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Chromosomal distribution of telomeric and telomeric-associated sequences in *Hordeum chilense* by in situ hybridization

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The chromosomal distribution of telomeric repeat pAtT4 from *Arabidopsis thaliana* and telomeric associated repetitive sequence HvT01 from *Hordeum vulgare* have been studied by FISH (fluorescence in situ hybridization) in two accessions (H1 and H7) of *Hordeum chilense*. The telomeric sequence pAtT4 is present at the end of all chromosome arms in H1 and H7 accessions. In contrast, the telomeric associated sequence homologous to HvT01 showed variability for size, intensity and position of the signals for each line. In H1, HvT01 was present in every chromosome whereas only four chromosomes were labeled in H7 accession. Physical distribution of GAA-satellite sequence on both H1 and H7 metaphase chromosomes was also studied. Polymorphism for hybridization signals between the two accessions for GAA-banding pattern was also found. Based on differences in position and intensity of the hybridization signals found for both GAA and HvT01-homologous sequences, karyotypes for the in situ hybridization patterns are presented for H1 and H7 accessions of *H. chilense*.

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The telomeric regions of eukaryotic chromosomes, including the telomeric repeat (TR) and telomere-associated sequences (TAS), have important functions in the protection, replication and stabilisation of the chromosome ends and they provide also important landmarks for the termini of genetic and physical maps of chromosomes. In higher plants, telomere repeat has been first cloned from *Arabidopsis thaliana* (Richards and Ausubel 1988) and the consensus sequence of the telomeric oligonucleotide was determined as 5′-TTTAGGG-3′.

The *Arabidopsis* telomere clone has been used for in situ hybridization to chromosomes of several monocots and dicots species (Simpson et al. 1990; Ganal et al. 1992; Schwarzacher and Heslop-Harrison 1991; Wang et al. 1991) and in all of them the sequence hybridize exclusively to the outhermost ends of chromosomes indicating a high level of conservation among the telomeric repeats in higher plant species except some species such as *Allium* spp. (Pich et al. 1996), *Aloe* spp. (Adams et al. 2000) and *Cestrum* (Sykorova et al. 2003). By opposite, telomeres-associated sequences (TAS) are organised in long arrays of hypervariable tandemly repeat units and they have subtelomeric position (Rayburn and Gill 1987; Wu et al. 1991).

The HvRT is a family of subtelomeric tandemly repeated sequences isolated from barley which shows a characteristic, but different, species-specific distribution among *Hordeum* species (Belostotsky and Ananiev 1990).

These subtelomeric repeat sequences are much more divergent than evolutionary conserved telomeric sequences and they have been cloned and used as molecular markers to cap genetic maps in wheat (Mao et al. 1997) and barley (Kilian et al. 1999).

Hordeum chilense Roem. et Schult. is a wild South American diploid barley included in the section *Anisolepis*. It occurs exclusively in Chile and Argentina and it is highly polymorphic both morphologically and biochemically (Bothmer et al. 1995). This wild barley species is used in the development of hexaploid tritordeum (amphiploids between *H. chilense* and tetraploid wheats) to be used as a new cereal (Martín 1998) and to introgress into wheat novel agronomic traits such as resistance to both root-knot nematode *Meloidogyne naasi* and *Septoria*, tolerance to salt, and high carotenoid pigment content (Martín et al. 2000).

To investigate the structure of telomeres in *H. chilense*, clone pAtT4, containing the telomeric repeat of *A. thaliana* and a PCR product corresponding to the barley subtelomeric HvT01 sequence were used as hybridization probes to chromosomes of H1 and H7 accessions of *H. chilense*. We also studied the in situ hybridization pattern of the GAA-satellite sequence (Dennis et al. 1980) on chromosomes of both H1 and H7 accessions. The pattern of hybridization obtained with this sequence allowed identification of all seven chromosome pairs in the two *H. chilense* accessions.

MATERIAL AND METHODS

Root tips were collected from germinated seeds of accessions H1 and H7 of *Hordeum chilense* ($2n = 2x = 14$). These root tips were pretreated for 3 h in a 0.05% colchicine solution at 25°C and fixed in 100% ethanol-acetic acid, 3:1 (v/v), for at least a week at room temperature. Root tips were then stained in acetocarmine for 3 min, scraped out the meristems and squashed in 45% acetic acid on ethanol-cleaned slides. The preparations were frozen in liquid nitrogen and the coverslips subsequently removed. The air-dried slides were stored at 4°C until used.

Telomere clone used was pAtT4 probe from *A. thaliana* (Richards and Ausubel 1988). A PCR product of the barley subtelomeric tandem repeat HvT01, was obtained by amplification by the polymerase chain reaction of genomic DNA from the barley cv. Betzes using primers made according to the published sequence (Belostotsky and Ananiev 1990) and kindly supplied by Dr. Shubert and Zuzana Jasencakova. Briefly, the thermal cycling protocol consisted of an initial heating activation step of 94°C for 10 min followed by 35 amplification cycles (15 s denaturation at 94°C, 15 s annealing at 50°C, 30 s of extension at 72°C), and a final extension of 20 min at 72°C using a system 9600 cycler from PE Biosystems.

The pAtT4 probe was labeled with biotin-11-dUTP (Roche Corporate, Postfach, Basel, Switzerland) by nick translation and the PCR product corresponding to the barley satellite HvT01 probe was labeled with both biotin-11-dUTP and digoxigenin-11-dUTP, (Roche Corporate, Postfach, Basel, Switzerland) by nick translation, respectively. Biotin-labeled pAtT4 and digoxigenin-labelled HvT01 probes were used simultaneously in double hybridization experiments to detect both telomeric and subtelomeric regions, respectively.

Preparations were also hybridized with biotin-labeled HvT01 probe. After examination of metaphases hybridized with that probe, they were re-probed with digoxigenin-labeled GAA-satellite sequence for identifying chromosomes with positive signals, as described by Pedersen and Langridge (1997). GAA-satellite sequence isolated from barley (Pedersen et al. 1996) was kindly provided by Dr. S. K. Rasmussen from the Risϕ National Laboratory, Roskilde (Denmark).

The hybridization mixture consisted on 50% formamide, $2 \times$SCC, 5 ng of biotin-labeled or digoxigenin-labeled probe, 10% dextran sulfate, 0.14 μg of yeast tRNA, 0.1 μg of sonicated salmon sperm DNA and 0.005 μg of glycogen. In situ hybridization protocol was performed as previously described by Cabrera et al. (2002).

Both biotin-labeled and digoxigenin-labeled probes were detected with Streptavidin-Cy3 conjugate (Sigma, St. Louis, MO, USA) and anti-digoxigenin-FITC (Roche Corporate, Postfach, Basel, Switzerland), respectively. Chromosomes were counterstained with DAPI (4′,6-diamidino-2-phenylindole) and mounted in Vectashield. Signals were visualized using a Leica epifluorescence microscope. Images were captured with a SPOT CCD camera using the appropriate SPOT 2.1 software (Diagnostics Instruments, Inc., Sterling Heights, Michigan, USA) and processed with PhotoShop 4.0 software (Adobe Systems Inc., San Jose, California, USA). Images were printed on a Hewlett Packard Deskjet HP 840C Color Printer. In situ hybridization pictures of *H. chilense* chromosomes shown in Fig. 2 were obtained from the negative after conversion to gray scale.

RESULT AND DISCUSSION

FISH with clone pAtT4, containing the telomeric repeat of *A. thaliana*, to somatic metaphase chromosome spreads of *H. chilense* accessions H1 and H7 are shown in Fig. 1a and 1d, respectively. Hybridization signals were found at the ends of all seven chromosomes pairs in the two accessions showing that the telomere sequence of *Arabidopsis* is also found in the genome of *H. chilense*. This finding supports previous studies, which have shown that the DNA sequences constituting functional telomeres are highly conserved in plants species (Richards and Ausubel 1988; Schwarzacher and Heslop-Harrison 1991; Cox et al. 1993). As it has been previously found in barley (Wang et al. 1991) and tomato (Ganal et al. 1991), in situ hybridization signals with pAtT4 clone showed different intensities among the chromosomes within the genome and between chromosome arms in both H1 and H7 accessions indicating differences in copy numbers of the telomeric repeat in the two accessions. Variation in size and signal intensity between telomeres of homologous chromosomes **1H^{ch}** and **2H^{ch}** in H1 accession (Fig. 1a) and **3H^{ch}** and **6H^{ch}** in H7 accession (Fig. 1d) have also been found in the present work.

In situ hybridization using a PCR-generated probe of the barley subtelomeric tandem repeat HvT01 to *H. chilense* metaphase chromosomes showed the presence of hybridization signals on the chromosomes ends of both H1 and H7 accessions (Fig. 1b and 1e) indicating that sequences homologous to the barley HvT01 satellite sequence are also present in the chromosomes of *H. chilense*. In barley, in situ hybridization of satellite HvT01 to metaphase chromosomes showed the presence of hybridization signals

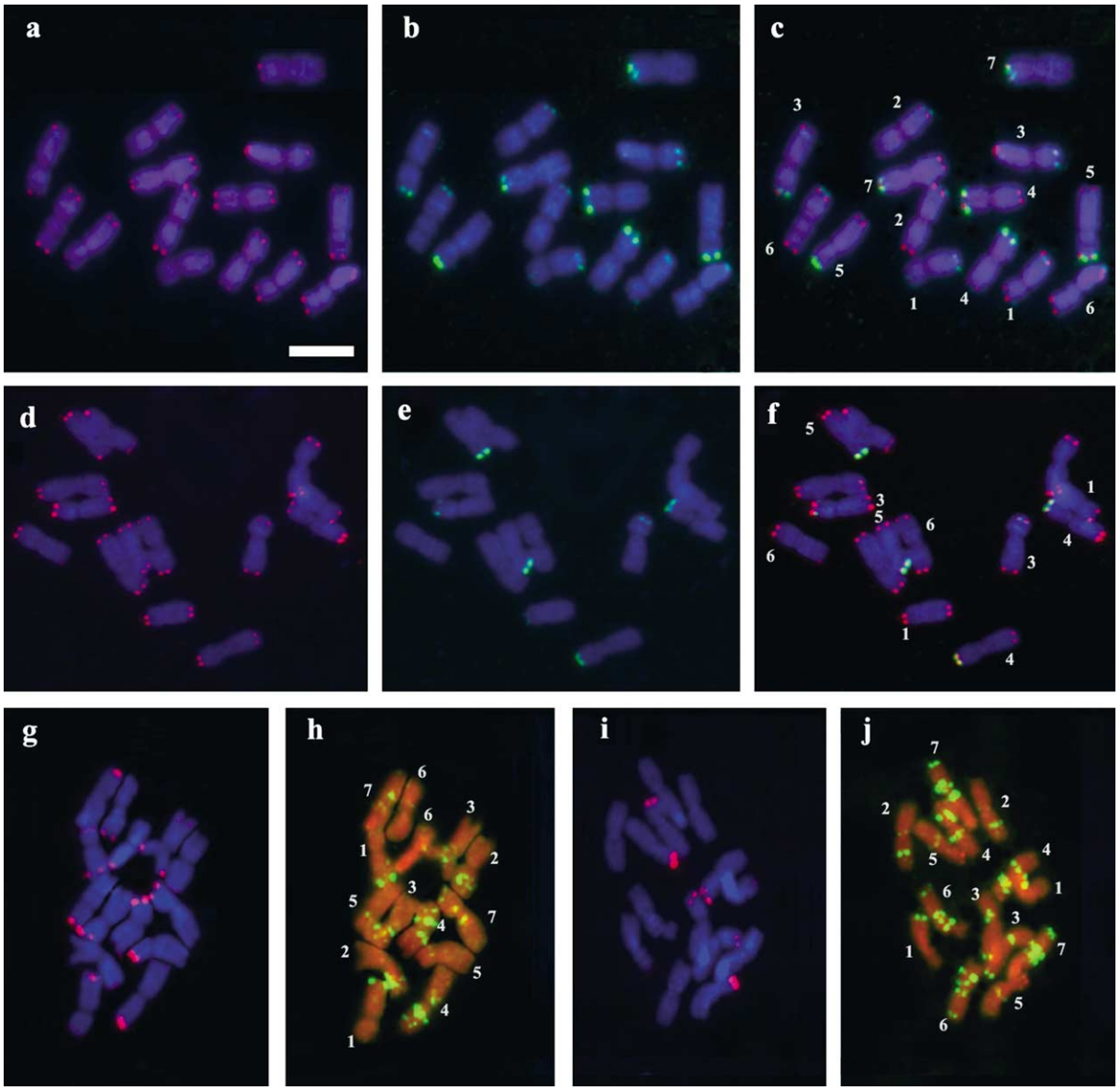

Fig. 1a–j. In situ hybridization of the barley satellite HvT01, repeat pAtT4 sequence and GAA satellite sequence in accesions H1 and H7. (**a**) In situ hybridization of the pAtT4 sequence to accesion H1 and (**d**) accesion H7; double exposure of pAtT4 probe detected with sulforhodamine (red) and DNA-specific dye, 4′,6-diamidino-2-phenylindole (DAPI). (**b**) In situ hybridization of the HvT01 sequence to accesion H1 and (**e**) accesion H7; double exposure of HvT01 probe detected with fluorescein isothiocynte (FITC) fluorescence (yellow/green) and DNA-specific dye, 4′,6-diamidino-2-phenylindole (DAPI). (**c**) Figure (**a**) and (**b**) superposed. (**f**) Figure (**d**) and (**e**) superposed. (**g**) In situ hybridization of the HvT01 sequence to accesion H1 and (**i**) accesion H7; double exposure of HvT01 probe detected with sulforhodamine (red) and DNA-specific dye, 4′,6-diamidino-2-phenylindole (DAPI). (**h**) In situ hybridization of the GAA satellite sequence to accesion H1 and (**j**) accesion H7; double exposure of GAA probe detected with fluorescein isothiocynte (FITC) fluorescence (yellow/green) and DNA-specific dye, propidium iodade (PI). Scale bar 10 μm.

on all except one chromosome arms (Röder et al. 1993). Differences in the distribution of HvT01-homologous sequence between H1 and H7 chromosomes have been found in the present work. The sequence is present in all seven pairs of chromosomes in H1 accession (9 of the 14 chromosome arms showed hybridization signals) whereas only four chromosome arms in accession H7 were labeled with the barley HvT01 sequence (Fig. 1e and 1i, respectively).

Identification of chromosomes with positive HvT01 signals was carried out after reprobing chromosome preparations with GAA-satellite sequence on both H1

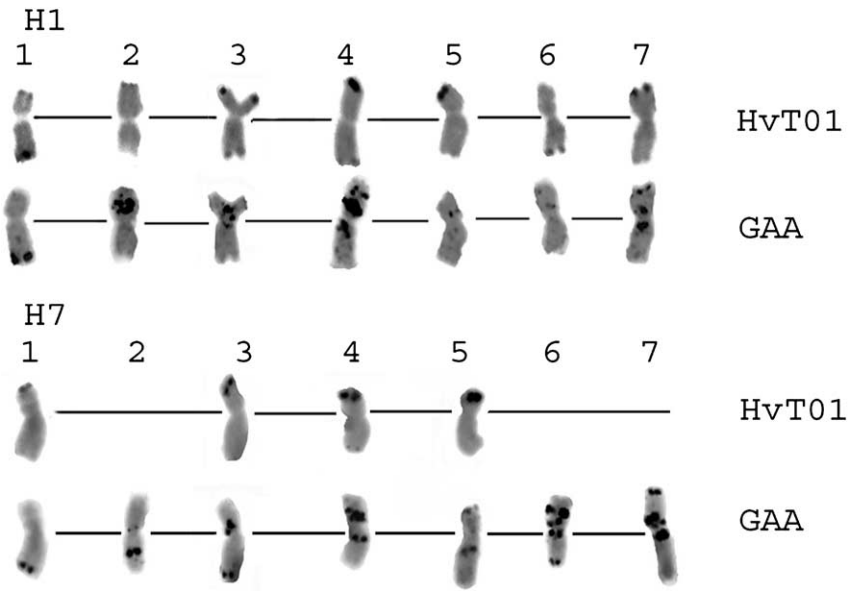

Fig. 2. FISH patterns of the subtelomeric HvT01and GAA-satellite probes on the *H. chilense* chromosomes in the two accesions H1 and H7.

and H7 accessions (Fig. 1h and 1j). Based on differences in position and intensity of the hybridization signals found for both GAA and HvT01-homologous sequences in H1 and H7 accessions, karyotypes for the in situ hybridization patterns using both probes are present for each line (Fig. 2). In H1, HvT01-homologous sequence is present in all chromosome arms except **2H^{ch}L**, **5H^{ch}L**, **6H^{ch}S** and **7H^{ch}L**. In H7, signals are present in the short arm of chromosomes **1H^{ch}**, **3H^{ch}**, **4H^{ch}** and **5H^{ch}**. No HvT01 hybridization signals were detected on any of the long arms of the seven chromosome pairs in H7. There is lack of chromosomes 2, 6 and 7. The highest signal intensities were observed on the short-arm of chromosomes **4H^{ch}** and **5H^{ch}** in both H1 and H7 accessions which imply that these short-arms contain the highest number of copies of subtelomeric repeats.

Analysis of subtelomeric regions in rye (Bedbrook et al. 1980; Vershinin et al. 1995), onion (Barnes et al. 1985), tomato (Ganal et al. 1992), rice (Wu and Tanksley 1993) and barley (Röder et al. 1993) has revealed that they are organized in long arrays of tandem repeat units often very close to the telomeres. The results of double in situ hybridization using both pAtT4 and satellite HvT01 sequences to *H. chilense* chromosomes allowed the detection of both telomeric and subtelomeric probes simultaneously (Fig. 1c and 1f). In some of the chromosome arms, the subtelomeric location of the HvT01 sequence cannot be distinguished from the telomeric location of the pAtT4. However, clear double hybridization signals corresponding to in situ location of both probes were observed very close to each other at the end of some chromosome arms in H1 accession. The close physical proximity of tandemly arranged repetitive HvT01 and telomere sequences found in the present work agree with that previously published in yeast (Chan and Tye 1983), *Plasmodium* (Corcoran et al. 1988) and barley (Kilian et al. 1999) suggesting that close association between TAS and TR seems to be a characteristic of the subtelomeric regions in eukaryotes.

The GAA probe was found to hybridize to specific areas along the chromosomes of both H1 and H7 accessions, allowing the identification of all seven pairs of the *H. chilense* chromosomes in the two accessions (Fig. 2). Similarities in GAA banding patterns between the two accessions for the chromosomes **1H^{ch}**, **3H^{ch}**, **4H^{ch}** and **7H^{ch}** have been found. In contrast, polymorphic differences were found between the two accessions for chromosomes **2H^{ch}**, **5H^{ch}** and **6H^{ch}**. The GAA-satellite sequence has previously been used for in situ hybridization in wheat and barley enabling the identification of 16 chromosome pairs in wheat (Pedersen and Langridge 1997) and all the chromosomes of barley (Pedersen et al. 1996). The identification of the chromosomes by these authors was based on the previously described similarity between N-banding patterns and in situ hybridization with the GAA-satellite sequence (Dennis et al. 1980). N-banding patterns of *H. chilense* accessions H1 and H7 have been previously published (Gonzalez and Cabrera 1999) and a good correspondence exists between GAA-banding pattern obtained in the present work and N-banding pattern of the two accessions. However, polymorphic differences between the

two accessions for N-banding prevent the definite identification of some N-banded chromosomes in these lines. In the present work, identification of H1 and H7 chromosomes hybridized with pAtT4, HvT01 or GAA-satellite sequences was confirmed after re-probing the chromosome preparations with pAs1 probe (data not shown) which was found to hybridize to multiple sites on the seven $\mathbf{H^{ch}}$ chromosomes, allowing the identification of all seven pairs of the *H. chilense* chromosomes (Cabrera et al. 1995). Comparisons between GAA banding pattern obtained in the present work and N-banded karyotypes previously reported (Gonzalez and Cabrera 1999) led to the correct identification of some misidentified *H. chilense* chromosomes. N-banded $\mathbf{2H^{ch}}$, $\mathbf{6H^{ch}}$ and $\mathbf{7H^{ch}}$ chromosomes in H1 accession are actually chromosomes $\mathbf{7H^{ch}}$, $\mathbf{2H^{ch}}$ and $\mathbf{6H^{ch}}$ and N-banded $\mathbf{2H^{ch}}$, $\mathbf{3H^{ch}}$, $\mathbf{5H^{ch}}$ and $\mathbf{7H^{ch}}$ chromosomes in H7 are actually $\mathbf{7H^{ch}}$, $\mathbf{2H^{ch}}$, $\mathbf{3H^{ch}}$ and $\mathbf{5H^{ch}}$, respectively.

Differences in positions and intensity of hybridization signals of both HvT01 telomeric associated sequence and GAA-satellite sequence on metaphase chromosomes of H1 and H7 accessions indicated that these two *H. chilense* accessions are polymorphic for the physical localization of these repetitive sequences and agree with morphological and biochemical polymorphic variation previously found between the two accessions. These results confirmed that H1 and H7 accessions belongs to distinct ecotype groups as found previously (Vaz Patto et al. 2001) based on morphological and AFLPs markers and offers an additional tool help on the introgression of *H. chilense* chromosomes into wheat.

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REFERENCES

Adams, S. P., Leitch, I. J., Bennet, M. D. et al. 2000. *Aloe* L. – a second plant family withough (TTTAGGG)(n) telomeres. – Chromosoma 109: 201–205.

Barnes, S. R., James, A. M. and Jamieson, G. 1985. The organisation, nucleotide sequence, and chromosomal distribution of a satellite DNA from *Allium cepa*. – Chromosoma 92: 185–192.

Bedbrook, J. R., Jones, J., O'Dell, M. et al. 1980. A molecular descripption of telomeric heterochromatin in *Secale* species. – Cell 19: 545–560.

Belostotsky, D. A. and Ananiev, E. V. 1990. Characterization of relic DNA from barley genome. – Theor. Appl. Genet. 80: 374–380.

Bothmer, R. von, van Jacobsen, N., Baden, C. et al. 1995. An ecogeographical study of the genus *Hordeum*, 2 ed. Systematic and ecogeographical studies on crop genepools 7. – Int. Plant Genetic Resour. Inst., Rome, p. 1–129.

Cabrera, A., Friebe, B., Jiang, J. et al. 1995. Characterization of *Hordeum chilense* chromosomes by C-banding and in situ hybridization using highly repeated DNA probes. – Genome 38: 435–442.

Canbrera, A., Martin, A. and Barro, F. 2002. In-situ comparative mapping (ISCM) of Glu-1 loci in *Triticum* and *Hordeum*. – Chromosome Res. 10: 49–54.

Chan, C. S. M. and Tye, B. K. 1983. Organization of DNA sequences and replication origins at yeast telomeres. – Cell 33: 563–573.

Corcoran, L. M., Thompson, J. K., Walliker, D. et al. 1988. Homologous recombination within subtelomeric repeat sequences generates chromosome size polymorphisms in *P. falciparum*. – Cell 53: 807–813.

Cox, A. V., Bennett, S. T., Parokonny, A. S. et al. 1993. Comparison of plant telomere localizations using a PCR-generated synthetic probe. – Ann. Bot. 72: 239–247.

Dennis, E. S., Gerlach, W. L. and Peacock, W. J. 1980. Identical polypyrimidine-polypurine satellite DNAs in wheat and barley. – Heredity 44: 349–366.

Ganal, M. W., Lapitan, N. L. and Tanksley, S. D. 1991. Macrostructure of the tomato telomeres. – Plant Cell 3: 87–94.

Ganal, M. W., Broun, P. and Tanksley, S. D. 1992. Genetic mapping of tandemly repeated telomeric DNA sequences in tomato (*Lycopersicon esculentum*). – Genomics 14: 444–448.

Gonzalez, M. J. and Cabrera, A. 1999. Identification of wheat and tritordeum chromosomes by genomic in situ hybridization using total *Hordeum chilense* DNA as probe. – Genome 42: 1194–2000.

Kilian, A., Kudrna, D. and Kleinhofs, A. 1999. Genetic and molecular characterization of barley chromosome telomeres. – Genome 42: 412–419.

Mao, L., Devos, K. M., Zhu, L. et al. 1997. Cloning and genetic mapping of wheat telomere-associated sequences. – Mol. Gen. Genet. 254: 584–591.

Martín, A. 1998. *Tritordeum*: the first ten years. – Rachis 7: 12–15.

Martín, A., Cabrera, A., Hernández, P. et al. 2000. Prospect for the use of *Hordeum chilense* in durum wheat breeding. – In: Royo, C., Nachit, M. M., Fonzo, Nd et al. (eds), Durum wheat improvement in the Mediterranean region: new challenges. IAMZ, Zaragoza, Spain, p. 111–115.

Pedersen, C. and Langridge, P. 1997. Identification of the entire chromosome complement of bread wheat by two-colour FISH. – Genome 40: 589–593.

Pedersen, C., Rasmussen, S. K. and 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.

Pich, U., Fuchs, J. and Schubert, I. 1996. How do Alliaceae stabilize their chromosome ends in the absence of TTTAGGG sequence? – Chromosome Res. 4: 207–213.

Rayburn, A. L. and Gill, B. S. 1987. Molecular analysis of the D-genome of the Triticeae. – Theor. Appl. Genet. 73: 385–388.

Richards, E. J. and Ausubel, F. M. 1988. Isolation of a higher eukaryotic telomere from *Arabidopsis thaliana*. – Cell 53: 127–136.

Röder, M. S., Lapitan, N. L. V., Sorrells, M. et al. 1993. Genetic and physical mapping of barley telomeres. – Mol. Gen. Genet. 238: 294–303.

Schwarzacher, T. and Heslop-Harrison, J. S. 1991. In situ hybridization to plant telomeres using synthetic oligomers. – Genome 34: 317–323.

Simpson, P. R., Newman, M. A., Davis, D. R. et al. 1990. Identification of translocations in pea by in situ hybridization with chromosome-specific probes. – Genome 33: 745–749.

Sykorova, E., Lim, K. Y., Chase, M. W. et al. 2003. The absence of *Arabidopsis*-type telomeres in *Cestrum* and closely related genera Vestia and Sessea (Solanaceae): first evidence from eudicots. – Plant J. 34: 283–291.

Vaz Patto, M. C., Aardse, A., Buntjer, J. et al. 2001. Morphology and AFLP markers suggest three *Hordeum chilense* ecotypes that differ in avoidance to rust fungi. – Can. J. Bot. 79: 204–213.

Vershinin, A. V., Schwarzacher, T. and Heslop-Harrison, J. S. 1995. The large-scale genomic organization of repetitive DNA families at the telomeres of rye chromosomes. – Plant Cell 7: 1823–1833.

Wang, S., Lapitan, N. L. V. and Tsuchiya, T. 1991. Characterization of telomeres in *Hordeum vulgare* chromosomes by in sity hybridization I. Normal diploid barley. – Jpn. J. Genet. 66: 313–316.

Wu, H. K., Chung, M. C., Wu, T. et al. 1991. Localization of specific repetitive DNA sequences in individual rice chromosomes. – Chromosoma 100: 330–338.

Wu, K. S. and Tanksley, S. D. 1993. Abundance, polymorphism and genetic-mapping of microsatellites in rice. – Mol. Gen. Genet. 241: 225–235.