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José Lima-Brito, Ana Carvalho, Antonio Martín, J. S. Heslop-Harrison ...+1 more authors

Institutions: University of Trás-os-Montes and Alto Douro, Spanish National Research Council, University of Leicester

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RESEARCH ARTICLE

Morphological, yield, cytological and molecular characterization of a bread wheat × tritordeum F_1 hybrid

J. LIMA-BRITO[1]*, A. CARVALHO[1], A. MARTIN[2], J. S. HESLOP-HARRISON[3] and H. GUEDES-PINTO[1]

[1]*Department of Genetics and Biotechnology, CGB-ICETA/University of Trás-os-Montes and Alto Douro, Apt. 1013, 5000-911 Vila Real, Portugal*
[2]*Instituto de Agricultura Sostenible (CSIC), Apartado 4084, 14080 Córdoba, Spain*
[3]*Department of Biology, University of Leicester, Leicester LE1 7RH, UK*

Abstract

The morphological, yield, cytological and molecular characteristics of bread wheat × tritordeum F_1 hybrids ($2n = 6x = 42$; $AABBDH^{ch}$) and their parents were analysed. Morphologically, these hybrids resembled the wheat parent. They were slightly bigger than both parents, had more spikelets per spike, and tillered more profusely. The hybrids are self-fertile but a reduction of average values of yield parameters was observed. For the cytological approach we used a double-target fluorescence *in situ* hybridization performed with total genomic DNA from *Hordeum chilense* L. and the ribosomal sequence pTa71. This technique allowed us to confirm the hybrid nature and to analyse chromosome pairing in this material. Our results showed that the expected complete homologous pairing (14 bivalents plus 14 univalents) was only observed in 9.59% of the pollen mother cells (PMCs) analysed. Some PMCs presented autosyndetic pairing of H^{ch} and A, B or D chromosomes. The average number of univalents was higher in the wheat genome (6.8) than in the H^{ch} genome (5.4). The maximum number of univalents per PMC was 20. We only observed wheat multivalents (one per PMC) but the frequency of trivalents (0.08) was higher than that of quadrivalents (0.058). We amplified 50 RAPD bands polymorphic between the F_1 hybrid and one of its parents, and 31 ISSR polymorphic bands. Both sets of markers proved to be reliable for DNA fingerprinting. The complementary use of morphological and yield analysis, molecular cytogenetic techniques and molecular markers allowed a more accurate evaluation and characterization of the hybrids analysed here.

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Introduction

Interspecific hybridization has been used by plant breeders in order to interchange genetic material between different plant species or to produce new species (see Gupta and Priyadarshan 1982; Fedak 1992; Martín *et al*. 1999). In the tribe Triticeae of family Poaceae, different hybrid combinations of *Triticum, Hordeum* and *Secale* have been produced (Lima-Brito *et al*. 1996; Martin *et al*. 1996). These materials could be interesting as starting material for the production of addition and/or substitution lines, widening the genetic basis of crops, and allowing the study of the interaction between different genomes in the same cytoplasmic background. However, most of the interspecific hybrids are sterile, restricting their direct use in plant breeding programmes. Usually, this sterility is correlated with irregular meiotic behaviour owing to the absence of chromosome pairing during metaphase (Anamthawat-Jónsson and Bödvarsdóttir 1998).

Characterization and identification of true hybrids at an early stage is mandatory in studies involving interspecific hybridization. Morphological and cytological analyses are used to ascertain hybridity in many crop species, including Triticeae (Fernández-Escobar and Martin 1985; Lima-Brito *et al*. 1996). While the sensitivity of morphological markers to environmental and developmental variations limits their applicability, molecular markers can be profitably utilized to screen and identify true hybrids at an early stage.

*For correspondence. E-mail: jbrito@utad.pt.

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Fluorescence *in situ* hybridization using both repetitive DNA sequences and total genomic DNA as probes is a valuable method for identifying genomes or alien and recombinant chromosomes (Schwarzacher *et al*. 1992; Anamthawat-Jónsson and Heslop-Harrison 1993) and to discriminate the parental origin of all chromosomes in multigeneric hybrids (Lima-Brito *et al*. 1996, 1997).

In the last few decades, new DNA molecular markers, based on the PCR technique, such as random amplified polymorphic DNA (RAPD; Williams *et al*. 1990; Welsh and McClelland 1990) and inter simple sequence repeats (ISSRs; Zietkiewicz *et al*. 1994), among others, have become excellent tools for plant breeders (Hernández and Martín 2003). When there is insufficient information about the genome sequence of a wild species, or there are economic constraints, one of the most adequate marker systems is RAPD amplification (Hernández and Martín 2003). This technique gives fast results but also has limitations, such as dependence on the genetic background, low reproducibility, and level of polymorphism obtained (Zietkiewicz *et al*. 1994; Godwin *et al*. 1997; Fernández *et al*. 2002).

In contrast to RAPD amplification, the ISSR markers are more feasible and reproducible (Godwin *et al*. 1997), and the distribution of ISSRs in the eukaryotic genome makes them highly informative (Tautz and Renz 1984). They are also highly polymorphic and their use is cost effective, requiring no prior information of the sequence (Bornet *et al*. 2002). In cereals, ISSR markers have been used to study genetic diversity and phylogenetic relationships (Kantety *et al*. 1995; Nagaoka and Ogihara 1997; Pejic *et al*. 1998; Blair *et al*. 1999; Joshi *et al*. 2000; Qian *et al*. 2001; Matos *et al*. 2001; Fernández *et al*. 2002), for gene mapping (Kojima *et al*. 1998), for gene tagging in molecular assisted selection (Akagi *et al*. 1996; Kaushik *et al*. 2003), and for DNA fingerprinting (Carvalho *et al*. 2005).

The *Hordeum chilense* × wheat amphiploid, named tritordeum ($2n = 6x = 42$; AABBH^{ch}H^{ch}), is one of the basic materials for the genetic breeding of wheat through interspecific crosses, since it contains the genetic variability of *H. chilense* (Martin *et al*. 1996). In this study, we evaluate the morphological and yield characters and analyse the meiotic behaviour of the F_1 hybrid between the Portuguese bread wheat (Barbela) and tritordeum. We also characterize these F_1 hybrids using RAPD and ISSR markers, and compare them to the parental lines.

Materials and methods

Plant material and chromosome preparations

The F_1 hybrids of bread wheat (female parent) × tritordeum (male parent) ($2n = 6x = 42$; AABBDH^{ch}) were produced at the University of Trás-os-Montes and Alto Douro (UTAD) after crossing the Portuguese bread wheat 'Barbela' ($2n = 6x = 42$; AABBDD), selected at UTAD, Portugal, with the tritordeum advanced line HT9 ($2n = 6x = 42$; AABBH^{ch}H^{ch}), selected at Instituto de Agricultura Sostenible (CSIC), Spain. For preparing meiotic chromosome spreads, anthers containing pollen mother cells (PMCs) at metaphase I previously fixed in ethanol : acetic acid (3:1) were used. A total of 105 PMCs were analysed.

Morphological characterization

The following morphological and yield characters were evaluated for 15 F_1 hybrid plants at maturity stage: length of main culm, spike length, number of spikelets, number of seeds, number of seeds per spikelet, and weight of seeds of the main spike. The tiller number was also scored. The values given are averages for the plants analysed.

The statistical analyses of the data were made by using the StatView program. The F values significance, due to the different effects and their interaction, was established for probabilities higher than 5% ($P > 0.05$), between 1% and 5% ($P < 0.05$), between 0.1% and 1% ($P < 0.01$), and lower than 0.1% ($P < 0.001$).

Probe preparation and fluorescence in situ hybridization

For *in situ* hybridization, we followed the methods described by Fernández-Calvin *et al*. (1995) and Lima-Brito *et al*. (1996). Chromosome pairing analysis was done after *in situ* hybridization performed with total genomic DNA from *H. chilense* and the ribosomal sequence pTa71, a 9-kb *Eco*RI fragment isolated from *Triticum aestivum* L. em. Thell (Gerlach and Bedbrook 1979). Both were labelled by nick translation with biotin-16-dUTP and digoxigenin-11-dUTP (Roche, Basel, Switzerland), respectively, and used as probes. Unlabelled total genomic DNA from bread wheat 'Chinese Spring' was sheared by autoclaving for use as DNA blocker. The antibodies used for probe detection were sheep anti-digoxigenin conjugated to rhodamine (Roche) and avidin-D conjugated with fluorescein (FITC; Vector, Burlingham, USA). Slides were mounted with Vecta Shield with DAPI (4′, 6′-diamidino-2-phenylindole; Vector) and observed on a Zeiss Axioplan 2 epifluorescence microscope equipped with appropriate light filters. The images were captured by an AxionCAM (Zeiss, Göttingen, Germany) with AxionVision software and prepared for printing with Adobe Photoshop.

RAPD and ISSR amplification

Total genomic DNA from the F_1 hybrid and its parents was extracted from young leaves with the DNA extraction kit NucleoSpin Plant (Macherey-Nagel, Düren, Germany). The RAPD amplification reactions were carried out in 50 μl containing 20 ng/μl of template DNA, 10× buffer $(NH4)_2SO_4$; Fermentas, St. Leon-Rot, Germany), 2.5 mM $MgCl_2$ (Fermentas), 2.5 mM dNTPs, 0.25 μM primer and 0.025 U *Taq* DNA polymerase (Fermentas). A total of twenty 10-mer oligonucleotides from set E (Operon Technologies, Alameda, USA) were tested. The RAPD amplifications occurred under the following conditions: an initial denaturation

step at 94°C for 6 min and 40 cycles at 94°C for 1 min, 36°C for 1 min and 72°C for 2 min; the final elongation step was at 72°C for 6 min.

The ISSR amplification reactions were carried out in 20 μl containing 20 ng/μl of template DNA, 5 μM primer, 10 μl of *Taq*-PCR master mix (Qiagen, Hilden, Germany) and 8 μl of ultra-pure distilled water (Qiagen). Twenty ISSR primers from the set 100/9 (University of British Columbia, Vancouver, Canada) were tested. For ISSR amplifications we used the following conditions: an initial denaturation step of 94°C for 5 min, followed by 45 cycles of denaturation at 94°C for 30 s, a primer annealing step at 52°C for 45 s, and an extension at 72°C for 2 min; then a final extension was carried out at 72°C for 5 min. Both RAPD and ISSR amplification reactions were carried out on a Biometra Thermocycler UNOII, and each reaction was repeated twice.

Amplification products were analysed on 1.5% agarose gels. The gels were stained with ethidium bromide, and then photographed with 667 Polaroid films under UV light and scored for presence/absence of polymorphic bands. Each amplification product (band) was considered an RAPD or ISSR marker, and only reproducible bands were considered for the presence (1) / absence (0) analysis.

Results

Morphological and yield characterization

Table 1 summarizes the average and range values scored for the different morphological and yield characters in the F_1 hybrids from the 'Barbela' × HT9 cross, and their parents. The F_1 hybrids showed an average value of main culm length very similar to but slightly higher than that observed in wheat and tritordeum parents (table 1). Our data revealed that the hybrids tillered profusely, presenting an average value of secondary culms higher than that observed in its parents (table 1). The spike morphology of the F_1 hybrids was wheat-like (figure 1,a). The average main spike length was also significantly higher than that of both parents. Spikelet number in the main spike was significantly higher than that of the female parent. The F_1 hybrid spikelets (figure 1,b) and seeds (figure 1,c) had a wheat-like morphology. In the yield characters, namely number of seeds, number of seeds per spikelet and weight of seeds of main spike of the hybrids, the hybrids had significantly lower values compared to both parents.

Chromosome pairing analysis

We analysed 105 PMCs of the hybrid at metaphase I after fluorescence *in situ* hybridization performed with genomic DNA from *H. chilense* and the rDNA sequence pTa71, simultaneously, as probes (figure 2). This technique allowed discrimination of the parental genomes, identification of seven rDNA loci (chromosome pairs 1B and 6B, chromosomes 5D, $5H^{ch}$ and $6H^{ch}$), and analysis of the meiotic behaviour of the parental genomes separately (figure 2; table 2).

Only 9.59% of the PMCs presented complete homologous chromosome pairing: 14 bivalents (AABB) and 14 univalents (seven from D genome and seven from H^{ch} genome). The total average number of bivalents was higher than 14, and some PMCs presented an average number of univalents higher than expected (figure 2,a; table 2). This high frequency of univalents at metaphase I induced secondary arm-to-arm chromosome associations of two types: wheat–H^{ch} (figure 2,b) and H^{ch}–H^{ch} (not shown).

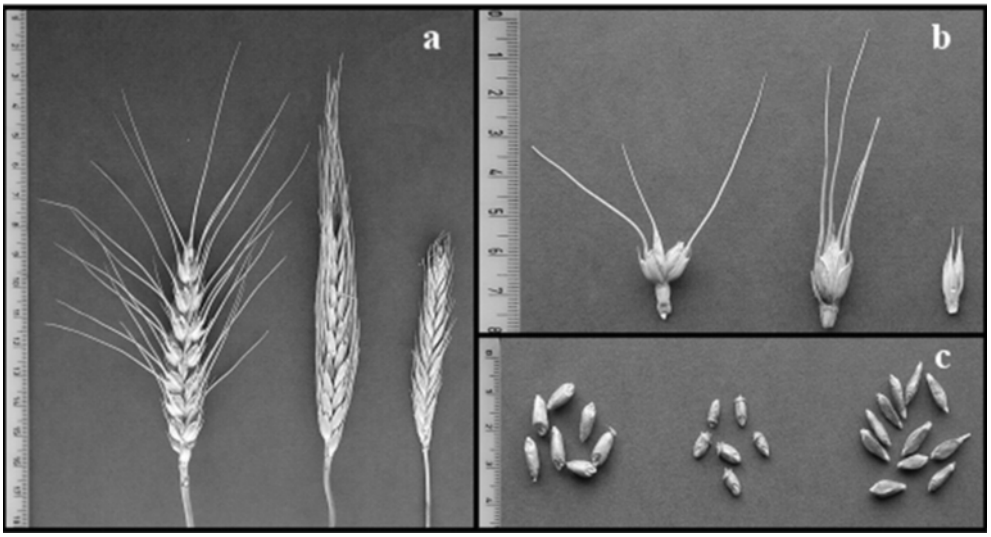

Figure 1. Morphological comparison of the F_1 hybrid $AABBH^{ch}$ and the parent species: (a) spikes from bread wheat 'Barbela' (left), F_1 'Barbela' × HT9 hybrid (centre), and tritordeum HT9 line (right); (b) spikelets, in the same order; (c) seeds, in the same order.

Table 1. Morphological and yield characters in F_1 'Barbela' × HT9 and the parents.

Genotype	Length of main culm (cm)	No. tillers	Main spike				
			Length (cm)	No. spikelets	No. seeds	No. seeds per spikelet	Weight seeds (g)
'Barbela'	$107.625^{ab} \pm 9.464$	$3.000^{a} \pm 1.414$	$8.800^{a} \pm 0.606$	$18.750^{a} \pm 0.957$	$28.250^{c} \pm 5.377$	$1.467^{c} \pm 0.264$	$1.274^{c} \pm 0.433$
	(94–115.5)	(1–4)	(18–20)	(18–20)	(22–35)	(1.16–1.75)	(0.87–1.75)
HT9	$104.978^{ab} \pm 7.931$	$7.333^{b} \pm 3.162$	$8.096^{a} \pm 0.687$	$25.963^{b} \pm 1.255$	$18.148^{b} \pm 13.496$	$0.701^{b} \pm 0.524$	$0.363^{b} \pm 0.251$
	(91.5–117)	(1–17)	(6.5–9.5)	(24–28)	(0–57)	(0–2.19)	(0–0.97)
F_1 (B × HT9)	$111.557^{b} \pm 5.403$	$9.571^{c} \pm 2.507$	$10.743^{b} \pm 0.950$	$26.286^{b} \pm 1.799$	$1.000^{a} \pm 1.915$	$0.037^{a} \pm 0.070$	$0.049^{a} \pm 0.098$
	(102.7–118.6)	(6–13)	(9.9–12.3)	(24–28)	(0–5)	(0–0.179)	(0–0.26)

For a given character, values with the same superscript letter label are not significantly different at 5% level.

Table 2. Numbers of meiotic configurations per PMC observed on 105 PMCs, at metaphase I, from 'Barbela' × HT9 hybrids.

Genome	I	II			III	IV			Chiasmata per PMC	% CAA
		Rod	Ring	Total		Open	Closed	Total		
AABBD (Barbela)	6.8 ± 2.1 (2–13)	3.3 ± 2.1 (0–9)	10.5 ± 2.3 (3–15)	13.9 ± 1.1 (10–16)	0.08 ± 0.3 (0–1)	0.029 ± 0.2 (0–1)	0.029 ± 0.2 (0–1)	0.058 ± 0.2 (0–1)	24.77 ± 2.8 (17–31)	70.78
H^{ch} (HT9)	5.4 ± 1.8 (1–7)	0.12 ± 0.3 (0–1)	0.7 ± 0.9 (0–3)	0.8 ± 0.9 (0–3)	0	0	0	0	1.5 ± 1.7 (0–6)	35.17
AABBDH^{ch} (hybrid)	12.2 ± 3.2 (3–20)	2.5 ± 1.8 (0–7)	10.7 ± 1.8 (7–15)	14.7 ± 1.6 (10–18)	0.08 ± 0.3 (0–1)	0.029 ± 0.2 (0–1)	0.029 ± 0.2 (0–1)	0.058 ± 0.2 (0–1)	26.25 ± 3.6 (17–35)	62.49

I, Univalents; II, bivalents; III, trivalents; IV, quadrivalents; %CAA mean percentage of chromosome arm association.

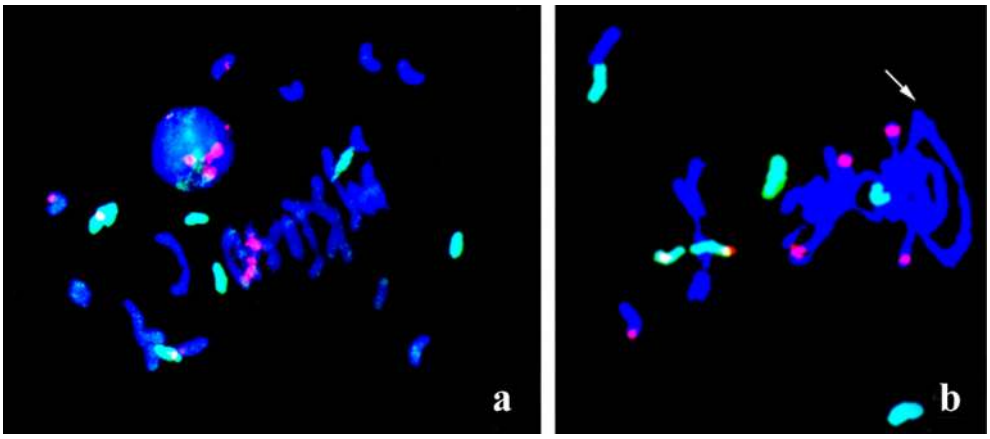

Figure 2. PMCs at metaphase I of F_1 'Barbela' × HT9 ($2n = 6x = 42$; AABBDH^{ch}) after counterstaining with DAPI and fluorescence *in situ* hybridization presenting: (a) 20 univalents (seven from *H. chilense* in green and 13 from wheat in blue); (b) a closed wheat quadrivalent (arrow) and a wheat – *H. chilense* secondary chromosome association. In both PMCs seven rDNA loci (red) were detected by pTa71 probe.

A small number of wheat multivalents (figure 2,b) were detected, but never exceeded one per PMC (table 2). The least average values of chiasmata per PMC (1.5) and percentage of chromosome arm associations (35.17) were observed in the H^{ch} genome (table 2).

RAPD and ISSR analysis

The total number of amplified bands, number of polymorphic bands, percentage of polymorphism and number of exclusive bands obtained per each RAPD and ISSR primer are shown in table 3. Among the 20 RAPD primers tested, only 11 produced bands polymorphic between the F_1 hybrid and one parent (table 3). An average of 10.8 bands per primer were amplified (ranging from 400 to 3000 bp) and 41.2% were polymorphic (table 3). The oligonucleotide E5 presented the highest percentage of RAPD polymorphism (figure 3; table 3). All oligonucleotides, except E15, presented RAPD polymorphic bands specific for the F_1 hybrid and for the female parent 'Barbela' (table 3). Nine oligonucleotides amplified a total number of 16 exclusive polymorphic bands, ranging from 400 to 3000 bp, in the F_1 hybrid and one of its parents (table 3).

Among the tested ISSR primers, only six amplified polymorphic ISSR loci (table 3). An average number of 6.5 bands per primer were amplified (ranging from 1750 to 450 bp) and the mean percentage of ISSR polymorphism was 81.51% (table 3). The oligonucleotide 856 amplified the highest number of ISSR loci but it was primer 825 that gave the highest percentage of polymorphism (table 3). Most of the polymorphic ISSR bands amplified were common and specific to the F_1 hybrid and the male parent (table 3). All the polymorphic ISSR loci amplified by the oligonucleotides 825 and 888 were common and specific to the F_1 hybrid and the male parent (table 3). Also, all the polymorphic ISSR exclusive bands (ranging from 1450 to 450 bp) were specific to the F_1 hybrid and the male parent.

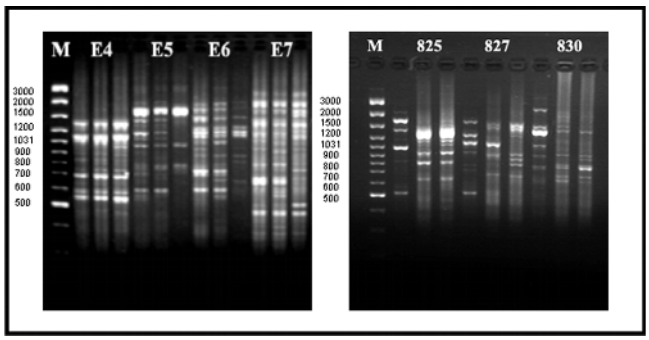

Figure 3. RAPD (left) and ISSR (right) amplification products from the F_1 'Barbela' × HT9 hybrid and its parents, visualized on 1.5% agarose gel stained with ethidium bromide. M, Molecular weight marker Gene Ruler 100 bp Plus (Fermentas).

Discussion

Alloploidy, the result of fusion of two species through interspecific hybridization, followed by chromosome doubling, has been considered a successful and revolutionary mechanism of plant evolution (Ozkan *et al*. 2001). This process could induce genetic effects like structural heterosis, which can result in bigger size and wide adaptation of the resulting polyploid species (Fernández-Escobar and Martín 1985; Stoinova 1994; Martín *et al*. 1999).

In the F_1 hybrid of 'Barbela' × HT9 analysed here, the average values of the morphological characters were higher than those of their parents, and there were statistically significant differences among them (table 1), confirming the

Table 3. RAPD and ISSR markers in 'Barbela' × HT9 F_1 hybrid and the parents.

				P				
Marker type	Primer 5′ → 3′	N	U	F_1♀	F_1♂	Total	%P	NEB
RAPDs	E3 CCAGATGCAC	12	7	4	1	5	41.66	1
	E4 GTGACATGCC	9	8	1	0	1	11.11	2
	E5 TCAGGGAGGT	10	2	8	0	8	80	1
	E6 AAGACCCCTC	11	4	6	1	7	63.63	3
	E7 AGATGCAGCC	15	11	3	1	4	26.66	1
	E11 GAGTCTCAGG	13	6	6	1	7	53.85	1
	E12 TTATCGCCCC	10	5	5	0	5	50	1
	E14 TGCGGCTGAG	14	9	4	1	5	35.71	4
	E15 ACGCACAACC	6	5	0	1	1	16.66	0
	E18 GGACTGCAGA	11	7	3	1	4	36.36	2
	E20 AACGGTGACC	8	5	2	1	3	37.5	0
	Total	119	69	42	8	50	M = 41.20	16
ISSRs	825 $(AC)_8T$	7	1	0	6	6	85.71	3
	827 $(AC)_8G$	5	0	1	4	5	100	1
	830 $(TG)_8G$	6	3	1	2	3	50	0
	856 $(AC)_8YA$*	10	3	1	6	7	70	4
	888 $BDB(CA)_7$*	5	0	0	5	5	100	2
	889 $DBD(AC)_7$*	6	1	3	2	5	83.33	1
	Total	39	8	6	25	31	M = 81.51	11

N, Total number of bands; U, number of monomorphic bands; P, number of polymorphic bands common to F_1 hybrid and female parent (F_1♀) or F_1 hybrid and male parent (F_1♂); %P, percentage of polymorphism; NEB, number of exclusive bands; M, mean percentage of polymorphism. *Type of degenerate nucleotide: B = C, G or T; D = A, G or T; Y = pyrimidine (C, T).

data reported by Fernández-Escobar and Martin (1985) and Stoinova (1994). Morphologically, the spikes, spikelets and seeds of the F_1 hybrids resembled those of the wheat parent (figure 1). The hybrids were self-fertile, as reported by Martin and Cubero (1981). There was statistically significant reduction in the average values of the yield parameters number and weight of seeds and number of seeds per spikelet (table 1). The same was found in F_1 hybrids from a durum wheat 'Candial' × tritordeum cross (Carvalho *et al*. 2003).

Use of fluorescence *in situ* hybridization with *H. chilense* genomic DNA and pTa71 probes allowed discrimination of parental genome in all PMCs of the hybrids (figure 2), as well as their chromosome pairing analysis (table 2). This technique was also successfully employed in the study of chromosome pairing of multigeneric hybrids involving wheat, rye and *H. chilense* (Lima-Brito *et al*. 2002) and in *H. chilense* × *Aegilops tauschii* amphiploid (Carvalho *et al*. 2005).

The F_1 hybrid of 'Barbela' × HT9 ($AABBDH^{ch}$) investigated here constitutes an interesting material for studying the possibility of recombination between the D and H^{ch} genomes. Analysis of chromosome pairing in these hybrids revealed instability and some meiotic irregularities. Most of the PMCs observed did not show complete homologous chromosome pairing, which can probably explain the reduction in yield parameters referred to above. The maximum number of bivalents per PMC reached 14.7 owing to the autosyndetic pairing between *H. chilense* chromosomes (table 2). This result and the presence of wheat multivalents (figure 2,b) demonstrated that chromosome pairing between different homology groups had occurred. Additionally, the reduced percentage of PMCs with complete homologous chromosome pairing suggests ineffective action of the wheat genetic system *Ph*, probably owing to interactions of the different genomes present in this hybrid. The two types of secondary arm-to-arm chromosome associations (wheat–H^{ch} and H^{ch}–H^{ch}) or pseudobivalents observed are not related

to intergenomic recombination since they do not involve chiasma production. However, the presence of a high frequency of univalents at metaphase I (figure 2,a) could induce Robertsonian translocations after centric breakage and fusion. Indeed, the absence of chromosome pairing between *Hordeum* and *Triticum* has been considered an obstacle to the introgression of *H. chilense* chromosomes into wheat (Martín and Sánchez-Monge 1980).

Our molecular characterization revealed 41.2% polymorphism of RAPD markers and 81.51% polymorphism of ISSR markers between the F_1 hybrid of 'Barbela' × HT9 and one of the parents (table 3). The difference is perhaps explained by the difference in the DNA segments targeted by the two methods, and is consistent with some previous studies which reported that ISSR markers are more polymorphic than RAPD markers (Zietkiewicz *et al*. 1994; Godwin *et al*. 1997; Nagaoka and Ogihara 1997; Fernández *et al*. 2002). All the RAPD primers, except E15, amplified more polymorphisms specific for the F_1 hybrid and for the female parent (table 3), which means that the RAPD primers used here were more appropriate to amplify polymorphic bands in the wheat parent. In contrast, most of the polymorphic ISSR loci amplified in this study were specific for the F_1 hybrid and for the male parent, tritordeum HT9.

This result contradicts some of our previous work (Carvalho *et al*. 2005), where we used oligonucleotides with $[(AG)_8]$ and $[(GA)_8]$ repeats in their sequence and found that most polymorphic ISSR loci amplified were specific for the F_1 hybrid and for the female parent, 'Barbela' wheat. This discrepancy can be explained because the sequences of the ISSR primers used in the present work have $[(AC)_8]$ and $[(CA)_8]$ repetitions which are probably more appropriate for amplification of ISSR regions in the male parent tritordeum. Given that the F_1 hybrid and its male parent share the A and B wheat genomes, we suggest that the oligonucleotides used here were more specific for the H^{ch} genome, and even the exclusive bands (NEB, see table 3) were also amplified in the male parent. Oligonucleotides with the $[(AG)_8]$ and $[(GA)_8]$ repetitions may be more suitable for amplification of ISSR markers polymorphic between the F_1 hybrid and the female parent, which share the A, B and D wheat genomes. Also in the RAPD analysis we observed that primer E15, whose sequence is rich in CA, did not amplify polymorphic bands in the female parent. In general, a good oligonucleotide for DNA fingerprinting must amplify polymorphisms in the F_1 interspecific hybrid and in both parents. In this study, the primers E4, E5, E12, 825 and 888 did not fit this situation.

The exclusive polymorphic RAPD and ISSR bands are interesting, since they could be converted to STS (sequence tagged site) markers which could have great value for detection of mixes between cultivars and for DNA fingerprinting (Fernández *et al*. 2002). Fernández *et al*. (2002) used RAPD and ISSR markers for DNA fingerprinting, because they provide a quick, reliable and highly informative system and can also be used to establish genetic relationships. Although in our work the ISSR markers showed higher percentage of polymorphism than RAPD markers, we believe that both could be useful for DNA and genomic fingerprinting.

Previous studies have reported analysis of amphiploids and interspecific hybrids belonging to the tribe Triticeae using morphological and/or cytological approaches (Fernández-Escobar and Martin 1985; Stoinova 1994; Lima-Brito *et al*. 1996). Martín *et al*. (1999) used the genomic *in situ* hybridization (GISH) technique in combination with morphological and RAPD analyses for characterization of the amphiploid *Agropyrum cristatum* and its parents. Carvalho *et al*. (2005) used ISSR markers to characterize three different hybrid combinations. In this work, we have demonstrated that complementary use of morphological and yield analysis, molecular cytogenetics techniques and molecular markers allows a more accurate evaluation and characterization of F_1 hybrids, and this could be useful for studies on other agriculturally important plant species.

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