Genetic characterization of a reciprocal translocation present in a widely grown barley variety

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Abstract Artificially induced translocation stocks have been used to physically map the barley genome; however, natural translocations are extremely uncommon in cultivated genotypes. Albacete is a barley variety widely grown in recent decades in Spain and carrying a reciprocal translocation which obviously does not affect its agronomical fitness. This translocation has been characterized by a combination of cytological and molecular genetic approaches. Firstly, recombination frequencies between markers on chromosomes 1H and 3H were estimated to determine the boundaries of the reciprocal interchange. Secondly, 1H-3H wheat barley telosome addition lines were used to assign selected markers to chromosome arms.

Thirdly, fluorescence in situ hybridization (FISH) with rDNA probes (5S and 18S-5.8S-26S) and microsatellite probes [(ACT)₅, (AAG)₅ and (CAG)₅] was used to determine the locations of the translocation breakpoints more precisely. Fourthly, fine-mapping of the regions around the translocation breakpoints was used to increase the marker density for comparative genomics. The results obtained in this study indicate that the translocation is quite large with breakpoints located on the long arms of chromosomes 1H and 3H, between the pericentromeric (AAG)₅ bands and above the (ACT)₅ interstitial distal bands, resulting in the reciprocal translocation 1HS.1HL-3HL and 3HS.3HL-1HL. The gene content around the

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translocation breakpoints could be inferred from syntenic relationships observed among different species from the grass family Poaceae (rice, *Sorghum* and *Brachypodium*) and was estimated at approximately 1,100 and 710 gene models for 1H and 3H, respectively. Duplicated segments between chromosomes Os01 and Os05 in rice derived from ancestral duplications within the grass family overlap with the translocation breakpoints on chromosomes 1H and 3H in the barley variety Albacete.

Keywords Reciprocal translocation · Barley · Translocation breakpoint · Fluorescence in situ hybridization · Comparative genomics

Introduction

Reciprocal translocations, interchanges of chromosome segments between two non-homologous chromosomes, are one of the most common structural chromosomal rearrangements occurring in plant species. Translocations in plants have been widely described by Burnham (1956). Permanent translocation heterozygotes were first observed by Gates (1908) in *Oenothera* species, which received extensive cytological and genetic studies by Cleland (1922) and Belling and Blakeslee (1926). McClintock (1930) was the first to supply cytological evidence of interchanges in economically important crops such as maize. Translocations have been widely utilized in both applied and fundamental scientific research for chromosome mapping, in particular for assigning linkage groups to chromosomes, development of physical maps in plants (Kim et al. 1993; Künzel et al. 2000; Sorokin et al. 1994; Marthe and Künzel 1994), and to improve our understanding of meiotic chromosome pairing behaviour (Rickards 1983). They have been reported in a number of crop species, such as rye (Benito et al. 1994), soybean (Mahama and Palmer 2003), *Prunus* (Jáuregui et al. 2001), *Lens* (Tadmor et al. 1987), pea (Kosterin et al. 1999), wheat (Naranjo et al. 1987) and *Brassica napus* (Osborn et al. 2003).

Chromosomal interchanges, and translocations in general, can be artificially induced in somatic or meiotic cells by ionizing radiation or mutagens. They may also occur spontaneously, although there are few cases of spontaneous reciprocal translocations described in cultivated barley. Konishi and Linde-

Laursen (1988) investigated 1,240 cultivated barley lines and 120 wild barley lines to detect spontaneous reciprocal translocations, which were identified both by semi-sterility associated to test crosses and by subsequent Giemsa banding technique. Of the 1,240 cultivated barley lines, four Ethiopian landraces carrying a reciprocal translocation had the same breakpoints at the centromere involving chromosomes 2H and 4H (2HS·4HS and 2HL·4HL), suggesting that the chromosomes involved in the rearrangement had a common origin. Of the 120 wild barley genotypes, three carried translocations between chromosomes 2H and 4H, 3H and 5H, and 3H and 6H, respectively. Xu and Kasha (1991) identified a chromosomal interchange between chromosomes 3H and 4H using N-banding and in situ hybridization techniques in a wild barley cross. The plant heterozygous for the interchange was derived from the backcross of Su Pie with pollen from a triploid interspecific F1 hybrid of Su Pie × tetraploid *Hordeum bulbosum* accession GBC141.

One of the consequences of a reciprocal translocation is the suppression of genetic recombination in a translocation heterozygote in the interstitial zone (the chromosome segment between the centromere and the breakpoint). The suppression depends on the centromere co-orientation frequency at metaphase I (alternate or adjacent segregations) and upon the chiasmata (crossover) frequency expected in both interstitial segments (Hanson and Kramer 1949; Burnham and Hagberg 1956; Kasha and Burnham 1965; Sybenga 1975). Recombination suppression in the interstitial zone affects the linkage relationships in a translocation heterozygote which results in “pseudo-linkage” between the genes of the two chromosomes involved in the reciprocal translocation and subsequent disturbed linkage maps. Another consequence of reciprocal translocations is the occurrence of gametic sterility, which depends on the frequency of the alternate or adjacent orientation in the quadrivalent. In barley, an excess of alternate over adjacent segregation of the chromosomes at meiotic metaphase I would explain the average 25% sterility caused by the interchange (Kakeda and Miyahara 1995).

Plant accessions carrying chromosome rearrangements such as translocations have been identified in the past by their effects on partial pollen and seed sterility (i.e. Jáuregui et al. 2001). However, depending on the chromosome breakpoints, a translocation can result in the disruption or misregulation of normal

gene functions. Thus, there is special interest in the characterization of the physical locations of the translocation breakpoints on the genome, allowing physical mapping of genes involved in the translocation on the chromosomes. These can be delimited by a combination of cytogenetics and molecular genetics. In barley, chromosome identification can be achieved by using *in situ* hybridization with ribosomal RNA probes (Brown et al. 1999). In addition, with labelled simple sequence repeat (SSR) markers it is now possible to cover the physical map with many landmarks distributed along all chromosome arms (Cuadrado and Jouve 2007). Undoubtedly, this rich set of chromosome markers should help to identify the barley breakpoints more precisely than by using conventional staining techniques.

In the present paper, we aimed to determine the positions of the translocation breakpoints in the Spanish six-row barley variety Albacete. Translocations may have dramatic consequences such as modified phenotypes. In fact, Albacete is the only extensively cultivated barley variety known to carry a reciprocal translocation between chromosomes 1H and 3H without any major reduction in fitness. It is adapted to low-yielding West Mediterranean areas and it has been the most widely grown cultivar in the driest Spanish areas for the last few decades (over a million hectares per year). The translocation was first identified by Cistué (unpublished) on meiotic analysis of semi-sterile F1 hybrids involving this variety.

In tracing the possible origin of the reciprocal translocation in Albacete, we used syntenic relationships between different families of the grass family Poaceae. For the location and characterization of the translocation breakpoints, we used a combination of molecular genetic and cytological techniques. Dense genetic linkage maps of the chromosomes involved in the reciprocal translocation will be used to identify markers in the vicinity of the translocation. Pseudo-linkage arising from suppressed recombination in the interstitial space may result in increased similarities between markers located on the two chromosomes involved in the reciprocal translocation and can be used to fine-map the recombination breakpoints. Further validation and physical characterisation of the translocation breakpoints will be achieved by the use of wheat–barley telosome addition lines and fluorescence *in situ* hybridization with rDNA probes (5S and 18S-5.8S-26S) and microsatellite probes

[(ACT)₅, (AAG)₅ and (CAG)₅] of Albacete and doubled haploid lines derived from crosses between Albacete and cultivars with a standard chromosome arrangement.

Materials and methods

Linkage analysis

Two bi-parental doubled haploid (DH) mapping populations were used. The first mapping population, Albacete × Barberousse (AB), consisted of 231 DH lines derived from anther-culture from the cross between the translocation-carrying six-row winter variety Albacete and the six-row winter variety Barberousse. DNA isolation and genotyping data, SSR and Diversity Arrays Technology (DArT[®]) were performed according to Farré et al. (2011). The second mapping population, (Albacete × Plaisant) × Plaisant (APP), consisted of 94 DH lines derived from the cross of a DH line produced from the Albacete × Plaisant F1, which carried the Albacete reciprocal translocation, backcrossed to Plaisant. DNA was extracted from leaf tissue using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). A set of 3072 expressed sequence tag (EST)-based high-confidence single nucleotide polymorphism (SNP) markers were genotyped using GoldenGate BeadArray technology (Illumina) as previously described (Close et al. 2009).

The allocation of markers to linkage groups as well as their genetic map positions were based on the published barley consensus map (Wenzl et al. 2006) for the AB population and Close et al. (2009) for the APP population. The position of the translocation breakpoints was first inferred according to the methods described by Farré et al. (2011).

Wheat–barley telosome addition lines

The chromosome arm locations of seven SSR markers on the chromosomes of the barley cultivar Betzes were checked on the Chinese Spring–Betzes (CS-B) wheat–barley telosome addition lines for chromosomes 1H and 3H. CS-B telosome addition lines for chromosomes 1H and 3H and chromosome arms 1HS, 3HS and 3HL were used to assign selected markers to chromosome arms. No telosome addition line for chromosome arm 1HL was available. Genomic DNA

from these addition lines were kindly provided by Rafiqul Islam (School of Agriculture, Food and Wine, The University of Adelaide, Australia) and Marion Röder (Leibniz Institute of Plant Genetics and Crop Plant Research, IPK, Germany).

Fluorescence in situ hybridization

Plant material and root tip and chromosome preparation

Root tips were obtained from seedlings of *Hordeum vulgare* cv. Albacete. Seeds were germinated on moist filter paper for 24 h at 25°C and then kept at 4°C for 72 h followed by 25°C for 24 h to synchronize cell division. The seedlings were then transferred to ice-cold water for 24 h to accumulate metaphases before tissue fixation in ethanol–glacial acetic acid (3:1) (Cuadrado and Jouve 2007). Chromosome preparations were prepared as described by Schwarzacher et al. (1989). Briefly, root tips were macerated with an enzymatic mixture and then squashed in a drop of 45% acetic acid. After removing the cover slips by quick freezing, the slides were air-dried.

DNA probes and in situ hybridization

The three oligodeoxyribonucleotide probes [(ACT)₅, (CAG)₅ and (AAG)₅] were provided by Roche labelled at their 5' and 3' ends with digoxigenin or biotin. The novel and rapid non-denaturing FISH (ND-FISH) technique developed by Cuadrado and Jouve (2010) was carried out to detect SSR-enriched chromosome regions.

The other probes, pTa71 (a plasmid containing the 18S-5.8S-25S rDNA and the intergenic spacer of *Triticum aestivum*) and pTa794 (which contains a 410-bp *Bam*HI fragment of 5S rDNA isolated from *T. aestivum*) were labelled by nick translation and PCR, respectively (Leitch and Heslop-Harrison 1992, 1993; Pedersen and Linde-Laursen 1994; Brown et al. 1999). Chromosome and probe denaturation and the in situ hybridization steps were carried out as described in Cuadrado et al. (2000).

Fluorescence microscopy and imaging

Slides were examined with a Zeiss Axiophot epifluorescence microscope. The separate images from each

filter set were captured using a cooled CCD camera (Nikon DS) and processed using Adobe Photoshop, employing only those functions that are applied equally to all pixels in the image.

Results

Similarities between markers on chromosomes 1H and 3H in the AB population

In order to identify DArT and SSR markers in the vicinities of the reciprocal translocation breakpoints, we explored recombination frequencies, between the markers located on chromosomes 1H and 3H. The allocation of markers to linkage groups as well as their genetic map position were based on the published barley consensus map (Wenzl et al. 2006). A total of 82 out of 305 polymorphic markers were mapped on chromosomes 1H and 3H for the AB population. A graphical representation of the recombination frequencies was presented by Farré et al. (2011) which shows that markers on chromosome 1H and 3H were strongly related. This suggests the presence of a reciprocal translocation between these chromosomes. So, for the AB population, the markers with recombination frequencies close to zero were located in the consensus map around 59–64 cM for 1H (HvM20 and EBmac0501) and 66–70 cM for 3H (Bmac209, Bmac067, Bmag0006 and Bmag0136), which corresponds with the pericentromeric regions of both chromosomes 1H and 3H.

1H and 3H wheat–barley telosome addition lines

Wheat–barley chromosome addition lines were then used to assign the chromosome arm location of the SSR markers found to be closely linked to the translocation breakpoints using their presence/absence as detected in hexaploid wheat *T. aestivum* cv. Chinese Spring (CS) and barley *H. vulgare* cv. Betzes using PCR. For chromosome 3H, three out of four SSR markers were found to be located on the short arm and the other one on the long arm (positions reported by Künzel and Waugh 2002). Using 1H wheat–barley addition lines, the two SSR markers used mapped on different chromosome arms. HvM20 amplified in both CS and Betzes and, therefore, did not have any diagnostic value.

Fluorescence in situ hybridization analysis

Further progress in localizing the translocation breakpoints can be expected from direct cytological observations of barley chromosomes by FISH using probes that were used successfully as anchored chromosomal markers. The following locations of pTa794 (5SrDNA) were reported: interstitial on chromosome arms 2HL and 3HL, distal on 4HL and proximal on 7HS (Leitch and Heslop-Harrison 1993). In addition to the two NOR-bearing barley chromosomes, 5HS and 6HS, the locations of four minor rDNA loci with pTa71 (18S-5.8S-26S rDNA) were reported on chromosome arms 1HS, 2HS, 4HS and 7HS, differentiated by their position and intensity (Pedersen and Linde-Laursen 1994). The use of these two ribosomal probes should thus allow easy identification of all barley chromosomes including chromosomes 1H and 3H.

First, two-colour FISH was carried out with pTa71 and pTa794 in pollen mother cells of a hybrid Albacete × Plaisant. The expected chromosome pairing occurred during meiotic I prophase for a heterozygous genotype for the chromosomal arrangement, in which five bivalents and one quadrivalent were observed (Fig. 1a). Bivalents for the two satellite chromosomes, 5H and 6H, with the strongest pTa71 signals, and the bivalents formed by chromosomes labelled with both probes, 2H, 4H and 7H, were identified despite the weak signals observed in Fig. 1. Thus, chromosome 1H, with the stronger pTa71 signal between the non-satellite chromosomes, and 3H are the chromosomes involved in the quadrivalent (Fig. 1b, c).

We subsequently analysed mitotic metaphases of Albacete (Fig. 1d–h). Chromosomes 2H, 4H, 5H, 6H and 7H showed the expected and distinctive FISH pattern of ribosomal probes. In addition, one pair of submetacentric chromosomes with a rather strong pTa71 signal on its short chromosome arm and pTa794 signal on its long chromosome arm, and another pair of metacentric chromosomes without any ribosomal signals, confirmed the presence of a reciprocal translocation resulting in two chromosomal combinations 1HS-3HL and 3HS-1HL, respectively (Fig. 1f). These two chromosomes are shown in Fig. 1i. The translocation breakpoints are therefore located below the pTa71 signal on 1HS and above the pTa794 signal on 3HL.

More information about the exact position of the translocation breakpoints can be obtained by

identifying relocated FISH landmarks on chromosomes 1H and 3H. With this aim, three microsatellite probes, (ACT)₅, (CAG)₅ and (AAG)₅, were chosen for their characteristic patterns on these chromosomes (Cuadrado and Jouve 2007). As expected, (ACT)₅ resulted in a distinct pattern of FISH signals on chromosomes 2H, 3H, 4H, 5H, and 6H. The characteristic signals on the long arm of chromosome 3H were found on the translocated chromosomes 1HS-3HL (Fig. 1g–i). These results suggest that the translocation breakpoints are located below the pTa71 signal on 1HS and above the (ACT)₅ signal on 3HL (Fig. 1e). The (AAG)₅ microsatellite probe facilitates the identification of all barley chromosomes thanks to a rich pattern of signals of different intensities located in the pericentromeric and interstitial chromosomal regions (Fig. 1h). The characteristic patterns obtained in the translocated chromosomes are similar to the patterns found in other barley varieties with normal 1H and 3H chromosomes and suggest that the translocation breakpoints are located below the pericentromeric AAG signals on the long arms of chromosomes 1HL and 3HL (Fig. 1i, j). Results obtained with the (CAG)₅ probe support the detection and identification of the translocations 1HS.1HL-3HL and 3HS.3HL-1HL. The signals obtained in the centromere of translocated chromosomes 3HS-1HL were stronger than on 1HS-3HL (Fig. 1j).

Fine-mapping of the translocation breakpoint region

In order to increase the marker density around the translocation breakpoints, the APP population genotyped with SNP was used. As (AAG)₅ and (ACT)₅ microsatellite probes have proven to be useful for determining the translocation breakpoints on 1H and 3H chromosomes, they were also chosen for detailed cytogenetic characterization of eight out of 94 DH lines from the APP population (Fig. 2). These DH lines were selected according to the Albacete SNP allele distribution along 1H and 3H, aiming to narrow down the position of the translocation breakpoints. The allocation of markers to linkage groups as well as their genetic map position were based on the published barley consensus map (Close et al. 2009). 755 out of 3072 SNP markers were polymorphic for the APP population; of these, 212 markers mapped on chromosomes 1H and 3H. From

Fig. 1 FISH results with rDNA probes (5S and 18S-5.8S-26S) and microsatellite probes [(ACT)₅, (AAG)₅ and (CAG)₅]. **a–c** In situ hybridization of rDNA probes pTa71 (red) and pTa794 (green) in pollen mother cells of hybrid Albacete × Plaisant (arrows indicate the quadrivalent): **a** DAPI staining for DNA; **b** pTa71 and pTa794 probes; **c** combination of DAPI and pTa71 and pTa794 probes. **d–h** Root-tip metaphase chromosomes from barley (*Hordeum vulgare* cv. Albacete) after DAPI staining and in situ hybridization with biotin-labelled probes (detected by red Cy3) or digoxigenin-labelled probes (detected by green FITC):

d DAPI staining for DNA; **e** rDNA probes and (ACT)₅ (arrows indicate the characteristic ACT signals on 3HL and arrowheads the 45S rDNA loci on 1HS); **f** pTa71 and pTa794 (arrows indicate the 5S rDNA loci on 3HL and arrowheads the 45S rDNA loci on 1HS); **g** (ACT)₅ (arrows indicate the characteristic ACT signals on 3HL); **h** (AAG)₅ and (ACT)₅ (arrowheads indicate the characteristic ACT signals on 3HL; lines indicate the pericentromeric AAG signals on chromosomes 1H and 3H); **i, j** translocated chromosomes hybridized with pTa71, pTa794, (AAG)₅, (ACT)₅ and (CAG)₅. Scale bar represents 10 μm

Fig. 2 it can be observed that eight markers around 50–52.5 cM on 1H (11_20427, 11_20660, 12_11209, 12_31208, 11_31381, 11_20912, 11_21312

and 12_30350) and 30 markers around 43.2–55.6 cM on 3H (12_21533, 11_11002, 11_21101, 11_11086, 11_11501, 11_10137, 11_10328, 11_20970, 12_30039,

Fig. 2 Fine-mapping of the translocation breakpoint region using eight selected lines from the APP mapping population. (*top*) Graphical genotypes of the eight selected DH lines. *Blue and yellow colours* indicate Albacete and Plaisant parental alleles, respectively. Genomic regions in the vicinity of the reciprocal translocation breakpoints are indicated with *grey colour*. *Horizontal dashed lines* are drawn every 10 cM with the distance proportional to the number of SNP markers. Line

12_30130, 12_30318, 12_31008, 12_31372, 11_10365, 12_10155, 12_31502, 11_10008, 11_20102, 11_10224, 11_20333, 11_20428, 11_20439, 11_10456, 11_20796, 11_20856, 11_20890, 11_21062, 11_21147, 11_11124 and 11_11337) were close to translocation breakpoints. This set of SNP markers was used to determine the gene content around the translocation breakpoints with rice, *Brachypodium* and *Sorghum* using Strudel, a standalone Java desktop application that allows the simultaneous multi-way comparison of several genomes (<http://bioinf.scri.ac.uk/strudel/>; Bayer et al. 2011). Inferred gene contents for rice, *Brachypodium* and *Sorghum* yielded around 1,100, 600 and 550 for the region on 1H and 710, 410 and 430 gene models for 3H, respectively.

Discussion

The combination of cytogenetics and molecular genetics allowed us to determine the translocation breakpoints on chromosomes 1H and 3H of the barley variety Albacete more accurately than with conventional staining techniques. The success of the

numbers are shown using *green and red colours* according to the presence or not of the reciprocal translocation, respectively (line no. 30200 was used as a positive control and 3.1 24 as a negative). (*bottom*) In situ hybridization of (ACT)₅ (*red or green*) and (AAG)₅ (*red*) in root-tip metaphase chromosomes 1H and 3H after DAPI staining and in situ hybridization with biotin-labelled probes (detected by red Cy3) or digoxigenin-labelled probes (detected by green FITC)

combination of methods depends on the locations of the translocation breakpoints and on the presence of differential signals on the chromosomes involved (Xu and Kasha 1991).

In the present study, the positions of the translocation breakpoints were determined using a number of approaches. First, an analysis involving recombination frequencies between markers was used to determine markers located near the translocation breakpoints with a high degree of precision (Farré et al. 2011). SNP and microsatellite markers putatively flanking the translocation breakpoints were identified in the AB and APP mapping populations in the pericentromeric regions of chromosomes 1H and 3H. Secondly, 1H and 3H wheat–barley telosome addition lines were used to assign a number of markers closely linked to the translocation breakpoints to their proper chromosome arms and to validate the genetic mapping results. For the genomic SSR markers on 3H chromosome, the positions are in good agreement with the positions reported by Künzel and Waugh (2002). They placed 24 microsatellite loci onto the physical restriction fragment length polymorphism map of barley chromosome 3H using the map position of the translocation

breakpoints as reference (Künzel et al. 2000). Our genomic SSR markers on chromosome 3H were identified to be flanking the centromere (positioned at 55 cM) in the Lina $\times$ *Hordeum spontaneum* Canada Park genetic map, where Bmac0067, Bmag0136 and Bmag0006 on the short arm and Bmac0209 on the long arm cover a genetic distance of 5 cM. It is well known that genetic map distances between markers along chromosomes correlate poorly with physical distances, particularly in the large cereal genomes where closely linked markers which genetically map near the centromere represent considerable physical distances (Schwarzacher 2003). As a consequence, most of the chromosomal gene content is trapped in chromosome segments with severely suppressed recombination. According to Künzel and Waugh (2002), the region flanked by our four SSR markers covers 36% of the entire 3H chromosome length on the physical map. For chromosome 1H, no studies were found relating genetic to physical distance using the genomic SSR markers we identified close to the translocation breakpoint. Although, being pericentromeric, we may expect results similar to those reported for 3H, in general, Triticeae centromeres are characterized by suppressed recombination (Schwarzacher 2003; Rostoks et al. 2002). In barley, suppressed recombination rates in proximal segments and high recombination rates in distal regions were demonstrated by Pedersen et al. (1995). Later, Künzel et al. (2000) reported that most recombination was confined in a relatively small chromosomal region, mostly at the arm ends, alternating abruptly with regions of severely suppressed recombination.

Our approach allowed us to identify and genetically delimit the physical regions in which the reciprocal translocation took place. However, as the regions involved in the translocation are located in the vicinity of the centromeres, given the recombination suppression they represent fairly large physical regions on both chromosomes 1H and 3H. FISH was used to validate the positions of the translocation breakpoints on the chromosomes involved in the chromosomal interchanges, and was found to be a useful approach to characterizing non-recombinant regions.

The proposed positions of the breakpoints on the long arms of chromosomes 1H and 3H can be attributed to the combined use of rDNA and micro-satellite probes. Once the translocation between chromosome 1H and 3H was confirmed using the

ribosomal probes (pTa71 and pTa794) and (ACT)₅, the reasons that led us to conclude that the breakpoints are on the long arms of chromosomes 1H and 3H (resulting in the reciprocal translocation 1HS.1HL-3HL and 3HS.3HL-1HL) are multiple. Firstly, the signals obtained using the (AAG)₅ probe (which were similar to banding patterns obtained by C-banding) on the short arms of both chromosomes are the same as those observed in other barley varieties, which indicates that the short arms are complete until the centromere and not involved in the reciprocal interchange (Pedersen and Linde-Laursen 1994; Cuadrado and Jouve 2007). However, a discrepancy in the number of bands on 3HL among different barley varieties was found; some barley lines such as Plaisant, Gaelic and Hispanic carried two bands compared with just one for varieties such as Albacete, Dobra and Golden Promise (Á. Cuadrado, personal communication). The lack of a band near the centromere could be a polymorphism in these varieties (unpublished data). Besides that, it is important to note that the signal intensity of the pericentromeric band present on 3HL is stronger than the one present on 1HL, which is characteristic for all barley varieties. Therefore, these results suggest that the translocation breakpoints are located below this band. Secondly, the differences in intensity found in the centromeres of the translocated chromosomes with the (CAG)₅ probe are consistent with those obtained for Plaisant (a variety with a standard chromosome arrangement). Signals on chromosome 3H were stronger than on 1H (Cuadrado and Jouve 2007), suggesting that the translocation breakpoints are located below the centromere on both chromosomes. Thirdly, the interchanged chromosomes 1HS.1HL-3HL and 3HS.3HL-1HL are more similar in total length and more submetacentric and metacentric, respectively, than published 'normal' barley karyotypes in which centromeres of chromosome 1H, the smallest barley chromosome, and 3H are located at 41 and 44% FL, respectively (arm ratios for 1H and 3H were estimated at 1.4 and 1.3 milliGenomes [mGN], respectively, and chromosome arms are the following: 51 mGN [1HS], 72 mGN [1HL], 64 mGN [3HS] and 83 mGN [3HL]; Taketa et al. 2003). To summarize, the different patterns of these SSR probes have proven to be of great value for localizing and validating the translocation breakpoints on 1H and 3H chromosomes in the Spanish barley variety Albacete. The results obtained in this study suggest

that the translocation breakpoints are located on the long arms of both chromosomes between the (AAG)₅ and (ACT)₅ pericentromeric bands for 3HS.3HL-1HL and below the (AAG)₅ pericentromeric band for 1HS.1HL-3HL, leading to a interstitial zone with suppressed recombination.

For barley and other not-yet-sequenced species, synteny conservation with related Poaceae species sequenced genomes such as rice, *Brachypodium*, maize or *Sorghum* (Mayer et al. 2011) can be explored and exploited for studying genome evolution and identification of candidate genes for traits of interest. An interesting point is thus to use the shared syntenic relationships observed among different species from the grass family Poaceae to determine the gene content around the translocation breakpoints. To explore genome co-linearity of barley with rice, *Brachypodium* and *Sorghum*, we employed Strudel on a reduced set of SNP markers selected using a cytogenetic and molecular characterization of the APP population.

The gene contents, as inferred from rice Os01/Os10 and Os05 chromosomes, around the translocation breakpoints were estimated as approximately 1,100 and 710 for chromosomes 1H and 3H, respectively. As a proportion of the genes represent retroelement-like components and pseudo-genes, the final number of functional genes in barley must be smaller. Inferred gene contents for *Sorghum* and *Brachypodium* yielded similar figures, around 600 (Sb09/Sb01) and 550 (Bradi02/Bradi03) genes for the region on 1H, and 410 (Sb02) and 430 (Bradi03) gene models for 3H. This gene content is in good agreement with the gene content reported by Mayer et al. (2011), who estimated that the barley genome contains in the order of 32,000 genes. With such a large gene content the use of a candidate gene approach aimed at identifying which gene(s) may have been altered by the translocation conferring a higher drought tolerance to Albacete is not practical.

Whole-genome duplication analyses have been documented in grass species. Thiel et al. (2009) identified shared duplicated segments that originate from the last common ancestor and that remain in the barley genome. A large degree of synteny existed between barley chromosome Hv1H and rice chromosome Os05 as well as between Hv3H and Os01. The barley chromosome Hv3H corresponded to the ancestral chromosome type of rice chromosome Os01. The structure of chromosome 1H could be explained by

combining the ancestral type of two rice chromosomes, where the first (A10) appears to be nested in the second (A05). An interesting observation arising from the comparative mapping of several Poaceae species is the presence of duplicated segments between chromosomes Os01 and Os05 in rice derived from ancestral duplications within the grass family (Guyot and Keller 2004) which are overlapping with the translocation breakpoints on chromosomes 1H and 3H in Albacete. Moreover, there are also large grass ancestral genome duplications affecting rice chromosomes Os03 and Os07 homologous to barley chromosomes 2H and 4H. Smaller ancestral duplications are present in regions of the rice genome homologous to barley chromosomes 3H-5H and 3H-6H, which are also involved in spontaneous reciprocal translocations in barley (Konishi and Linde-Laursen 1988).

In conclusion, the widely grown Spanish barley variety Albacete carries a large pericentric chromosome rearrangement between chromosomes 1H and 3H without any major change in fitness. SNP and microsatellite markers located in the proximity of the reciprocal translocation breakpoints were identified in both AB and APP mapping populations within the pericentromeric regions of both chromosomes. The physical characterization suggested that the translocation breakpoints are located on the long arms of both chromosomes between the (AAG)₅ and (ACT)₅ pericentromeric bands for 3HS.3HL-1HL and below the (AAG)₅ pericentromeric band for 1HS.1HL-3HL. The gene content in this region was estimated at approximately 750 and 2,100 gene models for 1H and 3H respectively. The presence of duplicated segments between chromosomes Os01 and Os05 in rice derived from ancestral duplications within the grass family (Guyot and Keller 2004) overlaps with the translocation breakpoints on 1H and 3H chromosomes in the barley variety Albacete. However, no current evidence is available as to whether similar gene contents on different chromosomes arising from ancestral duplications play a role in spontaneous chromosomal exchanges through non-homologous chromosome pairing.

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References

- Bayer M, Milne I, Stephen G, Shaw P, Cardle L, Wright F, Marshal D (2011) Comparative visualization of genetic and physical maps with Strudel. *Bioinformatics* 27(9): 1307–1308
- Bellin J, Blakeslee AF (1926) On the attachment of non-homologous chromosomes at the resolution division in certain 25-chromosome *Daturas*. *Proc Natl Acad Sci USA* 12:7–11
- Benito C, Llorente F, Henriques-Gil N, Gallego FJ, Zaragoza C, Delibes A, Figueiras AM (1994) A map of rye chromosome 4R with cytological and isozyme markers. *Theor Appl Genet* 87:941–946
- Brown SE, Stephens JL, Lapitan NL, Knudson DL (1999) FISH landmarks for barley chromosomes (*Hordeum vulgare* L.). *Genome* 42:274–281
- Burnham CR (1956) Chromosomal interchanges in plants. *Bot Rev* 22:419–552
- Burnham CR, Hagberg A (1956) Cytogenetic notes on chromosomal interchanges in barley. *Hereditas* 42:467–482
- Cleland RE (1922) The reduction division in the pollen mother cells of *Oenothera fransciscana*. *Am J Bot* 9:391–413
- Close TJ, Bhat PR, Lonardi S, Wu Y, Rostoks N, Ramsay L, Druka A, Stein N, Svensson JT, Wanamaker S, Bozdag S, Mikeal L, Roose ML, Moscou MJ, Chao S, Rajeev K, Varshney RK, Szűcs P, Sato K, Hayes PM, Matthews DE, Kleinhofs A, Muehlbauer GJ, DeYoung J, Marshall DF, Madishetty K, Fenton RD, Condamine P, Graner A, Waugh R (2009) Development and implementation of high-throughput SNP genotyping in barley. *BMC Genomics* 10:582
- Cuadrado A, Jouve N (2007) The nonrandom distribution of long clusters of all possible classes of trinucleotide repeats in barley chromosomes. *Chromosome Res* 15:711–720
- Cuadrado A, Jouve N (2010) Chromosomal detection of simple sequence repeats (SSRs) using nonamplifying FISH (ND-FISH). *Chromosoma* 119:495–503
- Cuadrado A, Schwarzacher T, Jouve N (2000) Identification of different chromatin classes in wheat using in situ hybridization with the simple sequence repeat oligonucleotides. *Theor Appl Genet* 101:711–717
- Farré A, Lacasa Benito I, Cistué L, de Jong JH, Romagosa I, Jansen J (2011) Linkage map construction involving a reciprocal translocation. *Theor Appl Genet* 122:1029–1037
- Gates RR (1908) A study of reduction in *Oenothera rubrinervis*. *Bot Gaz* 46:1–34
- Guyot R, Keller B (2004) Ancestral genome duplication in rice. *Genome* 47:610–614
- Hanson D, Kramer H (1949) The genetic analysis of two chromosome interchanges in barley from F2 data. *Genetics* 34:687–700
- Jáuregui B, Vicente MC, Messeguer R, Felipe A, Bonnet A, Salesses G, Arús P (2001) A reciprocal translocation between ‘Garfi’ almond and ‘Nemared’ peach. *Theor Appl Genet* 102:1169–1176
- Kakeda K, Miyahara S (1995) Cytogenetical analyses of reciprocal translocations in barley. *Bull Fac Bioresour* 14:1–24
- Kasha K, Burnham CR (1965) The location of interchange breakpoints in barley I. Linkage studies and map orientation. *Can J Genet Cytol* 7:62–77
- Kim NS, Armstrong K, Knott DR (1993) Molecular detection of Lophopyrum chromatin in wheat-Lophopyrum recombinants and their use in the physical mapping of chromosome 7D. *Theor Appl Genet* 85:561–567
- Konishi T, Linde-Laursen I (1988) Spontaneous chromosomal rearrangements in cultivated and wild barleys. *Theor Appl Genet* 75:237–243
- Kosterin OE, Pukhnacheva NV, Gorel FL, Berdnikov VA (1999) Location of the breakpoints of four reciprocal translocations involving linkage group V and their influence on recombination distances between neighboring markers. *Pisum Genet* 31:13–20
- Künzel G, Waugh R (2002) Integration of microsatellite markers into the translocation-based physical RFLP map of barley chromosome 3H. *Theor Appl Genet* 105:660–665
- Künzel G, Korzun L, Meister A (2000) Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. *Genetics* 154:397–412
- Leitch IJ, Heslop-Harrison JS (1992) Physical mapping of the 18S–5.8S–26S rDNA genes in barley by in situ hybridization. *Genome* 35:1013–1018
- Leitch IJ, Heslop-Harrison JS (1993) Physical mapping of four sites of 5S rDNA sequences and one site of the alpha-amylase-2-gene in barley (*Hordeum vulgare*). *Genome* 36:517–523
- Mahama AA, Palmer RG (2003) Translocation in soybean classical genetic linkage groups 6 and 8. *Crop Sci* 43:1602–1609
- Marthe F, Künzel G (1994) Localization of translocation breakpoints in somatic metaphase chromosomes of barley. *Theor Appl Genet* 89:240–248
- Mayer KFX, Martis M, Hedley PE, Simkova H, Liu H, Morris JA, Steuernagel B, Taudien S, Roessner S, Gundlach H, Kubalaková M, Suchánková P, Murat F, Felder M, Nussbaumer T, Graner A, Slase J, Endo T, Sakai H, Tanaka T, Itoh T, Sato K, Platzer M, Matsumoto T, Scholz U, Dolezel J, Waugh R, Stein N (2011) Unlocking the barley genome chromosomal and comparative genomics. *Plant Cell* 23: 1249–1263
- McClintock B (1930) A cytological demonstration of the location of an interchange between two non-homologous chromosomes of *Zea mays*. *Proc Natl Acad Sci USA* 16:791–796
- Naranjo T, Roca P, Goicoechea PG, Giraldez R (1987) Arm homoeology of wheat and rye chromosomes. *Genome* 29:873–882
- Osborn TC, Buttrill DV, Sharpe AG, Pickering KJ, Parking IAP, Parker JS, Lydiat D (2003) Detection and effects of a homeologous reciprocal translocation in *Brassica napus*. *Genetics* 165:1569–1577
- Pedersen C, Linde-Laursen I (1994) Chromosomal locations of four minor rDNA loci and a marker microsatellite sequence in barley. *Chromosome Res* 2:654–671

- Pedersen C, Giese H, Linde-Laursen I (1995) Towards an integration of the physical and the genetic chromosome map of barley by in situ hybridization. *Hereditas* 123: 77–88
- Rickards GK (1983) Orientation behaviour of chromosome multiples of interchange (reciprocal translocation) heterozygotes. *Annu Rev Genet* 17:443–498
- Rostoks N, Park YJ, Ramakrishna W, Ma J, Druka A, Shiloff BA, SanMiguel PJ, Jiang Z, Brueggeman R, Sandhu D, Gill K, Bennetzen JL, Kleinjohs A (2002) Genomic sequencing reveals gene content, genomic organization, and recombination relationships in barley. *Funct Integr Genomics* 2:51–59
- Schwarzacher T (2003) Meiosis, recombination and chromosomes: a review of gene isolation and fluorescent in situ hybridization data in plants. *J Exp Bot* 54(380):11–23
- Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS (1989) In situ hybridization localization of parental genomes in a wide hybrid. *Ann Bot* 64:315–324
- Sorokin A, Marthe F, Houben A, Pich U, Graner A, Künzel G (1994) Polymerase chain reaction mediated localization of RFLP clones to micro isolated translocation chromosomes of barley. *Genome* 37:550–555
- Sybenga J (1975) Meiotic configurations. Monographs on theoretical and applied genetics. Springer, Berlin
- Tadmor Y, Zamir D, Ladizinsky G (1987) Genetic mapping of an ancient translocation in the genus *Lens*. *Theor Appl Genet* 73:883–892
- Taketa SI, Linde-Laursen I, Künzel G (2003) Cytogenetic diversity. In: von Bothmer R, van Hintum TH, Kniipffer H, Sato K (eds) *Diversity in barley (Hordeum vulgare)*. Elsevier Science BV, The Netherlands, pp 97–119
- Thiel T, Graner A, Waugh R, Grosse I, Close TJ, Stein N (2009) Evidence and evolutionary analysis of ancient whole-genome duplication in barley predating the divergence from rice. *BMC Evol Biol* 9:209
- Wenzl P, Li H, Carling J, Zhou M, Raman H, Paul E, Hearnden P, Maier C, Xia L, Caig V, Ovesná J, Cakir M, Poulsen D, Wang J, Raman R, Smith KP, Muehlbauer GJ, Chalmers KJ, Kleinjohs A, Huttner E, Kilian A (2006) A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. *BMC Genomics* 7:206
- Xu J, Kasha KJ (1991) Identification of a barley chromosomal interchange using N-banding and in situ hybridization techniques. *Genome* 35:392–397