Meiotic pairing in a trigeneric hybrid *Triticum tauschii-Agropyron cristatum-Hordeum chilense*

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The chromosome affinity among the P genome from *Agropyron* and the D and H^{ch} genomes of *Triticum* and *Hordeum* in the absence of the *Ph* system controlling homoeologous chromosome pairing provides information for breeding purposes. Hybrids between two accesions of the diploid barley *Hordeum chilense* ($H^{ch}H^{ch}$) and the fertile amphiploid *Triticum tauschii-Agropyron cristatum* (DDPP) were made, giving rise to the trigeneric hybrid genomic combinations $H^{ch}DP$ from which such information could be obtained. The meiotic pairing of these hybrids using fluorescence genomic in situ hybridization (FISH) on metaphase I pollen mother cells yield data on allosyndetic and autosyndetic chromosome pairing between the three genomes. The results showed that the *A. cristatum* tetraploid parent of the amphiploid DDPP is a segmental alloploid. A higher pairing between the D and H^{ch} genomes than between them and the P genome from *A. cristatum* is observed.

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The tribe Triticeae is an excellent example of the integration of academic studies with their application in plant breeding. Genome analysis based on hybridization and chromosome pairing has provided useful phylogenetic information (KIHARA 1954; SAKAMOTO 1973; DEWEY 1984) and up to 31 major basic genomes have been designated in species of the Triticeae (WANG et al. 1996). Both interspecific and intergeneric hybridization in the Triticeae have been useful sources of genetic variability for plant breeders. Consequently, information on both genetic variability and chromosome homology are of great interest. *Hordeum*, *Triticum* and *Agropyron* are genera of Triticeae that are of the greatest importance for breeding as they include barley, wheat and crested wheatgrass. Therefore, additional information about the genomic relationship among them are of theoretical and practical value.

Hordeum chilense Roem. et Schult. is a wild South American diploid barley included in the Section *Anisolepis* (BOTHMER et al. 1995). It occurs exclusively in Chile and Argentina and it is highly polymorphic both morphologically and biochemically. It is a weak perennial that is a component of natural pastures where it is very much appreciated by cattle. The high crossability of *H. chilense* with other members of the Triticeae and its agronomically interesting characteristics, such as resistance to biotic and abiotic stresses and possession of storage endosperm proteins that are of potential value in wheat breeding, made this species an interesting subject for cytological analysis and offered the pos-sibility of obtaining novel genomic combinations (MARTÍN et al. 1996).

Triticum tauschii (Coss.) Schmal, (*Aegilops squarrosa* L.) the contributor of the D genome to bread wheat (KIHARA 1954) provides hexaploid wheat with the wide adaptation which made this amphiploid the most successful crop in the past 10,000 years. For this reason, *T. tauschii* is included in programs designed to increase the variability in bread wheat (MUJEEB-KAZI 1995).

Agropyron (Gaertn.) traditionally included most of the species with one spikelet per node, encompassing over 100 species. It is now restricted to a group of caespitose, cross-pollinated, long-lived perennial species with closely spaced, solitary spikelets and strongly keeled glumes, all of which have been found to be homoploids based on the P genome (genome designations are based on WANG et al., 1996). Thus, the genus became reduced to fewer than 10 species at three ploidy levels, $2n = 14$, 28 and 42. Hybrids and amphiploids between *T. aestivum* (L.) and *Agropyron* species have been obtained (LI and DONG 1994; LIMIN and FOWLER 1990) with the final aim of introducing into *Triticum* traits from *Agropyron*. Although a great deal of cytogenetic data has been acquired on the genus and new hybrids have been developed during the last 20 years (SHARMA 1995), some critical data are still missing, e.g. the chromosome pairing among *Triticum* and *Agropyron* genomes in hybrids without *Ph* genes or in hybrids and amphiploids with diploid *Triticum* species. Given the importance

of *A. cristatum* as a forage crop in western North America, these data could be important as a measure of both biological relationship and for breeding purposes.

Understanding of the genomic relationships among *Hordeum*, *Triticum* and *Agropyron* is relevant to chromosome manipulation and breeding of the Triticeae crops. To assist such endeavours, we present this paper on the meiotic behavior of the trigeneric hybrid between *H. chilense*, *T. tauschii* and *A. cristatum*.

MATERIALS AND METHODS

Plant material

Two *Hordeum chilense* Roem. et Schult. ($H^{ch}H^{ch}$, $2n = 2x = 14$) accessions (H1 and H17) from the collection maintained in Córdoba (Spain) were used in this work. These accessions were selected as representative of two very distinctive groups in the species. Emasculated spikes of *H. chilense* were pollinated with tetraploid amphiploid *Triticum tauschii-Agropyron cristatum* (DDPP, $2n = 4x = 28$). Gibberellic acid (GA3 at 75 ppm) was injected into the florets with an hypodermic syringe 1 day after pollination. Embryos were rescued about 21 days after pollination, and cultured on orchid agar medium in the dark at 10°C until there was clear development. Then they were transferred to a growth chamber at 15°C day/10°C night, with 12 hrs of fluorescence light, and finally transplanted to pots when they had developed 2–3 leaves.

Cytological analysis

Somatic chromosome number were obtained from root-tip cells. Meiotic pairing was analyzed at metaphase I of the hybrids from spikes fixed in ethanol:acetic acid (3:1) and stained with the Feulgen procedure or by fluorescence genomic in situ hybridization (FISH). For FISH, total genomic DNA of *T. tauschii* and *A. cristatum* were labeled by nick translation with biotin-11-dUTP and digoxigenin-11-dUTP (Boehringer Mannheim), respectively. The hybridization procedure was as described previously (MARTÍN et al. 1998). Post-hybridization washes were carried out according the protocol of FERNÁNDEZ-CALVÍN et al. (1995). The detection of the biotin-labeled *T. tauchii* genomic DNA with streptavidin-sulforhodamin and digoxigenin-labeled *A. cristatum* genomic DNA with antidigoxigenin-FITC (fluorescein isothiocyanate, Boerhinger Mannhein) in PBS (phosphate buffered saline) was made simultaneously. Hybridization probes were visualized with a Leica epifluorescence microscope following excitation using

appropriate light filters. The presence of *T. tauschii* and *A. cristatum* chromosomes are shown by red and yellow respectively, while the unlabelled *H. chilense* chromosomes appear pale brown.

RESULTS

From 239 pollinated florets, 75 embryos were cultured. Most of them grew and were transferred to pots, but only 14 reached flowering. *Hordeum chilense* accession H1 as mother plant was more fertile than H17 (36 % vs. 13 %). One hybrid had H17 as mother plant the remaining 13 plants came from H1. These 14 plants were cloned and some of them were treated with colchicine but no seed set was obtained. Two untreated hybrid clones from each *H. chilense* accession were used for meiotic analysis. All untreated clones were transferred to soil for field evaluation. Seven hybrid plants showed the expected chromosome number of 21 while the remaining 7 plants had 22 chromosomes.

Morphology

The trihaploid hybrid $H^{ch}DP$ is perennial and tillered profusely. Some plants have been maintained vegetatively for several years. In general, the hybrid's morphology favors the male parent, the amphiploid DDPP. Hybrid spikelet structure was of the male parent, intermediate between *Agropyron* and *Aegilops* although closer to *Agropyron*. Spike length was similar to that of the *Hordeum* parent (Fig. 1a). Some hybrids were more vigorous than both parents. Tillers per plant varied from 113 to 1025. Spikelets per spike ranged from 17.2 to 23.6. The euhaploid 21 chromosome plants were more vigorous than the aneuhaploids. On average, the euploid plants showed 490 spikes per plant against 225 of the plants with 22 chromosomes. This difference is significant. The difference on spikelets per spike follows the same trends, 20 spikelets on the 21 chromosome plants vs. 17.6 on hybrids with 22 chromosomes.

Cytology

In Fig. 1b, a somatic metaphase of the hybrid $H^{ch}DP$ is presented. The larger size of some *Agropyron* chromosomes is evident. Satellited chromosome regions from *H. chilense* and *A. cristatum* can be observed.

Meiotic chromosome associations were first analyzed on MI plates from pollen mothers cells (PMC) after Feulgen staining of the anthers (Table 1). In the *H. chilense* (H1) × DDPP hybrid up to a pentavalent was observed. In the $H^{ch}DP$ hybrid from *H. chilense* (H17) the highest chromosome configuration observed was a trivalent (Fig. 1c). Bridges and fragments have been observed (Fig. 1d) at very low frequency.

FISH analysis was carried out to discern the origin of the bivalents observed. Table 2 shows the results of FISH analysis on the H^{ch}DP hybrid obtained from *H. chilense* accession H17 used as mother plant. Intragenomic pairing between *A. cristatum* chromosomes was higher than that found for *H. chilense* or *T. tauschii* chromosomes indicating the presence of duplications in the *A. cristatum* chromosomes. No D-D bivalents were found. Intergenomic pairing was observed between the three genomes. The frequency of H^{ch}-D bivalents was the highest observed and similar to that found for the P-P pairing. The number of H^{ch}-P and D-P bivalents was practically negligible.

The trivalent observed was determined to be from *Agropyron* chromosomes. Fig. 2(a and b) shows bivalents between H^{ch}-D and H^{ch}-P genomes, respectively.

DISCUSSION

The crossability of *H. chilense* with other Triticeae species is known to be high. Our results accord with this knowledge, but the low survival rate of plants after embryo culture (under 20 %), is lower than in other interspecific crosses carried out by us under similar conditions.
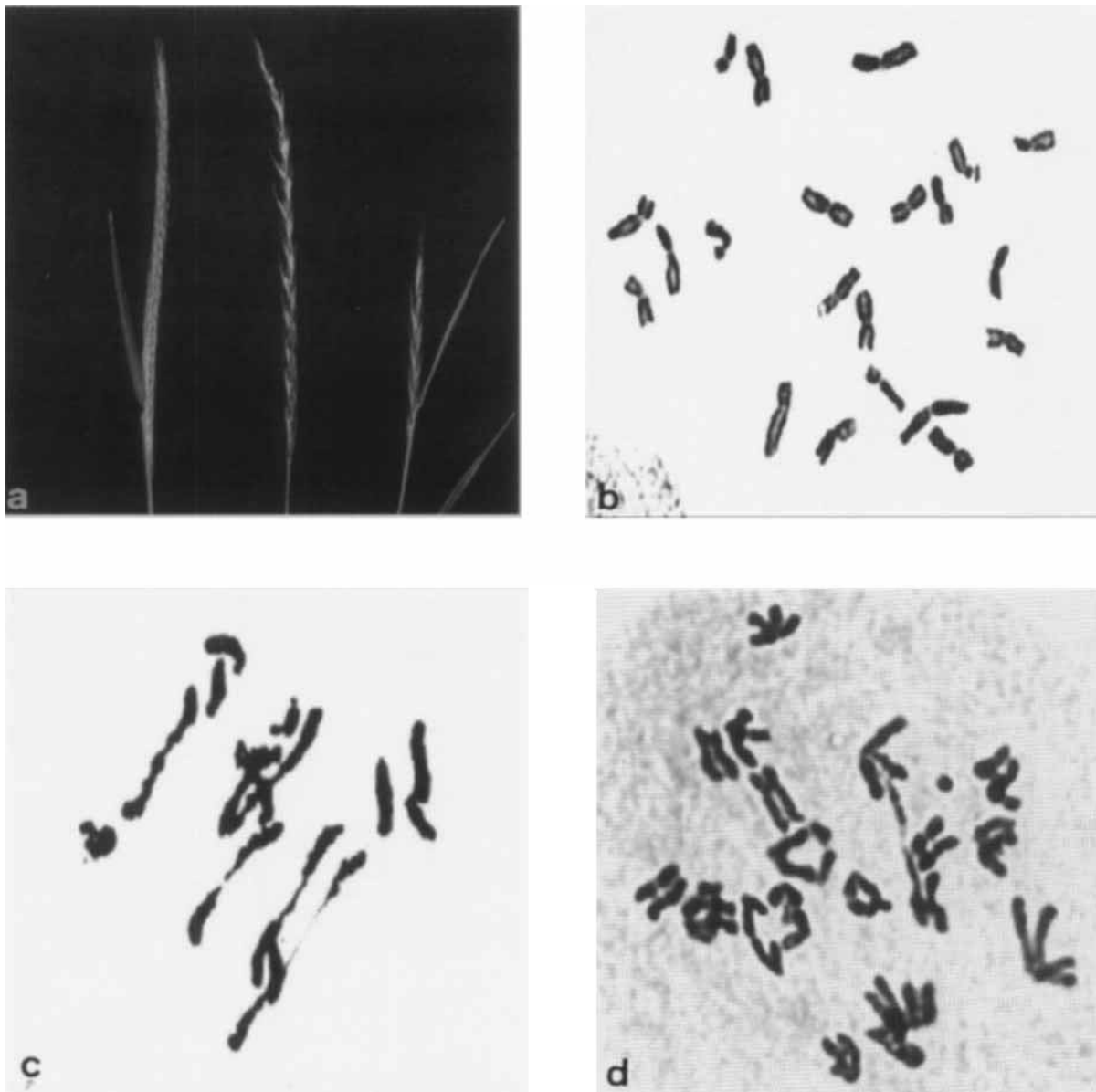

Fig. 1a–d. **a** From left to right, spikes of *Hordeum chilense*, hybrid H^{ch}DP, amphiploid *T. tauschii* × *A. cristatum*. **b** Somatic metaphase of the H^{ch}DP hybrid showing 21 chromosomes. **c** Meiotic metaphase I with 4 rod bivalents and a trivalent. **d** Meiotic anaphase I showing a bridge and a fragment.

Table 1. *Meiotic pairing in trigeneric hybrids Triticum-Agropyron-Hordeum (range in brackets)*

Hybrid	No. of PMCs examined	I	II			III	IV	V
			rod	ring	total			
H1DP	104	14.96 (9–21)	2.80 (0–6)	0.20 (0–2)	3.00 (0–6)	0.16 (0–1)	0.01 (0–1)	0.01 (0–1)
H17DP	158	17.51 (10–21)	1.73 (0–4)	0.02 (0–1)	1.75 (0–4)	0.02 (0–1)	0 –	0 –

Table 2. *Number of different meiotic configurations and distinguishable associations observed a metaphase in 178 cells analyzed by FISH in Triticum-Agropyron-Hordeum hybrid ($H^{ch}DP$)*

I	II										III
	H^{ch}-H^{ch}	D-D	P-P	Total$_1$	H^{ch}-D	H^{ch}-P	D-P	Total$_2$	Total $_{1+2}$		
18.52 (13–21)	0.01 (0–1)	– –	0.63 (0–2)	0.64 (0–2)	0.64 (0–4)	0.01 (0–1)	0.01 (0–1)	0.65 (0–4)	1.30 (0–1)		0.01 (0–1)

In the fertile amphiploid *T. tauschii-A. cristatum* (MARTÍN et al. 1998) a high number of *A. cristatum* multivalents were found at metaphase I. It was postulated in that work that the tetraploid *A. cristatum* plant used as male parental was a segmental allotetraploid (STEBBINS 1947). The major segmental changes from both genomes were attributed to reciprocal translocations, as SCHULZ-SCHAEFFER et al. (1963) found in a hexaploid population of this species. Most probably the reason of the low survival rate of embryos found in the present experiment was the high production of unbalanced gametes of the *T. tauschii-A. cristatum* amphiploid which should be considered to be of genomic constitution DDP_1P_2. This is also the most probable explanation for the high number of plants with 22 chromosomes obtained in this experiment, as well as the differences in vigor of the hybrid plants. The observed bridge and fragment in anaphase I of meiosis in the hybrids $H^{ch}DP$ (Fig. 1d) seems to indicate that a paracentric inversion is also contributing towards the differentiation of P_1 and P_2 genomes. Reciprocal translocation could be frequent in tetraploid *A. cristatum* and could explain the high frequency of multivalents observed on hybrids between wheat and this species.

Until the application of in situ hybridization to the analysis of meiotic chromosome pairing, it was difficult to distinguish in some cases, as in wheat-*Agropyron* hybrids (JAUHAR 1992), between allosyndetic or autosyndetic pairing. High level of meiotic pairing in hybrids between wheat and *Agropyron* species have frequently been reported and the interaction of genes from *Agropyron* with the *Ph* system of wheat has been considered as the most plausible

explanation. AHMAD and COMEAU (1991), studying the meiotic pairing of the hybrid between *T. aestivum* and tetraploid *A. fragile*, found differences in the chromosome pairing among different ABDPP hybrid plants. Up to hexavalent configurations were observed by these authors who concluded that *A. fragile* has a genetic system that modifies *Ph* gene activity. The same conclusion was reached by CHEN et al. (1989) and JAUHAR (1992) from hybrids between *T. aestivum* and tetraploid *A. cristatum*, although the latter author assigned part of the pairing to P-P associations. JAUHAR (1992) did not give the accession number of the tetraploid wheatgrass used in the cross (as in our case it came from Logan), but the meiotic behaviour of the two hybrid plants analyzed was similar to that found in this paper. The hybrids differed in the amount of pairing, one showing almost twice the amount of pairing of the other. These results are quite similar to ours (Table 1) and those reported by AHMAD and COMEAU (1991). As expected from the results obtained in the meiotic analysis of the amphiploid DDP_1P_2 (MARTÍN et al. 1988), the trivalents observed in meiotic cells of the $H^{ch}DP$ hybrid are ascribable to P chromosomes. The observed pairing differences between the two $H^{ch}DP$ hybrids (Table 1) could be explained as result of different duplications or deletions on P chromosomes of the male gametes. Pairing between P and D, and P and H^{ch} genome chromosomes (Table 2) was possible but their contribution to the total observed pairing was low. From these results we conclude that before hypothesizing the presence of a *Ph*-suppressor to explain the high chromosome pairing in wheat × tetraploid *Agropyron* hybrids, the autotetraploid nature of the latter species should be clearly established.
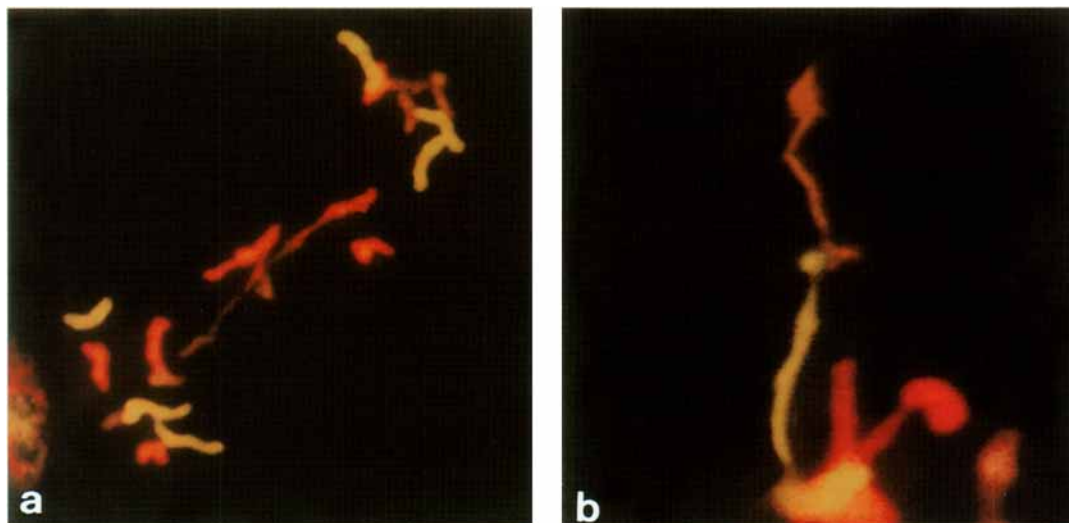
Fig. 2a and b. Meiotic metaphase I of the trigeneric hybrid *Hordeum-Triticum-Agropyron* after FISH analysis. **a** A bivalent between H^{ch} and D chromosomes. **b** A bivalent between H^{ch} and P chromosomes.

The observed pairing between the D and H^{ch} chromosomes (0.64 bivalents per cell) was similar to that observed (0.66) in the *H. chilense* × *T. tauschii* hybrid (MARTÍN 1983). This pairing between D and H^{ch} chromosomes clearly indicates that there is more affinity between these two genomes than between either of them and the *Agropyron* genome. Furthermore, D-H^{ch} pairing was higher than that observed between *H. chilense* and *H. bulbosum* or *H. chilense* and *H. vulgare* hybrids, respectively (PADILLA and MARTÍN 1983; THOMAS and PICKERING 1985). In situ hybridization of repetitive DNA sequence pAsI isolated from *T. tauschii* to *H. chilense* chromosomes revealed that multiple sites of hybridization were found on the seven H^{ch} chromosomes, indicating that the *H. chilense* genome contains a significant amount of repetitive DNA that is homologous to the pAsI sequence (CABRERA et al. 1995). These results support the chromosome affinity found in the present work and contradicts the phylogenetic relationships of the monogenomic species of the Triticeae derived from morphologic, biogeographic or molecular data (HSIAO et al. 1995). The D-H^{ch} chromosome pairing opens the possibility for genetic transfer from *H. chilense* to bread wheat by spontaneous recombination between chromosomes of the two genomes.

The high morphological differences observed among eupolyhaploids hybrids $H^{ch}DP$ (spikes per plant varied from 190 to 1025) could also be explained as result of the composition of P genome in DP gametes. These gametes may carry duplications and deficiencies as result of the segregation of normal and translocated chromosomes in anaphase I of meiosis. These deletions and duplications on the trigeneric hybrid could be the reason of the absence of fertility after colchicine treatment and therefore the failure to produce a fertile trigeneric amphiploid.

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