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THE CYTOGENETICS AND MOLECULAR CHARACTERISTICS OF A TRANSLOCATED CHROMOSOME 1AS.1AL-1DL WITH A *GLU-D1* LOCUS IN DURUM WHEAT

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Abstract: Wheat quality depends directly on the grain protein content and protein composition. High and low molecular weight glutenin subunits play an important role in determining the visco-elastic properties of gluten. In an attempt to improve the breadmaking quality of hexaploid triticale, a fragment of wheat chromosome 1D, containing the *Glu-D1* allele encoding the 5+10 subunits, was translocated to the long arm of chromosome 1A by Lukaszewski and Curtis [1]. The 1A.1D translocation chromosome was transferred to tetraploid wheat [2], making the *Glu-D1* locus available for the improvement of durum wheat. The goal of this study was to evaluate using cytogenetics and molecular approaches the amount of chromatin introgressed in durum wheat. Fluorescence *in situ* hybridization with total genomic DNA (GISH) of *Aegilops squarrosa* L. indicated that the translocated chromosome 1A.1D had a terminal 1DL segment of about 35-40% of the recombinant arm length. Several pairs of microsatellite primers from chromosome 1A and 1D were used to genetically characterize the recombinant chromosome. The mapping data indicated that a 1AL segment, at least 150 cM long, was substituted by a 1DL segment with a minimal length of 72 cM, and that the translocation breakpoint was near the 1A centromeric region. The genetic and physical data highlight a substantial discrepancy between the recombinational and physical map distances. We are using a targeted strategy via the *Ph* pairing manipulation system to generate small intercalary 1D chromosome segments in a durum wheat background.

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INTRODUCTION

The grain quality of wheats depends directly on the grain storage protein content and protein composition [3]. High molecular weight (HMW) and low molecular weight (LMW) glutenin subunits play an important role in determining the visco-elastic properties of gluten. HMW glutenin subunits are encoded by complex loci (*Glu-A1*, *Glu-B1*, *Glu-D1*) located on the long arm of the homoeologous group-1 chromosomes of hexaploid wheat [4]. Subunits coded by the *Glu-A1* locus are seldom expressed in most of the durum wheat cultivars, so variation is only present at the *Glu-B1* locus with only a few alleles. An approach to improve durum wheat quality could be the exploitation of the useful allele existing at the *Glu-1* loci of common wheat. Its effects on gluten quality have been studied in detail. The chromosomes of tetraploid wheat show regular pairing with the A and B genomes of hexaploid wheat, therefore each allelic variant of the *Glu-A1* and *Glu-B1* loci of hexaploid wheat can easily be transferred to durum wheat. The exploitation of allelic variation at *Glu-D1* can also be made possible by using aneuploids and *ph* mutant lines to induce homoeologous pairing and recombination between the 1D chromosome of common wheat and the 1A or 1B chromosomes of durum wheat. The transferred segment with the desired gene should be as small as possible in order to reduce or eliminate the fertility problems usually associated with intergenomic translocation.

In an attempt to improve the breadmaking quality of hexaploid triticale, a fragment of the long arm of wheat chromosome 1D was translocated to the long arm of chromosome 1R [5] and to the long arm of chromosome 1A [1] using various aneuploids and induced homoeologous recombination. The translocated fragment contained the *Glu-D1* locus encoding the HMW glutenin subunits 5+10, which accounts for the largest part of the total variation in gluten quality of common wheat. The translocated 1D segment on the 1A chromosome of hexaploid triticale Rhino-6 was analyzed using C-banding by Lukaszewski and Curtis [1], who demonstrated that the introgressed segment involved the 1.5 C-band from chromosome 1DL, and consequently was identifiable by C-banding. However, it was not clear whether the induced 1A.1D transfer in Rhino-6 was interstitial or terminal; moreover, the transfered segment size and the physical and genetic breakpoint were not determined. With the aim of transferring both the *Glu-D1* encoding HMW and the *Glu-D3* encoding LMW glutenin subunits to durum wheat, Ceoloni *et al.* [6, 7] were able to isolate translocated tetraploid lines with *Gli-D1/Glu-D3* and *Glu-D1* protein phenotypes. The recombinant lines were characterized via protein analysis and fluorescence *in situ* hybridization [8]. We carried out similar work by using the translocated hexaploid triticale obtained by Lukaszewski and Curtis [1] with the objective of

transferring the *Glu-D1* locus to tetraploid wheat, thereby making a different source of this gene available for the improvement of cultivated durum wheat. The goal of this study was to evaluate using cytogenetics and molecular approaches the amount of chromatin introgressed in the durum wheat.

MATERIALS AND METHODS

Plant material

The hexaploid triticale Rhino-6, which was provided by Dr. A. J. Lukaszewski, Department of Botany and Plant Science, University of California, Riverside, USA, was the source of the *Glu-D1* locus present on the translocation 1AS.1AL-1DL. The recipient tetraploid durum parent was the Italian semi-dwarf cultivar Creso. Rhino-6 was crossed to durum wheat and the resulting 35-chromosome pentaploid hybrids were backcrossed to Creso. The selected 28-chromosome translocation heterozygotes were backcrossed again twice to recover the Creso genetic background. This was followed by selfing. BC_3F_2 plants were analysed for grain yield and quality components [2] and used to characterise the recombinant chromosome.

In situ hybridisation on mitotic chromosomes

Seeds of the recombinant durum wheat lines were germinated on moist filter paper for a few days at 25°C in the dark. To increase the proportion of metaphases, the root-tips were treated with an aqueous solution of 0.05% colchicine for 4h or, alternatively, immersed in ice water for 24h. They were subsequently fixed in ethanol:acetic acid (3:1, v/v). Chromosome preparation and fluorescent *in situ* hybridisation followed (techniques as described by Schwarzacher *et al.* [9] and Galasso *et al.* [10]). Genomic *in situ* hybridisation was applied to pre-treated root-tip chromosome spreads, simultaneously using the following as probes: digoxigenin-11-dUTP labelled genomic DNA from *Aegilops squarrosa* L. (DD genomes) and biotin-14-dATP labelled genomic DNA from durum wheat cv. Creso (AABB). Hybridisation was carried out overnight at 37°C, and the slides were subsequently rinsed thoroughly and carefully to reduce cross-hybridisation. Digoxigenin- and biotin-labelled genomic probes were detected using antidigoxigenin-fluorescein and streptavidin-Cy3, respectively. Photographs were taken on Fujicolor 400 print film. Subsequently, the same metaphases were re-hybridised *in situ* with a digoxigenin-labelled pAs1 clone, a highly repeated sequence isolated from *Ae squarrosa* by Rayburn and Gill [11].

Microsatellite markers

Small-scale DNA extractions from leaves for microsatellite analysis were carried out using the Dneasy Plant Mini Kit (Qiagen, Germany). Microsatellite loci mapping on 1A and 1D chromosomes were PCR-amplified using the primer pairs GWM99, GWM106, GWM135, GWM164, GWM232, GWM337, GWM357,

GWM458, GWM642, GWM750 (12) and GDM111, GDM126 (18). PCR were performed in a volume of 25 µl in a Perkin-Elmer DNA Thermal Cycler (PTC 200). The reaction mixtures contained 0.2 µM of each primer, 0.2 mM of each deoxynucleotide, 1.5 mM $MgCl_2$, 1 unit *Taq* polymerase, and 50-100 ng of template DNA in 10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1 mg/ml gelatin buffer. After 3 min at 95 °C, 35 cycles were performed with 1 min at 94°C, 1 min at the appropriate annealing temperatures [12], 2 min at 72°C, and a final extension step of 10 min at 72°C. The PCR products were separated on 6% polyacrylamide non denaturing gels and stained with ethidium bromide, using a 100bp DNA Ladder as a marker.

Electrophoretic analysis

The gliadin and HMW glutenin components of grain storage proteins were identified. Total seed protein extraction from crushed single grain halves and sodium dodecyl sulphate polyacrylamide-gel electrophoresis (SDS-PAGE) on 10% gels were carried out according to Payne *et al.* [13]. Monomeric prolamins were extracted with 1.5 M dimethylformamide at a 1:5 w/v ratio. After centrifugation (15 min at 10,000 g), the clear supernatant was used for electrophoretic separation, as described by Lafiandra and Kasarda [14].

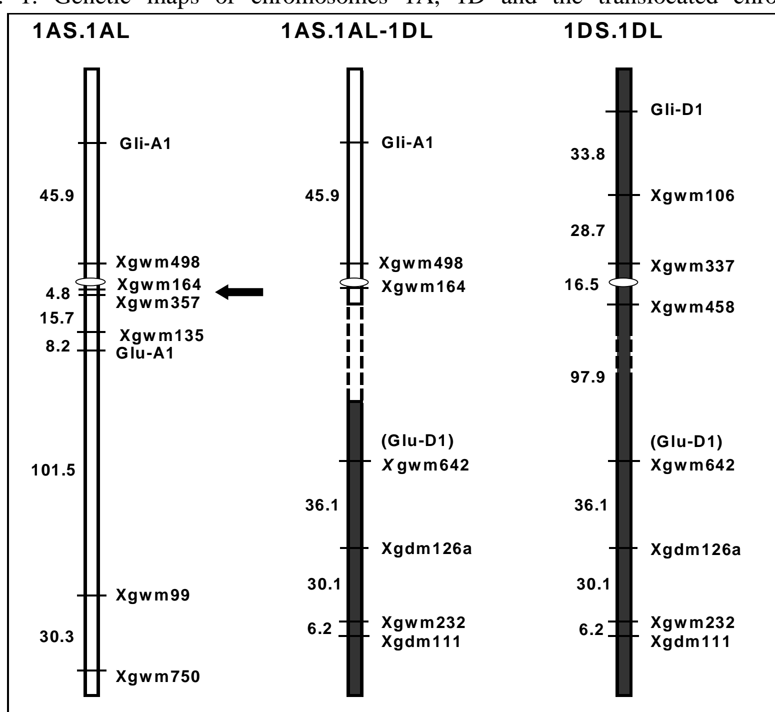
RESULTS AND DISCUSSION

The amount of chromatin introgressed in the translocated durum wheat was evaluated using molecular and cytogenetics approaches. The BC_3F_2 plants, obtained by backcrossing the translocated hexaploid triticale Rhino-6 with the recurrent tetraploid durum wheat cv. Creso [2], had 28 chromosomes and were segregating for the recombinant 1A.1D chromosome. In each backcrossing generation, the nongerm portions of each half-seed were screened for the storage proteins by SDS-PAGE, and the presence of the 5+10 HMW glutenin subunits, coded by the *Glu-D1* locus, indicated the presence of the 1D translocated segment. Plants with the 5+10 glutenin subunits were always used as the female and Creso as the male parent. Out of 99 BC_3F_2 seeds screened for the presence of the *Glu-D1* locus, 67 were found with 5+10 HMW glutenin bands and 32 without. As Creso has a null allele at the *Glu-A1* locus, the microsatellite marker locus *Xgwm135-1A*, located at 8.2 cM from *Glu-A1* on the 1AL chromosome arm [12], was then analysed on 67 BC_3F_2 plantlets in order to distinguish homozygous from heterozygous plants for the translocated 1A.1D chromosome. The DNA from 12 plants with 5+10 HMW glutenin subunits did not generate PCR products and those plants were therefore considered homozygous for the recombinant chromosome.

To genetically characterize the translocated chromosome, a total of 7 pairs of microsatellite primers (GWM106, GWM337, GWM458, GWM642, GDM126, GWM232, GDM111) from chromosome 1D [12, 18] were used to amplify the genomic DNA of the homozygous translocated durum wheat plants and the

durum wheat Creso, the common wheat Chinese Spring, and the translocated hexaploid triticale Rhino-6, which were used as controls. Three microsatellite primers that amplify loci on the chromosome arm 1DS (*Xgwm106* and *Xgwm337*) and on the pericentromeric region of 1DL (*Xgwm458*) only generated PCR products on Chinese Spring, indicating that the short arm and the centromere of the 1D chromosome were not involved in the translocation. Four microsatellite primers (GWM642, GDM126, GWM232 and GDM111), from chromosome arm 1DL, amplified the PCR products in the translocated durum wheat, in the translocated triticale and in Chinese Spring, but not in the normal durum wheat cv. Creso. These four microsatellite markers defined a 1DL chromosome segment with a minimal length of 72.4 cM involved in the translocation. Figure 1 shows the genetic maps of the 1A and 1D chromosomes

Fig. 1. Genetic maps of chromosomes 1A, 1D and the translocated chromosome



1AS.1AL-1DL. The genetic map distances (in cM) and relative positions of the microsatellite markers are from Roder *et al.* [12 and Roder, personal communication]; positions of *Gli-A1* and *Gli-D1* are from Van Deynze *et al.* [15]. The translocation of the 1D chromosome origin is shown as a grey block. *Glu-1* is substituted in the translocation according to the SDS-PAGE of storage proteins. The breakpoint on the 1A chromosome, indicated by an arrow, has been placed within the interval flanked by the discriminating wheat microsatellite markers.

and the translocated chromosome 1AS.1AL-1DL; the genetic map distances, in cM, and the relative positions of microsatellite marker loci are from Roder *et al.* [12 and Roder, personal communication]; the positions of *Glu-A1* and *Glu-D1* are from Van Deynze *et al.* [15].

With the aim of determining the size of the substituted 1A chromosome segment, 6 pairs of microsatellite primers (GWM99, GWM135, GWM164, GWM357, GWM498 and GWM750) from chromosome 1A were used to amplify the genomic DNA of the recombinant durum wheat and the control lines. Four microsatellite primers (GWM357, GWM135, GWM99 and GWM750) only amplified PCR-products in Chinese Spring and Creso, indicating that a 1AL segment, at least 155.7 cM long, was substituted by the 1DL segment in the recombinant durum wheat and triticale. The microsatellite primers GWM164 and GWM498 amplified PCR products in all the lines, indicating that the short arm and the centromere of the 1A chromosome were not involved in the translocation. According to these results, the translocation breakpoint was located just beyond the centromere, within the interval flanked by the discriminating wheat microsatellite loci *Xgwm16A* and *Xgwm357*. It seems, that the 1D segment transferred to wheat included the complete 1D long arm, and therefore that it was a near centromeric translocation.

Double-target genomic *in situ* hybridisation was also performed to determine the translocation breakpoint and the physical size of the introgressed 1D chromosome segment in the recombinant durum wheat. Labelled total genomic DNA from *Ae. squarrosa* was used as a probe in combination with labelled genomic DNA from durum wheat. After *in situ* hybridisation, all the durum wheat chromosomes were easily distinguished (in orange-red colour) from the introgressed 1D fragment (green colour) (Fig. 2a). Observation and measurements of the labelled chromosome (1AL-1DL) indicated that the relative length of the introgressed 1D segment is about 35-40% of the long arm of the recombinant chromosome (Fig. 2a). The 1AL-1DL physical breakpoint lies within the distal region of the long arm, with the 1D segment on the end of the translocated chromosome arm. The *Glu-D*1 gene is, therefore, located towards the end of the recombinant 1AL-1DL chromosome arm. In addition, FISH with a pAs1 probe was used to characterise the 1DL chromosome segment involved in the translocation with the 1AL chromosome. In common wheat, the pAs1 probe mainly hybridises with the D-genome chromosomes, each characterised by several diagnostic landmarks [16]. In particular, the 1D durum wheat chromosome shows one pAs1 site on the distal short arm (1AS) and one interstitial site on the long arm (1DL). In our experiments, the pAs1 hybridisation pattern indicated that the pAs1 interstitial site was included in the introgressed 1DL segment and located proximal to the translocation breakpoint (Fig. 2b).

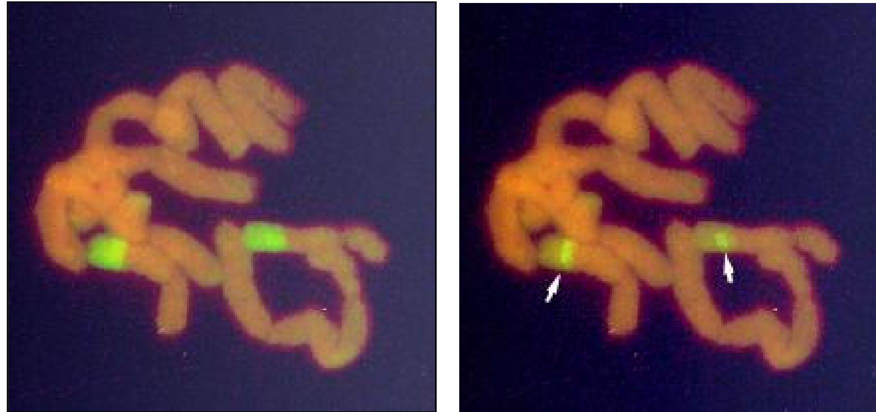


Fig. 2. Incomplete mitotic metaphase of a recombinant durum wheat genotype after *in situ* hybridisation simultaneously using biotin-labelled genomic DNA from durum wheat (orange-red colour) and digoxigenin-labelled DNA from *Ae. squarrosa* (green clour) as probes (a). The length of the introgressed 1D segment (bright green) is about 35-40% of the recombinant 1AL-1DL chromosome arm. Re-probing of the same metaphase with digoxigenin-labelled pAs1 probe (b). Arrows indicate the pAs1 sites.

The genetic and physical data highlight the substantial discrepancy between recombinational and physical map distances. It is well known that distances on genetic maps can differ markedly from those observed on physical maps in both plants and animals. Many markers which genetically map near the centromere or are closely linked to each other, are actually located at a considerable distance from the centromere or from each other [17].
Plants homozygous for the recombinant chromosome had normal plant vigour and seed set only slightly lower than normal disomic plants. The effect on grain quality as measured by the SDS sedimentation value makes the 1AS.1AL-1DL a very interesting chromosome translocation in the efforts to widen the genetic variation of durum wheat. We are using a targeted strategy using the *Ph* pairing manipulation system to generate small intercalary 1D chromosome segments in a durum wheat background starting from the 1AL-1DL translocation arm. We expect to have the *Glu-D1* locus with only small flanking regions to successfully exploit the beneficial effect of the locus for durum wheat improvement.

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