RESEARCH ARTICLE

Chromosomal organization of repetitive DNAs in *Hordeum bogdanii* and *H. brevisubulatum* (Poaceae)

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Abstract

Molecular karyotypes of *H. bogdanii* Wilensky, 1918 (2n = 14), and *H. brevisubulatum* Link, 1844 ssp. *brevisubulatum* (2n = 28), were characterized by physical mapping of several repetitive sequences. A total of 18 repeats, including all possible di- or trinucleotide SSR (simple sequence repeat) motifs and satellite DNAs, such as pAs1, 5S rDNA, 45S rDNA, and pSc119.2, were used as probes for fluorescence in situ hybridization on root-tip metaphase chromosomes. Except for the SSR motifs AG, AT and GC, all the repeats we examined produced detectable hybridization signals on chromosomes of both species. A detailed molecular karyotype of the I genome of *H. bogdanii* is described for the first time, and each repetitive sequence is physically mapped. A high degree of chromosome variation, including aneuploidy and structural changes, was observed in *H. brevisubulatum*. Although the distribution of repeats in the chromosomes of *H. brevisubulatum* is different from that of *H. bogdanii*, similar patterns between the two species imply that the autopolyploid origin of *H. brevisubulatum* is from a *Hordeum* species with an I genome. A comparison of the I genome and the other *Hordeum* genomes, H, Xa and Xu, shows that colocalization of motifs AAC, ACT and CAT and colocalization of motifs AAG and AGG are characteristic of the I genome. In addition, we discuss the evolutionary significance of repeats in the genome during genome differentiation.

Introduction

Species in Triticeae have large genomes, 75% of which consists of repetitive sequences (Flavell et al. 1974, 1977). Many repetitive sequences, such as microsatellite and satellite DNA, can generate fluorescence in situ hybridization (FISH) patterns on individual chromosomes that are specific to a single species (Tsujimoto et al. 1997, Cuadrado et al. 2008). The FISH patterns generated by these repetitive probes always produce a stable and unique karyotype for a given species (Badaeva et al. 1996, 2002). The FISH patterns from a few repeats correspond to the heterochromatin regions in chromosomes (Cuadrado et al. 1995, Pedersen and Langridge 1997). Thus, the genomic organization of a given species can be characterized using FISH-based cytological analysis. Phylogenies can even be derived from repeat-based comparative FISH karyotyping (Cuadrado and Jouve 2002, Jiang and Gill 2006, Heslop-Harrison and Schwarzacher 2011).

The genus *Hordeum* Linnaeus, 1753 in the tribe Triticeae is divided into 32 species and is distributed in southern South America, South Africa, and the northern hemisphere (Bothmer et al. 1995). Cytotypes in this genus exist at three ploidy levels (diploid, tetraploid and hexaploid) with a basic chromosome number of $x = 7$ (Bothmer et al. 1995). Hybrid analysis and C-band analysis (Bothmer et al. 1986, 1995, Linde-Laursen et al. 1992) have revealed four genome groups in *Hordeum* that are designated as H (*Hordeum bulbosum* Linnaeus, 1756; *Hordeum vulgare* Linnaeus, 1753), Xa (*Hordeum marinum* Hudson, 1778), Xu (*Hordeum murinum* Linnaeus, 1753), and I (all the remaining species) (Blattner 2009). The H genome chromosomes of *H. vulgare* were first characterized using FISH probes of all possible classes of trinucleotide SSRs (simple sequence repeat). This provided detailed information on the sequence content of barley chromatin and saturated the physical map of all the barley chromosomes (Cuadrado and Jouve 2007a). Later, cytogenetic diversity between *H. vulgare* and *H. bulbosum*, was revealed using probes of SSRs, 5S rDNA, 45S rDNA, and 120-bp repeats from *Secale cereale* Linnaeus, 1753 (Carmona et al. 2013a). Karyotypes of the species of the *H. marinum* complex were determined using several tandem repeats. The results revealed the genome structure of different *H. marinum* taxa and demonstrated the allopolyploid origin of tetraploid forms of *H. gussoneanum* Parlatore, 1845 (Carmona et al. 2013b). In addition, the genomic constitution of *H. murinum* was characterized using multiple repetitive sequences. The results identified all the individual chromosomes within the *H. murinum* complex, elucidated its genomic structure and phylogeny, and explained the appearance of different cytotypes (Cuadrado et al. 2013). Thus far, detailed information on the chromosome organization of multiple repetitive sequences is available for the H, Xa, and Xu genomes. However, this information is still lacking for the I genome.

Hordeum bogdanii Wilensky, 1918, and *H. brevisubulatum* , should include the I genome based on the description of Blattner (2009). *H. bogdanii* is a rather common Asiatic species with a distribution that ranges from western Iran to eastern China and a diploid form of 2n = 2x = 14 (Yang et al. 1987). Conversely, the *H. brevisubulatum* complex is distributed from Western Turkey to eastern China and consists of diploids,

tetraploids, and hexaploids (Bothmer 1979, Linde-Laursen et al. 1980). Polyploids of *H. brevisubulatum* are thought to be autoploid based on morphological (Bothmer 1979, Dewey 1979) and hybrid meiotic analysis (Landström et al. 1984).

In this paper, the molecular karyotype of the I genome is described in detail based on physical mapping of all possible dinucleotide and trinucleotide SSRs along with 5S rDNA, 45S rDNA, pSc119.2, and pAs1 repeats on mitotic chromosomes in *H. bogdanii* and *H. brevisubulatum*. The results provide more information on the genomic differentiation in *Hordeum* at the chromosomal level and will also help elucidate the functional and evolutionary implications of different repetitive sequences as genomes differentiate during speciation.

Material and methods

Plant material

Hordeum bogdanii was collected in Germu, Qinghai, China. *Hordeum brevisubulatum* ssp. *brevisubulatum* was collected in Tongde, Qinghai, China. More than 50 or more than 100 individuals of both species were collected in the field. Samples used for cytogenetic investigation were randomly selected from different individuals. Approximately 20 individuals were used for chromosome preparation. Only the investigated samples that displayed clear FISH patterns were present in this study.

Slide preparation

Seeds of *H. bogdanii* and *H. brevisubulatum* were germinated on moist filter paper in petri dishes at room temperature. Root tips with a length of approximately 1–2 cm were excised, pretreated in N_2O gas for 2 h as described by Kato (1999), and fixed in 3:1 (v/v) 100% ethanol:glacial acetic acid. Each root tip was squashed in a drop of 45% acetic acid.

DNA probes and labelling

Synthetic dinucleotide SSRs $(AT)_{15}$, $(AG)_{15}$, $(AC)_{15}$, and $(GC)_{15}$ and trinucleotide SSRs $(AAG)_{10}$, $(AAC)_{10}$, $(AAT)_{10}$, $(ACG)_{10}$, $(ACT)_{10}$, $(AGG)_{10}$, $(CAC)_{10}$, $(CAG)_{10}$, $(CAT)_{10}$ and $(GGC)_{10}$ were end-labelled with fluorescein amidate (FAM, green, Sangon Biotech Co., Ltd., Shanghai, China). For the repetitive sequences pAs1 (Rayburn and Gill 1986), pSc119.2 (Bedbrook et al. 1980), and 45S rDNA, oligonucleotide probes described by Danilova et al. (2012) and Tang et al. (2014) were used. The oligonucleotides pSc119.2 and 45S rDNA were end-labelled with FAM (green), and pAs1 was end-labelled with TAMRA (red). The 5S rDNA was amplified by polymerase chain

reaction (PCR) using genomic DNA of *H. bogdanii* as described by Fukui et al. (1994) and was labelled with fluorescein-12-dUTP (green) using a random primer labelling method. Genomic DNA of *H. bogdanii* was labelled with tetramethy1-rhodamine-5-dUTP using the random primer method, as described by Dou et al. (2009).

FISH and microphotometry

FISH experiments were conducted using the method of Dou et al. (Dou et al. 2009) with minor modifications. Samples on prepared slides were denatured in 0.2 M NaOH in 70% ethanol at room temperature for 10 min, rinsed in 70% cold ethanol (stored at minus 20°C) for approximately 30 minutes, and air dried. The hybridization mixture, 10 μl per slide, was prepared by adding 50% de-ionized formamide, 50% dextran sulphate, 2 × SSC (0.3 M NaCl, 0.03 M Na3-citrate), 1 μg/μl denatured salmon sperm DNA and 10 ng of probe. The hybridization mixture with oligonucleotide probes was placed directly onto the denatured slide preparation. The hybridization mixture with the 5S rDNA probe was denatured in boiling water for 5 minutes. Hybridization was conducted overnight at 37°C in a moistened chamber. Chromosomes were counterstained with 4',6-diamidino-2-phenylindole (DAPI). Images were captured with a cooled CCD camera (Photometrics CoolSNAP) using a fluorescence microscope (Leica) and were processed with the Meta Imaging System (Universal Imaging Corporation). Finally, images were adjusted using Adobe Photoshop 6.0 for contrast and background optimization.

Results

Chromosomal organization of repeats in *H. bogdanii*

A stable chromosome number of 2n = 14 was detected in all tested samples of *H. bogdanii*. The repetitive sequence pAs1 produced multiple sites that were subtelomeric, intercalary, or pericentromeric on all chromosomes. The hybridization pattern of pAs1 was informative enough to distinguish each chromosome of *H. bogdanii*. Thus, chromosome localization of other repeats was conducted using pAs1 as a landmark (Fig. 1). The chromosomal distribution of each sequence was described in detail (Fig. 2).

The four possible dinucleotide SSR probes $(AG)_{15}$, $(AC)_{15}$, $(AT)_{15}$, and $(GC)_{15}$ were used to characterize the chromosomes of *H. bogdanii*. Only $(AC)_{15}$ produced detectable hybridization signals, which appeared in subtelomeric regions on all chromosomes and in pericentromeric regions on a few chromosomes. The signals were strongest in subtelomeric regions of chromosomes B, C and D (Fig. 2).

All 10 trinucleotide SSRs produced detectable hybridizations (Fig. 2). The probe $(AAC)_{10}$ revealed many intense and rich pericentromeric hybridization signals on all seven pairs of chromosomes, intercalary signals on chromosomes B and F, and subtelo-
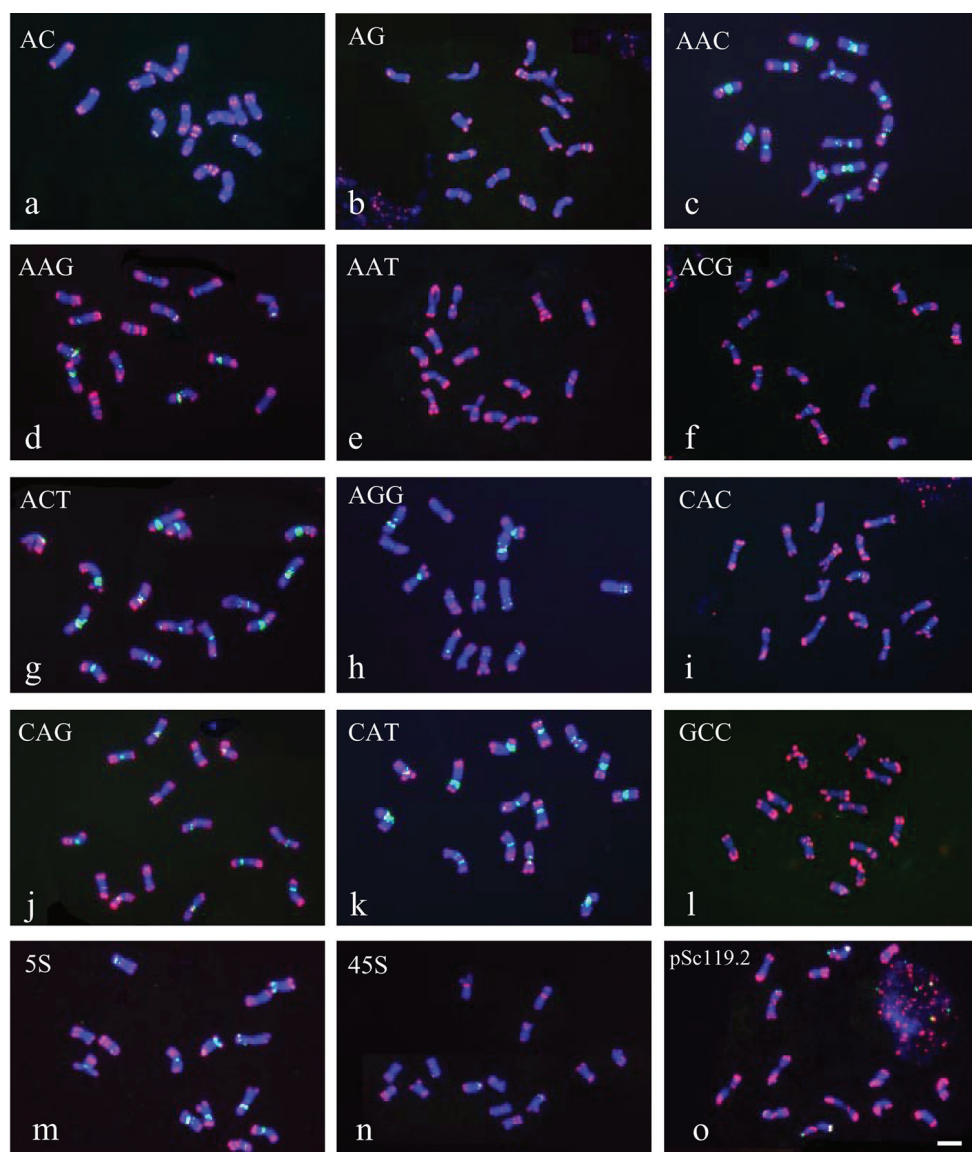
Figure 1. FISH patterns of mitotic metaphase chromosomes of *H. bogdanii* detected by pAs1 (red) combined with the several other repeats (green): **a** $(AC)_{15}$ **b** $(AG)_{15}$ **c** $(AAC)_{10}$ **d** $(AAG)_{10}$ **e** $(AAT)_{10}$ **f** $(ACG)_{10}$ **g** $(ACT)_{10}$ **h** $(AGG)_{10}$ **i** $(CAC)_{10}$ **j** $(CAG)_{10}$ **k** $CAT)_{10}$ **l** $(GCC)_{10}$ **m** 5S rDNA **n** 45SrDNA **o** pSc119.2. Bar =10 μm.

meric signals on chromosomes D and E. The FISH patterns of $(ACT)_{10}$ and $(CAT)_{10}$ appeared to be identical to those of $(AAC)_{10}$. An identical FISH pattern was shared by $(AAG)_{10}$ and $(AGG)_{10}$, which produced intense pericentromeric hybridizations, inter-calary hybridizations, or both in six pairs of chromosomes. The $(CAC)_{10}$ probe produced faint signals in four chromosome pairs and strong signals in three pairs in peri-
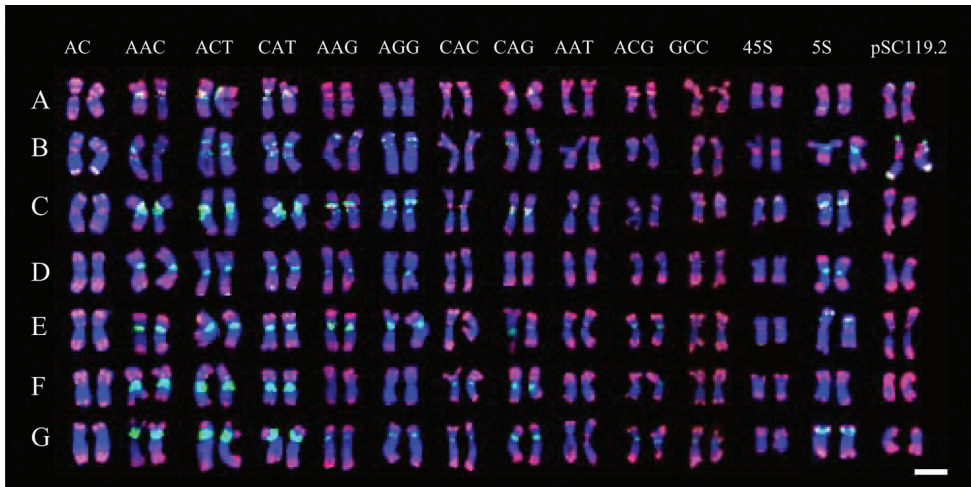
Figure 2. Molecular karyotypes of *H. bogdanii* probed by pAs1 (red) combined with the other several repeats (green). Seven pairs of chromosomes are designated from **A–G** for distinguishing them from the numerals of the 7 homologue groups used in barly. Bar =10 μm.

centromeric regions, intercalary regions, or both. The distribution of the $(CAG)_{10}$ signal overlapped with that of the $(CAC)_{10}$ signal, but the $(CAG)_{10}$ signal had a stronger hybridization intensity. $(AAT)_{10}$ produced faint signals in three pairs of chromosomes in pericentromeric, intercalary, and subtelomeric regions. Hybridization of $(ACG)_{10}$ was detected in six pairs of chromosomes and was localized primarily in centromeric regions. The distribution of $(GCC)_{10}$ was more dispersed; however, more intense hybridizations were still observed in centromeric regions on three pairs of chromosomes.

Three 45S rDNA sites were detected in two pairs of chromosomes. One carried a distinct site on the subtelomeric region of the short arm; the other harboured two faint hybridization sites in the ends of both arms. 5S rDNA was distributed in all chromosomes except for one. Nine distinct 5S rDNA sites were exclusively determined and were localized on centromeric, pericentromeric, intercalary, or subtelomeric regions. Three pairs of chromosomes carried only one 5S rDNA site, and another three harboured two 5S rDNA sites. Two 45S rDNA and three 5S rDNA sites were reported in an accession of *H. bogdanii* (Taketa et al. 2001). The fact that many more 45 S rDNA and 5S rDNA sites were revealed in this study suggests the presence of intra-specific polymorphisms. Four distinct hybridization sites were probed by pSc119.2, and these were localized on subtelomeric regions of three pairs of chromosomes.

Chromosomal organization of repeats in *H. brevisubulatum*

A chromosome number of 2n = 28 was detected in nearly all tested individuals of *H. brevisubulatum*. However, chromosome numbers of 2n = 26 and 2n = 27 (Fig. 3a, d)
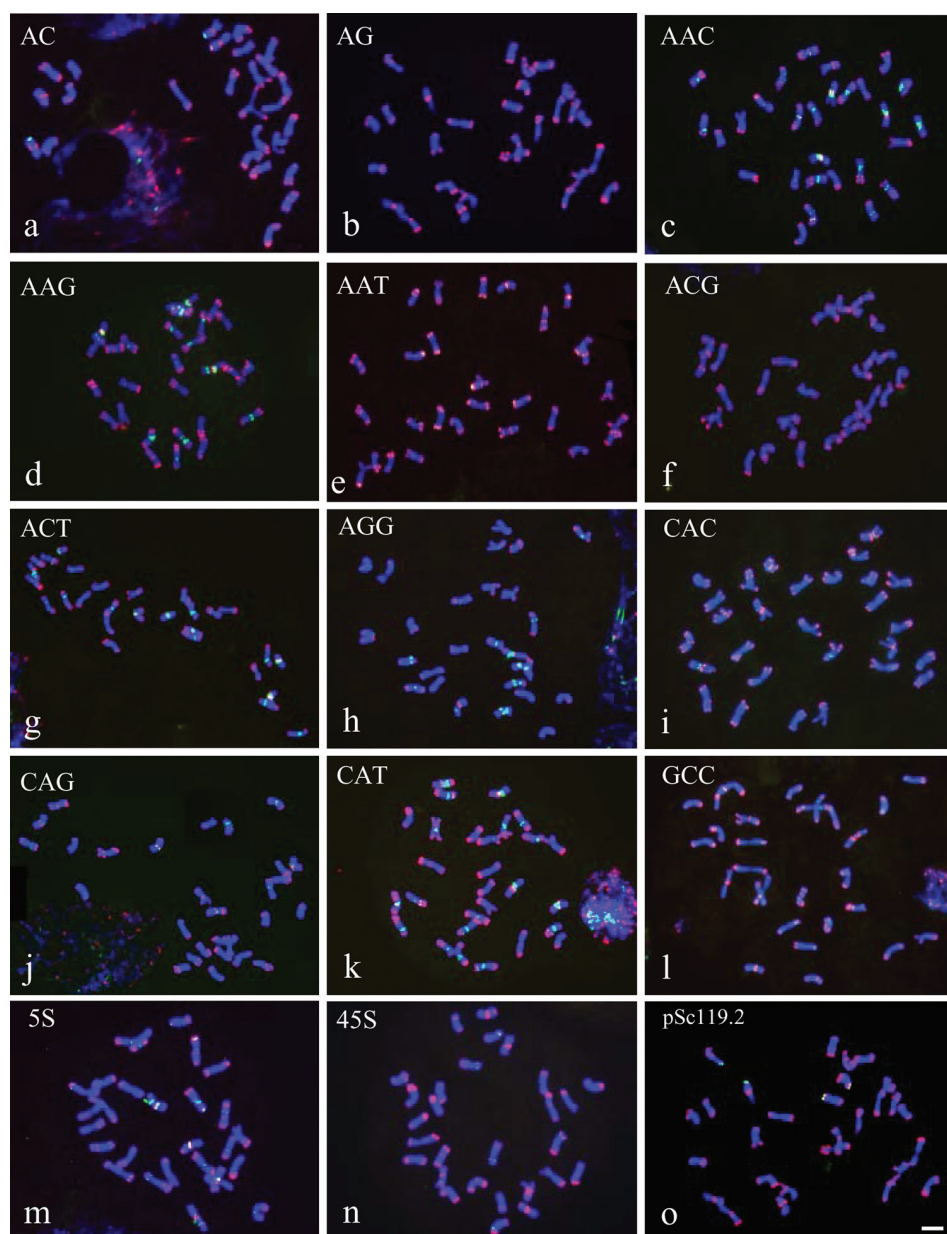
Figure 3. FISH patterns of mitotic metaphase chromosomes of *H. brevisubulatum* detected by pAs1 (red) combined with several other repeats (green): **a** $(AC)_{15}$ **b** $(AG)_{15}$ **c** $(AAC)_{10}$ **d** $(AAG)_{10}$ **e** $(AAT)_{10}$ **f** $(ACG)_{10}$ **g** $(ACT)_{10}$ **h** $(AGG)_{10}$ **i** $(CAC)_{10}$ **j** $(CAG)_{10}$ **k** $(CAT)_{10}$ **l** $(GCC)_{10}$ **m** 5S rDNA **n** 45SrDNA **o** pSc119.2. Bar =10 μm.

were also observed in a few cases. Moreover, a monotelosomic chromosome was clearly identified in one case (Fig. 3k). Karyotyping was tentatively conducted using pAs1 combined with other repeats. Highly variable karyotypes were revealed in *H. brevisubulatum*
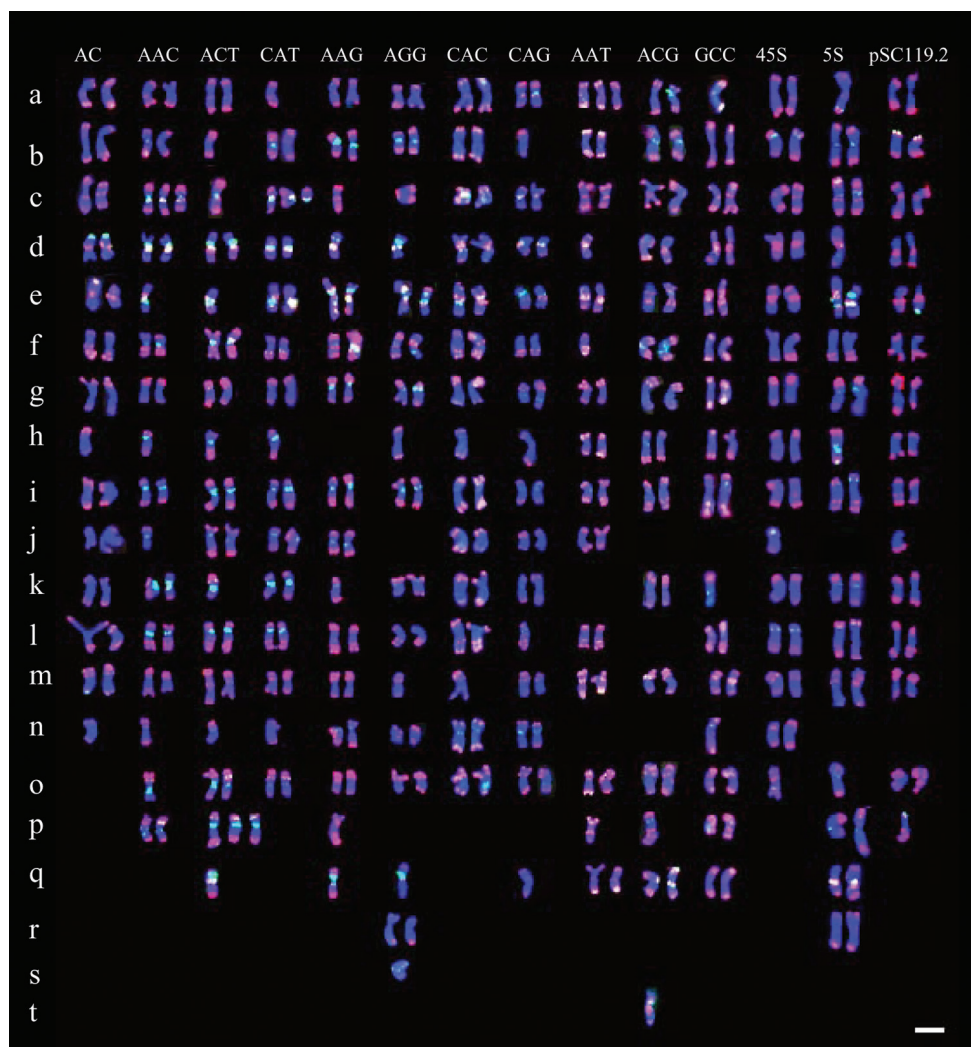
Figure 4. Molecular karyotypes of *H. brevisubulatum* probed by pAs1 (red) combined with several other repeats (green). Chromosome types are designated by lowercase roman letters from a to t to distinguish them from the symbols designating *H. bogdanii*. Bar =10 μm.

(Fig. 4). Variations in chromosome number, such as monosomy, nullisomy, and trisomy, were clearly shown to have occurred. Chromosome structural aberrations, such as deletions, translocations, or inversions, are likely to be present, although these aberrations were not evident from the FISH results alone. Thus, approximately 20 chromosomal variants designated from a to t were roughly identified in the individuals that were investigated (Fig. 4).

All possible di- and trinucleotide SSR probes except for $(AG)_{15}$, $(AT)_{15}$ and $(GC)_{15}$ produced hybridizations on chromosomes of *H. brevisubulatum*. The $(AC)_{15}$ probe
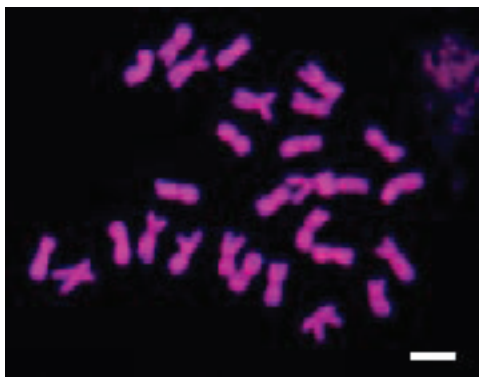
Figure 5. GISH patterns of mitotic metaphase chromosomes of *H. brevisubulatum* probed by the genomic DNA of *H. bogdanii*.

was primarily detected in subtelomeric regions. Nearly half of the total chromosomes carried detectable signals, and signals of seven or eight chromosomes appeared more distinct. The chromosomal distribution of $(AAC)_{10}$, $(ACT)_{10}$, and $(CAT)_{10}$ was similar to that in *H. bogdanii*. The $(AAC)_{10}$, $(ACT)_{10}$, and $(CAT)_{10}$ repeats were still colocalized and were detected on 22–26 chromosomes. $(AAG)_{10}$ and $(AGG)_{10}$ appeared to be colocalized and were primarily distributed in pericentromeric regions on 13–16 chromosomes. $(CAC)_{10}$ and $(CAG)_{10}$ had similar distributions to those observed in *H. bogdanii*. Signals of $(CAC)_{10}$ and $(CAG)_{10}$ were detectable in 19–22 chromosomes and were stronger on eight or nine chromosomes. The FISH pattern of $(AAT)_{10}$ in *H. brevisubulatum* was distinctly different from that in *H. bogdanii*. $(AAT)_{10}$ produced a stronger hybridization signal in *H. brevisubulatum* than in *H. bogdanii*, primarily in subtelomeric regions on nearly all chromosomes. Signals of $(ACG)_{10}$ were detectable in 13 chromosomes. As with $(AAT)_{10}$, stronger hybridization signals of $(GCC)_{10}$ were detected in *H. brevisubulatum* than in *H. bogdanii*.

45S rDNA produced hybridization signals in subtelomeric regions on seven or eight chromosomes. 5S rDNA was detected in centromeric, pericentromeric, intercalary, or subtelomeric regions on 24 chromosomes. The present karyotypes of 5S rDNA and 45S rDNA in *H. brevisubulatum* differed from those of *H. brevisubulatum* based on the accessions reported by Taketa et al. (1999). A heterogeneous composition of 5S and 45S rDNA was observed in a total of five 5S rDNA sites and 18 45S rDNA sites (Taketa et al. 1999). However, many more 5S rDNA sites than 45 S rDNA sites were detected in our study. This pattern suggests that intraspecific polymorphism may exist among different populations. Distinct pSc119.2 signals were detected in subtelomeric regions on four or five chromosomes.

Furthermore, the technique of genomic *in situ* hybridization (GISH) was used to determine the polyploidy origin of *H. brevisubulatum*. The result showed that the chromosomes of *H. brevisubulatum* were evenly painted by using labelled genomic DNA of *H. bogdanii* as the probe (Fig. 5).

Discussion

Chromosomal identification and genomic characterization of *H. bogdanii*

Fifteen of the 18 repetitive sequences produced detectable hybridization in mitotic metaphase chromosomes in *H. bogdanii*. A few repeats, including highly polymorphic sites, can be used to uniquely identify each chromosome. The satellite DNA pAs1 and 5S rDNA can be used to distinguish each chromosome in *H. bogdanii*. The SSRs AAC, ACT, CAT, AAG and AGG are also ideal markers for chromosome identification because of their abundance and their large number of polymorphic sites across individual chromosomes (Fig. 6). Thus, individual chromosomes of *H. bogdanii* can be easily identified using the above repeats. In this study, pAs1 repeats were used as a reference marker, and each repetitive sequence was accurately physically mapped.

Most of the repeats produced multiple hybridizations. However, high-intensity hybridizations were always observed on pericentromeric or subtelomeric regions. This implies that the *H. bogdanii* genome contains more repetitive sequences in subtelomeric and pericentromeric parts of the chromosome than in interstitial regions. The distributions of AAC, ACT, and CAT were revealed to be colocalized and were identical in intensity in this study, suggesting that AAC, ACT, and CAT may be evenly distributed in an intermingled way. The same was true for AAG and AGG. Although CAC and CAG were found to be colocalized, different hybridization intensities suggest that their distribution was close rather than intermingled.

Differences between the I and H genomes can be observed by comparing their distribution of their repetitive sequences. A distinct difference can be observed in a few trinucleotide SSR motifs. In *H. vulgare* (H genome), the AAC, AAG, and AGG motifs are colocalized around the centromere in all chromosomes; ACT produces multiple signals in six chromosome pairs; and CAT produces a strong signal and a weak signal in 4H and 5H, respectively (Cuadrado and Jouve 2007a) (Fig. 6). Another distinct difference is exhibited by the dinucleotide AG, which was intensely detected around the centromere in *H. vulgare* (Hagras et al. 2005, Carmona et al. 2013a) but was absent in *H. bogdanii*. Additional differences between *H. bogdanii* and *H. vulgare* can be seen in the distribution patterns of AAT, GCC, CAG, 45S rDNA, 5S rDNA and pSc119.2. Physical mapping of a few sequences in *H. marinum* (Xa genome) showed that AAC and AAG produced intense and rich patterns of multiple SSR signals that were particularly concentrated in the pericentromeric region and that ACT and CAT were weakly distributed (Carmona et al. 2013b). The pSc119.2 repeat produced subtelomeric signals on nearly all chromosomes in *H. marinum* (Taketa et al. 2000); however, fewer of these signals were present in *H. bogdanii*. Karyotype analysis of diploid *H. murinum* (Xu) showed that AG produced intense signals around the centromere in four chromosome pairs and that AAG produced more rich and intense signals than AAC, ACT, and CAT in pericentromeric regions (Cuadrado et al. 2013, Carmona et al. 2013b). In addition, no pSc119.2 hybridization signals were detected in diploid *H. murinum* (Taketa et al. 2000).
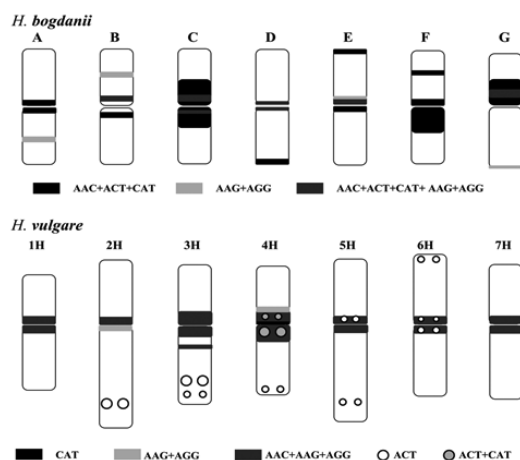
Figure 6. Idiogram of chromosomes of *H. bogdanii* (I genome) and *H. vulgare* (H genome) showing the distributions of the SSRs AAC, ACT, CAT, AAG and AGG. Chromosomal information of *H. vulgare* is taken from Cuadrado et al. (2007a)

Thus, the genomic composition of the I genome in *H. bogdanii* revealed by the distribution of several repeats was highly different from that of the H, Xa, and Xu genomes in *Hordeum*.

Autopolyploid origin of *H. brevisubulatum*

The *Hordeum brevisubulatum* species complex has a range of cytotypes and can occur in diploid, tetraploid, and hexaploid forms (Bothmer 1979, Dewey 1979, Linde-Laursen et al. 1980). Earlier studies have shown that polyploids in the *H. brevisubulatum* complex may be autoploids (Bothmer 1979, Dewey 1979). Data from meiotic pairing in the hybrids indicate that autoploidy characterizes the entire complex, with one "basic" genome (Landström et al. 1984). The karyotype of tetraploid *H. brevisubulatum* was not shown to be a strictly doubled karyotype from a diploid form. However, similar chromosomal distributions of many repetitive sequences between *H. bogdanii* and *H. brevisubulatum* were revealed in this study. Specifically, colocalized distributions of AAC, ACT, and CAT in pericentromeric regions in nearly all chromosomes and colocalized distributions of AAG and AGG in most chromosomes were observed in *H. brevisubulatum*. Nearly double the number of 45S rDNA and 5S rDNA sites in *H. bogdanii* were also detected in *H. brevisubulatum*. In addition, no genomic differentiation was detected in *H. brevisubulatum* by GISH with labelled genomic DNA of *H. bogdanii*. This pattern strongly suggests an autopolyploid origin of *H. brevisubulatum* from an I genome.

Several examples of structural rearrangements of chromosomes at the population level in the *H. brevisubulatum* complex have been reported (Landström et al. 1984). In

this study, high levels of chromosomal variation, including variation in chromosome number and chromosome structure, were observed in the population under investigation. This observation implies that there is a high degree of instability of the autoploid genome in *H. brevisubulatum.* Karyotype variations have recently been reported in re-synthesized and naturally formed allopolyploid species and in natural hybrids produced by polyploid homoploid hybridization (Chester et al. 2012, Lipman et al. 2013). Aneuploidy, inter- and intragenomic rearrangements, and the loss of repeats were frequently detected in early generations. As a natural species, *H. brevisubulatum* should be the product of a long evolutionary history. *Hordeum brevisubulatum* has been considered to be self-incompatible (Bothmer 1979). Although autopolyploidy may result from genome doubling within a single individual, most natural autopolyploids likely formed via some degree of hybridization, involving, for example, individuals from genetically differentiated populations (Stebbins 1985, Soltis and Soltis 1989). Thus, more studies are needed to determine whether karyotype variation in *H. brevisubulatum* might be affected by hybridization between genetically differentiated biotypes.

Major genetic changes, including the loss of homologs and DNA sequences, have been documented in recently formed polyploid species (Soltis et al. 2004, Abbott and Lowe 2004, Matyášek et al. 2007). Compared with the composition of the I genome in *H. bogdanii*, the distributions of a few repetitive sequences in *H. brevisubulatum* might be strongly altered by increases or decreases in copy number. The trinucleotide SSR motifs AAC, ACT, and CAT are primarily located in pericentromeric regions in all the chromosomes in *H. bogdanii*, but they are absent in a few chromosomes in *H. brevisubulatum*, possibly because of decreases in copy number. The distributions of AAG and AGG are similar. In contrast, the higher abundance of ATT and GCC in *H. brevisulatum* indicates that there is an increase in copy number that accompanies polyploidization.

Evolutionary trends of repetitive DNA sequences during genome differentiation

Repetitive DNA sequences are the main components of heterochromatin and are subject to rapid change. Such changes in the distribution of repetitive DNA sequences are one of the driving forces of genome evolution and speciation. One proposed function of repetitive sequences may be related to higher-order molecular structure (Redi et al. 2001). However, the molecular mechanisms by which genomes change are unknown (Plohl et al. 2002).

The SSR motif AAC was shown to be distributed mainly in the pericentromeric regions of the I, H, Xa, and Xu genomes. In addition, the wide distribution of AAC has been detected in wheat and the genus *Secale* (Cuadrado et al. 2000 Cuadrado and Jouve 2002). This wide distribution suggests that AAC repeats have an ancient origin in Triticeae species. The distribution of AAC in the I genome was revealed to be evenly co-localized with ACT and CAT. However, the distributions of AAC, ACT, and CAT are largely differentiated in the H, Xa, and Xu genomes. Variation in the distribution of repetitive sequences may be related to the amplification and deletion of repeat copies.

AAC, ACT, and CAT motifs in the I genome may have been co-amplified or deleted as a single repetitive unit. However, AAC, ACT, and CAT in other genomes have evolved independently. AAG exhibits the most chromosomal variation both within and between taxa (Pedersen et al. 1996, Pedersen and Langridge 1997, Cuadrado et al. 2008, Carmona et al. 2013a). AAG has been suggested to be more predisposed to being amplified or deleted relative to other repetitive sequences as a consequence of independent events in different lineages (Carmona et al. 2013b). The fact that AAG is evenly colocalized with AGG in the I genome suggests that the same evolutionary mechanism also drives variation in the chromosomal distribution of AAC, ACT, and CAT.

AC repeat sites are remarkably similar and have been shown to exhibit uniformly dispersed hybridization along the euchromatic portion of metaphase chromosomes in humans and barley and in the metaphase and polytene chromosomes of *Drosophila melanogaster* (Meigen, 1830) (Cuadrado and Jouve 2007b). In this study, AC repeat sites had a dispersed distribution. High-intensity hybridization bands for AC repeats were observed in subtelomeric and pericentromeric regions on a few chromosomes in the I genome. This atypical distribution suggests that there is a more complicated organization or function of AC repeats in plant genomes.

The sequence pAs1 contains Afa family sequences. The Afa family sequence was abundant in the four basic genomes of the genus *Hordeum*, and the hybridization patterns differed among the diploid species (Taketa et al. 2000). The distribution of the Afa family is more dispersed than those of the other tandem repetitive sequences. This dispersed distribution implies that the Afa family may be more commonly distributed in gene rich regions. In our study, despite the autopolyploidy origin of *H. brevisubulatum* from an I genome species, the distribution of pAs1 sequences in *H. brevisubulatum* was highly variable relative to those in diploid *H. bogdanii*. The Afa family of sequences may play an important role in the differentiation of either genome or species in the genus *Hordeum*.

Variation in the abundance and distribution of repetitive sequences and the shared distribution of the same repeats between different genomes suggest that repetitive sequences play a key role in both the structure and function of the genomes of higher eukaryotes (Cuadrado et al. 2008). To date, most information on the distribution of repeats in *Hordeum* genomes comes from physical mapping analysis of mitotic metaphase chromosomes, which are highly packaged. Physical mapping of repetitive sequences on extended chromosomes, such as during the pachytene stage of meiosis, may provide more valuable information on the chromosomal distribution of repeats and may help elucidate the evolution and genomic function of repetitive sequences.

Conclusion

Fifteen repetitive sequences, including SSR motif AC and all possible trinucleotide motifs and satellite DNAs pAs1, 5S rDNA, 45s rDNA, and pSc119.2, were accurately physically mapped on individual chromosomes in the I genome in *H. bogdanii*. High

genome instability was revealed in tetraploid *H. brevisubulatum*. The similar distribution of the repeats in both species suggests an autopolyploid origin of *H. brevisubulatum* from an I genome species. Comparative cytogenetic analysis between the I genome and other genomes in *Hordeum* showed that the distribution of a few repeats differed. Colocalization of motifs AAC, ACT, and CAT and colocalization of motifs AAG and AGG is characteristic of the I genome.

Acknowledgements

References

Abbott RJ, Lowe AJ (2004) Origins, establishment and evolution of new polyploid species: *Senecio cambrensis* and *S. eboracensis* in the British Isles. Biological Journal of the Linnean Society 82: 467–74. doi: 10.1111/j.1095-8312.2004.00333.x

Badaeva ED, Amosova AV, Muravenko OV, Samatadze TE, Chikida NN, Zelenin AV, Gill BS (2002) Genome differentiation in *Aegilops*. 3. Evolution of the D-genome cluster. Plant Systematics and Evolution 231(1-4): 163–190. doi: 10.1007/s006060200018

Badaeva ED, Friebe B, Gill BS (1996) Genome differentiation in *Aegilops*. 1. Distribution of highly repetitive DNA sequences on chromosomes of diploid species. Genome 39(2): 293–306. doi: 10.1139/g96-040

Bedbrook JR, Jones J, O'Dell M, Thompson RD, Flavell RB (1980) A molecular description of telomeric heterochromatin in *Secale* species. Cell 19: 545–560. doi: 10.1016/0092-8674(80)90529-2

Blattner FR (2009) Progress in phylogenetic analysis and a new infrageneric classification of the barley genus *Hordeum* (Poaceae: Triticeae). Breeding Science 59(5): 471–480. doi: 10.1270/jsbbs.59.471

Bothmer R (1979) Revision of the Asiatic taxa of *Hordeum* sect. *Stenostachys*. Botanisk Tidsskrift 74: 117–146.

Bothmer R, Jacobsen N, Baden C, Jorgensen RB, Linde-Laursen I (1995) An ecogeographical study of the genus *Hordeum*, 2nd edn. Systematic and ecogeographical studies on crop Gene pools 7. IBPGR, Rome.

Bothmer R, Flink J, Landström T (1986) Meiosis in interspecific *Hordeum* hybrids. I. Diploid combinations. Canadian Journal of Genetics and Cytology 28(4): 525–535. doi: 10.1139/g86-077

Carmona A, Friero E, de Bustos A, Jouve N, Cuadrado A (2013a) Cytogenetic diversity of SSR motifs within and between *Hordeum* species carrying the H genome: *H. vulgare* L. and *H. bulbosum* L. Theoretical and Applied Genetics 126(4): 949–961. doi: 10.1007/s00122-012-2028-y

Carmona A, Friero E, de Bustos A, Jouve N, Cuadrado A (2013b) The evolutionary history of sea barley (*Hordeum marinum*) revealed by comparative physical mapping of repetitive DNA. Annals of Botany 112(9): 1845–1855. doi: 10.1093/aob/mct245

Chester M, Gallagher JP, Symonds VV, Cruz da Silva AV, Mavrodiev EV, Leitch AR, et al. (2012) Extensive chromosomal variation in a recently formed natural allopolyploid species, *Tragopogon miscellus* (Asteraceae). Proceeding of the National Academy of Sciences of the United States of America 109(4): 1176–1181. doi: 10.1073/pnas.1112041109

Cuadrado A, Cardoso M, Jouve N (2008) Physical organisation of simple sequence repeats (SSRs) in Triticeae: structural, functional and evolutionary implications. Cytogenetic and Genome Research 120(3-4): 210–219. doi: 10.1159/000121069

Cuadrado A, Carmona A, Jouve N (2013) Chromosomal Characterization of the Three Subgenomes in the Polyploids of *Hordeum murinum* L.: New Insight into the Evolution of This Complex. PLoS ONE 8(12): e81385. doi: 10.1371/journal.pone.0081385

Cuadrado A, Ceoloni C, Jouve N (1995) Variation in highly repetitive DNA composition of heterochromatin in rye studied by fluorescence in situ hybridization. Genome 38: 101–1069. doi: 10.1139/g95-142

Cuadrado A, Jouve N (2002) Evolutionary trends of different repetitive DNA sequences during speciation in the genus *Secale*. Journal of Heredity 93(5): 339–345. doi: 10.1093/jhered/93.5.339

Cuadrado A, Jouve N (2007a) The nonrandom distribution of long clusters of all possible classes of trinucleotide repeats in barley chromosomes. Chromosome Research 15(6): 711–720. doi: 10.1007/s10577-007-1156-8

Cuadrado A, Jouve N (2007b) Similarities in the chromosomal distribution of AG and AC repeats within and between *Drosophila*, human and barley chromosomes. Cytogenetic and Genome Research 119(1-2): 91–99. doi: 10.1159/000109624

Danilova TV, Friebe B, Gill BS (2012) Single-copy gene fluorescence in situ hybridization and genome analysis: *Acc-2* loci mark evolutionary chromosomal rearrangements in wheat. Chromosoma 121(6): 597–611. doi: 10.1007/s00412-012-0384-7

Dewey DR (1979) The *Hordeum vioceum* complex of Iran. American Journal of Botany 66: 166–172. doi: 10.2307/2442520

Dou QW, Chen ZG, Liu YA, Tsujimoto H (2009) High frequency of karyotype variation revealed by sequential FISH and GISH in plateau perennial grass forage *Elymus nutans*. Breeding science 59(5): 651–656. doi: 10.1270/jsbbs.59.651

Flavell RB, Bennett MD, Smith JB, Smith DB (1974) Genome size and the proportion of repeated nucleotide sequence DNA in plants. Biochemical Genetics 12(4): 257–269. doi: 10.1007/BF00485947

Flavell RB, Rimpau J, Smith DB (1977) Repeated sequence DNA relationships in four cereal genomes. Chromosoma 63: 205–222. doi: 10.1007/BF00327450

Fukui K, Kamisugi Y, Sakai F (1994) Physical mapping of 5S rDNA loci by direct-cloned biotinylated probes in barley chromosomes. Genome 37(1): 105–111. doi: 10.1139/g94-013

Hagras A, Kishii M, Tanaka H, Sato K, Tsujimoto H (2005) Genomic differentiation of *Hordeum chilense* from *H. vulgare* as revealed by repetitive and EST sequences. Genes & Genetic Systems 80(3): 147–159. doi: 10.1266/ggs.80.147

Heslop-Harrison J, Schwarzacher T (2011) Organisation of the plant genome in chromosomes. The Plant Journal 66(1): 18–33. doi: 10.1111/j.1365-313X.2011.04544.x

Jiang J, Gill BS (2006) Current status and the future of fluorescence in situ hybridization (FISH) in plant genome research. Genome 49(9): 1057–1068. doi: 10.1139/G06-076

Kato A (1999) Air drying method using nitrous oxide for chromosome counting in maize. Biotechnic & Histochemistry 74(3): 160–166. doi: 10.3109/10520299909047968

Landström T, Bothmer R, Dewey DR (1984) Genomic relationships in the *Hordeum brevisubulatum* complex. Canadian Journal of Genetics and Cytology 26(5): 569–577. doi: 10.1139/g84-090

Linde-Laursen I, Bothmer R, Jacobsen N (1980) Giemsa C-banding in Asiatic taxa of *Hordeum* section *Stenostachys* with notes on chromosome morphology. Hereditas 93(2): 235–254. doi: 10.1111/j.1601-5223.1980.tb01364.x

Linde-Laursen I, Bothmer R, Jacobsen N (1992) Relationships in the genus *Hordeum*: Giemsa C-banded karyotypes. Hereditas 116(1–2): 111–116. doi: 10.1111/j.1601-5223.1992.tb00213.x

Lipman MJ, Chester M, Soltis PS, Soltis DE (2013) Natural hybrids between *Tragopogon mirus* and *T. miscellus* (Asteraceae): a new perspective on karyotypic changes following hybridization at the polyploid level. American Journal of Botany 100(10): 2016–2022. doi: 10.3732/ajb.1300036

Matyášek R, Tate JA, Lim YK, Šrubařová H, Koh J, Leitch AR, Kovařík A (2007) Concerted evolution of rDNA in recently formed *Tragopogon* allotetraploids is typically associated with an inverse correlation between gene copy number and expression. Genetics 176(4): 2509–2519. doi: 10.1534/genetics.107.072751

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(1): 93–104. doi: 10.1139/g96-013

Pedersen C, Langridge P (1997) Identification of the entire chromosome complement of bread wheat by two-colour FISH. Genome 40(5): 589–593. doi: 10.1139/g97-077

Plohl M, Luchetti A, Mestrovic N, Mantovani B (2002) Satellite DNAs between selfishness and functionality: structure, genomics and evolution of tandem repeats in centromeric (hetero) chromatin. Gene 409: 72–82. doi: 10.1016/j.gene.2007.11.013

Rayburn AL, Gill BS (1986) Isolation of a D-genome specific repeated DNA sequence from *Aegilops squarrosa*. Plant Molecular Biology Reporter 4: 102–109. doi: 10.1007/BF02732107

Redi C, Garagna S, Zacharias H, Zuccotti M, Capanna E (2001) The other chromatin. Chromosoma 110(3): 136–147. doi: 10.1007/s004120000114

Soltis DE, Soltis PS (1989) Genetic consequences of autopolyploidy in *Tolmiea* (Saxifragaceae). Evolution 43: 586–94. doi: 10.2307/2409061

Soltis DE, Soltis PS, Pires JC, Kovarik A, Tate J, Mavrodiev E (2004) Recent and recurrent polyploidy in *Tragopogon* (Asteraceae): cytogenetic, genomic and genetic comparisons. Biological Journal of the Linnean Society 82: 485–501. doi: 10.1111/j.1095-8312.2004.00335.x

Stebbins GL (1985) Polyploidy, hybridization, and the invasion of new habitats. Annals of the Missouri Botanical Garden 72: 824–32. doi: 10.2307/2399224

Taketa S, Ando H, Takeda K, Harrison G, Heslop-Harrison J (2000) The distribution, organization and evolution of two abundant and widespread repetitive DNA sequences in the genus *Hordeum.* Theoretical and Applied Genetics 100(2): 169–176. doi: 10.1007/s001220050023

Taketa S, Ando H, Takeda K, Bothmer R (2001) Physical locations of 5S and 18S-25S rDNA in Asian and American diploid *Hordeum* species with the I genome. Heredity 86(5): 522–530. doi: 10.1046/j.1365-2540.2001.00768.x

Taketa S, Harrison G, Heslop-Harrison J (1999) Comparative physical mapping of the 5S and 18S-25S rDNA in nine wild *Hordeum* species and cytotypes. Theoretical and Applied Genetics 98(1): 1–9. doi: 10.1007/s001220051033

Tang Z, Yang Z, Fu S (2014) Oligonucleotides replacing the roles of repetitive sequences pAs1, pSc119. 2, pTa-535, pTa71, CCS1, and pAWRC. 1 for FISH analysis. Journal of Applied Genetics 55(3): 313–318. doi: 10.1007/s13353-014-0215-z

Tsujimoto H, Mukai Y, Akagawa K, Nagaki K, Fujigaki J, Yamamoto M, Sasakuma T (1997) Identification of individual barley chromosomes based on repetitive sequences: conservative distribution of Afa-family repetitive sequences on the chromosomes of barley and wheat. Genes & Genetic Systems 72(5): 303–309. doi: 10.1266/ggs.72.303

Yang J, Yen C, Lu B, Bothmer R (1987) The genus *Hordeum* (Gramineae) in China. Variation and distribution. Willdenowia 16: 479–490.