Doubled haploid production in advanced back cross generations and molecular cytogenetic characterization of rye chromatin in triticale × wheat derived doubled haploid lines

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Key words: Haploid, Triticale × wheat backcross, FISH, GISH, Rye chromatin

Abstract: The rye genome has shown potential for improvement of bread wheat, where wheat-rye substitutions and translocations have been and are frequently used in resistance breeding. Crosses belongs to different generations viz., BC1F1, BC1F2, BC1F3, BC1F4 and BC2F3 of triticale × wheat derived were used for different haploid induction parameters using Gogon grass (Imperata cylindrica) as a pollen source. The percentage of pseudo seed formation ranged from 34.55% for BC1F2 to 63.77 for BC1F1 crosses, the haploid embryo formation ranges from 9.43% for BC1F1 to 30.2% for BC1F2, the haploid plant generation ranges from 19.36% for BC1F2 to 63.25% for BC1F1. Four doubled haploids were developed from ITSN 105/58/C2 VL 802/C2 VL 802 of BC2F3 underwent molecular cytogenetic analyses using the probes, viz., rye genomic rDNA, pSc 119 and pAs1. FISH and GISH analysis revealed an IBL.1RS translocation and substitution of 5R chromosome instead of the 5D chromosomes in these doubled haploids.

Abbreviations

FISH: Fluorescence in situ hybridization
GISH: Genotypic in situ hybridization
2,4-D: 2,4-dinitrophenylhydrazine
SSC: Saline sodium citrate,
BSA: Bovine serum albumin,
DAPI: 4’,6-diamidino-2-phenylindole

Introduction

Among the food grain crops of the world, wheat (Triticum aestivum L. em Thell) is pre-eminent regarding its antiquity and importance as a food of humankind (Arjona et al., 2020). There has been a tremendous increase in wheat production in India since the time of the Green Revolution. This has been possible with the introduction of the dwarf wheat genotypes that carrying the dwarfing genes viz. Rht-B1b and Rht-D1b from Norin-10-Brevor-14 background (Borner et al., 1996) and the genes for photo-insensitivity from Mexican spring wheat. Wheat improvement has led the country’s efforts to reach the status of self-sufficiency in food grain production (Rani and Mor, 2020). However, at present, production is affected by climate change and increase in pest and diseases infestation (Dar et al., 2020; Wani et al., 2020). To address this problem, new disease resistant genes need to be introgressed from cultivars/wild resources (Pietrusinska et al., 2018; Klymiuk et al., 2019). In the mountainous regions of India, wheat is generally grown under diverse and rainfed conditions (Dar et al., 2020) Thus, drought becomes the major constraint, followed by frost stress and susceptibility to various diseases, which drastically reduce wheat production in these areas. Therefore, the breeding objectives must be essentially comprised of the development of high yielding varieties resistant to abiotic (drought and frost) and biotic (rusts and powdery mildew) stresses prevalent in this region. Winter wheat and rye are the important sources can be used for the transfer of resistance for biotic and abiotic stresses to spring wheat.

Triticale (× Triticosecale Wittmack) may be used as a bridging species to accomplish the transfer of the rye chromatin into the background of wheat. Because of rye
chromosome complement, triticales have many agronomic attributes not found in wheat (Merker, 1984). To isolate promising recombinants from the segregating populations, careful selection of parents is required on the part of breeders for triticale × wheat hybridization programs. To achieve faster desirable results, doubled haploid breeding has a significant advantage over conventional breeding, where it leads to the production of completely homozygous plants in just a single step (1 year). In contrast, the conventional breeding approach takes about 7–8 years to isolate stable lines from the crosses. Bread wheat has been improved using the rye genome as a potential resource, where wheat-rye translocations and substitutions have been and are commonly used in resistance breeding (Rabinovich, 1998), and the 1B/1R wheat-rye translocation is incorporated in most of the wheat currently grown around the world (Heslop-Harrison et al., 1990). Doubled haploid breeding helps to attain homozygous populations from the triticale × wheat hybrids and their backcrosses following chromosome elimination approach through the use of Imperata cylindrica-mediated systems. It is further required to enhance the precision and efficiency of selection amongst the newly developed wheat doubled haploids to identify alien chromatin/genes introgressed with minimal introgression of undesirable genes. The molecular cytogenetic approach gives various powerful and novel tools such as genomic in situ hybridization (GISH) and fluorescence in situ hybridization (FISH) which can help in the physical mapping of introgressed chromatin/genes into wheat genome. The present investigation envisages the physical mapping of introgressed chromatin/genes into wheat genome. The present investigation envisages the development of triticale × wheat-derived doubled haploids involving triticale and elite wheat lines following intergeneric hybridization with I. cylindrica and identification and characterization of rye chromatin introgressed into wheat genome (developed through DH breeding) through FISH and further isolation of wheat like doubled haploids having the desired rye chromatin with minimal undesirable genes.

Materials and Methods

Plant material

Triticale × wheat (Tab. 1) derived populations of different backcross generations used for doubled haploid production following chromosome elimination approach by using Imperata cylindrica pollen and further utilized to detect rye chromatin introgression using GISH and FISH techniques. The present work was carried out in the Molecular Cytogenetics Laboratory of Department of Crop Improvement, CSK HP Agricultural University, Palampur, H.P, India.

Wide hybridization procedure

Emasculation was done three days before anthesis by removing the anthers manually. The next day, fresh pollen from I. cylindrica was collected and applied to the feathery stigma of the emasculated spikes. On third day, the spikes were injected with a 2.4-D solution of 250 mg/L concentration (Pratap and Chaudhary, 2007) at the base of the uppermost internode using a syringe fitted with a fine hypodermic disposable needle. Petroleum jelly (Vaseline-Hindustan Lever, Ltd.) was used for sealing the injection holes. The injections were repeated for two more consecutive days to ensure proper seed and embryo formation. Murashige and Skoog medium (Murashige and Skoog, 1962) was used to rescue haploid embryos. This medium was supplemented with 0.5 mg/L kinetin, 150 mg/L glutamine, 20 mg/L each arginine, cysteine and leucine, and solidified agar. The pollinated spikes were harvested from the tiller base after 18–20 days of pollination. The embryo carrying seeds were identified using the technique of Bains et al. (1998). The embryos were removed under strict aseptic conditions and placed on the culture medium in the test tubes. Cultured immature embryos were given cold treatment at 4°C temperature in dark for first 24 h. After that, they were incubated in the dark in the Plant Growth Chamber at 25 ± 1°C for regeneration for about a week till the roots and shoots initiated. The regenerated plantlets were then transferred to the other section of the Plant Growth Chamber at 25 ± 1°C with 10/14 h light/dark profile for plants’ proper development. The haploid plantlets were transferred to rooting medium for profuse rooting, then potted in soil mixture for hardening and later treated with 0.1% colchicine solution for chromosome doubling. The haploid plantlets were treated with colchicine at three to five tiller stages according to the method given by Inagaki (1985) with slight modifications. Each haploid plant’s crown was submerged in a 0.1% colchicine solution supplemented with 1.5% dimethyl sulphoxide at 20°C for 5 h. The treated plants were kept in the running tap water for 20 min, then potted in soil and maintained in the cage house up to maturity.

Recording of observations

Observations were recorded with respect to haploid induction traits on per cent basis as follows:

\[
\text{Pseudo seed formation frequency} = \frac{\text{Number of pseudo seeds formed}}{\text{Total number of florets pollinated}} \times 100
\]

\[
\text{Embryo formation frequency} = \frac{\text{Number of pseudo seeds carrying embryo}}{\text{Total number of pseudo seeds formed}} \times 100
\]

\[
\text{Haploid plantlet regeneration frequency} = \frac{\text{Number of haploid plantlets developed}}{\text{Total number of embryos cultured}} \times 100
\]

These data were transformed to definite value using Arcsine transformation. The significant difference for various haploid induction parameters, namely pseudo seed formation, embryo formation and haploid regeneration frequencies was analysed by simple t-test.

Molecular cytogenetics

Genomic and fluorescence in situ hybridization procedure

Four doubled haploid lines derived from (ITSN 105/58 × VL 802) × VL 802 crosses of BC₂F₃ generation of the present investigations were utilized 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) to identify and characterize the introgressed rye chromatin and isolate wheat like recombinants with less undesirable genes. Molecular Probes (Tab. 2) 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 (Yamamoto and Mukai, 1989). All the probes were labelled with the haptens viz., biotin-16-dUTP (Vitamin H) and digoxigenin-11-dUTP (Steroid) following the nick translation protocol given by (Maniatis et al., 1975). Detection of the labelled sites was executed by the fluorophores viz., fluorescein-conjugated streptavidin and rhodamine-conjugated anti-digoxigenin.

Hybridization signals were detected with an Olympus fluorescence microscope equipped with a filter for FITC (fluorescein isothiocyanate), a filter for rhodamine and a triple-band filter set for FITC, DAPI (4’,6-diamidino-2-phenylindole) and rhodamine. Images were captured using an Olympus CCD (charge-coupled device) camera.

**Table 1**

Wheat and triticale lines used in the present investigation

| S.No. | Genotype | Parentage | Source |
|-------|----------|-----------|--------|
| 1     | HPW 89   | INTERMEDIIO RODI/HD 2248 | CSKHPKV, Palampur |
| 2     | HPW 155  | BT 2549/FATH | -do- |
| 3     | HPW42    | VEE’S/4/PVN’S/CBB//CNO’S/3/JAR/ORZ’S’ | -do- |
| 4     | DH 776   | Pnijoumeé × HPW 143 | -do- |
| 5     | W5       | Selection Local Potia | DHY |
| 6     | VL 802   | CPAN3018/CPA N 3004//PBW65 | VPKAS, Almora |
| 7     | HS 396   | -          | -do- |
| 8     | RL-14-1  | -          | -do- |

**Triticale**

| S.No. | Genotype | Parentage | Source |
|-------|----------|-----------|--------|
| 1     | TL 1210  | CINNAMON/RAJ 821//IN 19-Turkey 602/3/AYMC | PAU, Ludhiana |
| 2     | TL 2920  | PBW 189/WHITE RYE/JNIT 128 | -do- |
| 3     | TL 2900  | -          | -do- |
| 4     | TL2117   | -          | -do- |
| 5     | ITSN 65  | -          | -do- |
| 6     | ITSN 105/58 | -          | -do- |

**Imperata cylindrica**

Wildly growing weedy grass in the surroundings of Experimental Fields at Palampur

**Table 2**

Molecular probes

| S. No. | Probe | Source |
|--------|-------|--------|
| 1      | Rye Genome | Total rye genome DNA from Himalayan collection |
| 2      | pTa 71 | 45S rDNA from Triticum aestivum |
| 3      | pSc 119 | Secale cereale |
| 4      | pAs 1  | Aegilops squarrosa |

**Statistical Analysis**

The data were transformed to definite value using Arcsine transformation (Warton and Hui, 2011). The significant difference for various haploid induction parameters, namely pseudo seed formation, embryo formation and haploid regeneration frequencies was analysed by simple t-test.

**Results**

**Doubled haploid breeding**

The pseudo seed formation data are provided below (Tab. 3). The data were transferred from percentage to absolute value for easy analysis using arcsine transformation. The pseudo seed formation was lower in the crosses of BC1F2, whereas higher in the crosses of BC1F4 generations. Variation in the pseudo seed formation might be due to genotype specificity in the crosses. The embryo formation was lower in the crosses of BC1F1, whereas higher in the crosses of BC2F3 generations. It may happen because all the pseudo seeds might not be containing the haploid embryo due to the chromosomal disharmony between the triticale × wheat derivatives and Imperata cylindrica. Although successful wheat haploid formation after crossing with I. cylindrica has been reported through cytological evidence of fertilization of parental gametes and further complete elimination of I. cylindrica chromosomes. The haploid plant regeneration was lower in the crosses of BC3F2, whereas higher in the crosses of BC1F3 generations. The recovery of doubled haploids was less after colchicine treatment due to the mortality of haploid plants. The cross (ITSN 105/58 × VL 802) × VL 802 × VL 802 of BC2F3 generation was yielded four doubled...
haploids. The pseudo seed formation data are provided below (Tab. 3). The data were transferred from percentage to absolute value for easy analysis using Arcsine transformation. The pseudo seed formation was lower in the crosses of BC1F2, whereas higher in the crosses of BC1F4 generations. Variation in the pseudo seed formation might be due to genotype specificity in the crosses. The embryo formation was lower in the crosses of BC1F1, whereas higher in the crosses of BC1F3 generations. This may happen because all the pseudo seeds might not be containing the haploid embryo due to the chromosomal disharmony between the triticale × wheat derivatives and Imperata cylindrica. Although successful wheat haploid formation after crossing with I. cylindrica has been reported through cytological evidence as the elimination of complete set of I. cylindrica chromosomes. The haploid plant regeneration was lower in the crosses of BC1F3 and higher in the crosses of BC1F1 generations. The recovery of doubled haploids was less after colchicine treatment due to mortality of haploid plants. The cross (ITSN 105/58 × VL 802 × VL 802 × VL 802 of BC2F3 generation was yielded four doubled haploids.

**FISH and GISH analysis in triticale × wheat-derived doubled haploids**

The four doubled haploid lines TWDH 1, TWDH 2, TWDH 4 and TWDH 5 (Figs. 1–4) derived from the BC2F3 of ITSN

| S. No. | Generation/Crosses | (Triticale × wheat) derivatives × Imperata cylindrica | No. of florets pollinated | sf (%) | ef (%) | hpr (%) |
|-------|-------------------|----------------------------------------------------|--------------------------|--------|--------|--------|
|       |                   |                                                    |                          |        |        |        |
| BC1F1 |                   |                                                    |                          |        |        |        |
| 1     | ITSN 65 × HPW 155 × HPW 155 | 386                                             | 46.88 (206)              | 20.41* (25) | 50.00 (15) |
| 2     | ITSN 65 × HPW 89 × HPW 89     | 152                                             | 47.87 (82)               | 18.97* (9)  | 31.10 (3)  |
| 3     | TL 1210 × W 5 × W 5        | 311                                             | 49.85 (183)              | 16.27 (14) | 42.11 (9) |
| 4     | TL 1217 × HPW 42 × HPW 42   | 182                                             | 55.22* (123)             | 21.17* (16) | 51.89* (10) |
| 5     | TL 2920 × DH 776 × DH 776  | 348                                             | 63.77* (280)             | 15.03 (19) | 49.33 (11) |
| 6     | TL 2920 × HS 396 × HS 396  | 366                                             | 43.33 (173)              | 9.43 (5)  | 42.75 (2) |
| 7     | TL 2920 × W 5 × W5          | 130                                             | 59.33* (96)              | 17.62 (9) | 63.25* (7) |
| Mean  |                   |                                                    |                          | 52.32   | 16.99  | 47.20  |
|        |                   |                                                    |                          | 1.09    | 0.56   | 1.47   |
| BC1F2 |                   |                                                    |                          |        |        |        |
| 1     | ITSN 105/58 × HS 396 × HS 396 | 1742                                            | 34.55 (382)              | 12.91 (28) | 19.36 (3) |
| 2     | TL 2900 × VL 802 × VL 802   | 209                                             | 38.36 (80)               | 30.21 (21) | 65.96 (17) |
| Mean  |                   |                                                    |                          | 36.36   | 21.56  | 42.66  |
|        |                   |                                                    |                          | 0.38    | 1.62   | 4.36   |
| BC1F3 |                   |                                                    |                          |        |        |        |
| 1     | ITSN 105/58 × VL 802 × VL 802 | 1338                                            | 46.9 (715)               | 17.37 (65) | 38.60 (25) |
| 2     | ITSN 105/58 × HPW 89 × HPW 89 | 1400                                            | 51.24 (850)              | 17.27 (75) | 46.22 (39) |
| Mean  |                   |                                                    |                          | 49.07   | 17.82  | 42.41  |
|        |                   |                                                    |                          | 0.35    | 0.01   | 0.61   |
| BC1F4 |                   |                                                    |                          |        |        |        |
| 1     | ITSN 105/58 × VL 802 × VL 802 | 288                                             | 56.24* (200)             | 25.68* (38) | 45.00 (19) |
| 2     | TL 2900 × VL 802 × VL 802   | 2481                                            | 56.65* (1730)            | 19.79 (198) | 23.76 (31) |
| 3     | TL 2920 × VL 802 × VL 802   | 76                                              | 48.31 (42)               | 14.87 (3)  | 56.08* (2) |
| Mean  |                   |                                                    |                          | 53.73   | 20.11  | 41.61  |
|        |                   |                                                    |                          | 0.53    | 0.61   | 1.85   |
| BC2F3 |                   |                                                    |                          |        |        |        |
| 1     | ITSN 105/58 × VL 802 × VL 802 | 6463                                            | 42.54 (2955)             | 29.51 (718) | 26.61 (144) |
| 2     | ITSN105/58 × RL-14-1 × RL-14-1 × RL-14-1 | 1397                                           | 43.14 (653)              | 27.77 (142) | 51.77 (88) |
| Mean  |                   |                                                    |                          | 42.84   | 28.64  | 39.19  |
|        |                   |                                                    |                          | 0.03    | 0.08   | 1.16   |
105/58 × VL 802 × VL 802 were also subjected to molecular cytogenetic analysis using probes viz., rye genomic rDNA, pSc119 and pAs1 (Tab. 4). The lines TWDH 1, TWDH 2 (Figs. 5 and 6), and TWDH 4 were possessing 1BL.1RS translocation. The photographic plates of spikes and seeds of TWDHs are given in Figs. 1 to 4. The GISH analysis revealed that 1RS replaced the translocation chromosome’s short arms due to its obvious satellite. The line TWDH 1 (Fig. 2), apart from 1BL.1RS translocation, showed 5D (5R) chromosome substitution and the line TWDH 5 exhibited 5D (5R) chromosome substitution.

Discussion

Pseudo seed formation
The good triticale response to Imperata cylindrica induced haploid induction has also been reported in the few studies carried out earlier. Kishore et al. (2011) had reported 21.3% pseudo seed formation in BC1F1 and 46.8% in BC1F2, and Pratap and Chaudhary (2007) had reported similar results. Chaudhary et al. (2015) findings of enhanced production of pseudo seed (30.2 to 56.3%) were also corroborated with the present investigation. The successful seed set obtained in this investigation (62.79%) was close to the value reported by Matzk and Mahn (1994) with 12 wheat genotypes. Percent of seed set represents the efficiency of emasculation and pollination procedures. The lack of embryos in the F1s of triticale × wheat in the current study indicated that either fertilization did not take place or embryo development was stalled at an early stage. The problem seemed to be accentuated due to the chromosomal imbalance in the gametes produced by triticale × wheat F1s. With the idea of improving chromosomal balance in the gametes of
triticale × wheat crosses, the F1s were advanced to the subsequent generations by backcrossing to wheat or selfing. Advanced generations (BC1F1, BC1F2, BC1F3, BC1F4 and BC1F5) were used for attempting crosses with maize employing 250 ppm 2, 4-D hormonal treatment. This kind of hormonal modification was used by Pratap et al. (2004); Chaudhary et al. (2002) with 20% pseudo seed production. However, variation was observed even within a group of lines derived from the same triticale × wheat cross where some lines had very low to no response to maize-mediated induction. Again, a probable reason may be the imbalanced chromosome complement of these lines. These results are in correspondence with the previous studies by Gill et al. (2008). They reported low seed set and no embryo development in F1 lines crossed with maize. Overall, the approach has high feasibility as a rapid technique for generating chromosome transfers between triticale and wheat. Successful seed formation was reported by Pratap et al. (2005) in all the 15 triticale × wheat hybrids fertilized with maize, Imperata cylindrica, pearl millet, sorghum pollen which suggests that these species effect fertilization in triticale × wheat hybrids, thereby stimulating seed formation. Similar studies were conducted by Mahato and Chaudhary (2015) involving seven diverse durum wheat genotypes and using two composite varieties of Himalayan maize, viz., Bajaura Makka and Early Composite and a wild grass, I. cylindrica, as pollen sources. Their result showed that I. cylindrica performed better for haploid induction in durum wheat over maize in terms of pseudo seed formation (46.93%), embryo formation (38.06%), haploid regeneration (40.42%) and haploid formation efficiency (7.44%). Authors opined the use of durum wheat × I. cylindrica as a superior technique over the maize-mediated system, and its large-scale use could open a new horizon in the sphere of durum wheat doubled haploid breeding programme.

Embryo formation

Very few investigations have been reported to produce Doubled Haploid (DH) on triticale × wheat cross using I. cylindrica. Several technical problems affect the haploid embryo production efficiency. The genotypes pollinated with I. cylindrica pollen coupled with the post-pollination treatments efficiently produced haploids and doubled haploids. However, in cross combinations, the frequency of both haploid embryo development and DH production varied considerably. Imperiala cylindrica was crossable with all the triticale × wheat-derived F1 hybrids, as shown by pseudo seed formation and embryo formation in all wheat genotypes. These hybrids’ parental lines did not carry recessive crossability alleles kr1 and kr2 except for variety C306. Thus, the wheat × maize system, as demonstrated earlier (Singh et al., 2005; Bakos et al., 2005), is independent of crossability alleles kr1 and kr2. Similarly, I. cylindrica also independent of crossability problem observed by Jamwal et al. (2016) in triticale × wheat recombinants used for DH production. Many possible reasons for the effect of the environment on haploid embryo recovery have been reported in other similar investigation (Pienaar et al., 1996). The variation in glasshouse condition could affect the pollen viability and wheat fertilization. Similarly, environment influenced durum wheat embryo survival in a genotypically dependent manner (Donoughue and Bennett, 1994). As reported, the performance of cultivar ‘Rampton Rivet’ for embryo recovery was significantly better in a 20°C growth room than in an unheated glasshouse as compared to cultivars ‘Wakona’ and ‘Chinese Spring’. Campbell et al. (1998) evaluated the effects of temperature and light intensity on wheat genotypes crossed with maize pollen and showed that both could significantly affect haploid embryo numbers. They also reported that the light intensity of 1000 µmol/m² s produced the greatest number of embryos (38% of florets pollinated) compared to the optimal temperature (22/17°C) embryo recovery. Overall germination

FIGURE 5. Detection of 1BL:1RS translocation in triticale × wheat derived doubled haploid bread wheat line, TWDH 2 with the probe Bio: pAs1 (green), Dig: pSc 119 (red).

FIGURE 6. Detection of 1BL:1RS translocation and 5D (5R) substitution in triticale × wheat derived doubled haploid bread wheat line, TWDH 1 with the probe Bio: rye genomic (green), Dig: rDNA (red).
rates were still low (~40% of all embryos) irrespective of variety. Low germination rates (43%) of wheat × maize cross-derived embryos were consistently observed (Inagaki and Tahir, 1990). Similarly, the non-development of embryos into plants in this investigation appeared due to vitrification or hyperhydricity. Badiyal et al. (2016) study also corroborated with present investigation for the haploid embryo formation using I. cylindrica as a pollen source. The chromosome elimination study (Komeda et al., 2007) indicated that I. cylindrica chromosomes were eliminated during the first mitotic cell division, whereas maize chromosomes were eliminated after two to three cell divisions. This gives a clear picture of the haploid nature of embryos obtained.

**Haploid plant regeneration**

The present investigation of plant regeneration ranged from 19.36% to 65.69% for different triticale × wheat-derived generations. One of the factors limiting the further development of a germinated embryo to a plantlet is the embryos’ deficiency in one of their polarity (poles) to obtain shoot or root induction. In such cases, meristems are not properly formed (Lefèvre and Devaux, 1996). More florets can be pollinated for getting more haploid plants. Earlier investigations have revealed that spikelet positions (lower, middle, and upper) determined the success ratios for embryo initiation (Martins-Lopes et al., 2001). However, Bitsch et al. (1998) previously observed that embryo initiation was found to be distributed evenly all over the wheat spike. Similar results were obtained by Tayeng et al. (2012) in wheat × wheat recombinants using I. cylindrica as a pollen source. In a normal wheat × maize crossing process, 25–30 florets are generally pollinated per spike, and the synchronization of flowering decides the increase in the number of florets to be pollinated. Better space planting of the wheat plants might increase the number of spikes for pollination. Similar results of efficient haploid induction by I. cylindrica were obtained by Rather et al. (2014) for 21 F1 wheat crosses assessed for their haploid induction efficiency when crossed with four Indian and one Japanese accession of I. cylindrica. Authors concluded that both wheat and I. cylindrica genotypes influenced haploid induction and the accession Ic-Aru, performed better as a pollen source.

**FISH and GISH analysis in triticale × wheat-derived doubled haploids**

In the present investigation, doubled haploid line TWDH2 possessed IBL.1RS translocation, amber colour seed and good spike type and the line TWDH1 showed 1BL.1 RS translocation and 5R (5D) substitution. A similar result was obtained by Carvalho et al. (2009) and Efremova et al. (2014) in wheat × rye crosses using fluorescent in situ hybridization (FISH) performed with genomic DNA probes for genomic in situ hybridization (GISH) from rye. Among the 55 plants, a wheat-rye translocation was detected in one plant after GISH. Recombinant chromosomes were identified using probes pTa71 and pSc119.2. Badaeva et al. (2002) reported that the repetitive DNA probe pAs1 was not only hybridized well with D-genome chromosomes of wheat but was also successfully used in the identification of specific chromosomes having different colour bands. In our investigation, the identification of D chromosomes present in the line TWDH2 was made using the probe pAs1. Tan et al. (2009) used FISH and suggested that line 15-3-2 possessed all 14 D-genome chromosomes and chromosome 5U, concluding that line 15-3-2 was a new synthetic wheat-–Aegilops biuncialis partial amphiploid, and could be used to transfer the disease resistance genes to wheat. Georgiev (2008) reported that after GISH, it was obtained that the mutant forms K1 and K2 of Triticum aestivum carried the 1B/1R chromosome translocation. Altuntepe and Jauhar (2001) study of GISH in haploid plants derived from durum substitution lines also supports the current investigation results. An et al. (2015) developed WR49-1 using sequential GISH (genomic in situ hybridization), mc-FISH (multicolour fluorescence in situ hybridization), mc-GISH (multicolour GISH) and EST (expressed sequence tag)-based marker analysis, WR49-1 proved to be a new wheat-rye 6R disomic addition line. Yang et al. (2016) observed that the newly developed wheat-rye addition line N9436B possessed two rye chromosomes. A similar result of wheat-rye translocation T1RS.1BL was obtained by Ren et al. (2017) from the progeny of the crossing of the wheat cultivar Miyanyang11-1 and a Chinese local rye variety, Weining. Two novel translocation lines were identified by molecular cytogenetic analysis and the results also revealed that the pSc119.2 signals of 5AL were absent in both lines along with the pSc119.2 signals of 4AL of RT828-11. Liu et al. (2017) was also observed one 1B(1R) substitution line and five 1BL.1RS whole-arm translocation lines. Most of the recombinant lines were 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 (Jeberon et al., 2021).
Conclusions

Very few investigations have been conducted using *Imperata cylindrica* as a pollen source for crossing different generations of triticale × wheat derivatives. Therefore, from the above study, it can be inferred that *I. cylindrica* is a useful pollen source for efficient doubled haploid production in triticale × wheat advanced generations. Doubled haploids possessing an alien introgression of rye chromatin were identified through the molecular cytogenetic analysis viz., FISH and GISH technique, which are efficient techniques for detecting alien addition, translocation, and substitution lines. The developed doubled haploid lines can be released as varieties for use in future wheat breeding programmes.

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