J Appl Genetics (2013) 54:265–269
DOI 10.1007/s13353-013-0153-1

PLANT GENETICS • SHORT COMMUNICATION

Molecular cytogenetic characterisation of *Salix viminalis* L. using repetitive DNA sequences

Anna Viktória Németh · Dénes Dudits · Márta Molnár-Láng · Gabriella Linc

Received: 5 April 2013 / Revised: 14 May 2013 / Accepted: 15 May 2013 / Published online: 30 May 2013

Abstract *Salix viminalis* L. (2n=38) is a diploid dicot species belonging to the *Salix* genus of the *Salicaceae* family. This short-rotation woody crop is one of the most important renewable bioenergy resources worldwide. In breeding for high biomass productivity, limited knowledge is available on the molecular cytogenetics of willow, which could be combined with genetic linkage mapping. The present paper describes the adaptation of a fluorescence *in situ* hybridisation (FISH) protocol as a new approach to analyse the genomic constitution of *Salix viminalis* using the heterologous DNA clones pSc119.2, pTa71, pTa794, pAs1, Afa-family, pAl1, HT100.3, ZCF1 and the GAA microsatellite marker. Three of the nine probes showed unambiguous signals on the metaphase chromosomes. FISH analysis with the pTa71 probe detected one major 18S-5.8S-26S rDNA locus on the short arm of one chromosome pair; however, the pTa794 rDNA site was not visible. One chromosome pair showed a distinct signal around the centromeric region after FISH with the telomere-specific DNA clone HT100.3. Two chromosome pairs were found to have pAs1 FISH signals, which represent a D-genome-specific insert from *Aegilops tauschii*. Based on the FISH study, a set of chromosomes with characteristic patterns is presented, which could be used to establish the karyotype of willow species.

Keywords FISH · Genome constitution · Repetitive DNA clones · *Salix viminalis*

A. V. Németh · D. Dudits
Institute of Plant Biology, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

M. Molnár-Láng · G. Linc (✉)
Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár 2462, Hungary
e-mail: linc.gabriella@agrar.mta.hu

Salix viminalis L. (2n=38) is a diploid dicot species belonging to the *Salix* genus of the *Salicaceae* family. Willow is an arboreal, dioecious plant with male or female catkins. This woody species is used in many different fields, such as agriculture, the phytoremediation of soils and renewable energy production. Common willow has the ability to absorb some heavy metals like cadmium and is often planted to reclaim industrial sites. It is also planted as part of small-scale water treatment systems. Willow, which has a growth rate of about 1 m per year, represents a significant biomass crop due to its ease of propagation and ability to grow quickly in short-rotation coppice (SRC) cycles with only minimal fertiliser inputs (Hinchee et al. 2009). Cuttings are planted in early spring, from March to April. The willow biomass is harvested in winter (from December to February) every second or third year, and the biomass production may be as much as 20–40 tonnes per hectare. The burn heat of solid biofuel (such as fuel pellets or pills) is 29.2 MJ/kg (Kondor 2007). The active ingredient of aspirin, salicylic acid, was originally extracted from the bark of willow (Hanley et al. 2002).

To achieve the productivity level required for tree biofuel and bioenergy applications, significant research efforts are needed in order to improve tree genetics, breeding methods and silvicultural practices (Karp et al. 2011). In terms of genomic complexity, the low number of genetic markers available could restrict the genetic analysis of willow. A genetic linkage map of willow composed of amplified fragment length polymorphisms (AFLP) and restriction fragment length polymorphic (RFLP) markers was constructed by Tsarouhas et al. (2002). This map was based on a population derived from a cross between the male hybrid clone *Salix viminalis* × *Salix schwerinii* and the female clone *S. viminalis*. The RFLP probes were produced using a sequence of the *Populus* genome. The authors used this genetic map to identify quantitative trait loci (QTL) affecting growth-related traits. Barcaccia et al. (2003) reported a

linkage map of tetraploid willow constructed using AFLP and microsatellite polymorphic loci (SAMPL) markers. Two additional, more detailed willow linkage maps were established using single nucleotide polymorphism (SNP), microsatellite and AFLP markers (Berlin et al. 2010). The alignment of the willow linkage map to the poplar genome revealed a high degree of synteny and gene-order conservation across a significant part of the two *Salicaceae* genomes. In contrast to the progress in the molecular genetic mapping of the willow genome, the chromosomal structure of this species has not been studied extensively. Therefore, the adaptation of the fluorescence *in situ* hybridisation (FISH) protocol is presented as a new approach in the characterisation of willow chromosomes.

In situ hybridisation, a method routinely used in modern plant cell and molecular biology, is an efficient technique to localise nucleic acid sequences (DNA or RNA) in the cytoplasm, organelles, chromosomes or nuclei of biological material (Pardue and Gall 1969; Houben et al. 2005). FISH using repetitive DNA probes gives a specific chromosome pattern (Rayburn and Gill 1987; Pedersen and Langridge 1997). The method is suitable for chromosome identification in various plant species, such as those in the *Triticeae* tribe (Mukai et al. 1993; Rayburn and Gill 1985, 1986). The black cottonwood (*Populus trichocarpa*) was the first tree species for which the genome was sequenced, so the poplar species can be considered to be the model of forest trees in genomics and genetics research (Tuskan et al. 2006). The FISH signals produced by rDNA clones, *Arabidopsis*-type telomere repeat sequences and marker-selected BAC DNA clones have been identified in *Populus trichocarpa* (Islam-Faridi et al. 2009a, b).

The aim of the present study was to adapt the protocol of FISH-based chromosome karyotyping to willow plants. FISH based on labelled heterologous DNA probes allowed the identification of chromosomes or chromosome pairs as a first step in the prediction of the chromosome karyotype.

Due to the lack of any described standard protocol for the preparation of chromosome spread of willow, first, we optimised the procedure. Stem cuttings of *Salix viminalis* L. "ENERGO" plants were used for root induction in water at room temperature. Shoots with 1–2-cm roots were exposed to cold treatment at 4 °C for 4 days. After this treatment, the explants were stored at room temperature for 22 h. Root tips were collected and fixed in Carnoy's solution (ethanol and acetic acid, 3:1 v/v). Root meristem preparations and FISH analysis were carried out according to Linc et al. (1999), with minor modifications. The fixed roots were digested in 1 % pectolytic enzyme mixture: 0.3 % (w/v) cellulase, 0.3 % (w/v) pectolyase, 0.3 % (w/v) cytohelicase and squash preparations were made in 45 % acetic acid. The complete enzymatic digestion of willow root tips was a critical step in the preparation of suitable metaphase chromosome spreads and in generating good hybridisation signals. These treatments were previously applied successfully on several plant species, such as *Arabidopsis*, tomato, rice etc. (Fransz et al. 1996; Zhong et al. 1998; Sasaki et al. 2002). The digestion of root tissues with cytohelicase enzyme was found to be an essential component in the procedure for willow cytology. The quality of the chromosomes was checked by DAPI staining.

The metaphase chromosomes of willow are metacentric and of similar size, less than 5 μm on average and no secondary constructions are visible (Fig. 1a). These morphological features are insufficient to distinguish the 19 chromosome pairs or for the identification of individual chromosomes, which makes the chromosome cytology of willow challenging. Considering this limitation, here, we outline an adapted FISH protocol applied to discriminate the different willow chromosomes. Probe labelling and the FISH procedure were carried out according to Linc et al. (2012), using nine repetitive DNA clones and trinucleotide sequences (Table 1). The GAA microsatellite sequence was amplified from the genomic DNA of *Triticum aestivum* L. according to Vrána et al. (2000). The clone pSc119.2 contains a 120-bp repeat derived from an *Eco*RI relic DNA of rye cv. King II inserted into the plasmid pBR322 (Bedbrook et al. 1980). The pTa71 ribosomal DNA probe contains a 9.05-kbp fragment, which is a part of the rDNA repetitive unit consisting of one copy each of 18S rDNA, 5.8S rDNA, 25S rDNA and an intergenic spacer from wheat cv. Chinese Spring (Gerlach and Bedbrook 1979). The probe pTA794 contains a 410-bp *Bam*HI fragment including an intergenic spacer and is part of the 5S rDNA gene family isolated from wheat (Gerlach and Dyer 1980). The HT100.3 telomere repeat $(TTTAGGG)_n$ sequences were originally isolated and amplified from *Arabidopsis thaliana* L. (Juchimiuk-Kwasniewska et al. 2011). The pAl1 178 bp tandem repeat was cloned from *Arabidopsis thaliana* L. (Martinez-Zapater et al. 1986). The probe pAs1 contains a 1-kb DNA fragment isolated from *Aegilops tauschii* Coss. in the plasmid pUC8 (Rayburn and Gill 1986). The Afa-family repeats (Nagaki et al. 1995) were amplified from the genomic DNA of barley (*Hordeum vulgare*) L. The ZCF1 centromere-specific repeat (Cheng and Murata 2003) was isolated from genomic wheat DNA.

In the present study, three of the nine DNA clones showed unambiguous hybridisation signals in the characterisation of different regions of the willow chromosomes: pAs1, the HT100.3 telomere repeat sequence and the pTa71 rDNA clone. An important aspect of genome investigations at the molecular level is now to learn the composition of the repetitive DNA sequences. As the centromeric marker, we used the pAs1-labelled DNA clone that resulted in FISH signals near or around the centromeric region of two chromosome pairs (Fig. 1b, d). The pAs1 represents a D-genome-specific clone in the tribe *Triticeae* and contains

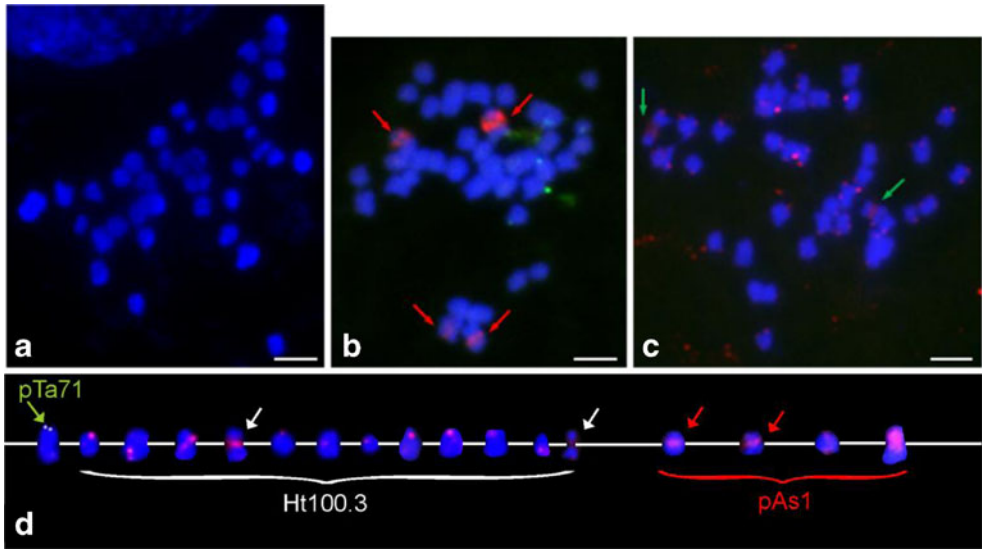

Fig. 1 Results of FISH using heterologous DNA probes on mitotic chromosome spreads of *S. viminalis*. Digoxigenin-16-dUTP and biotin-11-dUTP were detected using anti-digoxigenin-rhodamine Fab fragments (Roche) and streptavidin-FITC (Roche), respectively. *In situ* hybridisation signals were captured and visualised with a Zeiss Axio Scope 2 epifluorescence microscope equipped with filter sets appropriate for DAPI (filter set 1), FITC (filter set 10), rhodamine (filter set 15) and for the simultaneous detection of FITC and rhodamine (double filter set 24). Photographs were taken with a Spot CCD camera (Diagnostic Instruments, Inc., USA). The image processing was carried out using Image-Pro Plus 5.1 (Media Cybernetics, USA) software. All the images were only manipulated to enhance the contrast. **a** Morphology of *S. viminalis* mitotic chromosomes by DAPI staining. **b** FISH pattern with the pAs1 DNA fragment. The *red arrows* show two putative chromosome pairs. **c** HT100.3 DNA probe shows red FISH signals in telomeric chromosome regions. The *green arrows* indicate a chromosome pair with HT100.3 signals around the centromeres. Scale bar = 5 μm. **d** Representative *S. viminalis* chromosomes with characteristic FISH patterns. The *red arrows* show a putative chromosome pair with pAs1 FISH signals at the centromeric region, the *green arrow* shows the pTa71 FISH signal and the *white arrows* show the putative chromosome pair detected by the Ht100.3-specific probe. The *horizontal white line* indicates the predicted position of the centromere

a ~1-kb insert from *Aegilops tauschii*. Many families of repetitive sequences have been cloned from wheat or related species. These sequences, aligned in a tandem fashion, have been used as cytological markers to identify genomes, chromosomes and chromosomal regions. Among the woody species, this is the first report that shows two chromosome pairs with pAs1 FISH signals. However, after using the Afa-family as a labelled DNA probe, no FISH signal was detected. The Afa-family sequences are tandem repetitive sequences of 340 bp, which is basically a subclone of pAs1. The insert of pAs1 consisted of three units of the repetitive sequences. Each unit was 336 or 337 bp and was highly homologous with the others. Within the unit, there seemed to be conservative and variable regions: a base change from A to T or T to A frequently occurred. It may be important for the repetitive sequences to keep these structures for amplification and perpetuation in different genomes (Nagaki et al. 1995).

Table 1 Probe and DNA labelling combinations used for FISH mapping

DNA clone	Labelling	Signal on willow chromosomes
Afa family	Digoxigenin-16-dUTP	No
GAA	Biotin-11-dUTP	No
Ht100.3	Digoxigenin-16-dUTP	Yes
pAl1	Biotin-11-dUTP	No
pAs1	Digoxigenin-16-dUTP	Yes
pSc119.2	Biotin-11-dUTP	No
pTa71	Biotin-11-dUTP	Yes
pTa794	Biotin-11-dUTP	No
	Digoxigenin-16-dUTP + Biotin-11-dUTP	No
ZCF1	Biotin-11-dUTP	No

FISH signals obtained with the clone HT100.3 were observed on the telomere region of all the chromosomes with varying intensity. Furthermore, clear hybridisation signals were localised near the centromere region of two chromosomes, which may represent a chromosome pair (see Fig. 1c, d). The detection of a telomeric sequence motif in the centromeric region is in agreement with previous studies on human, animal and plant material, and telomeric-like repeats are also present at internal sites of the chromosomes (intrachromosomal or interstitial telomeric sequences, ITSs) (Ruiz-Herrera et al. 2008). Meyne and collaborators (1990) provided the first cytogenetic evidence for the presence of ITSs in the karyotypes of various vertebrate species (Meyne et al. 1990). It is most probable that the chromosome pair detected in willow contains heterochromatic ITSs (het-ITSs), which are large stretches of telomeric-like DNA, localised

mainly at the centromeres. FISH experiments on *Populus trichocarpa* chromosomes with *Arabidopsis*-type telomere repeat sequence probes resulted in signals at the distal ends of each chromosome. The authors did not observe hybridisation signals in the centromeric region (Islam-Faridi et al. 2009a, b). It was previously established that the presence of het-ITSs may cause chromosomal instability during species evolution (Ruiz-Herrera et al. 2008), so willow could be used as a general model system to study chromosomal aberrations involving het-ITSs in woody species.

Chromosome preparations were also hybridised with the pTa71 and pTa794 rDNA probes. In this case, FISH signals were only detected with pTa71 in the telomeric region of a single chromosome pair, as shown in Fig. 1d. The cloned ribosomal probe pTa71 represents the major 18S-5.8S-26S ribosomal genes of wheat (Gerlach and Bedbrook 1979). The same probe was used for FISH analysis on the chromosomes of birch trees (*Betula* L.) (Anamthawat-Jónsson 2003). A biotinylated rDNA probe from soybean was used by Brown and co-workers (1993) to compile a preliminary karyotype of white spruce (*Picea glauca*). A plasmid DNA with a 18S-28S rDNA insert from maize served as the hybridisation probe to characterise the chromosomes of American chestnut (*Castanea dentata*) as a forest tree species (Islam-Faridi et al. 2009a, b).

The present study, like the above cited works, demonstrated the potential advantage of the FISH approach in molecular studies on the genomes of forest trees. The combination of the genetic and physical mapping technologies is gaining increasing attention in research on the genomics of tree species and in related breeding programmes. The establishment of a basic FISH protocol is a prerequisite for these attempts. The present results obtained with heterologous probes could support future efforts to create the karyotype of energy willow. The isolation and use of species-specific DNA clones from the *S. viminalis* genome will be needed in order to improve the FISH technology. The application of these DNA probes as molecular cytogenetic markers will provide new information to study the FISH patterns of the *Salix* chromosomes. The detailed cytogenetic analysis of individual chromosomes will lead to progress in the genome studies on the willow genome.

Acknowledgements This study was funded by Kreátor 2005 Ltd. (Budapest), TÁMOP-4.2.2/B-10/1-2012-0025) and a János Bolyai Research Scholarship from HAS to G.L. The authors thank Barbara Harasztos for revising the manuscript linguistically.

References

Anamthawat-Jónsson K (2003) Preparation of chromosomes from plant leaf meristems for karyotype analysis and *in situ* hybridization. Methods Cell Sci 25:91–95

Barcaccia G, Meneghetti S, Albertini E, Triest L, Lucchin M (2003) Linkage mapping in tetraploid willows: segregation of molecular markers and estimation of linkage phases support an allotetraploid structure for *Salix alba* × *Salix fragalis* interspecific hybrids. Heredity 90:169–280

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

Berlin S, Lagercrantz U, von Arnold S, Öst T, Rönnberg-Wästljung AC (2010) High-density linkage mapping and evolution of paralogs and orthologs in *Salix* and *Populus*. Genomics 11:129

Brown GR, Amarasinghe V, Kiss G, Carlson JE (1993) Preliminary karyotype and chromosomal localization of ribosomal DNA sites in white spruce using fluorescence in situ hybridization. Genome 36:310–316

Cheng Z-J, Murata M (2003) A centromeric tandem repeat family originating from a part of ty3/*gypsy*-retroelement in wheat and its relatives. Genetics 164:665–672

Fransz PF, Alonso-Blanco C, Liharska TB, Peeters AJM, Zabel P, de Jong JH (1996) High-resolution physical mapping in *Arabidopsis thaliana* and tomato by fluorescence *in situ* hybridization to extended DNA fibres. Plant J 9:421–430

Gerlach WL, Bedbrook JR (1979) Cloning and characterization of ribosomal RNA genes from wheat and barley. Nucleic Acids Res 7:1869–1885

Gerlach WL, Dyer TA (1980) Sequence organization of the repeating units in the nucleus of wheat which contain 5S rRNA genes. Nucleic Acids Res 8:4851–4865

Hanley S, Barker JHA, Van Ooijen JW, Aldam C, Harris SL, Åhman I, Larsson S, Karp A (2002) A genetic linkage map of willow (*Salix viminalis*) based on AFLP and microsatellite markers. Theor Appl Genet 105:1087–1096

Hinchee M, Rottmann W, Mullinax L, Zhang C, Chang S, Cunningham M, Pearson L, Nehra N (2009) Short-rotation woody crops for bioenergy and biofuels applications. In Vitro Cell Dev Biol Plant 45:619–629

Houben A, Orford SJ, Timmis JN (2005) *In situ* hybridization to plant tissues and chromosomes. In Situ Hybridization Protoc 326:203–218

Islam-Faridi MN, Nelson CD, DiFazio SP, Gunter LE, Tuskan GA (2009a) Cytogenetic analysis of *Populus trichocarpa*—ribosomal DNA, telomere repeat sequence, and marker-selected BACs. Cytogenet Genome Res 125:74–80

Islam-Faridi N, Nelson CD, Sisco PH, Kubisiak LT, Hebard FV, Paris RL, Phillips RL (2009b) Cytogenetic analysis of American chestnut (*Castanea dentata*) using fluorescent in situ hybridization. Acta Hort 844:207–210, ISHS

Juchimiuk-Kwasniewska J, Brodziak L, Maluszynska J (2011) FISH in analysis of gamma ray-induced micronuclei formation in barley. J Appl Genetics 52:23–29

Karp A, Hanley SJ, Trybush SO, Macalpine W, Pei M, Shield I (2011) Genetic improvement of willow for bioenergy and biofuels. J Integr Plant Biol 53:151–165

Kondor A (2007) Adatok az „energia fűz" (*Salix viminalis* L.) gyomszabályozási lehetőségeiről. Agrártudományi Közlemények 26:108–112

Linc G, Friebe BR, Kynast RG, Molnár-Láng M, Köszegi B, Sutka J, Gill BS (1999) Molecular cytogenetic analysis of *Aegilops cylindrica* host. Genome 42:497–503

Linc G, Sepsi A, Molnár-Láng M (2012) A FISH karyotype to study chromosome polymorphisms for the *Elytrigia elongata* E genome. Cytogenet Genome Res 136:138–144

Martinez-Zapater JM, Estelle MA, Somerville CR (1986) A highly repeated DNA sequence in *Arabidopsis thaliana*. Mol Gen Genet 204:417–423

Meyne J, Baker RJ, Hobart HH, Hsu TC, Ryder OA, Ward OG, Wiley JE, Wurster-Hill DH, Yates TL, Moyzis RK (1990) Distribution

of non-telomeric sites of the $(TTAGGG)_n$ telomeric sequence in vertebrate chromosomes. Chromosoma 99:3–10

Mukai Y, Friebe B, Hatchett JH, Yamamoto M, Gill BS (1993) Molecular cytogenetic analysis of radiation-induced wheat-rye terminal and intercalary chromosomal translocations and the detection of rye chromatin specifying resistance to Hessian fly. Chromosoma 102:88–95

Nagaki K, Tsujimoto H, Isono K, Sasakuma T (1995) Molecular characterization of a tandem repeat, Afa family, and its distribution among *Triticeae*. Genome 38:479–486

Pardue ML, Gall JG (1969) Molecular hybridization of radioactive DNA to the DNA of cytological preparations. Proc Natl Acad Sci USA 64:600–604

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

Rayburn AL, Gill BS (1985) Use of biotin-labeled probes to map specific DNA sequences on wheat chromosomes. J Hered 76:78–81

Rayburn AL, Gill BS (1986) Molecular identification of the D-genome chromosomes of wheat. J Hered 77:253–255

Rayburn AL, Gill BS (1987) Isolation of D-genome specific repeated DNA sequence from *Aegilops squarrosa*. Plant Mol Biol Rep 4:102–109

Ruiz-Herrera A, Nergadze SG, Santagostino M, Giulotto E (2008) Telomeric repeats far from the ends: mechanisms of origin and role in evolution. Cytogenet Genome Res 122:219–228

Sasaki T, Matsumoto T, Yamamoto K, Sakata K, Baba T, Katayose Y, Wu J, Niimura Y, Cheng Z, Nagamura Y, Antonio BA, Kanamori H, Hosokawa S, Masukawa M, Arikawa K, Chiden Y, Hayashi M, Okamoto M, Ando T, Aoki H, Arita K, Hamada M, Harada C, Hijishita S, Honda M, Ichikawa Y, Idonuma A, Iijima M, Ikeda M, Ikeno M, Ito S, Ito T, Ito Y, Ito Y, Iwabuchi A, Kamiya K, Karasawa W, Katagiri S, Kikuta A, Kobayashi N, Kono I, Machita K, Maehara T, Mizuno H, Mizubayashi T, Mukai Y, Nagasaki H, Nakashima M, Nakama Y, Nakamichi Y, Nakamura M, Namiki N, Negishi M, Ohta I, Ono N, Saji S, Sakai K, Shibata M, Shimokawa T, Shomura A, Song J, Takazaki Y, Terasawa K, Tsuji K, Waki K, Yamagata H, Yamane H, Yoshiki S, Yoshihara R, Yukawa K, Zhong H, Iwama H, Endo T, Ito H, Hahn JH, Kim HI, Eun MY, Yano M, Jiang J, Gojobori T (2002) The genome sequence and structure of rice chromosome 1. Nature 420:312–316

Tsarouhas V, Gullberg U, Lagercrantz U (2002) An AFLP and RFLP linkage map and quantitative trait locus (QTL) analysis of growth traits in *Salix*. Theor Appl Genet 105:277–288

Tuskan GA, DiFazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, Putnam N, Ralph S, Rombauts S, Salamov A, Schein J, Sterck L, Aerts A, Bhalerao RR, Bhalerao RP, Blaudez D, Boerjan W, Brun A, Brunner A, Busov V, Campbell M, Carlson J, Chalot M, Chapman J, Chen GL, Cooper D, Coutinho PM, Couturier J, Covert S, Cronk Q, Cunningham R, Davis J, Degroeve S, Déjardin A, Depamphilis C, Detter J, Dirks B, Dubchak I, Duplessis S, Ehlting J, Ellis B, Gendler K, Goodstein D, Gribskov M, Grimwood J, Groover A, Gunter L, Hamberger B, Heinze B, Helariutta Y, Henrissat B, Holligan D, Holt R, Huang W, Islam-Faridi N, Jones S, Jones-Rhoades M, Jorgensen R, Joshi C, Kangasjärvi J, Karlsson J, Kelleher C, Kirkpatrick R, Kirst M, Kohler A, Kalluri U, Larimer F, Leebens-Mack J, Leplé JC, Locascio P, Lou Y, Lucas S, Martin F, Montanini B, Napoli C, Nelson DR, Nelson C, Nieminen K, Nilsson O, Pereda V, Peter G, Philippe R, Pilate G, Poliakov A, Razumovskaya J, Richardson P, Rinaldi C, Ritland K, Rouzé P, Ryaboy D, Schmutz J, Schrader J, Segerman B, Shin H, Siddiqui A, Sterky F, Terry A, Tsai CJ, Uberbacher E, Unneberg P, Vahala J, Wall K, Wessler S, Yang G, Yin T, Douglas C, Marra M, Sandberg G, Van de Peer Y, Rokhsar D (2006) The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). Science 313:1596–1604

Vrána J, Kubaláková M, Simková H, Číhalíkováí J, Lysák MA, Dolezel J (2000) Flow sorting of mitotic chromosomes in common wheat (*Triticum aestivum* L.). Genetics 156:2033–2041

Zhong X-B, Fransz PF, Wennekes-van Eden J, Ramanna MS, van Kammen A, Zabel P, de Jong H (1998) FISH studies reveal the molecular and chromosomal organization of individual telomere domains in tomato. Plant J 13:507–517