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

A. Farré[1,2]* · A. Cuadrado[3] · I. Lacasa-Benito[1] · L. Cistué[4] · I. Schubert[5] · J. Comadran[6] · J. Jansen[2] · I. Romagosa[1,7]

[1] Department of Plant Production and Forest Science, University of Lleida, Lleida, Spain

[2] Biometris, Wageningen University and Research Centre, Wageningen, the Netherlands

[3] Department of Cell Biology and Genetics, University of Alcalá de Henares, Alcalá de Henares, Spain

[4] Estación Experimental de Aula Dei, CSIC, Zaragoza, Spain

[5] Department of Cytogenetics, Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

[6] Genetics Programme, Scottish Crop Research Institute (SCRI), Dundee, Scotland

[7] Centre UdL-IRTA, University of Lleida, Lleida, Spain

*Author for correspondence

Email: Alba Farré – alba.farre@pvcf.udl.cat; Ángeles Cuadrado - angeles.cuadrado@uah.es; Isabel Lacasa-Benito - isa.lac.ben@gmail.com; Luís Cistué - lcistue@eead.csic.es; Ingo Schubert- schubert@ipk-gatersleben.de; Jordi Comadran - comadran.jordi@scri.ac.uk; Johannes Jansen - johannes.jansen@wur.nl; Ignacio Romagosa - iromagosa@pvcf.udl.cat

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 the last decades in Spain and carrying a reciprocal translocation which obviously does not affect its agronomical fitness. This has been characterized by a combination of cytological and molecular genetic approaches. First, similarities between markers on chromosomes 1H and 3H, involved in the translocation, 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 translocation breakpoints could be inferred from syntenic relationships observed among different species from the grass family Poaceae (rice, *Sorghum* and *Brachypodium*) which was estimated at approximately 2,100 and 750 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 generous cytological and genetic study 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 (Ahloowalia 1962; Alonso-Blanco *et al.* 1993; Benito et al. 1994; Catarino et al. 2006), soybean (Mahama et al. 2003), *Prunus* (Jáuregui et al. 2001), *Lens* (Tadmor et al. 1987), pea (Kosterin et al. 1999), wheat (Naranjo et al. 1987) and *Brassica napus* in which a significant higher seed yield effect was showed for the progeny segregating for a reciprocal translocation between N7 and N16 (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 by both semi-sterility associated to test crosses and 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 their rearrangement chromosomes 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 F_1 hibrid 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 suppresion depends on the centromere coorientation 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 suppresion 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 occurence of gametic sterility of 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 averaged about 25% of the sterility for the interchange (Kakeda and Miyahara 1995).

Plant accessions carrying chromosome rearrangements such as translocations have been identified in the past by its 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 function. Thus, special interest resides on the characterization of the physical location of the translocation breakpoints in the genome which can be physically delimited by the combination of cytogenetics with molecular genetics allowing the location of breakpoints for physical mapping of genes on chromosomes. In barley, chromosome identification can be achieved by using *in situ* hybridization with ribosomal RNA probes (Brown et al. 1999). In addition, with labelled SSRs it is now posible 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 barley breakpoints more precisely than conventional staining techniques.

In the present paper, we aim at determining the positions of the translocation breakpoints in the Spanish six-row barley variety 'Albacete' in order to know how large the segments are that are involved in the interchange between the two chromosomes. 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 decades (over a million ha per

124 year). The translocation was first identified by Cistué (personal communication) upon
125 meiotic analysis of semi-sterile F_1 hybrids involving this variety.

126 In tracing the possible origin of the reciprocal translocation in 'Albacete' we use
127 syntenic relationships between different families of the grass family Poaceae. For the
128 location and characterization of the translocation breakpoints we used a combination of
129 molecular genetical and cytological techniques. Dense genetic linkage maps of the
130 chromosomes involved in the reciprocal translocation will be used to identify markers
131 in the vicinity of the translocation. Pseudo-linkage arising from suppressed
132 recombination in the interstitial space may result in increased similarities between
133 markers located on the two chromosomes involved in the reciprocal translocation and
134 can be used to fine-map the recombination breakpoints. Further validation and physical
135 characterisation of the translocation breakpoints will be achieved by wheat-barley
136 telosome addition lines and fluorescence *in situ* hybridization with rDNA probes (5S
137 and 18S-5.8S-26S) and microsatellite probes ($(ACT)_5$, $(AAG)_5$ and $(CAG)_5$) of
138 'Albacete' and doubled haploid lines derived from crosses between 'Albacete' and
139 cultivars with a standard chromosome arrangement.

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Materials and methods

Linkage analysis

Two bi-parental doubled haploid (DH) mapping populations were used. The first mapping population, 'Albacete' × 'Barberousse' (AB), consists of 231 DH lines derived from anther-culture from the cross between the translocation-carrier six-rowed winter variety 'Albacete' and six-row winter variety 'Barberousse'. DNA isolation and genotyping data, SSR and DArT®, were performed according to Farré et al. (2011). The second mapping population, ('Albacete' × 'Plaisant') × 'Plaisant' (APP), consists of 94 DH lines derived from the cross of a DH genotype produced from the 'Albacete' × 'Plaisant' $F_{1,}$ which carried the 'Albacete' reciprocal translocation, backcrossed to 'Plaisant'. DNA was extracted from leaf tissue using kit DNeasy Plant Mini Kit (Quiagen, Valencia, CA, USA). A set of 3072 EST-based high confidence 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 position 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 by estimating the similarities between markers on chromosomes 1H and 3H on the AB DH populationThe similarities were measured by the simple matching coefficient, which for a doubled haploid population is equal to one minus the recombination frequency. The graphical representation of similarities between markers was drawn with the Pajek programme (Batagelj and Mrvar 1998).

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167 **Wheat-barley telosome addition lines**

168 The chromosome arm locations of seven SSR markers on the chromosomes of the
169 barley cultivar 'Betzes' were checked on the 'Chinese Spring'-'Betzes' (CS-B) wheat-
170 barley telosome addition lines for chromosomes 1H and 3H. CS-B telosome addition
171 lines for the chromosome 1H and 3H and chromosome arms 1HS, 3HS and 3HL were
172 used to determine the exact position of the centromere and to assign selected markers to
173 chromosome arms. No telosome addition line for chromosome arm 1HL was available.
174 Genomic DNA of plant material from these addition lines were kindly provided by
175 Rafiqul Islam (School of Agriculture, Food and Wine. The University of Adelaide,
176 Australia) and Marion Röder (Leibniz Institute of Plant Genetics and Crop Plant
177 Research. IPK, Germany).

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179 **Fluorescence *in situ* Hybridization**

180 *Plant material and root tip and chromosome preparation*

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

189

190 *DNA probes and in situ hybridization*

191 The three oligodeoxyribonucleotide probes ($(ACT)_5$, $(CAG)_5$ and $(AAG)_5$) were
192 provided by Roche labelled at their 5' and 3' with digoxigenin or biotin. The novel and
193 rapid non-denaturating FISH (ND-FISH) technique developed by Cuadrado and Jouve
194 (2010) was carried out to detect SSR-enriched chromosome regions.

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

202

203 *Fluorescence microscopy and imaging*

204 Slides were examined with a Zeiss Axiophot epifluorescence microscope. The separate
205 images from each filter set were captured using a cooled CCD camera (Nikon DS) and
206 processed using Adobe Photoshop, employing only those functions that are applied
207 equally to all pixels in the image.

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Results

Similarities between markers on chromosomes 1H and 3H

In order to identify DArT and SSR markers in the vicinities of the reciprocal translocation breakpoints, we explored similarities, *i.e.* simple matching coefficients, 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). Markers with identical segregation patterns were binned and of each bin only one marker was selected to obtain a set of markers well distributed along the chromosomes. A graphical representation of the simple matching coefficients was obtained for a total of 52 markers out of 82 for AB. Figure 1 shows that markers on chromosome 1H and 3H were strongly related. This suggests the presence of a reciprocal translocation between these chromosomes. In Figure 1, the thicker the connecting lines, the higher the simple matching coefficient. So, for the AB population, the markers with the highest simple matching coefficient (>0.90) were located in the consensus map around 59-64 cM and 66-70 cM for 1H (HvM20 and EBmac0501) and 3H (Bmac209, Bmac067, Bmag006 and Bmag0136), respectively, which corresponds with the pericentromeric regions of both chromosomes 1H and 3H. Weaker, yet significant, similarities extended for 34 cM on 1H and 30 cM on 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 *Triticum aestivum* cv. 'Chinese Spring' (CS) and barley *Hordeum vulgare* cv. 'Betzes' using PCR (Table 1). For chromosome 3H, 3 out of 4 SSR markers were found to be located on the short arm

237 and the other one on the long arm. Using 1H wheat-barley addition lines the two SSR
238 markers used mapped on different chromosome arms. HvM20 amplified in both CS and
239 Betzes and, therefore, did not have any diagnostic value.
240
241 **Fluorescence *in situ* hybridization analysis**
242 Further progress in localizing the translocation breakpoints can be expected from direct
243 cytological observations of barley chromosomes by FISH using probes that were used
244 successfully as anchored chromosomal markers. The following locations of pTa794
245 (5SrDNA) were reported: interstitial on chromosome arms 2HL and 3HL, distal on 4HL
246 and proximal on 7HS (Leitch and Heslop-Harrison 1993). In addition to the two NOR-
247 bearing barley chromosomes, 5HS and 6HS, the locations of four minor rDNA loci with
248 pTa71 (18S-5.8S-26S rDNA) were reported on chromosome arms 1HS, 2HS, 4HS and
249 7HS differentiated by their position and intensity (Pedersen and Linde-Laursen 1994).
250 Thus the use of these two ribosomal probes should allow easy identification of all
251 barley chromosomes including chromosomes 1H and 3H.
252 First, two-colour FISH was carried out with pTa71 and pTa794 in pollen mother cells of
253 a hybrid between 'Albacete'×'Plaisant'. The expected chromosome pairing occurred
254 during meiotic I prophase for a heterozygous genotype for the chromosomic
255 arrangement, in which five bivalents and one quadrivalent were observed (Fig. 2a).
256 Bivalents for the two satellited chromosomes, 5H and 6H, with the strongest pTa71
257 signals, and the bivalents formed by chromosomes labelled with both probes, 2H, 4H
258 and 7H, were identified despite the weak signals observed in the Figure 2. Thus,
259 chromosome 1H, with the stronger pTa71 signal between the non-satellited
260 chromosomes, and 3H are the chromosomes involved in the quadrivalent (Fig. 2b,c).

We subsequently analysed mitotic metaphases of 'Albacete' (Fig. 2 d-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. 2f). These two chromosomes are shown in Fig. 2i. Therefore, the translocation breakpoints are 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. To this aim three microsatellites 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 2g and 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. 2e). 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. 2h). 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. 2i-j). Results obtained with the $(CAG)_5$ probe suport 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 2j).

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 proved to be useful to determine the translocation breakpoints on 1H and 3H chromosomes, they were also chosen for detailed cytogenetic characterization of 8 out of 94 DH lines from the APP population (Figure 3). 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). From Figure 3 it can be observed that 8 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, 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) close to translocation breakpoints.

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 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 have been determined using a number of approaches. First, an analysis involving similarities between markers (simple matching coefficients) was used to determine markers located near the translocation breakpoints with a high degree of precision. This approach is equivalent to using recombination frequencies in linkage analysis (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 RFLP map of barley chromosome 3H using the map position of the translocation breakpoints as reference (Künzel et al. 2000). Our genomic SSR markers on 3H chromosome were identified flanking the centromere (positioned at 55 cM) in the 'Lina'×*Hordeum spontaneum* Canada Park genetic map, where Bmac0067, Bmag0136 and Bmag006 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 002 the region flanked by our four SSR 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 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 characterised 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 region involved in the translocation located in the vicinity of the centromeres, given recombination suppression, represents a fairly large physical region n both 1H and 3H chromosomes. FISH was used in an attempt to validate the positions of the translocation breakpoints on the chromosomes involved in the chromosomal interchanges, which was found to be a useful approach to characterize 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 microsatellite 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 concluding 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 as 'Albacete', 'Dobla' and 'Golden Promise'. The lacking of a band near the centromere could be a polymorphism in these varieties (unpublished data). Besides that, it is important to notice 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 an 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, compared with 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

382 (mGN), respectively, and chromosome arms are the following: 51 mGN (1HS), 72
383 mGN (1HL), 64 mGN (3HS) and 83 mGN (3HL); Taketa et al. 2003). To summarize,
384 the different patterns of these SSR probes have proven to be of a great value for
385 localizing and validating the translocation breakpoints on 1H and 3H chromosomes in
386 the Spanish barley variety 'Albacete'. The results obtained in this study suggest that the
387 translocation breakpoints are located on the long arms of both chromosomes between
388 the $(AAG)_5$ and $(ACT)_5$ pericentromeric bands for 3HS.3HL-1HL and below the
389 $(AAG)_5$ pericentromeric band for 1HS.1HL-3HL existing a interstitial zone with
390 suppressed recombination.
391 For barley and other not yet sequenced species synteny conservation with related
392 Poaceae species sequenced genomes such as rice, *Brachypodium*, maize or *Sorghum*
393 (Moore et al. 1995; Sandhu 2002) can be explored and exploited for studying genome
394 evolution and identification of candidate genes for traits of interest. So, an interesting
395 point is to use the shared syntenic relationships observed among different species from
396 the grass family Poaceae to determine the gene content around the translocation
397 breakpoints. We used a reduced set of SNP markers selected from the based on a
398 combination of a cytogenetical and molecular characterization of the APP population
399 lines to explore genome co-linearity of barley with rice, *Brachypodium* and *Sorghum*
400 Mayer et al. (2009) mentioned that the gene contents of 1H and 3H are estimated to be
401 approximately 5,400 and 6,500 genes, respectively, out of an overall 45,000 genes for
402 the entire barley genome. Their results also indicated that approximately 20% of all
403 genes on barley chromosome 1H are located in centromeric and subcentromeric regions
404 with very low recombination frequencies. Similar gene density can be expected for
405 barley chromosome 3H; Smilde et al. (2001) reported a 30-fold reduction of
406 recombination around the barley 3H centromere. The gene contents, as inferred from

407 rice Os01 and Os05 chromosomes, around the translocation breakpoints were estimated
408 as approximately 750 and 2,100 for 1H and 3H, respectively. As a proportion of the
409 genes represent retroelement-like components and pseudo-genes, the final number of
410 functional genes in barley must be smaller. Inferred gene contents for *Sorghum* and
411 *Brachypodium* yielded similar figures, around 1,250 and 1,200 genes for the region on
412 1H and 670 and 600 gene models for 3H, respectively. With such large gene content the
413 use of a candidate gene approach aiming at identifying which gene(s) may have been
414 altered by the translocation and that may confer higher drought tolerance to 'Albacete'
415 is not practical. An interesting observation arising from the comparative mapping of
416 several Poaceae species is the presence of duplicated segments between chromosomes
417 Os01 and Os05 in rice derived from ancestral duplications within the grass family
418 (Guyot and Keller 2004) which are overlapping with the translocation breakpoints on
419 chromosomes 1H and 3H in 'Albacete'. Moreover, there are also large grass ancestral
420 genome duplications affecting rice chromosomes Os03 and Os07 homologous to barley
421 chromosomes 2H and 4H. Smaller ancestral duplications are present in regions of the
422 rice genome homologous to barley chromosomes 3H-5H and 3H-6H, which are also
423 involved in spontaneous reciprocal translocations in barley (Konishi and Linde-Laursen
424 1988).
425 In conclusion, the widely grown Spanish barley variety 'Albacete' carries a large
426 pericentric chromosome rearrangement between chromosomes 1H and 3H without any
427 major change in fitness. SNP and microsatellite markers located in the proximity of the
428 reciprocal translocation breakpoints were identified in both AB and APP mapping
429 populations within the pericentromeric regions of both chromosomes. The physical
430 characterization suggested that the translocation breakpoints are located on the long
431 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. 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 evidences of whether similar gene contents on different chromosomes arising from ancestral duplications may play a role in spontaneous chromosomal exchanges through non-homologous chromosome pairing have been published.

Acknowledgments

This study has been supported by the Spanish Ministry of Science and Innovation (Project AGL2008-05541-C02 and the Agrogenomics CONSOLIDER).

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630 **Figure and table legend**

631

632 **Figure 1. Graphical representation of the simple matching coefficients using 52**
633 **markers for AB population.** The thickness of the lines connecting markers is
634 proportional to the magnitude of the corresponding simple matching coefficient; only
635 simple matching coefficients above 0.7 were indicated with a line. The centromere
636 position is indicated by a white circle. The genetic distances are expressed in map
637 distances (cM) according to the barley consensus map.

638

639 **Figure 2**. **FISH results with rDNA probes (5S and 18S-5.8S-26S) and microsatellite**
640 **probes ($(ACT)_5$, $(AAG)_5$ and $(CAG)_5$).** (a-c) *In situ* hybridization of rDNA probes
641 pTa71 (red) and pTa794 (green) in pollen mother cells of hybrid between 'Albacete' ×
642 'Plaisant' (Arrows indicate the quadrivalent). (d-h) Root-tip metaphase chromosomes

643 from barley (Hordeum vulgare cv. 'Albacete') after DAPI staining and *in situ*
644 hybridization with biotin-labelled probes (detected by red Cy3) or digoxigenin-labelled
645 probes (detected by green FITC): (d) DAPI staining for DNA; (e) rDNA probes and
646 $(ACT)_5$ (arrows indicate the characteristic ACT signals on 3HL and arrowheads the 45S
647 rDNA loci on 1HS); (f) pTa71 and pTa794 (arrows indicate the 5S rDNA loci on 3HL
648 and arrowheads the 45S rDNA loci on 1HS); (g) $(ACT)_5$ (arrows indicate the
649 characteristic ACT signals on 3HL); (h) $(AAG)_5$ and $(ACT)_5$ (arrowsheads indicate the
650 characteristic ACT signals on 3HL. Lines indicated the pericentromeric AAG signals on
651 chromosomes 1H and 3H); (i-j) translocated chromosomes hybridized with pTa71,
652 pTa794, $(AAG)_5$, $(ACT)_5$ and $(CAG)_5$. Scale bar represent 10 μm.
653
654 **Figure 3**. **Fine-mapping of the translocation breakpoint region using eight selected**
655 **lines from the APP mapping population**. Schematic representation of the eight
656 selected DH lines (top). Blue and yellow colours indicate 'Albacete' and 'Plaisant'
657 parental alleles respectively. Genomic region in the vicinity of the reciprocal
658 translocation breakpoints are indicated with grey colour. Horizontal dashed lines are
659 drawn every 10 cM with the distance proportional to the number of SNPs markers. Line
660 number are shown using green and red colours according to the presence or not of the
661 reciprocal translocation, respectively (line nº*30200* was used as a positive control
662 whereas *3.1 24* as a negative). On the bottom, *In situ* hybridization of $(ACT)_5$ (red or
663 green) and $(AAG)_5$ (red) in root-tip metaphase chromosomes 1H and 3H after DAPI
664 staining and *in situ* hybridization with biotin-labelled probes (detected by red Cy3) or
665 digoxigenin-labelled probes (detected by green FITC).
666
667 **Table 1. Presence (+) or absence (-) of 7 SSR markers using CS-B addition lines.**

Fig. 1

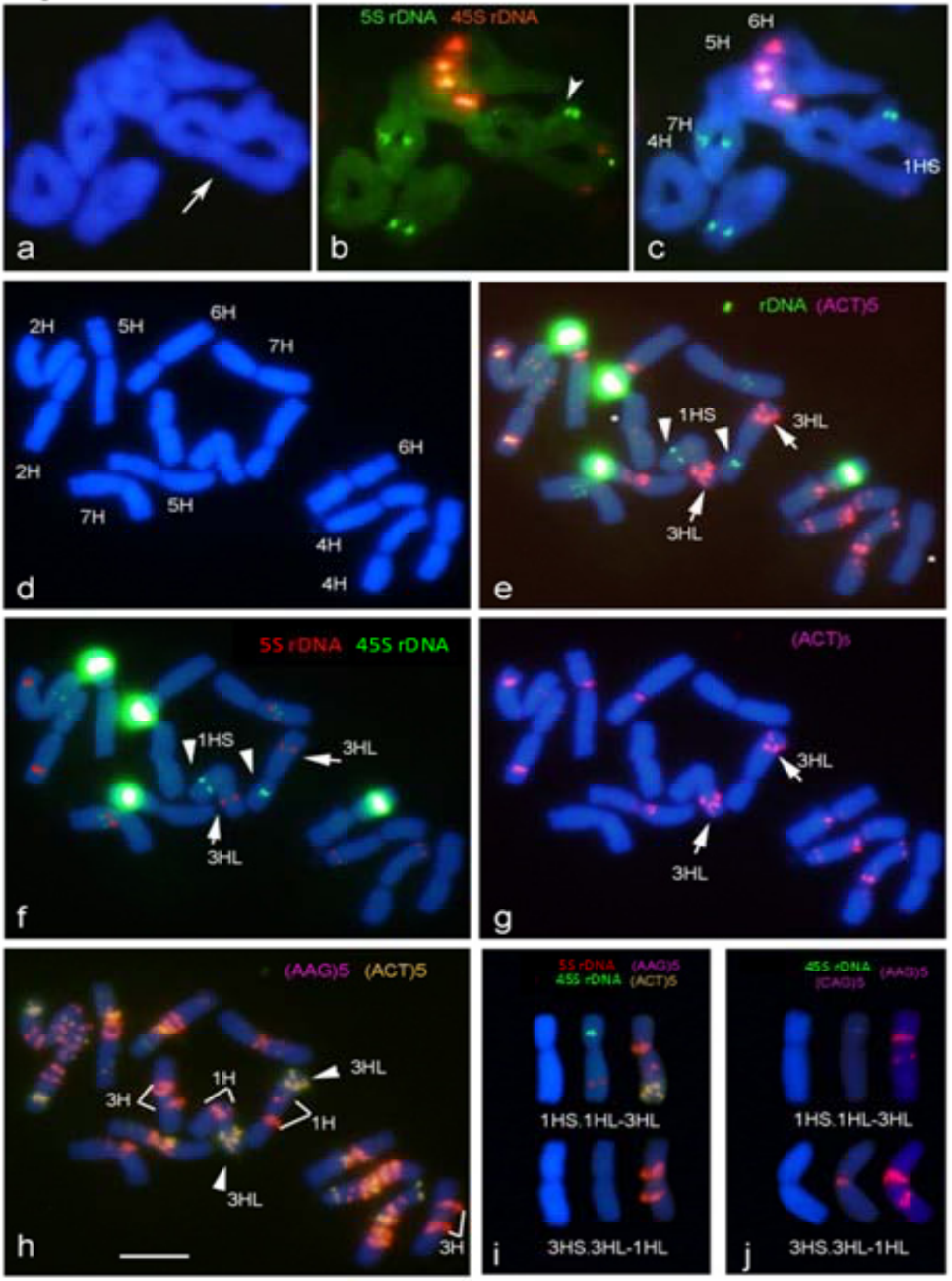
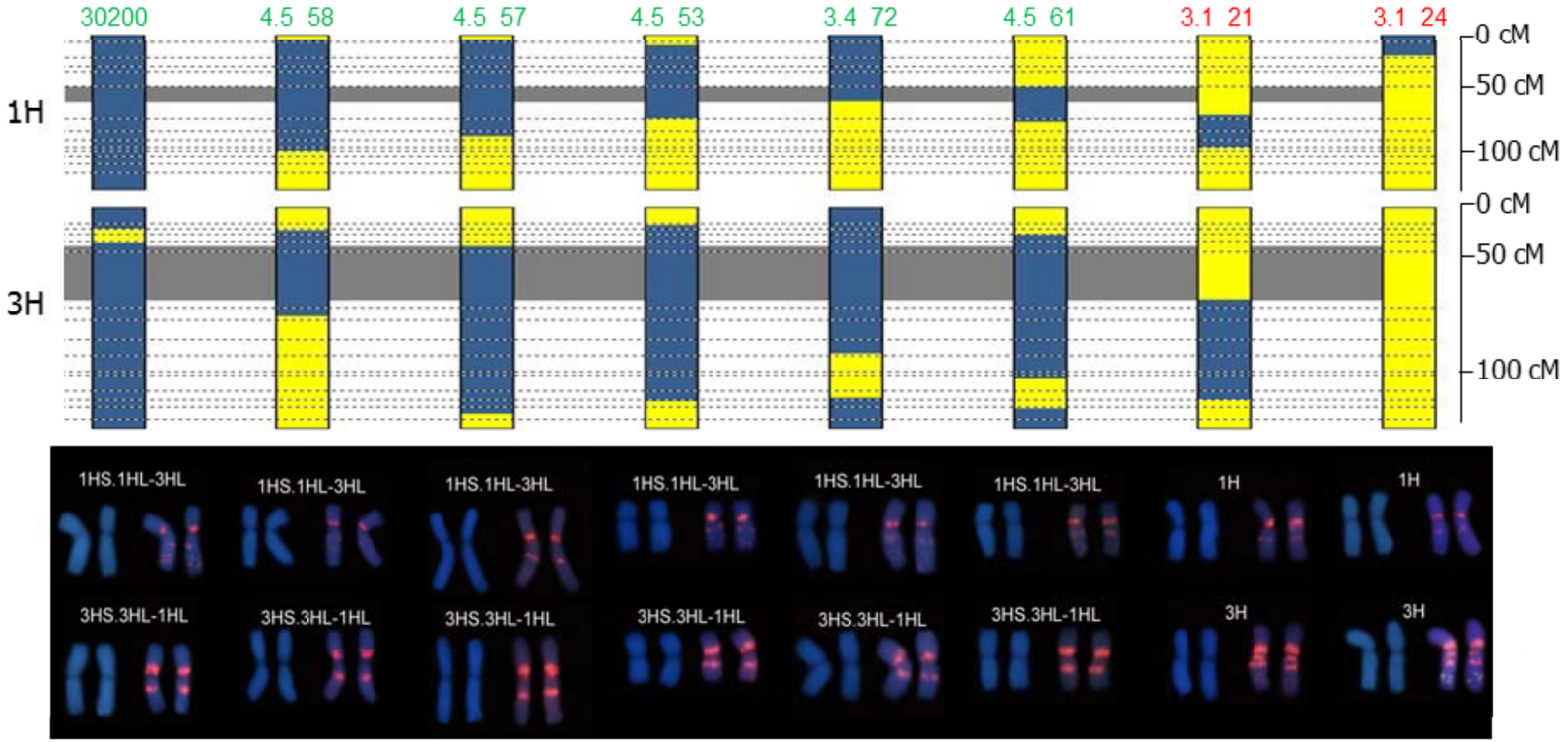

a
5S rDNA 45S rDNA
b
6H
5H
7H
4H
1HS
c
2H
5H
6H
7H
2H
6H
7H
5H
4H
4H
d
rDNA (ACT)5
1HS
3HL
3HL
e
5S rDNA 45S rDNA
1HS
3HL
3HL
f
(ACT)5
3HL
3HL
g
(AAG)5 (ACT)5
1H
3H
3HL
1H
3HL
3H
h
5S rDNA (AAG)5
45S rDNA (ACT)5
1HS.1HL-3HL
3HS.3HL-1HL
i
45S rDNA (AAG)5
(CAG)5
1HS.1HL-3HL
3HS.3HL-1HL
j

Fig. 2


30200
4.5 58
4.5 57
4.5 53
3.4 72
4.5 61
3.1 21
3.1 24
1H
3H
0 cM
50 cM
100 cM
0 cM
50 cM
100 cM
1HS.1HL-3HL
1HS.1HL-3HL
1HS.1HL-3HL
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3H
3H