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A fertile amphiploid between durum wheat (*Triticum turgidum*) and the × *Agroticum* amphiploid (*Agropyron cristatum* × *T. tauschii*)

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Agropyron (Gaertn) is a genus of Triticeae which includes the crested wheatgrass complex, i.e. *A. cristatum* (L.) as representative species containing the **P** genome. This species is an important source for increase the genetic variability of both durum and bread wheat. Among the possible interesting features to be introgressed into wheat are resistance to wheat streak mosaic virus, rust diseases, and tolerance to drought, cold and moderate salinity. By crossing tetraploid wheat (*Triticum turgidum* conv *durum*, 2n = 4x = 28; **AABB**) with a fertile allotetraploid (2n = 4x = 28; **DDPP**) between diploid wheat (*T. tauschii*) and crested wheatgrass (*A. cristatum* L.), amphiploid plants were obtained. Fluorescence in situ hybridization (FISH) using both genomic DNA from *A. cristatum* and the repetitive probe pAs1, proved that the plants were true amphiploids with a chromosome number 2n = 8x = 56 and genomic constitution **AABBDDPP**. Using total genomic in situ hybridization (GISH) to study meiotic metaphase I, data on allosyndetic and autosyndetic chromosome pairing were obtained. The amphiploids were perennial like the male parent but their morphology was close to that of the wheat parent. They were resistant to wheat leaf rust and powdery mildew under field conditions.

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The tribe Triticeae includes important agriculture crops such as wheat, barley and rye, as well as forage crops within the genera *Agropyron*. The tribe has been the subject of extensive genetic and cytogenetic research. Hybrids and amphiploids between *Triticum aestivum* and different *Agropyron* species have been obtained with the aim to introduce into *Triticum* characters from *Agropyron*, i.e. *A. cristatum* (CHEN et al. 1989; LIMIN and FOWLER 1990), *A. desertorum* (LI and DONG 1990; LIMIN and FOWLER 1990) and *A. michnoi* (LI and DONG 1991). The **P** genome of *Agropyron* is a potential source of novel genes for traits, such as disease resistance, tolerance to drought, cold and moderate salinity. Recently a fertile amphiploid between diploid wheat (*Triticum tauschii*) and *A. cristatum* has been obtained (MARTÍN et al. 1998). In this paper we report the synthesis of a fertile amphiploid between durum wheat (*T. turgidum* L. var. *durum*) and the allotetraploid *T. tauschii-A. cristatum* (2n = 4x = 28; **DDPP**).

MATERIAL AND METHODS

The self-fertile amphiploid **DDPP**, which was obtained by crossing tetraploid accessions of *A. cristatum* (2n = 4x = 28) and *T. tauschii* (MARTÍN et al. 1998) was used as male parent to pollinate emasculated spikes of durum wheat (*T. turgidum* var. *durum cv.* Gerardo, T15).

The three hybrid plants (T15 × **DDPP**) were fertile, with an average seed set of 7.2 seeds/spike. These seeds germinated and root tips were collected from each seedling when the roots were 1.5-2 cm long. These root tips were treated with 0.05% colchicine for about 3 hours at room temperature and then fixed in a mixture of 3:1 (ethanol: glacial acetic acid) for 20 days. The root tips were then pre-treated one minute with acetocarmine and the number of chromosomes was determined by microscopic examination of squash preparation in one drop of 45% acetic acid. The preparations were frozen in liquid nitrogen for one minute to remove the coverslips and they were stored until used. Plants with 56 chromosomes were selected and transferred to individual pots in a growth chamber.

For meiotic analyses, spikes were collected and fixed in 3:1 alcohol: acetic acid solution. The fixed spikes were treated with 70% and 100% ethanol, respectively during about 3 minutes each. The anthers were excised from the florets and placed on aceto-carmine during 3 minutes and squashed in 45% acetic acid. The preparations were frozen in liquid nitrogen for one minute to remove the coverslips and they were stored until used. The in situ hybridization protocol was carried out according to CABRERA et al. (1999). Probes used were the repetitive pAs1 sequence, isolated from *T. tauschii* (RAYBURN and GILL 1986) and total genomic DNA isolated from *A.*

cristatum. Mitotic chromosome preparations were hybridized simultaneously with both biotin-labeled pAs1 and digoxigenin-labeled DNA from *A. cristatum*. The hybridization sites were detected with anti-digoxigenin-FITC (fluorescein isothiocyanate, Roche Corporate, Postfach, Basel, Switzerland) and streptavidin-Cy3 conjugate (Sigma, St. Louis, MO, USA) respectively, and they were counterstained with DAPI (4′,6-diamidino-2-phenylindole). Meiotic chromosome preparations were hybridized with digoxigenin-labeled DNA from *A. cristatum* and the signals detected with anti-digoxigenin-FITC. The preparations were counterstained with PI (propidium iodide). The signals were visualized with an epifluorescence microscope Leica, images were captured with a SPOT CCD camera using the appropriate SPOT 2.1 software and processed with PhotoShop 4.0 software (Adobe Systems Inc., San Jose, CA, USA). Images were printed on a Hewlett Packard Deskjet HP 840 colour printer. The response of the amphiploids and their parents to wheat leaf rust and powdery mildew diseases were analyzed under field conditions. The infection by the two pathogen species occurred naturally and infection severity scores were based on the percentage leaf area covered by mildew and rust.

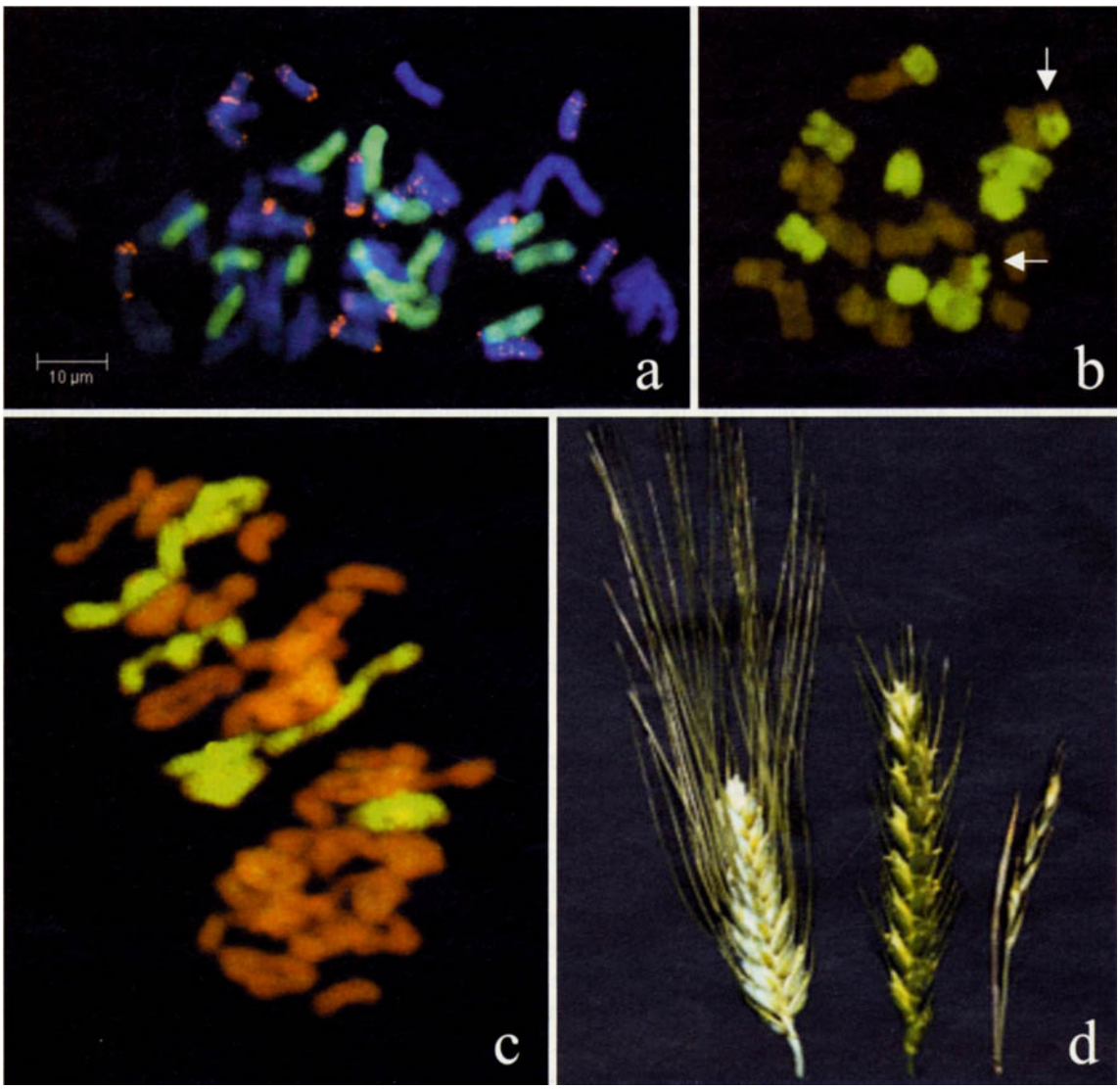

Fig. 1a–d. **a** Mitotic metaphase cell showing 28 chromosomes derived from durum wheat (blue), 14 from *A. cristatum* (green) and 14 from *T. tauschii* (blue with red pAs1 hybridization signals). **b** Anaphase I cell showing two *A. cristatum*-wheat recombinant chromosomes (arrows). **c** Meiotic metaphase I cell showing seven bivalents from *A. cristatum* (green) and three univalents from wheat (red). **d** From left to right, spikes of *T. turgidum*, amphiploid *T. turgidum* × **DDPP** and **DDPP**.

Table 1. *Number of different meiotic configurations and distinguishable associations observed at metaphase cells analyzed by FISH in three* ***AABBDDPP*** *amphiploids (range in brackets)*

Plant	PMC	*Agropyron*								Wheat	
		I	II						III	I	
			P-P		P-wheat						
			Rod	ring	Total 1	rod	ring	Total 2	Total 1+2		
T15 × DDPP-21	30	2.93 (0–10)	3.22 (0–7)	1.80 (0–7)	5.02 (0–7)	0.13 (0–2)	0.03 (0–1)	0.06 (0–1)	5.16	0.20 (0–1)	3.90 (1–11)
T15 × DDPP-8	15	3.60 (0–11)	3.60 (1–6)	1.40 (0–3)	5.00 (0–6)	0.20 (0–1)	0	0.20 (0–1)	5.20	0.06 (0–1)	6.60 (1–14)
T15 × DDPP-14	19	2.68 (0–10)	2.00 (0–5)	3.10 (0–6)	5.10 (0–6)	0	0	0	5.10	0.36 (0–2)	3.90 (0–10)
Mean	64	3.10			5.10			0.09	5.19	0.20	4.80

RESULTS AND DISCUSSION

Three amphiploid plants with chromosome number $2n = 8x = 56$ and genomic constitution **AABBDDPP**, out of eleven plants with chromosome number ranging from 50 to 57, were selected from crosses between the tetraploid *T. turgidum* and the **DDPP** allotetraploid. They were called (T15 × DDPP)-8, (T15 × DDPP)-14 and (T15 × DDPP)-21, respectively. We assume that these amphiploid plants have been produced naturally by the formation of unreduced gametes.

FISH using both total genomic DNA from *A. cristatum* and pAs1 repetitive probe allowed simultaneous discrimination of both the **P**- and **D**-genome chromosomes in the amphiploids. Fig. 1a shows a somatic metaphase spread from one of the three amphiploid plants with chromosome number $2n = 56$, in which 28 chromosomes derived from the durum wheat parent (blue colour), 14 chromosomes from *A. cristatum* (green colour) and 14 derived from *T. tauschii* (blue with red pAs1 hybridization signals). GISH was applied to meiotic metaphase I cells to distinguish between homoeologous (intergenomic) *vs.* homologous (intragenomic) chromosome associations on the three amphiploids obtained. Data obtained on chromosome pairing at metaphase I is shown in Table 1. In the three amphiploid plants the pairing values were similar. Pairing between **P** genome of *A. cristatum* and wheat genome chromosomes was observed, although at low frequency. Fig. 1b shows an anaphase I cell showing two wheat-*Agropyron* recombinant chromosomes.

The mean of chromosome pairing observed between **P**-genome chromosomes was 3.1 univalent, 5.19 bivalents and 0.2 trivalents. These trivalents always included at least one wheat chromosome. As have been found previously in the **DDPP** amphiploid (MARTÍN et al. 1998), pairing between *A. cristatum* and wheat chromosomes is possible although it contributes very little to the total chromosome pairing observed. From the breeder's point of view the wheat-*Agropyron* chromosome pairing, even this low, is relevant, since it indicates that recombination and therefore introgression of genetic material from *A. cristatum* into wheat is possible. The observation of unpaired chromosomes of wheat (4.8 univalents) was unexpected and it could be a consequence of chromosomal instability which was observed in the parental **DDPP** (MARTÍN et al. 1998). Fig. 1c shows a meiotic metaphase I cell from the amphiploid **AABBDDPP** showing 7 bivalents from *A. cristatum* and at least 3 univalents from wheat.

Table 2. *Pollen and seed fertilities in the **AABBDDPP** amphiploids*

Plant	No. of pollen exam.	% of pollen fertile	No. of seeds
(T15 × DDPP)-21	200	66.9	3
(T15 × DDPP)-14	320	71.2	7
(T15 × DDPP)-8	647	72.1	28
Mean	389	70	

Table 3. *Percentage of leaf area covered by leaf rust and powdery mildew in the **AABBDDPP** amphiploids and its parents in a field test under natural infection*

Plant	*P. triticina*	*B. graminis*
(T15 × DDPP)-21	0	0
(T15 × DDPP)-14	1	0
(T15 × DDPP)-8	1	0
Gerardo (T15)	20	20
A. cristatum	0	0
DDPP	0	0

The three amphiploids were fertile with a mean of 70% pollen (Table 2). Morphologically the amphiploids were more variable than the highly uniform **DDPP**, upright, tall, vegetatively vigorous, and they tillered profusely. The spike morphology (Fig. 1d) was generally intermediate between its parents but tended to resemble *T. turgidum*. The amphiploids tended to be perennial with hairy leaves like the male parent **DDPP**.

The amphiploids were resistant to powdery mildew (*Blumeria graminis* f. sp. *tritici*) and wheat leaf rust (*Puccinia triticina*) (Table 3). In all cases the amphiploids were more resistant than the wheat parent.

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