Front. Agric. China 2008, 2(3): 314–316
DOI 10.1007/s11703-008-0059-6

RESEARCH ARTICLE

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# Multicolor FISH analysis of rDNA and telomere on spinach



**Abstract** In this study, multicolor fluorescence *in situ* hybridization (FISH) analysis on metaphase chromosomes of spinach with biotin-labeled 25S rDNA, DIG-labeled telomere sequences and biotin-labeled and DIG-labeled 5S rDNA was performed. There were six 25S rDNA loci located on the satellites of the third, the fifth and the sixth chromosomes, and four 5S rDNA loci located on the long arms of the third and the fifth chromosomes. The telomere loci were located on the end of the sixth chromosome and also on both the end and centromeric regions of other chromosomes. This study is an important complement to both traditional karyotype analysis and FISH karyotype analysis in spinach.

**Keywords** spinach, multicolor FISH, rDNA, telomere

## 1 Introduction

With the progress in molecular cytogenetics technology, the traditional karyotype analysis technique has been gradually revealing its defects. Using fluorescence *in situ* hybridization (FISH) to map molecular markers on chromosomes can afford effective cytological markers for karyotype analysis and compensate for the inadequacies. Multicolor FISH (Mc-FISH), which can locate many different probes on chromosomes simultaneously, is an important branch of FISH techniques. Using Mc-FISH to construct FISH karyotype has been applied in many plant species, for example, *Lotus japonicus*, *Pinus* sp., *Picea abies* and *Silene latifolia* (Lengerova et al., 2004; Hizume et al., 2002). Ribosomal RNA genes (rDNAs) belong to highly conservative repeat sequence family with hundreds of copies and locate on one or more pairs of chromosomes (Pedersen and Linde-Laursen, 1994). FISH mapping of rDNA on chromosomes can provide important clues for molecular markers of karyotype analysis, evolution of karyotype and phylogenetics research. Currently, it is most widely used in the research of molecular markers of chromosomes. Telomeres are the special structures on the ends of chromosomes, usually composed of tandem repeat sequences, and extended to the 3′ end of chromosomes. They are widespread repetitive sequences existing in angiosperms, gymnosperms and ferns (Cox et al., 1993; Fuchs et al., 1995), mainly located on the ends or other districts of chromosomes. They are important molecular markers in FISH karyotype analysis.

Spinach belongs to Chenopodiaceae. Previous traditional karyotype analysis showed that its chromosome complement was composed of $2n = 2x = 12$ chromosomes, including two large metacentrics, two long subtelocentrics, two short subtelocentrics, two acrocentrics, and four submetacentrics. The fifth and sixth chromosomes harbor satellites. In this study, we corrected the numbers of the satellites by Mc-FISH mapping of rDNA and telomere on chromosomes of spinach. The results are an important supplement to the traditional karyotype analysis and provide evidence for molecular karyotype studies of spinach.

## 2 Materials and methods

### 2.1 Plant materials

Root tips and young leaves were collected from spinach in Jixian county, Tianjin, China.

### 2.2 Chromosome preparation

For FISH analyses, slides were prepared by wall degradation hypotonic method according to Chen et al. (1979) with minor modifications.

### 2.3 Probe labeling and fluorescence *in situ* hybridization

25S rDNA, 5S rDNA and telomere sequence were amplified from *Arabidopsis thaliana*. The 25S rDNA and 5S

Translated from *Hereditas* (*Beijing*), 2007, 29(11): 1405–1408 [译自: 遗传(北京)]

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rDNA were labeled with Biotin-dUTP (Roche) by randomly-primed DNA synthesis. Telomere sequence and 5S rDNA were labeled with DIG-dUTP (Roche) by randomly-primed DNA synthesis. The labeled 25S rDNA, telomere sequence, DIG-labeled and Biotin-labeled 5S rDNA probes were mixed by ratio 2:2:1:1 when FISH was carried out as described by Qi et al. (2002).

## 3 Results

### 3.1 Mc-FISH results

The results of Mc-FISH with 25S rDNA, 5S rDNA and telomere DNA on metaphase chromosomes of spinach are shown in Fig. 1. The green signals were 25S rDNA loci, the red signals were telomere sequence loci and the blue signals were 5S rDNA loci, which were all clear and steady. In signal intensity, 25S rDNA was the strongest, followed by the telomere sequences and 5S rDNA (the weakest). Fig. 1-(e) and Fig. 1-(f) are FISH images using biotin-labelled 25S rDNA and DIG-labelled 5S rDNA as probes, respectively. They prove that the number of 25S rDNA loci and 5S rDNA loci were 6 and 4.

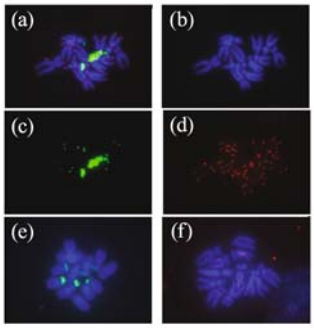


**Fig. 1** Multicolor fluorescence *in situ* hybridization with 25S rDNA, 5S rDNA and telomere DNA on metaphase chromosomes of spinach
Notes: (a) is FISH image using 25S rDNA, 5S rDNA and telomere DNA as probes, merged by (b), (c) and (d). (b), (c), (d), (e) and (f) represent DAPI-stained chromosomes; detection of the biotin-labeled signals; detection of the DIG-labeled signals; FISH image using biotin-labelled 25S rDNA as the probe, and FISH image using DIG-labelled 5S rDNA as the probe, respectively.

### 3.2 Karyotype analysis

Homologous chromosomes pairing and sorting results from Mc-FISH image of metaphase chromosomes in spinach are shown in Fig. 2. There are six 25S rDNA loci located on the secondary constriction (SC) districts and satellites of the third, fifth and sixth chromosomes respectively. The signals of the third chromosome were the smallest and weakest in size and intensity among all the chromosomes. Four 5S rDNA loci were located in the long arms of the third and fifth chromosomes. There were no significant difference in the size and intensity of these signals. The signals of telomere sequences were located on the ends of the sixth chromosomes and both the ends and centromere districts of other chromosomes. Especially, the signals of the sixth chromosome were the strongest of all.

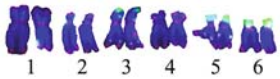


**Fig. 2** The karyotype of spinach

## 4 Discussion

### 4.1 Physical mapping of rDNA on chromosomes

Ribosomal DNAs of plants are genes coding for various ribosomal RNA precursors, including 45S rDNA and 5S rDNA. 45S rDNA is composed of 18S, 5.8S and 25S rDNA. So far, many studies have proved that, in many species, 45S rDNA is mainly located on the nuclear organization region (NOR), that is the SC region (Xu et al., 2007; Liu et al., 2005). 25S rDNA is a part of the 45S rDNA; it locates on the NOR too. The number of NORs is consistent with the number of the satellites, therefore the number of the 25S rDNA loci is the number of satellites. The previous traditional karyotype analysis indicated that there are two pairs of satellites in spinach, which belong to the fifth and sixth chromosomes while 25S rDNA physical mapping result indicated there are 3 pairs of satellites which belong to the third, fifth and sixth chromosomes. FISH is a semi-quantitative technique, although the result cannot directly reflect the actual copy number of the sequences, the size and intensity of the signals can indicate the relative copy number indirectly. The signals on the third chromosomes are weaker and smaller than those of the others, which means the satellites on the third chromosomes are smaller than the other satellites. The traditional karyotype analysis method mainly relies on the naked eye without using molecular markers. When the satellites of the chromosomes are too small to be recognized, and the SC regions are visible, it would be easily judged as the chromosomes do not harbor satellites. Such phenomenon can be found in other plants such as cotton (Bie et al., 2004).

5S rDNA of spinach is located on the long arms of the third and the fifth chromosomes which can also harbor 25S rDNA loci. Different species have different 5S rDNA loci. In higher plants, including angiosperms and gymnosperms, the locations of 5S rDNA are not conservative, and no species harbors the 5S rDNA loci in the NOR (Rogers and Bendich, 1987; Beech and Strobeck, 1993). But in partial moss plants and prokaryotic cells, 5S rDNA can be located in the NORs (Srivastava and Schlessinger, 1991; Sone et al., 1999).

### 4.2 Physical mapping of telomere sequences on chromosomes

The telomere sequences are widely distributed in most plant and animal species. They are an important functional component of chromosomes, whose main role is to maintain the integrity and individuality of chromosomes. All telomere sequences in one genome are composed of identical repetitive sequences. However, different species have different telomere sequences. The conservative sequence of telomere in mammals and vertebrates is TTAGGG. This sequence tandemly repeats 500–3000 times, and the total length would be 2–20 kb. Most plant species can harbor telomere sequences, including bryophytes, e.g., *Pellia epiphylla*, and gymnosperms, e.g., *Zamia furfuracea* and *Pinus sylvestris* (Fuchs et al., 1995). The conservative sequence of telomere in plant species is AAATGGG, which was first found in Arabidopsis, then called Arabidopsis-type telomere. However, some other plant species, e.g., *Allium cepa*, have tandem repeated DNA instead of the telomere sequences at the telomeric heterochromatic regions. In many animal species, the telomere sequences of human are localized at the interstitial and proximal regions in addition to chromosome ends (telomeres) (Meyne et al., 1990). In plants, the telomere sequences are localized not only at the chromosome end but also at other chromosome regions: the centromeric region in chromosomal race in *Vicia fagba* and *Cycas revolute* (Schubert, 1992; Schubert et al., 1995; Hizume et al., 1998), interstitial region in *Pinus sylvestris* and terminal heterochromatin in *Tradescantia commelinoides* (Cox et al., 1993). At molecular level the telomere sequences are reported in centromeric DNA and interstitial DNA of *Arabidopsis thaliana* (Richards et al., 1991; Regad et al., 1994). In our study, we applied the Arabidopsis-type telomere sequence, $(AAATGGG)_n$. The signals were located on the ends of the sixth chromosomes and both the ends and centromere districts of other chromosomes. That is, in spinach, the telomere sequences are mainly located on the ends of acrocentric chromosome and both the ends and centromere regions of other chromosomes. The intensity of signal on one end of the sixth chromosome is stronger than that of other signals. The main reason for this result would be that, this district contains both ends and centromere DNA, so the copy number of telomere sequences is larger than that of other districts.

## References


Beech R N, Strobeck C (1993). Structure of the intergenic spacer region from the ribosomal RNA gene family of white spruce (*Picea glauca*). Plant Mol Biol, 22(5): 887–892

Bie S, Wang K B, Wang C Y, Song G L, Kong F L, Liu F, Liu S H, Li S H, Zhang X D, Wang Y H (2004). Studies on 45S rDNA-FISH and karyotype of *Gossypium herbaceum* and *Gossypium arboreum*. Cotton Science, 16(4): 223–228 (in Chinese)

Chen R Y, Song W Q, Li X L (1979). A new method of plant mitosis chromosome spread. Acta Bot Sin, 21(3): 297–298 (in Chinese)

Cox A V, Bennett S T, Parokonny A S, Kenton A, Callimassia M A, Bennett M D (1993). Comparison of plant telomere locations using a PCR-generated synthetic probe. Ann Bot, 72(3): 239–247

Fuchs J, Brandes A, Schubert I (1995). Telomere sequence localization and karyotype evolution in higher plants. Plant Syst Evol, 196(4): 227–241

Hizume M, Kurose N, Shibata F, Kondo K (1998). Molecular cytogenetic studies on sex chromosomes and proximal heterochromatin containing telomere-like sequence in *Cycas revoluta*. Chromosome Sci, 2(1): 63–72

Hizume M, Shibata F, Matsusaki Y, Garajova Z (2002). Chromosome identification and comparative karyotypic analyses of four *Pinus* species. Theor Appl Genet, 105(4): 491–497

Lengerova M, Kejnovsky E, Hobza R, Macas J, Grant S R, Vyskot B (2004). Multicolor FISH mapping of the dioecious model plant, *Silene latifolia*. Theor Appl Genet, 108(7): 1193–1199

Liu B, Chen C B, Li X L, Qi L W, Han S Y (2005). Karyotype analysis and physical mapping of 45S rDNA in eight species of *Sphora*, *Robinia* and *Amorpha*. Acta Botanica Yunnanica, 27(3): 261–268 (in Chinese)

Meyne J, Baker R J, Hobart H H, Hsu T C, Ryder T C, Ward O G, Wiley J E, Wurster-Hill D H, Yates T L, Moyzis R K (1990). Distribution of non-telocentric sites of the $(TTAGGG)_n$ telomeric sequence in vertebrate chromosomes. Chromosoma, 99(1): 3–10

Pedersen C, Linde-Laursen I (1994). Chromosomal locations of four minor rDNA loci and a marker microsatellite sequence in barley. Chromosome Res, 2(1): 65–71

Qi Z X, Zeng H, Li X L, Song W Q, Chen R Y (2002). The molecular characterization of maize B chromosome specific AFLPs. Cell Res, 12(1): 63–68

Regad F, Lebas M, Lescure B (1994). Interstitial telomeric repeats within the *Arabidopsis thaliana* genome. J Mol Biol, 239(2): 163–169

Richards E J, Goodman H M, Ausubel F M (1991). The centromere region of *Arabidopsis thaliana* chromosome 1 contains telomere-similar sequences. Nucleic Acids Res, 19(12): 3351–3357

Rogers S O, Bendich A J (1987). Ribosomal RNA genes in plants: variability in copy number and in the intergenic spacer. Plant Mol Biol, 9(4): 509–520

Schubert I (1992). Telomeric polymorphism in *Vicia faba*. Biol Zbl, 111: 164–168

Schubert I, Rieger R, Fuchs J (1995). Alteration of basic chromosome number by fusion-fission cycles. Genome, 38(6): 1289–1292

Sone T, Fujisawa M, Takenaka M, Nakagawa S, Yamaoka S, Sakaida M, Nishiyama R, Yamato KT, Ohmido N, Fukui K, Fukuzawa H, Ohyama K (1999). Bryophyte 5S rDNA was inserted into 45S rDNA repeat units ager the divergence from higher land plants. Plant Mol Biol, 41(5): 679–685

Srivastava A K, Schlessinger D (1991). Structure and organization of ribosomal DNA. Biochimie, 73(6): 631–638

Xu Y H, Yang F, Cheng Y L, Ma L, Wang J B, Li L J (2007). Comparative analysis of rDNA distribution in metaphase chromosomes of Cucuibitaceae species. Hereditas (Beijing), 29(5): 614–620 (in Chinese)