

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

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25 **Abstract**

26

27 Artificially induced translocation stocks have been used to physically map the barley  
28 genome; however, natural translocations are extremely uncommon in cultivated  
29 genotypes. ‘Albacete’ is a barley variety widely grown in the last decades in Spain and  
30 carrying a reciprocal translocation which obviously does not affect its agronomical  
31 fitness. This has been characterized by a combination of cytological and molecular  
32 genetic approaches. First, similarities between markers on chromosomes 1H and 3H,  
33 involved in the translocation, were estimated to determine the boundaries of the  
34 reciprocal interchange. Secondly, 1H-3H wheat barley telosome addition lines were  
35 used to assign selected markers to chromosome arms. Thirdly, fluorescence *in situ*  
36 hybridization (FISH) with rDNA probes (5S and 18S-5.8S-26S) and microsatellite  
37 probes ((ACT)<sub>5</sub>, (AAG)<sub>5</sub> and (CAG)<sub>5</sub>) was used to determine the locations of the  
38 translocation breakpoints more precisely. Fourthly, fine-mapping of the regions around  
39 the translocation breakpoints was used to increase the marker density for comparative  
40 genomics. The results obtained in this study indicate that the translocation is quite large  
41 with breakpoints located on the long arms of chromosomes 1H and 3H, between the  
42 pericentromeric (AAG)<sub>5</sub> bands and above the (ACT)<sub>5</sub> interstitial distal bands, resulting  
43 in the reciprocal translocation 1HS.1HL-3HL and 3HS.3HL-1HL. The gene content  
44 around the translocation breakpoints could be inferred from syntenic relationships  
45 observed among different species from the grass family Poaceae (rice, *Sorghum* and  
46 *Brachypodium*) which was estimated at approximately 2,100 and 750 gene models for  
47 1H and 3H, respectively. Duplicated segments between chromosomes Os01 and Os05 in  
48 rice derived from ancestral duplications within the grass family overlap with the  
49 translocation breakpoints on chromosomes 1H and 3H in the barley variety ‘Albacete’.

50

51 **Keywords** Reciprocal translocation · Barley · Translocation breakpoint ·

52 Fluorescence In Situ Hybridization · Comparative genomics

53

## 54 **Introduction**

55 Reciprocal translocations, interchanges of chromosome segments between two non-  
56 homologous chromosomes, are one of the most common structural chromosomal  
57 rearrangements occurring in plant species. Translocations in plants have been widely  
58 described by Burnham (1956). Permanent translocation heterozygotes were first  
59 observed by Gates (1908) in *Oenothera* species which received generous cytological  
60 and genetic study by Cleland (1922) and Belling and Blakeslee (1926). McClintock  
61 (1930) was the first to supply cytological evidence of interchanges in economically  
62 important crops such as maize. Translocations have been widely utilized in both applied  
63 and fundamental scientific research for chromosome mapping, in particular for  
64 assigning linkage groups to chromosomes, development of physical maps in plants  
65 (Kim et al. 1993; Künzel et al. 2000; Sorokin et al. 1994; Marthe and Künzel 1994), and  
66 to improve our understanding of meiotic chromosome pairing behaviour (Rickards  
67 1983). They have been reported in a number of crop species, such as rye (Ahloowalia  
68 1962; Alonso-Blanco *et al.* 1993; Benito et al. 1994; Catarino et al. 2006), soybean  
69 (Mahama et al. 2003), *Prunus* (Jáuregui et al. 2001), *Lens* (Tadmor et al. 1987), pea  
70 (Kosterin et al. 1999), wheat (Naranjo et al. 1987) and *Brassica napus* in which a  
71 significant higher seed yield effect was showed for the progeny segregating for a  
72 reciprocal translocation between N7 and N16 (Osborn et al. 2003).

73 Chromosomal interchanges, and translocations in general, can be artificially induced in  
74 somatic or meiotic cells by ionizing radiation or mutagens. They may also occur  
75 spontaneously, although there are few cases of spontaneous reciprocal translocations  
76 described in cultivated barley. Konishi and Linde-Laursen (1988) investigated 1,240  
77 cultivated barley lines and 120 wild barley lines to detect spontaneous reciprocal

78 translocations, which were identified by both semi-sterility associated to test crosses  
79 and subsequent Giemsa banding technique. Of the 1,240 cultivated barley lines, four  
80 Ethiopian landraces carrying a reciprocal translocation had the same breakpoints at the  
81 centromere involving chromosomes 2H and 4H (2HS·4HS and 2HL·4HL), suggesting  
82 that their rearrangement chromosomes had a common origin. Of the 120 wild barley  
83 genotypes, three carried translocations between chromosomes 2H and 4H, 3H and 5H  
84 and 3H and 6H, respectively. Xu and Kasha 1991 identified a chromosomal interchange  
85 between chromosomes 3H and 4H using N-banding and *in situ* hybridization techniques  
86 in a wild barley cross. The plant heterozygous for the interchange was derived from the  
87 backcross of ‘Su Pie’ with pollen from a triploid interspecific F<sub>1</sub> hybrid of ‘Su Pie’ ×  
88 tetraploid *Hordeum bulbosum* accession GBC141.

89 One of the consequences of a reciprocal translocation is the suppression of genetic  
90 recombination in a translocation heterozygote in the interstitial zone (the chromosome  
91 segment between the centromere and the breakpoint). The suppression depends on the  
92 centromere coorientation frequency at metaphase I (alternate or adjacent segregations)  
93 and upon the chiasmata (crossover) frequency expected in both interstitial segments  
94 (Hanson and Kramer 1949; Burnham and Hagberg 1956; Kasha and Burnham 1965;  
95 Sybenga 1975). Recombination suppression in the interstitial zone affects the linkage  
96 relationships in a translocation heterozygote which results in “pseudo-linkage” between  
97 the genes of the two chromosomes involved in the reciprocal translocation and  
98 subsequent disturbed linkage maps. Another consequence of reciprocal translocations is  
99 the occurrence of gametic sterility of which depends on the frequency of the alternate or  
100 adjacent orientation in the quadrivalent. In barley, an excess of alternate over adjacent

101 segregation of the chromosomes at meiotic metaphase I would explain the averaged  
102 about 25% of the sterility for the interchange (Kakeda and Miyahara 1995).

103 Plant accessions carrying chromosome rearrangements such as translocations have been  
104 identified in the past by its effects on partial pollen and seed sterility (i.e. Jáuregui et al.  
105 2001). However, depending on the chromosome breakpoints, a translocation can result  
106 in the disruption or misregulation of normal gene function. Thus, special interest resides  
107 on the characterization of the physical location of the translocation breakpoints in the  
108 genome which can be physically delimited by the combination of cytogenetics with  
109 molecular genetics allowing the location of breakpoints for physical mapping of genes  
110 on chromosomes. In barley, chromosome identification can be achieved by using *in situ*  
111 hybridization with ribosomal RNA probes (Brown et al. 1999). In addition, with  
112 labelled SSRs it is now possible to cover the physical map with many landmarks  
113 distributed along all chromosome arms (Cuadrado and Jouve 2007). Undoubtedly, this  
114 rich set of chromosome markers should help to identify barley breakpoints more  
115 precisely than conventional staining techniques.

116 In the present paper, we aim at determining the positions of the translocation  
117 breakpoints in the Spanish six-row barley variety ‘Albacete’ in order to know how large  
118 the segments are that are involved in the interchange between the two chromosomes.  
119 Translocations may have dramatic consequences such as modified phenotypes. In fact,  
120 ‘Albacete’ is the only extensively cultivated barley variety known to carry a reciprocal  
121 translocation between chromosomes 1H and 3H without any major reduction in fitness.  
122 It is adapted to low-yielding West Mediterranean areas and it has been the most widely  
123 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<sub>1</sub> 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)<sub>5</sub>, (AAG)<sub>5</sub> and (CAG)<sub>5</sub>) of  
138 ‘Albacete’ and doubled haploid lines derived from crosses between ‘Albacete’ and  
139 cultivars with a standard chromosome arrangement.

140

141

142 **Materials and methods**

143 **Linkage analysis**

144

145 Two bi-parental doubled haploid (DH) mapping populations were used. The first  
146 mapping population, ‘Albacete’ × ‘Barberousse’ (AB), consists of 231 DH lines derived  
147 from anther-culture from the cross between the translocation-carrier six-rowed winter  
148 variety ‘Albacete’ and six-row winter variety ‘Barberousse’. DNA isolation and  
149 genotyping data, SSR and DArT®, were performed according to Farré et al. (2011). The  
150 second mapping population, (‘Albacete’ × ‘Plaisant’) × ‘Plaisant’ (APP), consists of 94  
151 DH lines derived from the cross of a DH genotype produced from the ‘Albacete’ ×  
152 ‘Plaisant’ F<sub>1</sub>, which carried the ‘Albacete’ reciprocal translocation, backcrossed to  
153 ‘Plaisant’. DNA was extracted from leaf tissue using kit DNeasy Plant Mini Kit  
154 (Quiagen, Valencia, CA, USA). A set of 3072 EST-based high confidence SNP markers  
155 were genotyped using GoldenGate BeadArray technology (Illumina) as previously  
156 described (Close et al. 2009).

157 The allocation of markers to linkage groups as well as their genetic map position were  
158 based on the published barley consensus map (Wenzl. et al. 2006) for the AB  
159 population and Close et al. 2009 for the APP population. The position of the  
160 translocation breakpoints was first inferred by estimating the similarities between  
161 markers on chromosomes 1H and 3H on the AB DH population. The similarities were  
162 measured by the simple matching coefficient, which for a doubled haploid population is  
163 equal to one minus the recombination frequency. The graphical representation of  
164 similarities between markers was drawn with the Pajek programme (Batagelj and Mrvar  
165 1998).

166

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).

178

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 25°C and then kept at 4°C for 72h followed  
183 by 25°C 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)<sub>5</sub>, (CAG)<sub>5</sub> and (AAG)<sub>5</sub>) were  
192 provided by Roche labelled at their 5' and 3' with digoxigenin or biotin. The novel and  
193 rapid non-denaturing 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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## 212 **Results**

### 213 **Similarities between markers on chromosomes 1H and 3H**

214 In order to identify DArT and SSR markers in the vicinities of the reciprocal  
215 translocation breakpoints, we explored similarities, *i.e.* simple matching coefficients,  
216 between the markers located on chromosomes 1H and 3H. The allocation of markers to  
217 linkage groups as well as their genetic map position were based on the published barley  
218 consensus map (Wenzl et al. 2006). Markers with identical segregation patterns were  
219 binned and of each bin only one marker was selected to obtain a set of markers well  
220 distributed along the chromosomes. A graphical representation of the simple matching  
221 coefficients was obtained for a total of 52 markers out of 82 for AB. Figure 1 shows that  
222 markers on chromosome 1H and 3H were strongly related. This suggests the presence  
223 of a reciprocal translocation between these chromosomes. In Figure 1, the thicker the  
224 connecting lines, the higher the simple matching coefficient. So, for the AB population,  
225 the markers with the highest simple matching coefficient ( $>0.90$ ) were located in the  
226 consensus map around 59-64 cM and 66-70 cM for 1H (HvM20 and EBmac0501) and  
227 3H (Bmac209, Bmac067, Bmag006 and Bmag0136), respectively, which corresponds  
228 with the pericentromeric regions of both chromosomes 1H and 3H. Weaker, yet  
229 significant, similarities extended for 34 cM on 1H and 30 cM on 3H.

230

### 231 **1H and 3H wheat-barley telosome addition lines**

232 Wheat-barley chromosome addition lines were then used to assign the chromosome arm  
233 location of the SSR markers found to be closely linked to the translocation breakpoints  
234 using their presence/absence as detected in hexaploid wheat *Triticum aestivum* cv.  
235 ‘Chinese Spring’ (CS) and barley *Hordeum vulgare* cv. ‘Betzes’ using PCR (Table 1).  
236 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 chromosomal  
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).

261 We subsequently analysed mitotic metaphases of 'Albacete' (Fig. 2 d-h). Chromosomes  
262 2H, 4H, 5H, 6H and 7H showed the expected and distinctive FISH pattern of ribosomal  
263 probes. In addition, one pair of submetacentric chromosomes with a rather strong  
264 pTa71 signal on its short chromosome arm and pTa794 signal on its long chromosome  
265 arm and another pair of metacentric chromosomes without any ribosomal signals,  
266 confirmed the presence of a reciprocal translocation resulting in two chromosomal  
267 combinations 1HS-3HL and 3HS-1HL, respectively (Fig. 2f). These two chromosomes  
268 are shown in Fig. 2i. Therefore, the translocation breakpoints are located below the  
269 pTa71 signal on 1HS and above the pTa794 signal on 3HL.

270 More information about the exact position of the translocation breakpoints can be  
271 obtained by identifying relocated FISH landmarks on chromosomes 1H and 3H. To this  
272 aim three microsatellites probes, (ACT)<sub>5</sub>, (CAG)<sub>5</sub> and (AAG)<sub>5</sub> were chosen for their  
273 characteristic patterns on these chromosomes (Cuadrado and Jouve 2007). As expected,  
274 (ACT)<sub>5</sub> resulted in a distinct pattern of FISH signals on chromosomes 2H, 3H, 4H, 5H,  
275 and 6H. The characteristic signals on the long arm of chromosome 3H were found on  
276 the translocated chromosomes 1HS-3HL (Fig 2g and i). These results suggest that the  
277 translocation breakpoints are located below the pTa71 signal on 1HS and above the  
278 (ACT)<sub>5</sub> signal on 3HL (Fig. 2e). The (AAG)<sub>5</sub> microsatellite probe facilitates the  
279 identification of all barley chromosomes thanks to a rich pattern of signals of different  
280 intensities located in the pericentromeric and interstitial chromosomal regions (Fig. 2h).

281 The characteristic patterns obtained in the translocated chromosomes are similar to the  
282 patterns found in other barley varieties with normal 1H and 3H chromosomes and  
283 suggest that the translocation breakpoints are located below the pericentromeric AAG  
284 signals on the long arms of chromosomes 1HL and 3HL (Fig. 2i-j). Results obtained  
285 with the (CAG)<sub>5</sub> probe support the detection and identification of the translocations

286 1HS.1HL-3HL and 3HS.3HL-1HL. The signals obtained in the centromere of  
287 translocated chromosomes 3HS-1HL were stronger than on 1HS-3HL (Fig 2j).

288

### 289 **Fine-mapping of the translocation breakpoint region**

290 In order to increase the marker density around the translocation breakpoints the APP  
291 population genotyped with SNP was used. As (AAG)<sub>5</sub> and (ACT)<sub>5</sub> microsatellite probes  
292 have proved to be useful to determine the translocation breakpoints on 1H and 3H  
293 chromosomes, they were also chosen for detailed cytogenetic characterization of 8 out  
294 of 94 DH lines from the APP population (Figure 3). These DH lines were selected  
295 according to the ‘Albacete’ SNP allele distribution along 1H and 3H aiming to narrow  
296 down the position of the translocation breakpoints. The allocation of markers to linkage  
297 groups as well as their genetic map position were based on the published barley  
298 consensus map (Close et al. 2009). From Figure 3 it can be observed that 8 markers  
299 around 50-52.5 cM on 1H (11\_20427, 11\_20660, 12\_11209, 12\_31208, 11\_31381,  
300 11\_20912, 11\_21312 and 12\_30350) and 30 markers around 43.2-55.6 cM on 3H  
301 (12\_21533, 11\_11002, 11\_21101, 11\_11086, 11\_11501, 11\_10137, 11\_10328,  
302 11\_20970, 12\_30039, 12\_30130, 12\_30318, 12\_31008, 12\_31372, 11\_10365,  
303 12\_10155, 12\_31502, 11\_10008, 11\_20102, 11\_10224, 11\_20333, 11\_20428,  
304 11\_20439, 11\_10456, 11\_20796, 11\_20856, 11\_20890, 11\_21062, 11\_21147,  
305 11\_11124 and 11\_11337) close to translocation breakpoints.

306

307 **Discussion**

308 The combination of cytogenetics and molecular genetics allowed us to determine the  
309 translocation breakpoints on chromosomes 1H and 3H of the barley variety 'Albacete'  
310 more accurately than with conventional staining techniques. The success of the  
311 combination of methods depends on the locations of the translocation breakpoints and  
312 on the presence of differential signals on the chromosomes involved (Xu and Kasha,  
313 1991).

314 In the present study, the positions of the translocation breakpoints have been determined  
315 using a number of approaches. First, an analysis involving similarities between markers  
316 (simple matching coefficients) was used to determine markers located near the  
317 translocation breakpoints with a high degree of precision. This approach is equivalent to  
318 using recombination frequencies in linkage analysis (Farré et al. 2011). SNP and  
319 microsatellite markers putatively flanking the translocation breakpoints were identified  
320 in the AB and APP mapping populations in the pericentromeric regions of  
321 chromosomes 1H and 3H. Secondly, 1H and 3H wheat-barley telosome addition lines  
322 were used to assign a number of markers closely linked to the translocation breakpoints  
323 to their proper chromosome arms and to validate the genetic mapping results. For the  
324 genomic SSR markers on 3H chromosome, the positions are in good agreement with the  
325 positions reported by Künzel and Waugh (2002). They placed 24 microsatellite loci  
326 onto the physical RFLP map of barley chromosome 3H using the map position of the  
327 translocation breakpoints as reference (Künzel et al. 2000). Our genomic SSR markers  
328 on 3H chromosome were identified flanking the centromere (positioned at 55 cM) in the  
329 'Lina'×*Hordeum spontaneum* Canada Park genetic map, where Bmac0067, Bmag0136  
330 and Bmag006 on the short arm and Bmac0209 on the long arm cover a genetic distance  
331 of 5 cM. It is well known that genetic map distances between markers along

332 chromosomes correlate poorly with physical distances, particularly in the large cereal  
333 genomes where closely linked markers which genetically map near the centromere  
334 represent considerable physical distances (Schwarzacher 2003). As a consequence, most  
335 of the chromosomal gene content is trapped in chromosome segments with severely  
336 suppressed recombination. According to Künzel and Waugh 002 the region flanked by  
337 our four SSR covers 36% of the entire 3H chromosome length on the physical map. For  
338 chromosome 1H, no studies were found relating genetic to physical distance using the  
339 genomic SSR we identified close to the translocation breakpoint. Although being  
340 pericentromeric we may expect results similar to those reported for 3H. In general,  
341 Triticeae centromeres are characterised by suppressed recombination (Schwarzacher  
342 2003; Rostoks et al. 2002). In barley, suppressed recombination rates in proximal  
343 segments and high recombination rates in distal regions were demonstrated by Pedersen  
344 et al. (1995). Later, Künzel et al. (2000) reported that most recombination was confined  
345 in a relatively small chromosomal region, mostly at the arm ends, alternating abruptly  
346 with regions of severely suppressed recombination.

347 Our approach allowed us to identify and genetically delimit the physical regions in  
348 which the reciprocal translocation took place. However, as the region involved in the  
349 translocation located in the vicinity of the centromeres, given recombination  
350 suppression, represents a fairly large physical region in both 1H and 3H chromosomes.  
351 FISH was used in an attempt to validate the positions of the translocation breakpoints  
352 on the chromosomes involved in the chromosomal interchanges, which was found to be  
353 a useful approach to characterize non-recombinant regions.

354 The proposed positions of the breakpoints on the long arms of chromosomes 1H and 3H  
355 can be attributed to the combined use of rDNA and microsatellite probes. Once the  
356 translocation between chromosome 1H and 3H was confirmed using the ribosomal

357 probes (pTa71 and pTa794) and (ACT)<sub>5</sub>, the reasons that led us to concluding that the  
358 breakpoints are on the long arms of chromosomes 1H and 3H (resulting in the  
359 reciprocal translocation 1HS.1HL-3HL and 3HS.3HL-1HL) are multiple. Firstly, the  
360 signals obtained using the (AAG)<sub>5</sub> probe (which were similar to banding patterns  
361 obtained by C-banding) on the short arms of both chromosomes are the same as those  
362 observed in other barley varieties which indicates that the short arms are complete until  
363 the centromere and not involved in the reciprocal interchange (Pedersen and Linde-  
364 Laursen 1994; Cuadrado and Jouve 2007). However, a discrepancy in the number of  
365 bands on 3HL among different barley varieties was found; some barley lines such as  
366 ‘Plaisant’, ‘Gaelic’ and ‘Hispanic’ carried two bands compared with just one for  
367 varieties as ‘Albacete’, ‘Dobla’ and ‘Golden Promise’. The lacking of a band near the  
368 centromere could be a polymorphism in these varieties (unpublished data). Besides that,  
369 it is important to notice that the signal intensity of the pericentromeric band present on  
370 3HL is stronger than the one present on 1HL, which is characteristic for all barley  
371 varieties. Therefore, these results suggest that the translocation breakpoints are located  
372 below this band. Secondly, the differences in intensity found in the centromeres of the  
373 translocated chromosomes with the (CAG)<sub>5</sub> probe are consistent with those obtained for  
374 Plaisant (a variety with an standard chromosome arrangement). Signals on chromosome  
375 3H were stronger than on 1H (Cuadrado and Jouve 2007) suggesting that the  
376 translocation breakpoints are located below the centromere on both  
377 chromosomes. Thirdly, the interchanged chromosomes 1HS.1HL-3HL and 3HS.3HL-  
378 1HL are more similar in total length and more submetacentric and metacentric,  
379 respectively, compared with published ‘normal’ barley karyotypes in which centromeres  
380 of chromosome 1H, the smallest barley chromosome, and 3H are located at 41 and 44%  
381 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)<sub>5</sub> and (ACT)<sub>5</sub> pericentromeric bands for 3HS.3HL-1HL and below the  
389 (AAG)<sub>5</sub> 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)<sub>5</sub> and (ACT)<sub>5</sub> pericentromeric bands

432 for 3HS.3HL-1HL and below the (AAG)<sub>5</sub> pericentromeric band for 1HS.1HL-3HL. The  
433 gene content in this region was estimated at approximately 750 and 2,100 gene models  
434 for 1H and 3H respectively. Presence of duplicated segments between chromosomes  
435 Os01 and Os05 in rice derived from ancestral duplications within the grass family  
436 (Guyot and Keller 2004) overlaps with the translocation breakpoints on 1H and 3H  
437 chromosomes in the barley variety ‘Albacete’. However, no current evidences of  
438 whether similar gene contents on different chromosomes arising from ancestral  
439 duplications may play a role in spontaneous chromosomal exchanges through non-  
440 homologous chromosome pairing have been published.

441

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445

446 **References**

447

448 Ahloowalia BS (1962) Study of a translocation in diploid rye. *Genetica* 33:128-144

449

450 Alonso-Blanco C, Goicoechea PG, Roca A, Giraldez R (1993) A cytogenetic map on  
451 the entire length of rye chromosome 1R, including one translocation breakpoint,  
452 three isozyme loci and four C-bands. *Theor Appl Genet* 85:735-744

453

454 Batagelj V, Mrvar A (1998) Pajek – Program for Large Network Analysis. *Connections*,  
455 21, 2, 47-57

456

457 Bayer M, Milne I, Stephen G, Shaw P, Cardle L, Wright F, Marshal D (2011)  
458 Comparative visualization of genetic and physical maps with Strudel.  
459 *Bioinformatics* 27 (9):1307-1308

460

461 Bellin J, Blakeslee AF (1926) On the attachment of non-homologous chromosomes at  
462 the resolution division in certain 25-chromosome *Daturas*. *Proc. Natl. Acad. Sci.*  
463 *U.S.A* 12:7-11

464

465 Benito C, Llorente F, Henriques-Gil N, Gallego FJ, Zaragoza C, Delibes A, Figueiras  
466 AM (1994) A map of rye chromosome 4R with cytological and isozyme markers.  
467 *Theor Appl Genet* 87:941-946

468

469 Brown SE, Stephens JL, Lapitan NL, Knudson DL. (1999). FISH landmarks for barley  
470 chromosomes (*Hordeum vulgare* L.) Genome 42:274-281  
471

472 Burnham CR (1956) Chromosomal interchanges in plants. Bot. Rev. 22:419-552  
473

474 Burnham CR, Hagberg A (1956) Cytogenetic notes on chromosomal interchanges in  
475 barley. Hereditas 42:467-482  
476

477 Catarino S, Alvarez E, Campa A, Vieira R, Roca A, Giraldez R (2006) Identification  
478 and physical mapping of induced translocation breakpoints involving chromosome  
479 1R in rye. Chromosome Research 14:755-765  
480

481 Cleland RE (1922) The reduction division in the pollen mother cells of *Oenothera*  
482 *fransciscana*. An. J. Bot. 9:391:413  
483

484 Close TJ, Bhat PR, Lonardi S, Wu Y, Rostoks N, Ramsay L, Druka A, Stein N,  
485 Svensson JT, Wanamaker S, Bozdag S, Mikeal L, Roose ML, Moscou MJ, Chao  
486 S, Rajeev K, Varshney RK, Szűcs P, Sato K, Hayes PM, Matthews DE, Kleinhofs  
487 A, Muehlbauer GJ, DeYoung J, Marshall DF, Madishetty K, Fenton RD,  
488 Condamine P, Graner A, Waugh R (2009) Development and implementation of  
489 high-throughput SNP genotyping in barley. BMC Genomics 10:582.  
490

491 Cuadrado A, Schwarzacher T, Jouve N (2000) Identification of different chromatin  
492 classes in wheat using in situ hybridization with the simple sequence repeat  
493 oligonucleotides. Theor Appl Genet 101:711-717

494

495 Cuadrado A, Jouve N (2007) The nonrandom distribution of long clusters of all possible  
496 classes of trinucleotide repeats in barley chromosomes. *Chromosome Research*  
497 15:711-720

498

499 Cuadrado A, Jouve N (2010) Chromosomal detection of simple sequence repeats  
500 (SSRs) using nondenaturing FISH (ND-FISH). *Chromosoma* 119:495-503

501

502 Farré A, Lacasa Benito I, Cistué L, de Hong JH, Romagosa I, Jans J (2011) Linkage  
503 map construction involving a reciprocal translocation. *Theor Appl Genet* 122:  
504 1029-1037

505

506 Gates RR (1908) A study of reduction in *Oenothera rubrinervis*. *Bot Gaz* 46:1-34

507

508 Guyot R, Keller B (2004) Ancestral genome duplication in rice. *Genome* 47:610-614

509

510 Hanson D, Kramer H (1949) The genetic analysis of two chromosome interchanges in  
511 barley from F2 data. *Genetics* 34:687-700

512

513 Jáuregui B, Vicente MC, Messeguer R, Felipe A, Bonnet A, Salesses G, Arús P (2001)  
514 A reciprocal translocation between 'Garfi' almond and 'Nemared' peach. *Theor*  
515 *Appl Genet* 102: 1169-1176

516

517 Kakeda K, Miyahara S (1995) Cytogenetical analyses of reciprocal translocations in  
518 Barley. *Bull. Fac. Bioresources* 14: 1-24

519

520 Kasha K, Burnham CR (1965) The location of interchange breakpoints in barley I.  
521 Linkage studies and map orientation. *Can. J. Genet. Cytol.* 7:62-77

522

523 Kim NS, Armstrong K, Knott DR (1993) Molecular detection of Lophopyrum  
524 chromatin in wheat-Lophopyrum recombinants and their use in the physical  
525 mapping of chromosome 7D. *Theor Appl Genet* 85: 561-567

526

527 Konishi T, Linde-Laursen I (1988) Spontaneous chromosomal rearrangements in  
528 cultivated and wild barleys. *Theor Appl Genet* 75:237-243

529

530 Kosterin OE, Pukhnacheva NV, Gorel FL, Berdnikov VA (1999) Location of the  
531 breakpoints of four reciprocal translocations involving linkage group V and their  
532 influence on recombination distances between neighboring markers. *Pisum*  
533 *Genetics* 31: 13-20

534

535 Künzel G, Korzun L, Meister A (2000) Cytologically Integrated Physical Restriction  
536 Fragment Length Polymorphism Maps for the Barley Genome Based on  
537 Translocation Breakpoints. *Genetics* 154:397-412

538

539 Künzel G, Waugh R (2002) Integration of microsatellite markers into the translocation-  
540 based physical RFLP map of barley chromosome 3H. *Theor Appl Genet* 105:660-  
541 665

542

543 Leitch IJ, Heslop-Harrison JS (1992). Physical mapping of the 18S-5,8S-26S rDNA  
544 genes in barley by in situ hybridization. *Genome* 35:1013-1018  
545

546 Leitch IJ, Heslop-Harrison JS (1993). Physical mapping of four sites of 5S rDNA  
547 sequences and one site of the alpha-amylase-2-gene in barley (*Hordeum vulgare*).  
548 *Genome* 36:517-523  
549

550 Lukaszewski AJ (2000) Manipulation of the 1RS.1BL translocation in wheat by  
551 induced homoeologous recombination. *Crop. Sci.* 40:216-225  
552

553 Mahama AA, Palmer RG (2003) Translocation in soybean classical genetic linkage  
554 groups 6 and 8. *Crop. Sci.* 43:1602-1609  
555

556 Marthe F, Künzel G (1994) Localization of translocation breakpoints in somatic  
557 metaphase chromosomes of barley. *Theor Appl Genet* 89:240-248  
558

559 Mayer K, Taudien S, Martis M, Simková H, Suchánková P, Gundlach H, Wicker T,  
560 Petzold A, Felder M, Steuernagel B, Scholz U, Graner A, Platzer M, Doležal J,  
561 Stein N (2009) Gene content and virtual gene order of barley chromosome 1H.  
562 *Plant Physiol* 151:496–505  
563

564 McClintock B (1930) A cytological demonstration of the location of an interchange  
565 between two non-homologous chromosomes of *Zea mays*. *Proc Natl Acad Sci*  
566 *U.S.A* 16:791-796  
567

568 Moore G, Devos KM, Wang Z, Gale MD (1995) Cereal genome evolution: grasses, line  
569 up and form a circle. *Curr Biol* 5:737-739  
570

571 Naranjo T, Roca P, Goicoechea PG, Giraldez R (1987) Arm homoeology of wheat and  
572 rye chromosomes. *Genome* 29: 873-882  
573

574 Osborn TC, Butrulle DV, Sharpe AG, Pickering KJ, Parking IAP, Parker JS, Lydiate D  
575 (2003) Detection and effects of a homeologous reciprocal translocation in  
576 *Brassica napus*. *Genetics* 165: 1569-1577  
577

578 Pedersen C, Linde-Laursen I (1994). Chromosomal locations of four minor rDNA loci  
579 and a marker microsatellite sequence in barley. *Chromosome Research* 2: 654-71  
580

581 Pedersen C, Giese H, Linde-Laursen I (1995) Towards an integration of the physical  
582 and the genetic chromosome map of barley by in situ hybridization. *Hereditas*  
583 123:77-88  
584

585 Rickards GK (1983) Orientation behaviour of chromosome multiples of interchange  
586 (reciprocal translocation) heterozygotes. *Ann Rev Genet* 17:443-498  
587

588 Rostoks N, Park YJ, Ramakrishna W, Ma J, Druka A, Shiloff BA, SanMiguel PJ, Jiang  
589 Z, Brueggeman R, Sandhu D, Gill K, Bennetzen JL, Kleinjofs A (2002) Genomic  
590 sequencing reveals gene content, genomic organization, and recombination  
591 relationships in barley. *Funct Integr Genomics* 2:51-59  
592

593 Sandhu D, Gill KS (2002) Gene-containing regions of wheat and the other grass  
594 genomes. *Plant Physiology* 128:803-811  
595

596 Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS (1989) In situ  
597 hybridization localization of parental genomes in a wide hybrid. *Ann Bot* 64:315-  
598 324  
599

600 Schwarzacher T (2003) Meiosis, recombination and chromosomes: a review of gene  
601 isolation and Fluorescent in situ hybridization data in plants. *J. Exp Bot* 54  
602 (380):11-23  
603

604 Smilde W, Haluskove J, Sasaki T, Graner A (2001) New evidence for the synteny of  
605 rice chromosome 1 and barley chromosome 3H from rice expressed sequence tags.  
606 *Genome* 44:361–367  
607

608 Sorokin A, Marthe F, Houben A, Pich U, Graner A, Künzel G (1994) Polymerase chain  
609 reaction mediated localization of RFLP clones to microisolated translocation  
610 chromosomes of barley. *Genome* 37:550-555  
611

612 Sybenga J (1975) Meiotic configurations. Monographs on theoretical and applied  
613 genetics. Springer, Berlin  
614

615 Tadmor Y, Zamir D, Ladizinsky G (1987) Genetic mapping of an ancient translocation  
616 in the genus *Lens*. *Theor Appl Genet* 73:883-892  
617

618 Taketa S, I. Linde-Laursen I, Künzel G (2003) Cytogenetic diversity. In: R. von  
619 Bothmer, Th. van Hintum, H.Kniipffer and K. Sato (eds) Diversity in Barley  
620 (*Hordeum vulgare*), Elsevier Science BV, The Netherlands, pp 97-119.

621 Wenzl P, Li H, Carling J, Zhou M, Raman H, Paul E, Hearnden P, Maier C, Xia L, Caig  
622 V, Ovesná J, Cakir M, Poulsen D, Wang J, Raman R, Smith KP, Muehlbauer GJ,  
623 Chalmers KJ, Kleinhofs A, Huttner E, Kilian A (2006) A high-density consensus  
624 map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural  
625 traits. BMC Genomics 7:206

626

627 Xu J, Kasha KJ (1991) Identification of a barley chromosomal interchange using N-  
628 banding and in situ hybridization techniques. Genome 35:392-397.

629

### 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)<sub>5</sub>, (AAG)<sub>5</sub> and (CAG)<sub>5</sub>).** (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)<sub>5</sub> (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)<sub>5</sub> (arrows indicate the  
649 characteristic ACT signals on 3HL); (h) (AAG)<sub>5</sub> and (ACT)<sub>5</sub> (arrowheads 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)<sub>5</sub>, (ACT)<sub>5</sub> and (CAG)<sub>5</sub>. 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 n°30200 was used as a positive control  
662 whereas 3.1 24 as a negative). On the bottom, *In situ* hybridization of (ACT)<sub>5</sub> (red or  
663 green) and (AAG)<sub>5</sub> (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

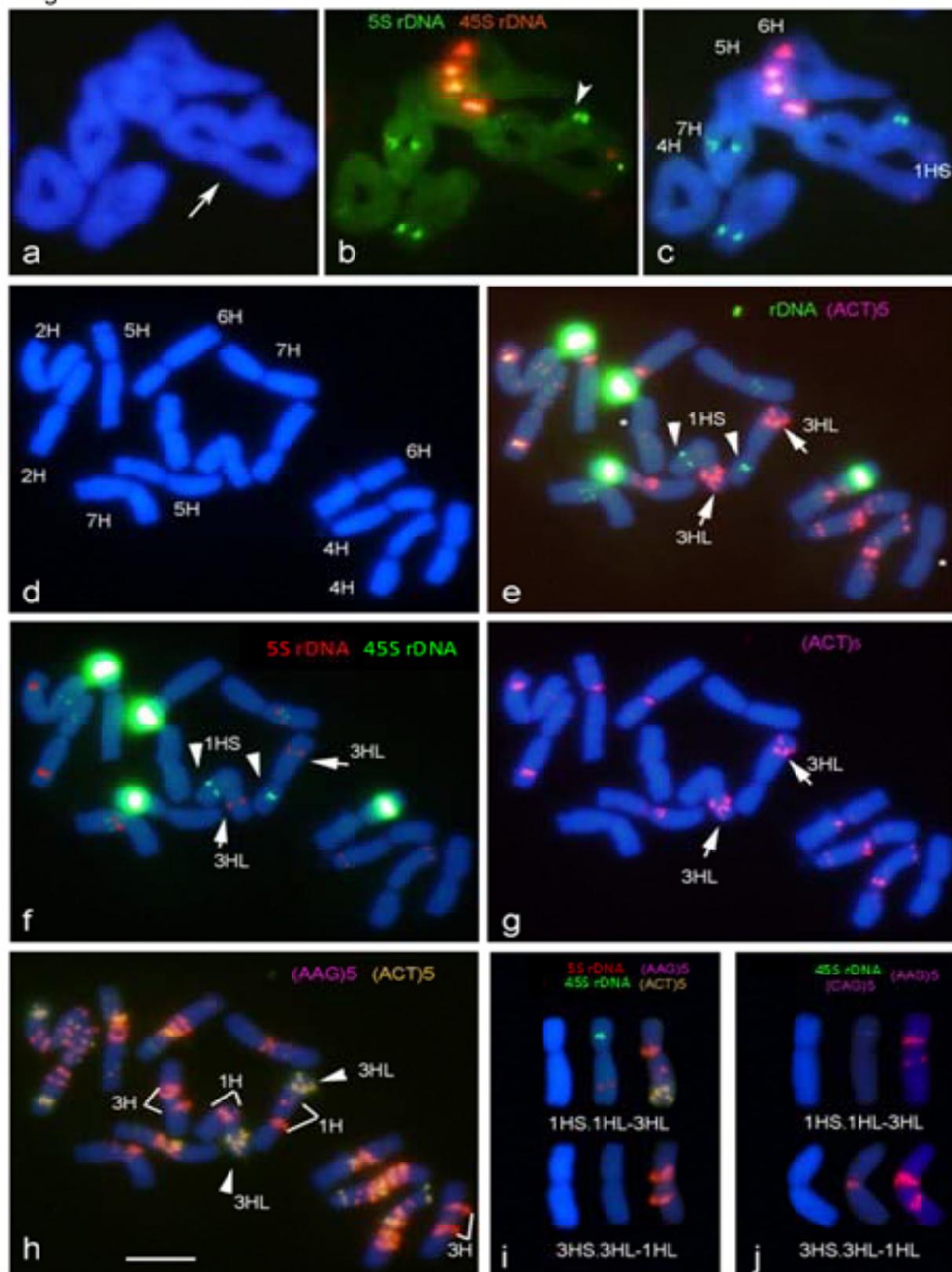


Fig. 2

