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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

Molecular cytogenetic study was conducted for identifying rye chromosome/chromatin translocation, substitution and addition in 43 triticale x wheat derived stable lines (BC_1F_7 and F_7 generations) six different crosses using FISH (Fluorescence in situ hybridization) and GISH (Genomic in situ hybridization) analyses with different probes. Rye chromosomes/chromatin was identified in 17 BC_1F_7 and 12 F_7 lines by these two analyses. The GISH and FISH techniques worked satisfactorily for identifying the presence of rye chromatin at metaphase. All the lines were associated with alien chromosome translocation, addition or substitution except a few lines. Most of the recombinants present in TW 1, TW 2 and TW 6 crosses were observed to be associated with important alien chromatin translocation like 1BL/1RS, substitutions 1R (1D), 5R (5D) and combination of both i.e., 1BL/1RS + 5R (5D) and in some cases presence of more than 4 rye chromosomes. The crosses TW 3 and TW 5 possessed 8 to 14 rye chromosomes in their chromosome constitution. So, their morphological expression was similar to triticale. These reconstituted lines will be of immense application for the future wheat breeding programmes.

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

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INTRODUCTION

Wheat–alien chromosome addition or substitution lines have been widely used as a bridge material in breeding programmes to generate translocation lines. These translocations might result from hybridization between similar-sized fragments belonging to homoeologous chromosomes of wheat–alien hybrids, Smith et al. (1968) or centric-breakage-fusion cycles (Sears, 1972), inducing Robertsonian translocations. Known wheat-rye translocations such as 1BL/1RS and 1AL/1RS, among others, have been introduced in *Triticum aestivum* in the globe to transfer interesting agronomic characteristics from rye to wheat (Graybosch, 2001). After the production of an interspecific or intergeneric hybrid, the confirmation of its nature is required, and the fluorescent *in situ* hybridization (FISH) technique was extensively used for that reason. FISH could be performed using only total genomic DNA, genomic *in situ* hybridization (GISH) from both parents, as probes, ensuring the clear parental genomes differentiation or it could include sequences of repetitive DNA. Additionally, this technique already provided breakpoints determination and the amount of alien chromatin in translocated chromosomes (Carvalho et al., 1997) and the detection of wheat–barley spontaneous translocations (Molnar-Lang et al., 2000; Prieto et al., 2001).

In wheat breeding programs worldwide, the rye chromosome arm 1RS has been widely used (Zhou et al., 2004). However, the genetic weakness caused predominantly by wider cultivation of the 1BL/1RS cultivars resulted from a narrow genetic base contributed by the 1RS chromosome arm from cv. Petkus rye in all wheat Villareal et al. (1998). Few new wheat lines possessing the 1BL/1RS translocation derived from Korean rye cultivar Paldanghomil, Chinese Weiling rye, and variation of cv. Petkus rye has been reported (Zhang and Ren, 2007; Ren et al., 2009). Based on the above interests, the present investigation was aimed to introgression the rye chromatin from triticale as a bridging species to bread wheat.

MATERIALS AND METHODS

Plant Materials

Triticale x wheat recombinants in different generations were derived from the triticale x wheat hybrids *viz*., ITSN 105/58 x VL 802 and TL 2908 x HS 396 and back crosses *viz*., (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 and (TL 2919 x PW 565) x PW 565 lines. Wheat like plants of 43 stable triticale x wheat derivatives in BC_1F_7 and F_7 generations developed through conventional approach were used in this study for molecular cytogenetic analysis following Genomic *in situ* Hybridization (GISH) and Fluorescence *in situ* Hybridization (FISH) approaches (Yamamoto and Mukai, 1989; Mukai et al., 1993) so as to detect and characterize the introgressed rye chromatin and sort out the wheat like recombinants with minimum linkage drag.

Molecular Probes and labelling

Labeling of DNA probes for FISH by nick translation

Molecular Probes *viz*., Genomic probe of rye, Ribosomal DNA probe (pTa 71) and repetitive DNA sequences probes (pSc119 and pAs1) were used to detect and characterize the alien introgressions (Table 1). The haptens *viz*., biotin (Vitamin H) and digoxigenin (Steroid) were utilized for labelling all the probes following the nick translation protocol given by (Maniatis et al., 1975). Detection of the labeled sites was executed by the fluorophores *viz*., fluorescein-conjugated streptavidin and rhodamine-conjugated anti-digoxigenin.

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

The seeds of selected 43 fixed lines were kept for germination in Petri plate lined with Whatman filter paper as a germination medium. After 2-3 days, the root tips of 2-3 cm long were excised and transferred to the 5 ml vial containing distilled water and the vial is kept in the ice and was placed at 40 °C for 18-20hr as a pretreatment. After the pretreatment, the roots were fixed in a freshly prepared solution of absolute ethanol and glacial acetic acid (3:1). The roots were kept in the acetocarmine for 15 min then the roots were placed in the Whatman filter paper and using the razor blade, the root cap was removed, and the small piece of meristem was squashed in 45% acetic acid. The prepared slide was observed in the phase-contrast microscope for a good spread of chromosomes in metaphase. Chromosome containing slide was placed on the dry ice with the coverslip

facing upside for 15-30 min. After dry ice treatment, the coverslip was removed using the blade and immediately kept in the 45% glacial acetic acid for 15-30 min. After 15-30 min, the slides were removed from 45% acetic acid and kept for drying for a while. The slides were then placed in the silica gel containing desiccator for overnight. The next day, slides were screened for FISH experiments. The slides with the moderate number of cells and a good spread of chromosomes in somatic metaphase were selected under the phase-contrast microscope. The selected slides were kept in the slides box, sealed and stored in -20 °C for further use.

Denaturing of Chromosomes

The selected slides for FISH (experiment were placed in the coupling jars, which contain 70% formamide prepared in 2x SSC kept in water bath, maintaining the temperature 69ºC for 2 min. After two min, the slides were transferred to -20ºC 70%, 95% and 100% ethanol each for five min for removing water and formamide. This treatment does not allow the denatured chromosome to reunite. After the ethanol treatment, the slides were removed one by one and dried using a blower.

DNA Probe Mixture preparation

The probe DNA preparation includes 50% formamide (3µl), 50% Dextran sulphate (2µl), 20x SSC (1µl), 5µg Salmon DNA (0.5 µl), 0.1 µg labeled DNA (2µl) and sterilized doubled distilled water (1.5 µl) were mixed in 1.5 ml microtubes. The probe mixture contained microtubes were kept at 100ºC boiling water to denature the labelled probe DNA for at least 10 min. After the required time, the probe mixture containing microtubes was kept in the icebox for 5 min and more. The probe mixture of 10 µl was added on the treated slide, the 18 x 18 mm coverslips were placed on the mixture, and the slides were placed in the humid chamber. The chamber was further placed in the incubator (37ºC) for 16 hr for proper hybridization. The next day, the humid chamber was taken from the incubator, and the slides were removed from the humid chamber and kept in the 2x SSC containing 100 ml beaker for removing of the coverslips. Then the slides were placed in the beakers containing 2x SSC for 5 min at room temperature. After five min the slides were transferred to slide basket kept in 50% formamide containing coupling jar kept at 40 ºC in the water bath for 15 min. Then the slides were transferred to 2x SSC and 1x SSC each for 15 min at room temperature. Finally, the slides were transferred to 4x SSC solution for 5 min at room temperature.

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 buffer solutions for 10 min at room temperature in the dark. Then the slides were transferred to 4x SSC solution having 0.1% Triton X-100 for 10 min at room temperature. Then 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 includes 25 µl DABCO (1,4-Diazabicyclo (2,2,2) octane) and 0.25 µl DAPI (per slide) was prepared and poured on the slide. The coverslips of size 24 x 32 mm were placed on the slides and kept in the dark for 30 min.

Microscopy and Photography

Hybridization signals were detected with Olympus fluorescence microscope equipped with a filter for FITC (Fluorescein Isothiocyanate), filter for Rhodamine and a triple-band filter set for FITC, DAPI (4',6-Diamidino-2-Phenylindole) and Rhodamine. Pictures were taken by Olympus CCD camera.

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, whereas other chromosome arms showed only a weak signal.

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

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 Dig: Rye genomic Bio: rye genomic, pAs1 Dig: r DNA, pSc 119	42	IBL/IRS translocation
21.	TW-4-2	Bio: Rye genomic, pAs1 Dig: r DNA, pSc 119	42	No translocation
22.	TW-4-9	Bio: Rye genomic Dig: Rye genomic	42	No translocation

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

Bright terminal bands revealed by DAPI staining on both of the 1R origin chromosome arms, and in wheat originated chromosome arms smaller DAPI-bright segments were visible. In lines TW-1-50 and TW-1-280, wheat chromosomes segments were not hybridized with the rye DNA probe, indicating the absence of rye chromosome segments. For identification of smaller rye chromosome segments introduced into wheat, large populations need to be screened.

For the cross (TL 2908 X VL 802) X VL 802 probes viz., rye genomic rDNA, pSc119 and pSc74 were used to detect the rye chromatin and different wheat genomes. The lines derived from this cross *viz*., TW-2-7, TW-2-27, TW-2-153, TW-2-160 TW-2-181 and TW-2-10 were constituted 1BL/1RS translocation in the background of the wheat genome. In this investigation, four triticale x wheat-derived lines, FISH with the biotin labelled probe of rDNA produced strong signals at the secondary constriction of the satellite mitotic metaphase chromosomes. The short arms of six somatic chromosomes in the chromosome complement of bread wheat were shown by FISH signals of the rDNA sites, which hybridized with the probe were six signals on the NOR regions. Lines TW-2-184 and TW-2-186 were identified as 1R (1D) substitutions (Figure 1b & 1c). Metaphases with 2n=42 mostly had four chromosomes with distinct satellites beyond the secondary constriction at the

NOR, co-located with the rDNA probe, all on triticum-origin chromosomes. Wheat loci showed strong terminal decondensation of the rDNA loci, with the condensation of the genes seen by a strong hybridization signal close to the centromere and greater diffusion near the satellite chromosome. Two major NORs were detected by in situ hybridization with the probe rDNA on chromosomes found as wheat chromosomes lB and 6B. Minor sites, most probably originating from wheat chromosomes 5D and 1A were usually exposed. Still, under the hybridization and detection (including photographic) conditions, we could not expect to observe smaller NORs as they would be below the visualization threshold. Such a GISH pattern of genome differentiation has allowed several distinctively coloured chromatin domains to be visible on interphase nuclei, which might reflect the somatic spatial arrangement of every single genome in the wheat-rye hybrids. We have cytologically confirmed by GISH the rye segment's presence in the wheat lines with the larger genome size as measured by flow cytometric analysis (Wetzel et al., 1998). The cross ITSN 105/58 X VL 802 derived lines TW-3-5, TW-3-8, TW-3-11, TW-3-24 and TW-3-26 (Figure 1d & 1e) were triticale types because they all were carrying more than 10 rye chromosomes shown by rye genomic DNA probe hybridization. The rDNA shows the NOR regions of the chromosomes. Using total genomic DNA as a probe, 14 chromosomes of rye origin were also identified. The bright green hybridization signals along the rye chromatin could be observed clearly, and rDNA signals in the terminal end of the chromosomes are also visible.

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.

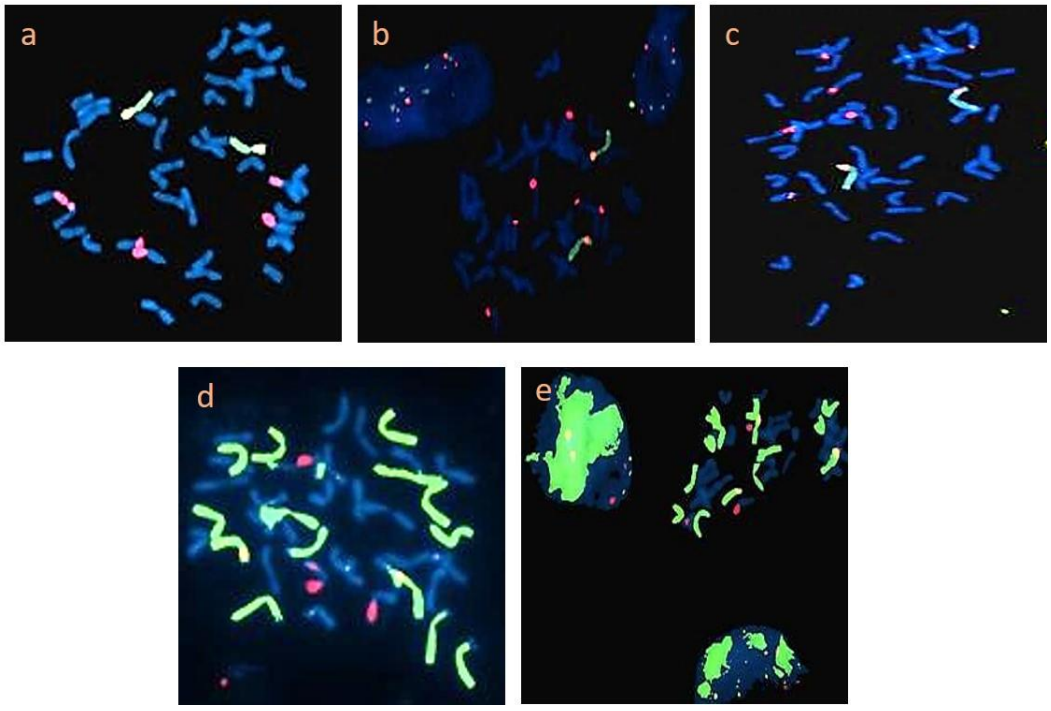

Figure 1. Detection of 1R substitution in triticale × wheat derived wheat lines with the probe Bio: rye genomic (green), Dig: rDNA(red). a), TW-1-12; b), TW-2-184; c), TW-2-186; d, TW-3-5; e), TW-3-11.

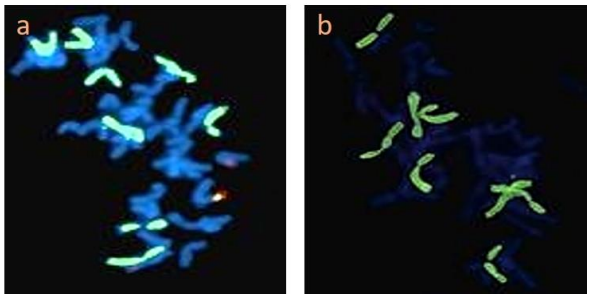

Figure 2. Detection of 4 pair of rye chromosome substitution in triticale x wheat derived bread wheat line, a), TW-5-31 & b), TW-5-6 with the probe Bio: rye genomic (green), Dig: rDNA (red)

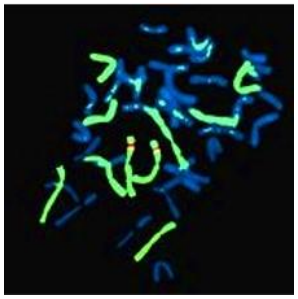

Figure 3. Detection of 5 pair of rye chromosome substitution in triticale x wheat derived bread wheat line, TW-5-31 with the probe Bio: rye genomic (green), Dig: rDNA (red)

DISCUSSION

TW 1- TW 3 Lines

To identify alien chromatin introgressions, GISH is considered an efficient method and identifies rye chromosome arms particularly effective in wheat background. This method can also indicate the number and chromosomal location of the rye chromosomes or chromosome segments present. 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), where they reported that wheat-rye chromosome translocations were commonly detected in the *Triticum aestivum* cv. Anyuepaideng– *Secale africanum* amphiploid. The sensitivity of this probe has enabled detection of previously undiscovered wheat-rye translocation lines. Similarly, Carvalho et al. (2001), also observed the introgression of rye-origin chromatin onto wheat chromosome arm 2DL in two of the lines using genomes of rye, combined with 45S rDNA and the repetitive sequences dpTa1 and pSc119.2 as probes in two sequential *in situ* hybridization method.

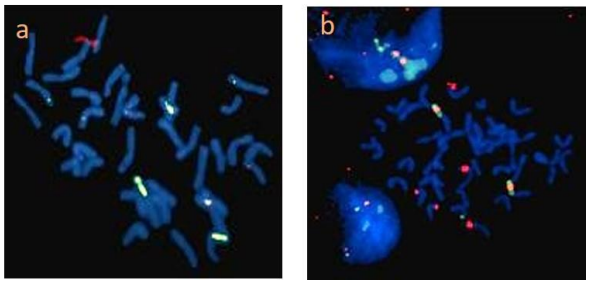

Figure 4. Detection of 1 BL/1 RS translocation in triticale x wheat derived bread wheat line, a), TW-2-10; b), TW-6-4 & TW-6-261 with the probe Bio: rye genomic (green), Dig: rDNA (red).

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 using 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. The labeled total genomic probe hybridized strongly to the rye chromosome arms in triticale x wheat derivatives in metaphase cells. The 1RS translocation was visible as a discrete entity throughout the cell cycle.

TW 2 lines are carrying IBL/IRS translocation, and IR(1D) substitutions in association with the small nuclear DNA amount variations found by [15] are real, and the difference among the wheat lines was found to be due to the substitution of a rye 1RS chromosome arm for the 1BS chromosome arm. The rye 1RS chromosome arm in wheat cultivars affects their performance positively and gives resistance against pathogen and insect attacks (Carver and Rayburn, 1994). In all our wheat x rye derivatives samples analyzed, the quality of *in situ* hybridization was highly satisfactory for diagnosing alien chromosomes/chromatin. The same results were obtained by Islam-Faridi and Mujeeb-Kazi (1995), in wheat/rye translocation lines. In our study, the technique is enough insightful and precise for detecting the presence of alien chromosome segments, representing the wonderful potential for resolving cryptic wheat/alien exchanges both at interphase and metaphase.

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* and the hexaploid *Trititrigia* 8801 were characterized by different repetitive sequences probes and found that all E-genome chromosomes could be correctly identified using a combination of pSc119.2, pTa535, pTa71, and pTa713 repeats.

TW 4-TW6 lines

The cross (TL 2900 x RL-14-1) x RL-14-1 only 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), where they detected *Haynaldia villosa* chromosomes in telosomic and translocation lines of common wheat using GISH, C-banding techniques and polyacrylamide gels electrophoresis. Mitotic GISH analysis showed that the 6VS/6AL translocation chromosome remained unchanged after being transferred into the new wheat background. *In situ* hybridization using *Secale africanum* genome as a probe revealed that probe pSaD15940 was exclusively hybridized throughout all rye chromosomes arms except for the terminal regions (Liu et al., 2008). This result also following the studies of Chaudhary (2008a and b), who detected 1BL/1RS translocation in different triticale x wheat-derived wheat lines.

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. Out of these three lines were pure AABBRR hexaploids without any D-genome chromosomes. For the remaining 42 lines (51.8%), a wide spectrum of 20 different recombination between chromosomes 1A and 1D was uncovered. Using C-banding, GISH, FISH and restriction fragment length polymorphism (RFLP). Wang et al. (2001), screened the amphidiploids common wheat-*R. ciliaris* and BC1F7 or BC2F6 derivatives and reported the presence of *R. ciliaris* chromatin introgressed into wheat. The genomic affinities of the added *R. ciliaris* chromosomes were revealed by FISH technique using the repetitive sequence pCbTaq4.14 as a probe. The rye chromosomes are predominantly recognized by their large heterochromatic regions of the telomeres.

The cross, (TL 2919 x PW 565) x PW565 all the lines are carrying 1BL/1RS translocation except TW-6-250 and TW-6-285 by studies of Mukai et al. (1993), where many wheat cultivars carry the 1RS arm, but in most cases, group-1 chromosomes, that is, 1B, 1A or 1D of wheat chromosomes has its original arm substituted by 1RS. Present result substantiates the report provided Angelova and Georgiev (2006), in which total genomic DNA from rye was labeled with biotin and used as a probe for *in situ* hybridization to show the sizes and translocation points of the rye chromosome segments in wheat mutant forms K1 and K2, which carry a translocation between wheat chromosome 1B, and the short arm of rye chromosome 1R (1B/1R). Ren et al. (2009), also developed a new 1BL/1RS line, R14, by means of crossing rye inbred line L155, selected from Petkus rye to several wheat cultivars. Mukai et al. (1993), in hexaploid wheat and Kubalakova et al. (2005), in durum wheat used FISH analysis of repeated DNA sequences also revealed minor differences in their genomic distribution between the hexaploid wheat and durum wheat. In triticale, the D genomes' stability seems more strongly affected by the R genomes than that of the A and B genomes (Dou et al., 2006). Therefore, it is reasonable that the whole or part of the D genomes was lost in these lines. Fu et al. (2010), screened 100 wheat lines derived from monosomic additions of chromosome 1R of rye using GISH technique and reported that lines 1R296, 1R330, and 1R725 contained translocations involving the whole short arm of chromosome 1R. However, 1R314 and 1R734 had a pair of wheat chromosomes with small, terminal, rye-derived chromosome segments. Similarly using MC-FISH, two stable wheat-rye primary T1RS/1BL translocated lines from the progeny of the crossing of the wheat cultivar Mianyang11-1 and a Chinese local rye variety, Weining (Ren et al., 2017). They have also suggested that using the combination of molecular and cytological analysis detection of small alien chromatin introgressions were efficient.

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

and these lines have been isolated using FISH and GISH technique. The isolated lines can be utilized in the future wheat improvement programs.

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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