Identification of intergenomic translocations involving wheat, *Hordeum vulgare* and *Hordeum chilense* chromosomes by FISH

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Intergenomic translocations between wheat, *Hordeum chilense* and *Hordeum vulgare* have been obtained in tritordeum background. Advanced lines from the crosses between three disomic chromosome addition lines for chromosome 2H¹, 3H¹, and 4H¹ of barley (*Hordeum vulgare*) in *Triticum aestivum* cv. Chinese Spring (CS) and hexaploid tritordeum (2n = 6x = 42, AABBBHCHHCh) were analyzed. Multicolor FISH using both genomic DNA from *H. chilense* and *H. vulgare* were used to establish the presence and numbers of *H. vulgare* introgressions into tritordeum. Interspecific *H. vulgare/H. chilense* and intergeneric wheat/H. vulgare and wheat/H. chilense translocations were identified. Frequencies of plants containing different kinds of intergenomic translocations between chromosome arms are presented. These lines can be useful for introgressing into tritordeum characters of interest from *H. vulgare*.

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Interspecific and intergeneric hybrids in the Triticeae are valuable for transfers of genes from one species to another and to extend the range of genetic variation available to plant breeders. For a long time, one goal of cereal breeders has been the production of amphiploids between wheat and barley in order to combine desirable features from both species. Since the first success achieved by Kruse (1973) crossing cultivated barley and wheat (*Hordeum vulgare* × *Triticum aestivum*, *T. turgidum*, and *T. monococcum*), numerous hybrid combinations between the two genera have been reported (see Fedak 1992, for a review), mainly involving non-cultivated *Hordeum* species. Among these, *H. chilense* is a wild barley from Chile and Argentina with high cross-ability with other Triticeae genera (Fedak 1992). Crosses between *H. chilense* and diploid, tetraploid and hexaploid wheats give rise to tetraploid, hexaploid and octoploid tritordeums, respectively (Martín and Sánchez-Monge Laguna 1982; Martín et al. 1987; Cabrera and Martin 1991). Hexaploid tritordeum (2n = 6x = 42, AABBBHCHHCh) is a subject for a breeding program with the goal of creating a new cereal crop (Martín 1988) and in this way we intend to incorporate the genetic variability of cultivated barley, *H. vulgare*, into the gene pool of tritordeum. For achieving this objective hybrids between barley and tritordeum at the three ploidy levels have been produced (Martín et al. 1995). Another different approach is to cross barley addition lines in *T. aestivum* with hexaploid tritordeum. We present here the identification by fluorescence in situ hybridization (FISH) of intergenomic translocations involving barley, wheat and *H. chilense* chromosomes obtained from crosses between barley addition lines in hexaploid wheat and tritordeum.

MATERIALS AND METHODS

Root tips were collected from germinating seeds of advanced lines from the cross between three disomic chromosome addition lines for chromosomes 2H¹, 3H¹, and 4H¹ of barley (*Hordeum vulgare*) in *Triticum aestivum* cv. Chinese Spring (CS) (Islam et al. 1975) and hexaploid tritordeum (2n = 6x = 42, AABBBHCHHCh). These root tips were pre-treated for 3 h in a 0.05 % colchicine solution at 25°C and fixed in 100% ethanol-acetic acid, 3:1 (v/v), for at least a week at room temperature. Root tips were then stained in aceticarmine for 3 min, scraped out the meristems and squashed in 45% acetic acid on ethanol-cleaned slides. The preparations were frozen in liquid nitrogen, the cover slips subsequently removed and then dehydrated for 3 min each in 70% and absolute ethanol at room temperature. The air-dried slides were stored at 4°C until used.

Probes used were total genomic *H. vulgare* DNA and total genomic *H. chilense* DNA and they were labelled by nick translation with biotin-11-dUTP (Roche Corporate, Basel, Switzerland) and digoxigenin-11-dUTP (Roche Corporate), respectively. Both probes were mixed to a final concentration of 5 ng/μl in the hybridization mixture. The in situ hybridization protocol was performed according to Cabrera et al. (2001).
Biotin-labelled *H. vulgare* DNA and digoxigenin-labelled *H. chilense* DNA were detected with Streptavidin-Cy3 conjugate (Sigma, St. Louis, MO, USA) and anti-digoxigenin-FITC (Roche Corporate), respectively. Chromosomes were counterstained with DAPI (4',6-diamidino-2-phenylindole) and mounted in Vectashield. Signals were visualized using a Leica epifluorescence microscope. Images were captured with a SPOT CCD camera using the appropriate SPOT 2.1 software (Diagnostics Instruments, Inc., Sterling Heights, Michigan, USA) and processed with Photoshop 4.0 software (Adobe Systems Inc., San Jose, California, USA). Images were printed on a Hewlett Packard Deskjet HP 840C Color Printer.

RESULTS AND DISCUSSION

Double in situ hybridization experiments using both total genomic DNA from *H. vulgare* and *H. chilense* as probes enabled the identification of different genomes in the material analysed. *H. vulgare* chromosomes were labelled by red fluorescence and *H. chilense* by yellow-green fluorescence whereas the remaining wheat chromosomes appeared as blue DAPI fluorescence (Fig. 1a, b and c). This differential pattern allowed to distinguish three types of chromosome translocations at somatic metaphase, namely those involving wheat–*H. vulgare* (Fig. 1a), wheat–*H. chilense* (Fig. 1a and c) and *H. chilense*–*H. vulgare* (Fig. 1b and c) chromosomes. The translocation break-points were clear and appeared as centric break-fusion products. Interchanges between A-genome chromosomes and both *Hordeum* species could not be distinguished from those between B and both *Hordeum* species owing the identical pattern of DAPI fluorescence shown by all wheat chromosomes; for this reason, both were pooled into the wheat type.

Translocations occurred spontaneously in the progeny of crosses between *H. vulgare* addition lines for chromosomes 2H', 3H' and 4H' in *T. aestivum* with hexaploid tritordeum. Plants with simple, double or triple intergenomic translocations were found, and the relative frequencies of these translocations were different for the three barley chromosomes involved (Table 1). The highest frequency of plants with translocations were found in the offspring of crosses involving barley addition line for chromosome 3H' with a 44.6% compared to a 10.7% and 4.8% for barley chromosomes 2H' and 4H', respectively. Translocations between wheat and barley chromosomes were only found in the progeny of crosses involving barley chromosome 2H', although at low frequency. However, a high percentage of plants with translocations involving barley chromosome 3H' and *H. chilense* was found in the descendants analyzed.

These results indicated those chromosome arms from barley 2H' and 3H' can be introgressed into tritordeum background. No translocation involving barley chromosome 4H' and both wheat and *H.
Table 1. Intergenomic translocations obtained in the descendence from the crosses between barley addition lines for chromosomes 2H\(^{b}\), 3H\(^{b}\) and 4H\(^{b}\) in *T. aestivum* cv. Chinese Spring and hexaploid tritordeum, respectively analyzed by FISH

<table>
<thead>
<tr>
<th>Barley chromosome</th>
<th>No. of plants analyzed</th>
<th>No. of plants with intergenomic translocations</th>
<th>wheat/H(^{b})</th>
<th>wheat/H(^{ch})</th>
<th>H(^{b}/H(^{ch})</th>
<th>double</th>
<th>triple</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>56</td>
<td>3 (5.3%)</td>
<td>1 (1.8%)</td>
<td>0</td>
<td>1 (1.8%)*</td>
<td>1 (1.8%)</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>65</td>
<td>0</td>
<td>0</td>
<td>9 (13.8%)</td>
<td>20 (30.8%)*#</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>21</td>
<td>0</td>
<td>1 (4.8%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* wheat/H\(^{b}\) and wheat/H\(^{ch}\)
*+ wheat/H\(^{ch}\) and H\(^{b}/H\(^{ch}\)

*chilense* chromosomes was obtained. Only one wheat–*H. chilense* translocation was found in the progeny of barley addition line for chromosome 4H\(^{b}\) in *T. aestivum* with tritordeum (Table 1).

Considering all single, double and triple translocations obtained in the offspring of crosses involving the three barley chromosomes, the most frequent translocation obtained was the inter-specific *H. vulgare*–*H. chilense* (52.54%) following by wheat–*H. chilense* (40.7%) and wheat–*H. vulgare* (6.8%) intergeneric translocations. The successful transfer of useful genes to tritordeum from alien species such as barley requires that the target genes or a segment of chromosome carrying them can be incorporated into the tritordeum chromosomes as recombinant segments or translocations. Earlier reports of hybrids involving *Hordeum* with wheat suggest a lack of pairing of *Hordeum* chromosomes with chromosomes of wheat (FEDAK 1985). Also, no chromosome pairing has been observed in the *H. chilense* × *H. vulgare* hybrid (THOMAS and PICKERING 1985). The material developed in the present work offers the opportunity for introgressing either parts or entire barley chromosomes in tritordeum background. As has been previously found in wheat-rye translocations (e.g. 1BL/1RS and 1AL/1RS), centric-break fusion events are an important source of introgression and they have significantly contributed to global wheat production (PENA et al. 1990). Most of the translocation lines obtained in the present work were fertile and could be also useful for introgression into wheat of genetic material from both *H. vulgare* and *H. chilense* genomes. Further effort are carried out to identify the wheat and *H. chilense* chromosomes as well as barley chromosome arms involved in the translocations.

The use of total genomic DNA as species-specific probes resulted in an efficient procedure to distinguish between different genomes in the material analyzed. Previous workers have successfully applied fluorescence in situ hybridization, including the multicolour type, for wheat and its relatives and to identify alien chromosomes and chromosome segments in wheat (LAPITAN et al. 1986; LEE et al. 1989; MUKAI and GILL 1991; SCHWARZACHER et al. 1992; SÁNCHEZ-MORÁN et al. 1999). In most of these experiments, total genomic DNA from the introgressed alien species was used as probe, together with excess amounts of unlabelled blocking from wheat, for DNA:DNA in situ hybridization (ANAMTHAWAT-JONSSON et al. 1990; HESLOP-HARRISON et al. 1990). In all our samples analyzed the quality of in situ hybridization was highly satisfactory and clearly differentiate between wheat, *H. vulgare* and *H. chilense* genomes without the need of blocking DNA. The use of genomic *H. chilense* DNA as probe without blocking produced hybridization bands on the A- and B-genome chromosomes from wheat as a result of cross-hybridization (Fig. 1c). The pattern of hybridization obtained is similar to that obtained with N-banding and allowed the identification of all A- and B-genomes chromosomes present in tritordeum and wheat (GONZÁLEZ and CABRERA 1999).

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**REFERENCES**


