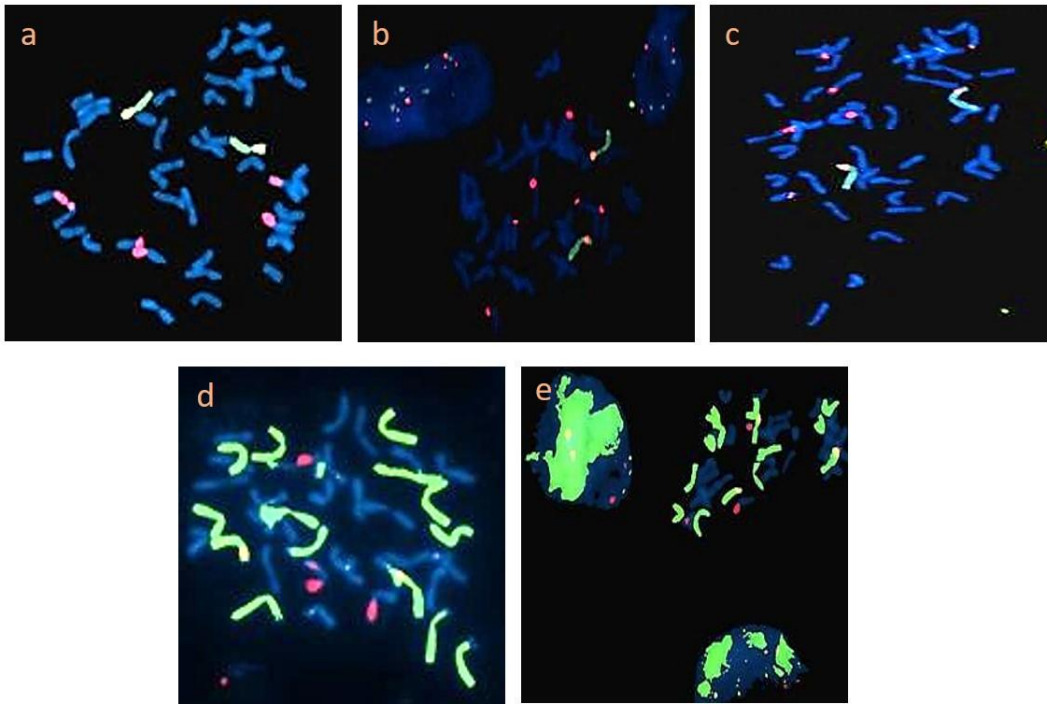

Figure 1. Detection of 1R substitution in triticale × wheat derived wheat lines

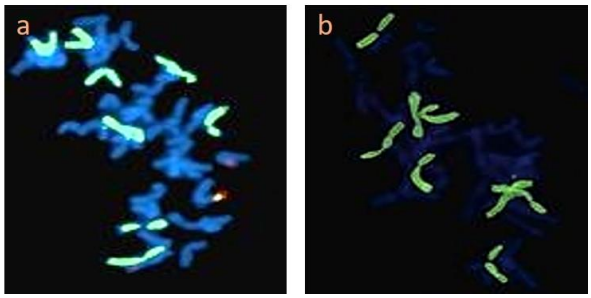

Figure 2. Detection of 4 pair of rye chromosome substitution

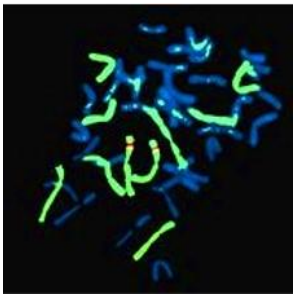

Figure 3. Detection of 5 pair of rye chromosome substitution

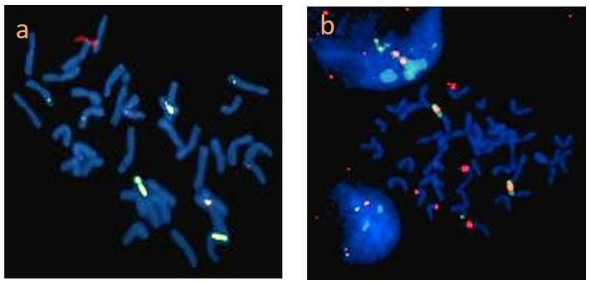

Figure 4. Detection of 1 BL/1 RS translocation

The cross (TL 2919 X PW565) X PW 565 probes viz., pSc119, rye genomic and rDNA were used to find out translocation, addition and substitution lines. The present investigation of *in situ* evaluations exploitation rye genomic DNA and the probes pSc119 revealed the 1BL/1RS translocation in TW-6-4, TW-6-7, TW-6-243, TW-6-245, TW-6-261 and TW-6-267 (Figure 4a & 4b) through strong green fluorescence. The red colour of rDNA identified the translocated arm of 1RS of rye chromosome.

DISCUSSION

TW 1- TW 3 Lines

To identify alien chromatin introgressions, GISH is identifying rye chromosome arms particularly effective in wheat background. In the present study of TW 1 lines, viz., Line TW-1-12 have IR(ID) substitution, and line TW-1-35 possesses IBL.IRS translocation corroborates with the study of Liu, et al. (2008). TW 2 lines are carrying IBL/IRS translocation, and IR(1D) substitutions in association with the small nuclear DNA amount variations.

The TW3 lines (ITSN 105/58 x VL 802) are carrying more than 10 rye chromosomes, which is similar to the study of Angelova, and Georgiev (2006), who used total rye genomic DNA as a probe and distinguished 12 rye chromosomes in 6x triticale. The green colour fluorescence shows lines TW-3-29 and TW-3-41 carrying a variable number of rye chromosomes. This is in accordance with Brasileiro-Vidal et al. (2005), where they have studied wheat x *Thinopyrum ponticum* cross and identified the whole genome of wheat in the derivatives. Li et al. (2018), used chromosome-specific FISH-based markers, two accessions of tetraploid *Thelongatum*.

TW 4-TW6 lines

The cross, (TL 2919 x PW 565) x PW565 all the lines are carrying 1BL/1RS translocation except TW-

The two lines TW-4-1 and TW-4-122 possesses the 1BL/1RS translocation and one pair of rye chromosome translocation. This finding is corroboration with Yu et al. (2001),

TW 5 lines are derived from the cross TL 2908 x HS 396 and the lines TW-5-41, TW-5-6 and TW-5-31 were carrying the 4,4 and 5 pairs of rye chromosome respectively and phenotypically they looked like triticale. Similar results were observed by Hohmann, et al. (1999), in wheat and rye cross that one 1D addition, six 1D (1R) substitutions and nine 1D (1B) substitutions line in the study undertaken by them. The rye chromosomes are predominantly recognized by their large heterochromatic regions of the telomeres.

6-250 and TW-6-285 by studies of Mukai et al. (1993). Present result substantiates the report provided Angelova and Georgiev (2006).

CONCLUSION

The rye is a potential source for resistance gene for diseases viz., powdery mildew, rust etc., and insects, but direct transfer of genes is difficult for that we can and these lines have been isolated using FISH and GISH technique.

use the triticale as a bridging species. In the present investigation using triticale several substitutions, addition and translocational lines have been obtained

AUTHOR CONTRIBUTIONS

MSJ, HKC and RKC conceptualized the manuscript. MSJ performed the experiment. MSJ, HKC and RKC wrote the manuscript. KA edited and updated the manuscript.

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DISCLOSURE STATEMENT

The author declares no competing interests

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