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Introgression of 1Dx5+1Dy10 into Tritordeum

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Abstract The uses of hexaploid tritordeum as a crop for human consumption require improvement of its bread-making quality. For this purpose chromosome 1D of bread wheat with the *Glu-D1* allele encoding for high-molecular-weight glutenin subunits Dx5+Dy10 was introgressed into tritordeum. Different primary tritordeums were crossed with wheats carrying subunits Dx5+Dy10. The hybrids were backcrossed to tritordeum and seeds for the next backcross (or selfing) were selected for the presence of chromosome 1D using SDS-PAGE. Forty two chromosome plants carrying subunits Dx5+Dy10 were obtained after two backcrosses and selfing. Chromosome characterization of these plants using fluorescence in situ hybridisation (FISH) proved that either chromosome substitution $1H^{ch}$/1D or 1A/1D had been obtained. A homozygous plant with a translocation of the entire 1DL arm to $1H^{ch}S$ was also obtained. The complete chromosome substitution lines have better agronomic characteristics than the lines with translocations.

Keywords Barley-wheat amphiploids · Chromosome substitution · *Hordeum chilense* · FISH · HMW

Introduction

Plant breeders have been interested in crossing wheat (*Triticum* spp.) and barley (*Hordeum vulgare*) since the beginning of the past century (Farrer 1904), but amphiploids, × *Tritordeum* Asch. et Graebn. (1902, http://www.bgbm.fu-berlin.de/iapt/nomenclature/code/tokyo-e/Art_h08.htm), were obtained only when wild relatives of *H. vulgare*, as for example *Hordeum chilense*, were used. *H. chilense* Roem. et Schult. is a wild South American diploid barley included in the Section Anisolepis (Bothmer et al. 1995). Hexaploid tritordeum [*H. chilense* × *Tritium turgidum* conv. *durum* (Desf.) MacKey, 2n = 6*x* = 42, $\mathbf{H^{ch}H^{ch}AABB}$] showed good fertility and chromosome stability as well as a morphology similar to that of bread wheat (Martín and Sánchez-Monge Laguna 1982). These and other favourable agronomic traits, such as high biomass yield, number of spikelets/spike, seed size and protein content, made us consider the potential of this amphiploid as a possible new crop (Martín and Cubero 1981) and later studies confirmed these expectations (Martín et al. 1999). However, their poor threshing (brittle rachis or tough glumes) have limited its use as a new crop. The yield of advanced tritordeum lines is similar to that of bread wheat, and free-threshing lines are at present available. Nevertheless, building lines with both traits is not a straightforward task.

The most accessible source of genetic variability in the tritordeum-breeding program is the synthesis of new amphiploids with a range of lines of *H. chilense* and durum wheat. Although the possibilities for chromosome manipulation by crossing hexaploid tritordeum with bread wheat, or octoploid tritordeum (2n = 8*x* = 56, $\mathbf{H^{ch}H^{ch}AABBDD}$) and durum wheat, are also explored. Both crosses easily produced hybrids of the genomic formula $\mathbf{AABBDH^{ch}}$ which, after backcrossing to hexaploid tritordeum, open the possibilities of introgressing the **D** genome into hexaploid tritordeum.

The acceptance of tritordeum as a new cereal will depend, besides yield or good agronomic performance in general, on the quality of the grain, either as a feed or as a food. As a feed, tritordeum has nutritional properties similar to its durum wheat parent (Cubero et al. 1986) with a higher pigment content (Alvarez et al. 1999). As a cereal for human consumption hexaploid tritordeum

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shows properties similar to bread wheat (Alvarez et al. 1995). Although hexaploid tritordeum has promising bread-making characteristics, it lacks the **D** genome, present in bread wheat, and therefore the Dx5+Dy10 glutenin subunits associated with high bread-making quality (Payne et al. 1987). In this paper we describe the introgression of chromosome 1D coding for the high-molecular-weight (HMW) glutenin subunits Dx5+Dy10 either substituting chromosome 1H or chromosome 1A, as well as the translocation 1HS/1DL, into hexaploid tritordeum.

Materials and methods

Crosses between bread wheat (2n = 6*x* = 42, **AABBDD**) cv 'Yecora' carrying the Dx5+Dy10 HMW glutenin subunits, and hexaploid tritordeums (2n = 6*x* = 42, **$H^{ch}H^{ch}$AABB**) were performed to obtain the hybrid **AABBDH^{ch}** in wheat cytoplasm. Recombinant tritordeums HTC174, HTC178, HTC503 and HTC721 were used as pollen donors in order to have hybrids with different genetic backgrounds to overcome any shortcoming due to hybrid necrosis. These hybrids were backcrossed two or three times to tritordeum.

Grains were divided into two pieces for chromosome counting and HMW glutenin subunit determination on the same seed. The embryo-less halves were used for endosperm storage protein analysis.

For somatic chromosome counting, root-tips were treated for 4 h with a 0.05% colchicine-aqueous solution, fixed in 3:1 ethanol-acetic and stained by the conventional Feulgen technique.

FISH

Somatic metaphase chromosome preparations and FISH protocol was carried out according to Cabrera et al. (2002). Both the GAA-satellite and pAs1 repetitive probes were used for chromosome identification. The barley clone pHvG38 (Pedersen et al. 1996) containing the GAA-satellite sequence was kindly provided by Dr. S.K. Rasmussen from the Risø National Laboratory, Roskilde (Denmark), and the pAs1 probe (1 kb) isolated from *Aegilops tauschii* Coss. by Rayburn and Gill (1986) was kindly provided by the Wheat Genetics Resource Centre, University of Kansas (USA). The GAA-satellite and pAs1 probes were labelled with digoxigenin-11-dUTP and biotin-11-dUTP respectively, by nick-translation.

The in situ hybridization pattern observed after probing the chromosome preparations with the GAA-satellite probe corresponds to that previously reported in wheat by Pedersen et al. (1996). The pAs1 probe especially hybridised on the D-genome chromosome from wheat and H^{ch}-genome chromosomes from tritordeum. The pAs1 banding-pattern observed on both the 1D- and H^{ch}-genome chromosomes is in general agreement with that found previously in wheat (Mukai et al. 1993; Pedersen and Langridge 1997) and *H. chilense* (Cabrera et al. 1995), respectively.

After examination of metaphases hybridized with the GAA-satellite or pAs1 probes, preparations were re-probed with digoxigenin-labelled genomic *H. chilense* DNA. The biotin-labelled pAs1-probe was detected with the Streptavidin-Cy3 conjugate (Sigma). The digoxigenin-labelled GAA-satellite sequence and the genomic *H. chilense* DNA were detected as was the anti-digoxigenin-FITC. Chromosomes were counterstained with DAPI (4′, 6-diamidino-2-phenylindole) or PI (propidium iodide) 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.). Images were printed on a Hewlett Packard Deskjet HP 840C Color Printer

Glutenin analysis

Proteins were extracted from crushed endosperm. Gliadin-free glutenin was solubilized with 250 µl of buffer containing 50% (v/v) propan-1-ol, 80 mM of Tris–HCl pH 8.5 and 2% (w/v) dithiothreitol at 60 °C for 30 min. After centrifugation, 200 µl of the supernatant were transferred to a new tube, mixed with 3 µl of 4-vinylpyridine, and incubated for 30 min at 60 °C. The samples were precipitated with 1 ml of cold-acetone. The dried pellet was solubilized in buffer containing 625 mM of Tris–HCl pH 6.8, 2% (w/v) SDS, 10% (v/v) glycerol, 0.02% (w/v) bromophenol blue and 2% (w/v) dithiothreitol in a 1:5 ratio (mg/µl) to wholemeal.

Reduced and alkylated proteins were fractionated by electrophoresis in vertical SDS-PAGE slabs in a discontinuous Tris–HCl-SDS buffer system (pH 6.8/8.8) at an 8% polyacrylamide concentration (w/v, C = 1.28%). The Tris–HCl/glycine buffer system of Laemmli (1970) was used. Electrophoresis was performed at a constant current of 30 mA/gel at 18 °C for 30 min after the tracking dye migrated off the gel. Gels were stained overnight with 12% (w/v) trichloroacetic acid solution containing 5% (v/v) ethanol and 0.05% (w/v) Coomassie Brilliant Blue R-250. De-staining was carried out with tap water.

Results and discussion

Three out of the four F_1 hybrids were necrotic. Only the hybrid 'Yécora' × HTC178 reached maturity and was able to be backcrossed with tritordeum. Hybrid necrosis has been observed in hybrids of similar genome constitution such as wheat × triticale and this fact has been one of the obstacles to interchange genetic information between both species (Bizimungu et al. 1998). In order to increase the chance of obtaining viable and fertile plants we used a mixture of pollen from different genotypes when backcrossing.

On the first backcross, BC_1F_1 plants expressing the Dx5+Dy10 glutenin subunits and showing normal development and morphology were selected. On the second backcross, BC_2F_1 in addition to the previous selection criteria, plants with a chromosome number close to 42 were selected.

The 1D chromosome transmission rate on the first and second backcross was 31.49% (57/181) and 25.5% (76/298) respectively, which facilitated the selection of plants carrying the subunits Dx5+Dy10. The elimination of the remaining D chromosomes was not rapid, most likely because of a high transmission rate. Of the 76 BC_2F_1 seeds expressing the subunits Dx5+Dy10, 23 had between 40 and 42 chromosomes. Thirteen of these plants were analysed by FISH and all, except one (THC214), had 30 or more wheat chromosomes (Table 1). Therefore the whole **A** and **B** genomes plus two or more D chromosomes were present.

The three BC_2F_1 plants with the higher fertility, THC80, THC94 and THC272, were selected. In addition THC214, which had 29 wheat chromosomes and 13 *H. chilense* chromosomes, was also selected, although it was male-sterile, under the presumption of it being a monosomic substitution 1H^{ch}/1D. THC214 was backcrossed to tritordeum again.

Table 1 Chromosome number and fertility of BC_2F_1 plants (bread wheat × tritordeum) expressing the Dx5+Dy10 subunits

Plant	Chromosome number	Wheat chromosomes	*H. chilense* chromosomes	Fertility
THC 20	42	32	10	Low
THC 80	42	32	10	Medium
TCH 94	41+1 telo	30 + 1 telo	11	Medium
THC 103	42	30	12	Low
THC 119	42	32	10	Low
THC 168	40 + 1 telo	30 + 1 telo	10	Low
THC 185	42	31	11	Male-sterile
THC 205	41	31	10	Low
THC 214	42	29	13	Male-sterile
THC 231	42	31	11	Low
THC 236	40	30 + 1 translocac chilense-wheat	9	Low
THC 238	41 + telo	31	10 + 1 telo	Male-sterile
THC 272	42	31	11	Low

Table 2 Segregation of HMW glutenins subunits codified by 1D or $1H^{ch}$ chromosomes, on BC_2F_2 (bread wheat × tritordeum). (p = present; a = absent)

BC_1F_1	BC_2F_2 plants						
	1D $1H^{ch}$ p p	1D $1H^{ch}$ p a	1D $1H^{ch}$ a p	1D $1H^{ch}$ a a	Total	1D %	$1H^{ch}$ %
THC80	5	8	1	5	19	68	47
THC94	6	3	1	6	16	56	25
THC272	5	9	2	4	20	70	55

Acquisition of substitution $1H^{ch}$/1D and the $1H^{ch}$S/1DL translocation

The progeny of THC80, THC94 and THC272 segregated for the HMW glutenin subunits coded on the $1H^{ch}$ and 1D chromosomes. Chromosome number and HMW profile was determined on 20 seeds from each selfing. On this basis, the transmission rate of chromosome 1D was determined as being higher than the transmission of chromosome $1H^{ch}$ (Table 2). Plant THC80-31 (BC_2F_2) was selected on the basis of fertility and good grain filling as well as the absence of HMW proteins coded on chromosome $1H^{ch}$ (Alvarez et al. 2001) and the presence of the subunits Dx5+Dy10, although its chromosome number was 41. Again, chromosome number and HMW profile was evaluated on the progeny of this plant. Fifty BC_2F_3 seeds were analysed and all the grains had the subunits Dx5+Dy10 and Bx17+By18, and lacked the subunits coded at 1A and $1H^{ch}$. The chromosome number of these plants ranged between 40 and 42. Twenty six plants had 42 chromosomes and a fertility between 65 and 90%. The chromosome number on ten seeds from each of the former BC_2F_3 plants was scored. Six plants in which no aneuploids were found were selected for FISH analysis. On the progeny of these plants, BC_2F_4, disomic substitutions (Fig. 1) and a plant heterozygous for a translocation, 1HS/1DL, were found. These plants were selfed and from its progeny a homozygous plant was found based on the LMW proteins coded by the 1DS chromosome arm (Fig. 2).

In Fig. 2, an electrophoregram of wheat and tritordeum, together with the substituted and translocated lines, are presented. In the box on lane 3, corresponding to the translocation T$1H^{ch}$S. 1DL, an appreciable change in the profile for the LMW glutenin subunits was seen, where the marked bands, coded on the short arm of chromosome 1D, were absent. The homozygotic nature of the translocation was confirmed by FISH (Fig. 1c).

Acquisition of substitution 1A/1D

As stated previously THC214 was male sterile and was backcrossed to tritordeum again. Four out of 26 BC3F1 seeds from this backcross expressed the subunits Dx5+Dy10, and two of them, with 42 chromosomes, were partially fertile and were named HT 284 and HT 285. The pro-geny of both plants (BC_3F_2) segregated for the subunits Dx5+Dy10 but all of them expressed $1H^{ch}$ and 1B glutenin subunits. The chromosome number of BC_3F_2 plants varied between 40 and 44 and the number of *H. chilense* chromosomes ranged between 12 and 14. Two of these plants, named HT284-28 and HT285-49, with 42 chromosomes (14 of them *H. chilense* chromosomes) carried a substitution of chromosome A or B for chromosome 1D. The high fertility of this plant, plus the expression of 1B and 1D glutenin subunits, suggest a balanced monosomic substitution 1A/1D.

HT285-49 progenies segregated for the Dx5+Dy10 subunits after five selfings. Nevertheless, on the progeny of HT284-28, BC_3F_4 plants were identified which were homozygous for the Dx5+Dy10 subunits (Fig. 2). To confirm the substitution 1A/1D, FISH with total ge-

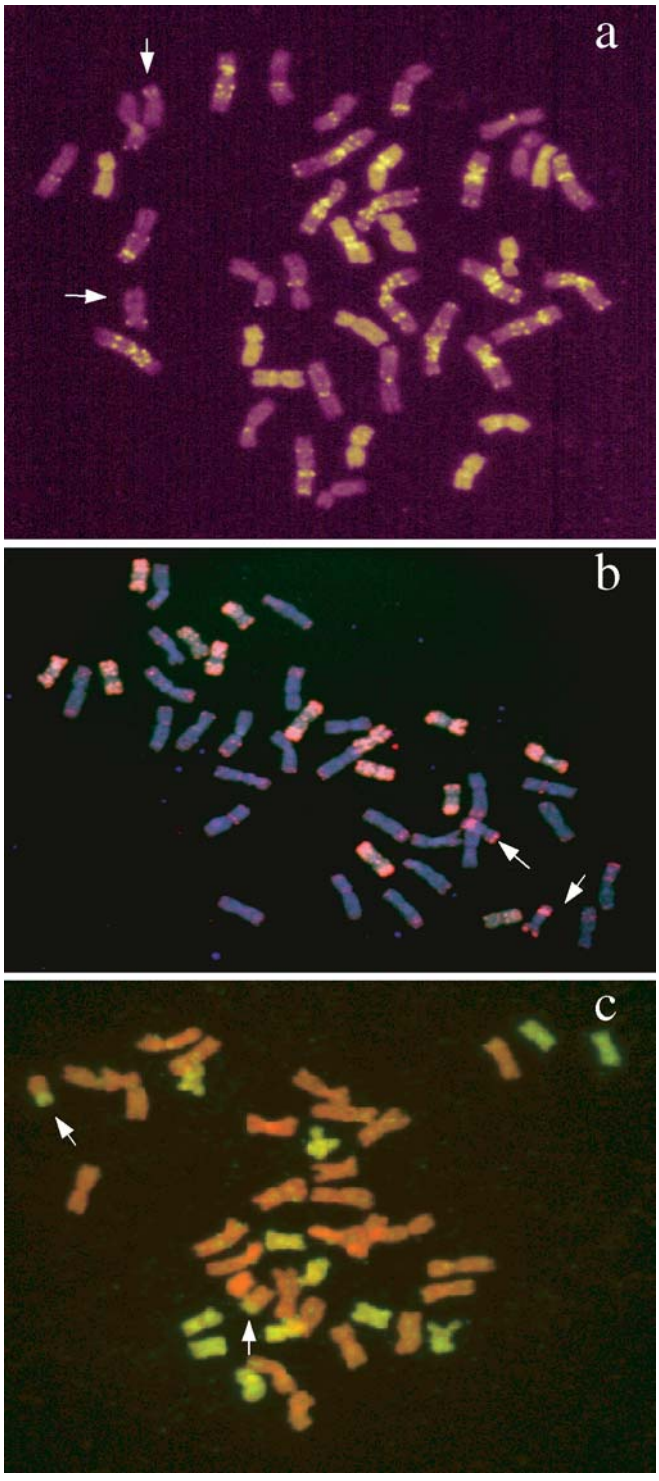

Fig. 1 FISH to metaphase chromosome spreads of **a** substitution 1H^{ch}/1D in tritordeum hybridized with a digoxigenin-labelled GAA sequence and re-probed with digoxigenin-labelled total genomic *H. chilense* DNA detected with FITC, showing one pair of chromosomes 1D (*arrowed*) and six pair of *H. chilense* chromosomes; **b** substitution 1A/1D hybridized with the biotin-labelled pAs1 probe (*red* Cy3 signals) and re-probed with digoxigenin-labelled total genomic *H. chilense* DNA (*green* FITC signals) showing one pair of chromosome 1D (*arrowed*) and the seven chromosome pairs of *H. chilense*, **c** translocation 1H^{ch}S. 1DL (*arrowed*) hybridized with digoxigenin-labelled total genomic *H. chilense* DNA detected with FITC. The cells were counterstained with DAPI (**a and b**) and PI (**c**)

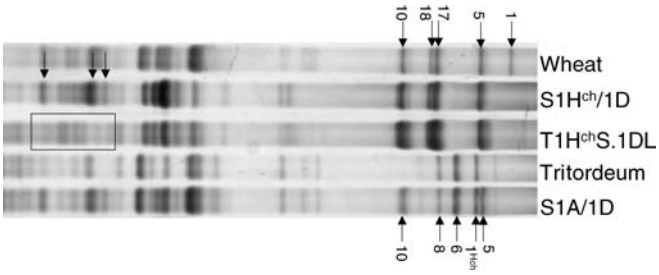

Fig. 2 SDS-PAGE analysis of wheat, tritordeum and substitution 1H^{ch}/1D, 1A/1D and translocation 1H^{ch}S.1DL. *Arrows* indicated the LMW glutenin subunits from chromosome 1D. The *box* indicated the absence of these bands in the translocation line

nomic DNA and the probe pAs1 was performed (Fig. 1c).

The high fertility and normal morphology of the 1H^{ch}/1D disomic substitution on tritordeum confirm the homoeology between *H. chilense* and *Ae. tauschii*, which has been previously established both at the cytological level by in situ hybridisation with probe pAs1 (Cabrera et al. 1995) and comparative mapping (Hernández et al. 2001).

Obtaining the translocation 1H^{ch}S/1DL is a first step to the production of the translocation of a small chromosome segment, 1D, in 1H^{ch}. The tritordeum with the subunits Dx5+Dy10, and a reduced amount of chromosome 1D, will allow normal chromosome pairing when breeding tritordeum, and therefore reducing sterility, usually associated with hybrids between tritordeum lines differing in a chromosome or chromosome arm.

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