Theor Appl Genet (2004) 109: 911–916
DOI 10.1007/s00122-004-1725-6

ORIGINAL PAPER

R. A. Pickering · S. Hudakova · A. Houben ·
P. A. Johnston · R. C. Butler

Reduced metaphase I associations between the short arms of homoeologous chromosomes in a *Hordeum vulgare* L. × *H. bulbosum* L. diploid hybrid influences the frequency of recombinant progeny

Received: 10 March 2004 / Accepted: 12 May 2004 / Published online: 26 June 2004

Abstract *Hordeum vulgare* (cultivated barley) × *H. bulbosum* (bulbous barley grass) hybrids have been used to obtain disease-resistant recombinant lines (RLs). The RLs contain chromatin transferred from the wild species mostly onto the long arms of recipient barley chromosomes. To determine whether differences in meiotic metaphase I (MI) associations between the long and short arms of homoeologous chromosomes can account for the preponderance of introgressions on the long arms, we carried out fluorescent in situ hybridisation on MI chromosome preparations obtained from pollen mother cells of a diploid interspecific hybrid. By using various probes, we established that MI associations between the long arms occurred more frequently than between the short arms for the five chromosomes tested.

Introduction

There are three gene pools of cultivated barley (*Hordeum vulgare* L.) that could be exploited to improve its agronomic performance (Bothmer et al. 1995). However, because of crossability barriers the opportunities for broadening the genetic base of the cultivated crop plant are restricted to the primary and secondary gene pools (Pickering 2000). These comprise *H. vulgare* ssp. *spontaneum* (C. Koch) Thell. and *H. bulbosum* L. (bulbous barley grass), respectively. Progress has been made recently regarding the introgression of useful agronomical traits from *H. bulbosum* into cultivated barley (Pickering 2000). For example, at Crop and Food Research, New Zealand, 72 recombinant lines (RLs) with *H. bulbosum* DNA introgressed into *H. vulgare* have been identified and fully or partially characterised. In general, the introgressions are distally located on the chromosomes and usually appear physically small, although there are variations in size (Zhang et al. 2001). The two largest introgressions observed were approximately half the physical size of the long arms of chromosomes 4H and 6H. The relative frequency of introgressions on particular chromosomes also varies. The commonest RLs, which are derived from several *H. vulgare* × *H. bulbosum* triploid and tetraploid hybrids involving different parental genotypes, are those involving chromosomes 2HL and 4HL (13 RLs), with least numbers for 1HS, 3HS, 3HL, 4HS and 5HS (Table 1); the reason for these differences remains unresolved. One possibility is that the frequencies of metaphase I (MI) associations between *H. vulgare* and *H. bulbosum* homoeologues differ. For example, in an *H. vulgare* ($2n=2x=14$) × *H. bulbosum* ($2n=4x=28$) triploid hybrid, 2H and 4H were the chromosomes that most frequently paired with their *H. bulbosum* homoeologues, although the methods available at that time were not refined enough for critical analysis (Xu and Snape 1988). In this paper we extend that study and present the MI-association frequencies of identifiable chromosome arms using fluorescent in situ hybridisation (FISH) on pollen mother cells (PMCs) of a diploid *H. vulgare* × *H. bulbosum* hybrid. The hybrid, 102C2, has been extensively investigated previously (Zhang et al. 1999, 2002) and, following colchicine treatment to double its chromosome number and restore fertility, has provided breeders with agronomically useful RLs (Zhang et al. 2001).

Communicated by J. W. Snape

R. A. Pickering (✉) · P. A. Johnston · R. C. Butler
New Zealand Institute for Crop and Food Research Limited,
Private Bag 4704 Christchurch, New Zealand
e-mail: pickeringr@crop.cri.nz
Tel.: +64-3-3256400
Fax: +64-3-3252074

S. Hudakova · A. Houben
Institute of Plant Genetics and Crop Plant Research (IPK),
Corrensstrasse 3,
06466 Gatersleben, Germany

Table 1 Numbers of recombinant progeny with introgressions on particular chromosomes obtained from *Hordeum vulgare* × *H. bulbosum* hybrids and the identifiable traits associated with the introgressions

Chromosome location of introgression	Number of recombinant progeny	Transferred trait
1HS	1	
1HL	3	Resistance to leaf rust
2HS	12	Resistance to leaf rust
		Resistance to powdery mildew
		Glossy spike and leaf sheath
2HL	13	Resistance to leaf rust
3HS	1	
3HL	0	
4HS	1	Resistance to scald
4HL	13	Resistance to Septoria speckled leaf blotch
		Black aleurone
		Pubescent leaf and leaf sheath
5HS	1	Response to DDT
5HL	7	Resistance to leaf rust
		Very susceptible to powdery mildew
		Winter growth habit
6HS	7	Resistance to BaYMV/BaMMV
6HL	5	
7HS	3	
7HL	5	Resistance to powdery mildew
		Resistance to stem rust

Materials and methods

A diploid hybrid (code 102C2, $2n=2x=14$) derived from the cross *H. vulgare* (cv. Emir, $2n=2x=14$) × *H. bulbosum* (HB2032, $2n=2x=14$) (Zhang et al. 1999) was grown in two different conditions.

1. Plants were cultivated in a heated glasshouse in New Zealand maintained at 21/15°C±2°C, 16/8 h. Natural daylight was supplemented when necessary with 400-W mercury and sodium vapour lamps to extend the day length to 16 h.
2. A growth room was also used for cultivation and was maintained at a temperature of 18/12°C±0.5°C, with a day length of 16 h provided by Sylvania Luxline T5 fluorescent lamps giving an irradiance of 960 μE/m^2/s at spike height.

Metaphase I associations in PMCs of 102C2 were determined to compare the data with previous results (Zhang et al. 1999; R.A. Pickering 1994, unpublished data). Spikes were fixed in 3:1 ethanol:acetic acid and anthers squashed in 1% aceto-carmine for microscopic examination.

FISH was performed on mitotic chromosome preparations of 102C2 (Pickering et al. 2000) to assess the suitability and signal strength of probes for identifying individual chromosome arms. The probes used were pSc119.2 (McIntyre et al. 1990), an oligonucleotide sequence $(CTT)_{10}$ (Pedersen et al. 1996), 5S rDNA sequence from *H. vulgare* (Menke et al. 1998), 45S rDNA sequence from *Arabidopsis thaliana* (Lysak et al. 2003) and C-hordein (Brown et al. 1999). Following confirmation of their suitability as diagnostic probes (Table 2), FISH was performed on PMCs of 102C2.

Fixed anthers were washed and PMCs at MI were identified by squashing half an anther in aceto-carmine and examining with light microscopy. Each of the remaining anthers in the floret was then incubated on a

Table 2 In situ hybridisation probes used for identifying individual chromosomes in a diploid *H. vulgare* × *H. bulbosum* hybrid

Marker	Identifiable *H. vulgare* chromosomes	Identifiable chromosomes of *H. bulbosum* (HB2032)
$(CTT)_{10}$	4H—very strong signal	$4H^b$—no signal
	5H—weak signal	$5H^b$—no signal
pSc119.2	No signals	$4H^bL$, $6H^bS$
		Distal signals
5S rDNA	2HL—weak signal	$2H^b$—no signal
	5H—no signal	$5H^bS$—strong signal
45S rDNA	5HS strong signal	$5H^bS$—no signal
	6HS strong signal	$6H^bS$—strong signal
C-hordein	1HS—weak signal	No signal

microscope slide in one drop of 2% pectinase + 2% cellulase (Sigma) for 1 h at 37°C in a humid chamber. The enzyme was removed by blotting and replaced with a drop of 45% acetic acid and left for 3–5 min at room temperature. The anthers were macerated and squashed under 18 mm × 18 mm cover slips, which were removed with a scalpel blade after immersion in liquid nitrogen. Slides were stored at 4°C for one to several days until use and were then treated for 20 min at 37°C in a humid chamber with 0.1 mg pepsin/ml 0.01 N HCl. Subsequent FISH methods were based on those of Pickering et al. (2000) for $(CTT)_{10}$ and Toubia-Rahme et al. (2003) for the remaining probes. To analyse chromosomes 1 and 2, anthers containing PMCs at MI were taken from three florets, whereas for chromosomes 4, 5 and 6, anthers from six florets were used.

pSc119.2, 5S rDNA and $(CTT)_{10}$DNA were labelled with digoxigenin- or biotin-labelled nucleotides by stan-

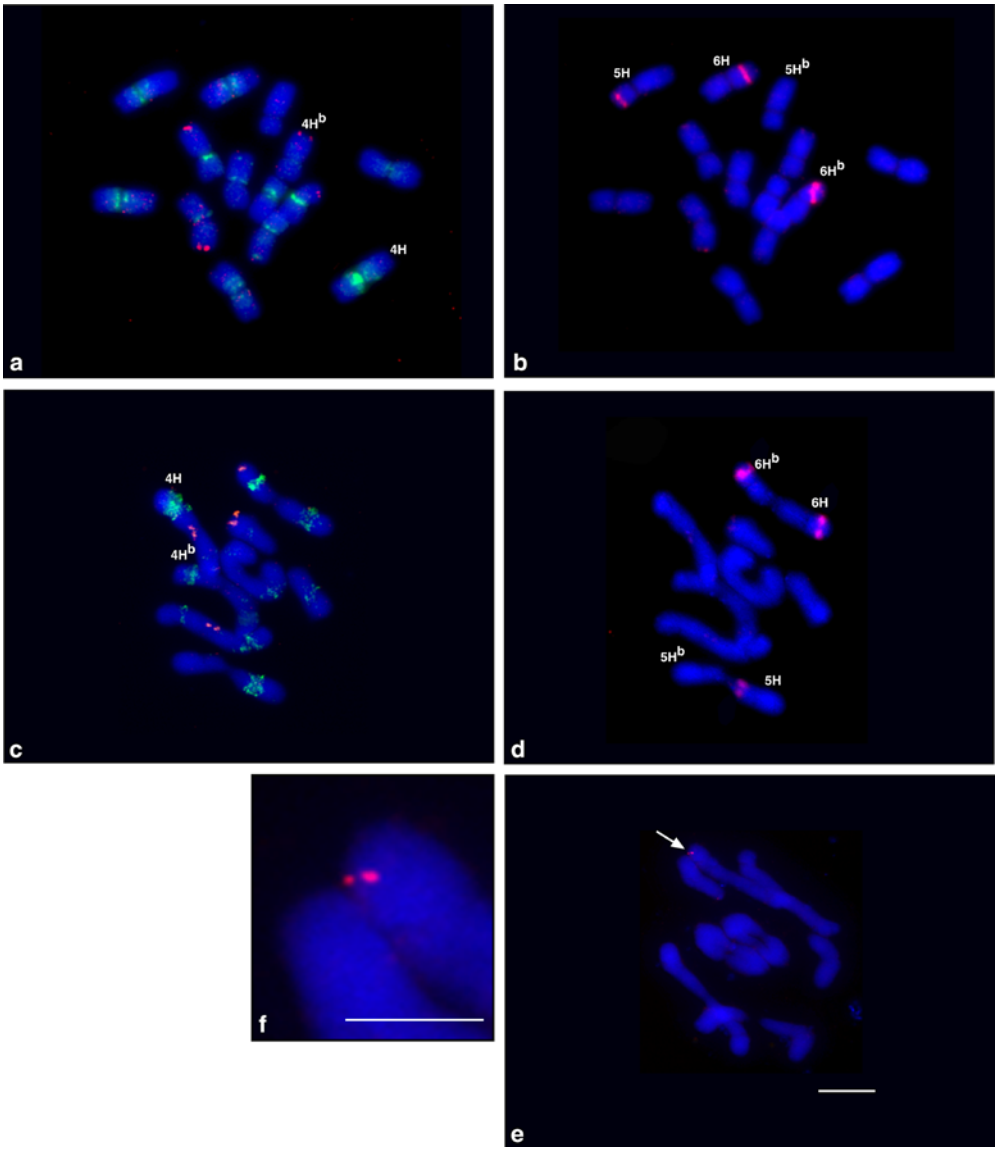

Fig. 1 Fluorescent in situ-hybridisation preparations of mitotic (**a, b**) and metaphase I (MI) (**c–f**) chromosomes from a diploid *Hordeum vulgare* × *H. bulbosum* hybrid comprising seven chromosomes from each parent. Chromosomes derived from *H. vulgare* and *H. bulbosum* are denoted as *H* and H^b, respectively. **a** Mitotic cell showing *green* $(CTT)_{10}$ and *red* (pSc119.2) *signals*. Note the strong CTT signals on 4H and their absence on $4H^b$ and, conversely, the absence and presence of 119.2 signals on 4HL and $4H^bL$, respectively. 119.2 signals are also visible on three other *H. bulbosum* chromosomes. **b** The same mitotic cell after re-probing with 45S rDNA. Note three *red signals* on chromosomes 5HS, 6HS and $6H^bS$, whereas $5H^b$, the only sub-metacentric chromosome, has no signals. **c** MI preparation with *green* $(CTT)_{10}$ and *red* (pSc119.2) *signals* illustrating associations between chromosomes 4HL (*bright CTT signals* on 4H) and $4H^bL$ (*red 119.2 signals* on $4H^bL$). 119.2 signals are present on three other *H. bulbosum* chromosomes paired with their *H. vulgare* homoeologues. **d** The same MI preparation after re-probing with 45S rDNA (three *red signals*) showing associations between 6HL and $6H^bL$, 5HS and $5H^bS$. **e** MI preparation with red (C-hordein) signal on chromosome 1HS (*arrow*) establishing associations between 1HL and $1H^bL$. **f** Enlargement of **e** showing the distal region of chromosome 1HS. *Bar* **a–e**=10 μm; *bar* **f**=5 μm

dard PCR in the presence of digoxigenin-11-dUTP or biotin-16-dUTP [2 mM each of dATP, dCTP, and dGTP; 1.9 mM cTTP, 0.1 mM digoxigenin-11-dUTP or 0.1 mM biotin-16-dUTP, pH 7.0; PCR digoxigenin labelling mix (Roche)]. The primers UP46 and UP47 were used to amplify 5S rDNA sequences according to Gottlob-McHugh et al. (1990). BAC T15P10 from *A. thaliana* containing 45S rDNA and a PCR product containing the C-hordein fragment were labelled with digoxigenin, using a nick translation kit (Roche), according to the manufacturer's instructions.

Detection of hybridisation signals was performed as described by Houben et al. (2001) using anti-digoxigenin rhodamine for digoxigenin-labelled probes and streptavidin Alexa 488 for biotin-labelled probes. Signal strength was generally adequate, but for C-hordein, hybridisation was extended to 3 days at 37°C, and signals were amplified using sheep–antidigoxigenin rhodamine-conjugated antibodies (Roche) and donkey–anti-sheep rhodamine-conjugated antibodies (Jackson Immuno Research). The slides were mounted in Vectashield (Vector Laboratories) with 1.0 μg/ml 4′,6-diamidine-2′-phenylindole dihydrochloride as a counterstain.

To complete the diagnosis of chromosomes and chromosome arms, sequential probing was usually performed after washing the slides to remove the probe (Heslop-Harrison et al. 1992).

Statistical comparisons between MI-association rates for the different chomosomes and environments were made using a log-linear model analysis for contingency tables of counts (McCullagh and Nelder 1989) and was carried out using GenStat Committee (2002).

Results

Overall metaphase I associations

Overall MI associations of 102C2 were similar to previous observations (Zhang et al. 2001; R.A. Pickering 1994, unpublished). Mean univalent, rod bivalent and ring bivalent formation was 0.50, 2.69 and 4.07, respectively, confirming that 102C2 is a relatively 'high-pairing' diploid hybrid (Zhang et al. 2001). There was some evidence for a difference in MI associations between the glasshouse and the growth room, but as only one slide was examined for the growth room, the evidence for this effect was not reliable.

Identification of chromosomes by FISH

By using in situ-hybridisation probes sequentially, most of the chromosomes could be identified in 102C2 (Table 2; Fig. 1a, b). CTT signals on *H. vulgare* resembled N-banding patterns (Pedersen et al. 1996), whereas the signals on the *H. bulbosum* chromosomes were mostly centromeric (Linde-Laursen et al. 1990), but weaker, and were absent altogether on two chromosomes. Using *H. vulgare–H. bulbosum* chromosome substitution lines, these two chromosomes were identified as $4H^b$ and $5H^b$ (R.A. Pickering, unpublished). pSc119.2 hybridised to distal sites on four *H. bulbosum* chromosomes, two of which, $4H^bL$ and $6H^bS$ (de Bustos et al. 1996; R.A. Pickering, unpublished), were used for diagnostic purposes in conjunction with other probes. There were no signals on any *H. vulgare* chromosomes. As expected from previous reports using 18S–26S rDNA sequences (de Bustos et al. 1996), 45S rDNA hybridised to chromosomes 5HS, 6HS and $6H^bS$. The 5S signal was distinctly seen on $5H^bS$ (de Bustos et al. 1996; Taketa et al. 1999), with a weaker signal on 2HL and occasional very weak signals on one or two other *H. vulgare* chromosomes (Fukui et al. 1993; Leitch and Heslop-Harrison 1993; Brown et al. 1999). The C-hordein probe was only observed on *H. vulgare* chromosome 1HS (Brown et al. 1999); no signals were detected on any *H. bulbosum* chromosomes.

Identification and MI-association frequency of homoeologous chromosomes

The long and short arms of chromosomes 4H, 5H and 6H were easily identified using 45S rDNA and $(CTT)_{10}$ probes at MI, and associations involving these arms were established (Table 3; Fig. 1c, d). For chromosomes 1H and 2H, using C-hordein (Fig. 1e, f) and 5S rDNA-specific probes, respectively, signals were usually weak and did not occur in every PMC. Numbers of analysed cells with detectable signals were, therefore, lower compared with chromosomes 4H, 5H and 6H but sufficient to obtain enough data to analyse arm associations.

Table 3 Proportions of metaphase I associations per pollen mother cell (*PMC*) for chromosomes 1H, 2H, 4H, 5H and 6H in a diploid *H. vulgare* × *H. bulbosum* hybrid. *L/L* Long-arm associations, *S/S* short-arm associations

Chromosome	2I	Rod bivalent L/L	Rod bivalent S/S	Ring bivalent
1H-H^b (29 PMCs)[a]	0.07	0.62 (0.93)	0.0 (0.31)	0.31
2H-$2H^b$ (53 PMCs)	0.0	0.15 (0.98)	0.02 (0.85)	0.83
4H-$4H^b$ (231 PMCs)	0.03	0.22 (0.88)	0.09 (0.75)	0.66
5H-$5H^b$ (261 PMCs)	0.06	0.63 (0.91)	0.03 (0.31)	0.28
6H-$6H^b$ (290 PMCs)	0.02	0.30 (0.88)	0.10 (0.68)	0.58

[a]Values in parenthesis indicate associations of the long and short arms as the sum of rod and ring bivalents

Long-arm associations were quite similar for all homoeologous chromosomes ($P>0.4$) but always higher than short-arm associations, especially for chromosomes 1H-1H^b and 5H-5H^b ($P<0.05$, Table 3). Short-arm associations varied among chromosomes ($P<0.001$), with the most frequent between 2HS-2H^{b}S and the least for homoeologous chromosomes 1HS-1H^{b}S and 5HS-5H^{b}S. 4HS-4H^{b}S and 6HS-6H^{b}S were somewhat intermediate in association frequency (Table 3).

Discussion

The predominance of long-arm MI associations over short-arm associations in the hybrid (Table 3) generally corresponds with the mostly higher frequency of RLs having introgressions on the long arms (Table 1). Lowest MI-association frequencies were between 1HS-1H^{b}S and 5HS-5H^{b}S. Burnham et al. (1954) observed the frequent occurrence in barley PMCs of six ring bivalents and one rod, which was most likely to have been the chromosome pair with the small satellite (i.e. 5H). Reduced recombination will influence association frequency and the fewest regions of high recombination ('hotspots') among all the *H. vulgare* chromosome arms were also recorded for 5HS (Künzel et al. 2000). In two trisomic series of *H. spontaneum* and *H. vulgare*, the greatest frequency of Y-type trivalents was observed for chromosome 5H (Tsuchiya 1960, 1967). Y-type trivalents can arise from chiasmata being restricted to one of the arms (Benavente and Orellana 1984), and we can speculate that MI association was reduced among the 5HS arms. Benavente and Orellana (1984) and Naranjo and Orellana (1984) also noted that in triploid and tetraploid rye, respectively, chiasmate associations of the long arms of several identifiable chromosomes exceeded the frequencies for the short arms. And among tomato telotrisomics, trivalents were more frequent for long arms than short arms (Khush and Rick 1968). Of the chromosomes that we analysed, 1HS and 5HS have the shortest chromosome arms in the barley genome (Fukui and Kakeda 1990; Jensen and Linde-Laursen 1992; Marthe and Künzel 1994) and 5H^{b}S the shortest arm in *H. bulbosum* (Symeonidis et al. 1985). Hence, in this *H. vulgare* × *H. bulbosum* hybrid there seems to be a close relationship between arm length and reduced recombination between the short arms of homoeologous chromosomes that would result in reduced MI association.

In conclusion, although MI association is an important factor influencing the relative frequencies of particular RLs, it does not fully explain all the differences in RL frequencies. For example, it is unclear why similar numbers of RLs are not obtained for the long arms of all chromosomes since the proportions of paired long arms were quite comparable, ranging only from 0.88 to 0.98 per PMC (Table 3). In addition, associations between 4HS-4H^{b}S and 4HL-4H^{b}L do not differ sufficiently to explain the paucity of 4HS RLs (Table 1), and the numbers of 1H RLs obtained are very low for both chromosome arms despite the dissimilarity between 1HS-1H^{b}S and 1HL-1H^{b}L associations (Table 3). We must, therefore, consider the heterogeneous distribution of recombination rates along individual barley chromosomes (Künzel et al. 2000), which could become obvious by critically mapping the introgressions in RLs to determine whether crossover sites have occurred in similar chromosomal regions. Another factor to be considered is the effect of parental genotype on chromosome associations since 102C2 is only one of several hybrids from which RLs have been obtained.

Acknowledgements We thank Professor D.L. Knudson (Colorado State University, USA) for kindly supplying the C-hordein probe, Professor I. Schubert (IPK, Gatersleben, Germany) for valuable discussions and the Foundation for Research Science and Technology for funding the New Zealand authors.

References

Benavente E, Orellana J (1984) Meiotic pairing of specific chromosome arms in triploid rye. Can J Genet Cytol 26:717–722

Bothmer R von, Jacobsen N, Baden C, Jørgensen RB, Linde-Laursen I (1995) An ecogeographical study of *Hordeum*. IPGRI, Rome

Brown SE, Stephens JL, Lapitan NLV, Knudson DL (1999) FISH landmarks for barley chromosomes (*Hordeum vulgare* L.). Genome 42:274–281

Burnham CR, White FH, Livers R (1954) Chromosomal interchanges in barley. Cytologia 19:191–202

Bustos A de, Cuadrado A, Soler C, Jouve N (1996) Physical mapping of repetitive DNA sequences and 5S and 18S–26S rDNA in five wild species of the genus *Hordeum*. Chromosome Res 4:491–499

Fukui K, Kakeda K (1990) Quantitative karyotyping of barley chromosomes by image analysis methods. Genome 33:450–458

Fukui K, Kamisugi Y, Sakai F (1993) Physical mapping of 5S rDNA loci by direct-cloned biotinylated probes in barley chromosomes. Genome 37:105–111

GenStat Committee (2002) GenStat, release 6.1, reference manual, parts 1–3. VSN International, Oxford

Gottlob-McHugh SG, Lévesque M, MacKenzie K, Olson M, Yarosh O, Johnson DA (1990) Organization of 5S rRNA genes in soybean *Glycine max* (L.) Merrill and conservation of the 5S rDNA repeat structure in higher plants. Genome 33:486–494

Heslop-Harrison JS, Harrison GE, Leitch IJ (1992) Reprobing of DNA:DNA in situ hybridization preparations. Trends Genet 8:372–373

Houben A, Field BL, Saunders VA (2001) Microdissection and chromosome painting of plant B chromosomes. Methods Cell Sci 23:115–124

Jensen J, Linde-Laursen I (1992) Statistical evaluation of length measurements on barley chromosomes with a proposal for a new nomenclature for symbols and positions of cytological markers. Hereditas 117:51–59

Khush GS, Rick CM (1968) Tomato telotrisomics: origin, identification, and use in linkage mapping. Cytologia 33:137–148

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 (1993) Physical mapping of four sites of 5S rDNA sequences and one site of the α-*amylase-2* gene in barley (*Hordeum vulgare*). Genome 36:517–523

Linde-Laursen I, von Bothmer R, Jacobsen N (1990) Giemsa C-banded karyotypes of diploid and tetraploid *Hordeum bulbosum* (Poaceae). Plant Syst Evol 172:141–150

Lysak MA, Pecinka A, Schubert I (2003) Recent progress in chromosome painting of *Arabidopsis* and related species. Chromosome Res 11:195–204

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

McCullagh P, Nelder JA (1989) Generalized linear models, 2nd edn. Chapman and Hall, London

McIntyre CL, Pereira S, Moran LB, Appels R (1990) New *Secale cereale* (rye) DNA derivatives of rye chromosome segments in wheat. Genome 33:635–640

Menke M, Fuchs J, Schubert I (1998) A comparison of sequence resolution on plant chromosomes: PRINS versus FISH. Theor Appl Genet 97:1314–1320

Naranjo T, Orellana J (1984) Meiotic behaviour of chromosomes 1R, 2R and 5R in autotetraploid rye. Chromosoma 89:143–150

Pedersen C, Rasmussen SK, Linde-Laursen I (1996) Genome and chromosome identification in cultivated barley and related species of the Triticeae (Poaceae) by in situ hybridization with the GAA-satellite sequence. Genome 39:93–104

Pickering R (2000) Do the wild relatives of cultivated barley have a place in barley improvement? In: Logue S (ed) Barley genetics VIII. Proceedings of the 8th international barley genetics symposium, vol I. Department of Plant Science, Waite Campus, Adelaide University, Australia, pp 223–230

Pickering RA, Malyshev S, Künzel G, Johnston PA, Korzun V, Menke M, Schubert I (2000) Locating introgressions of *Hordeum bulbosum* chromatin within the *H. vulgare* genome. Theor Appl Genet 100:27–31

Symeonidis LA, Moustakas MB, Coucoli HD (1985) Karyotype and seed protein profile analysis of diploid and tetraploid *Hordeum bulbosum* L. Phyton (Horn) 25:31–38

Taketa S, Harrison GE, Heslop-Harrison JS (1999) Comparative physical mapping of the 5S and 18S–25S rDNA in nine wild *Hordeum* species and cytotypes. Theor Appl Genet 98:1–9

Toubia-Rahme H, Johnston PA, Pickering RA, Steffenson BJ (2003) Inheritance and chromosomal location of *Septoria passerinii* resistance introgressed from *Hordeum bulbosum* into *Hordeum vulgare*. Plant Breed 122:405–409

Tsuchiya T (1960) Cytogenetic studies of trisomics in barley. Jpn J Bot 17:177–213

Tsuchiya T (1967) The establishment of a trisomic series in a two-rowed cultivated variety of barley. Can J Gen Cytol 9:667–682

Xu J, Snape JW (1988) The cytology of hybrids between *Hordeum vulgare* and *H. bulbosum* revisited. Genome 30:486–494

Zhang L, Pickering R, Murray B (1999) Direct measurement of recombination frequency in interspecific hybrids between *Hordeum vulgare* and *H. bulbosum*, using genomic in situ hybridization. Heredity 83:304–309

Zhang L, Pickering RA, Murray BG (2001) A *Hordeum vulgare* × *H. bulbosum* tetraploid hybrid provides useful agronomic introgression lines for breeders. NZ J Crop Hortic Sci 29:239–246

Zhang L, Murray BG, Pickering RA (2002) Variable patterns of chromosome synapsis at pachytene in *Hordeum vulgare* × *H. bulbosum* hybrids and their parents. Hereditas 137:90–95