Theor Appl Genet (1998) 96: 751–757 © Springer-Verlag 1998

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Visualization of a self-incompatibility gene in *Brassica campestris* L. by multicolor FISH

Received: 18 September 1997 / Accepted: 6 October 1997

Abstract The physical localization of the S-glycoprotein (SLG) locus in the chromosome of *Brassica campestris* L. 'pekinensis' cv 'Kukai' was visualized by multi-color fluorescent *in situ* hybridization (McFISH). 'Kukai', which is an F_1 hybrid between two parental lines, T-17 and T-18, has two *SLG* genes from both T-17 and T-18. In this study, a 1.3-kb DNA fragment was amplified from the genomic DNA of T-17 by PCR using a set of primers specific to the class-I *SLG*. From the genomic DNA of T-18, no DNA fragment was amplified using these primers. In the genomic Southern hybridization, a cloned PCR product hybridized with the genomic DNA of T-17 or F_1 but not with that of T-18. The PCR product had a sequence homology of approximately, 85% to another class-I *SLG* gene, *SLG-9*. Therefore, the PCR product from T-17 was named *SLG-17*, as it is thought to be a member of the class-I *SLG*. Using *SLG-17* as the probe, FISH was carried out to visualize the position of the *SLG* locus. McFISH was also carried out simultaneously using the *SLG-17* and *SLG-9* genes as probes. The *SLG-17* gene was detected as a doublet signal at the interstitial region close to the end of a small chromosome, with the signal site being identical to that of *SLG-9*. Therefore, it is concluded that the *SLG-17* gene is localized at the interstitial region close to the end of the chromosome derived from T-17 in *Brassica campestris* L. 'pekinensis' cv 'Kukai'.

Key words *Brassica campestris* · Multicolor · FISH · Self-incompatibility · S-glycoprotein (SLG) gene

Communicated by F. Mechelke

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Introduction

Some families of flowering plants have a self-incompatibility (SI) system to promote outbreeding and maintain the genetic diversity. The system prevents self-fertilization by rejecting pollens from a plant with the same genotype. SI in *Brassica* is of the sporophytic type and determined genetically by a single *S*-locus with multi alleles (Bateman 1955). More than 50 different *S*-alleles in *Brassica oleracea* have been identified (Brace et al. 1994) as well as 40 *S*-alleles in *B. campestris* (Nou et al. 1993).

S-glycoprotein (SLG) and S-receptor kinase (SRK) have been specifically identified as S-specific proteins. SLG and SRK, which are specific to each S-homozygote, are considered to be responsible for the self-incompatibility system (Hinata et al. 1993; Nasrallah and Nasrallah 1993). Their amino acid sequences and cDNA sequences have been analyzed in *B. oleracea* (Nasrallah et al. 1985; Nasrallah et al. 1987; Lalonde et al. 1989; Trick and Flavell 1989; Chen and Nasrallah 1990; Scutt and Croy 1990; 1992), *B. campestris* (Takayama et al.1987; Isogai et al. 1987; Yamakawa et al. 1994; Watanabe et al. 1994) and *B. napus* (Goring et al. 1992a, b). SRK is composed of the extracellular receptor, transmembrane and kinase domains. The amino acid sequence of the extracellular receptor domain, the S-domain, has high homology to that of the

corresponding SLG (Stein et al. 1991; Yamakawa et al. 1994; Watanabe et al. 1994). Two genes coding for SLG and SRK are linked to the *S*-locus, and the physical distance between the *SLG* locus and the *SRK* locus is within 25 kb in *Brassica napus* (Yu et al. 1996). In addition to the *SLG* and *SRK* genes, the expression of *S*-like genes, *SLR1* and *SLR2*, which are unlinked to the *S*-locus, was found in the stigma of *B. oleracea* and *B. campestris* (Lalonde et al. 1989; Watanabe et al. 1992). The homology among *SLR1s* was more than 90%, while the homology between *SLR1* and *SLG* is approximately 60% at the amino acid level (Trick and Flavell 1989; Isogai et al. 1991).

SLG genes are classified into two groups (Chen and Nasrallah 1990; Kusaba et al. 1997). One group is class-I *SLG* genes, which were isolated from S-homozygotes with a dominant self-incompatibility allele. Another group consists of class II *SLG* genes from S-homozygotes with a pollen-recessive *S*-allele. Recently, Kusaba et al. (1997) amplified each type of *SLG* gene from genomic DNA by the polymerase chain reaction (PCR) using primers specific to each class and then compared the sequences of the genes with those of *SLG* genes or *S*-like genes (Kusaba et al. 1997). The average homology among class-I *SLG*s was approximately 80% at the amino acid level. On the other hand, the homology between class-I *SLG* and class-II *SLG* is 60–70%. Interestingly, the class-II *SLG* has high sequence homology to *SLR2* (ca. 90%).

The fluorescent *in situ* hybridization (FISH) method was developed as a cytological technique to detect specific DNA sequences on chromosomes and nuclei. In Brassicaceae, FISH has been carried out using the *SLG* and *SLR1* probes isolated from *B. oleracea* in chromosomes of haploid *B. napus* (Fukui et al. 1998). Since *B. napus* is a self-compatible allotetraploid species with two progenitor species, *B. oleracea* and *B. campestris*, two kinds of *SLG* loci from these two progenitor species should exist in its chromosomes. However, the *SLG* locus was mapped on a single chromosome from *B. oleracea*. Although this result suggested that the probe is homologous to *SLG* from *B. oleracea*, the relation between the *SLG* probe and the *SLGs* of *B. napus* is ambiguous. The *SLG* locus from another progenitor species, *B. campestris*, is still unknown.

B. campestris L. 'pekinensis' cv 'Kukai' is an F_1 hybrid between parental lines, T-17 and T-18. Thus, 'Kukai' is a heterozygote with respect to *SLGs*. (In the investigation reported here, the *SLG* from genomic DNA of T-17 or T-18 was amplified by PCR with the sets of primers specific to each of the *SLGs*. The characteristics of the *SLGs* were examined by genomic Southern hybridization and sequence analysis. Using the identified *SLG* probe, we examined the position of the *SLG* locus on a metaphase chromosome of 'Kukai'. Furthermore, by means of the multi-color FISH (McFISH) method using *SLG* from T-17 and another class I *SLG* (*SLG9*) as probes, we were able to clearly detect the *SLG* locus in 'Kukai' on a prometaphase chromosome.

Materials and methods

Plant materials

B. campestris var 'pekinensis' cv 'Kukai' (Chinese cabbage) is a commercial variety (Takii seed Co, Kyoto, Japan) and an F_1 hybrid between T-17 and T-18 homozygotes. Genomic DNA was extracted from young leaves of an F_1 hybrid and the T-17 and T-18 homozygotes according to Rogers and Bendich (1985) for amplification of *SLG* genes by PCR and Southern hybridization. Samples for the FISH method were prepared from the root tips of the F_1 hybrid.

PCR

DNA fragments were amplified by PCR with class-I *SLG*-specific oligonucleotide primers PS5 and PS15 or class-II *SLG*-specific oligonucleotide primers PS3 and PS21 (Nishio et al. 1996). After DNA amplification, 10 μl of the sample was subjected to electrophoresis on 1% agarose gel in 1 × TBE buffer, and DNA was subsequently detected by staining with ethidium bromide.

Southern hybridization analysis

Five micrograms of DNA was digested with *Eco*RI, subjected to electrophoresis on a 0.8% (W/V) agarose gel and then transferred to a nylon membrane (Hybond-N, Amersham) with 10 × SSC. After hybridization with the digoxygenin-labeled PCR product of T-17, the nylon membrane was washed at high stringency (0.1 × SSC, 0.1% SDS, 68°C) and used for immunological detection of digoxygenin-labeled DNA according to the manufacturer's instructions (Boehringer).

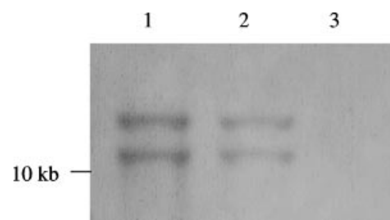

Fig. 1 Genomic Southern hybridization analysis with *SLG-17*. Genomic DNA was digested with *Eco*RI. The probe used was the cloned PCR product amplified from T-17 genomic DNA with class-I specific primers. *Lane 1* T-17 parental line, *2* F_1 hybrid, *3* T-18 parental line

Fig. 2 An alignment of the nucleotide sequences of *SLG-17* and *SLG-9*. Homologous nucleotides are indicated by *asterisks*. The positions of primers (PS5 and PS15) used to amplify the SLG-17 are *underlined*

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SLG17     1:ATGAAAGGCGTAAGAAAAACCTACGATAATTCTTACACCTTATCCTTTTTGCTTGTTTTTTTCGTCTTGA 70
SLG9      1:ATGAAAGGTGTACGAAACATCTATCACCATTCTTACACCTTC------TTGCTCGTCTTCTTTGTCATGA 64
            ******** *** **** * ***   *  *************        ***** ** ** ** *** ***
            PS5 primer
SLG17    71:TTCTATTTCGTCCTGCCTTTTCGATCAACACTTTGTCGGCTACAGAATCTCTTACAATCTCAAGTAACAG 140
SLG9     65:TTTTATTTCGCCCTGCCTTTTCGCTCAGCACTTTGTCGTCTACAGAATCTCTTACAATCTCAAGCAACAG 134
            ** ******* *********** *** ********** ************************* *****

SLG17   141:AACACTTGTATCTGGGGGTGATGTCTTTGAGCTCGGTTTCTTCAGAACCAACTCAAGTTCTCGTTGGTAT 210
SLG9    135:AACACTTGTATCTCCCGGTAATATCTTCGAGCTCGGCTTCTTCAGAACCAA------TTCTCGTTGGTAT 198
            *************    *** ** **** ******** **************       *************

SLG17   211:CTCGGGATATGGTACAAGAAATTGTGGGACAGAACCTATGTATGGGTTGCCAACAGAGATAGCCCCCTCT 280
SLG9    199:CTCGGGATGTGGTACAAGAAATTGTCCGGCAGAACCTATGTATGGGTTGCCAACCGAGATAACCCTCTCT 268
            ******** ***************    * ************************* ****** *** ****

SLG17   281:CCAATGCCATTGGAACCCTCAAAATCTCCAGCATGAATCTTGTCCTCCTTGATCACTCTAATAAATCTGT 350
SLG9    269:CCAATTCCATTGGAACCCTCAAAATCTCCAACATGAACCTTGTCCTCCTCGATCACTCTAATAAATCTGT 338
            ***** *********************** ****** *********** *******************

SLG17   361:TTGGTCGACGAATATAACTAGAGGAAATGAGAGATCTCCGGTGGTGGCAGAGCTTCTCGCTAATGGAAAC 420
SLG9    339:TTGGTCGACCAATCTTACTAGAGAAAATGTGAGATCTCCGGTGGTGGCAGAGCTTCTGGCTAACGGAAAC 408
            ************* * ******* ***** ************************** ***** ******

SLG17   431:TTCGTGATGCGAGACTCCAATAACAACGGCGCAAGTGGATTCTTGTGGCAAAGTTTCGATTACCCTACAG 490
SLG9    411:TTCGTGGTACGAG------------ACC---CAAGTGGATTCTTGTGGCAAAGTTTCGATTACCCTACAG 463
            ****** * ****             **     ***************************************

SLG17   491:ATACTTTGCTTCCAGAGATGAAACTGGGTTACGACCTCAAAACAGGGCTGAACAGGTTCCTTACATCATG 560
SLG9    464:ATACTTTGCTTCCAGAGATGAAACTGGGTTACGACCTCAAAACAGGGCTGAACAGGTTCCTTGTCTCATG 533
            ***************************************************************    *****

SLG17   561:GAGAAGTTCAGATGATCCGTCAAGCGGAGTTTACTCGTACAAGCTTGAACTCCGAAA---TTTTCCTGAG 627
SLG9    534:GAGAAGTTCAGATGATCCGTCAAGCGGGGATTTCTCGTATAAACTCGACATTCAAAGGGGGTTGCCTGAG 603
            ************************** * ** ****** ** ** **  * * **    **  ******

SLG17   628:TTTTATATATTTGATGTGGACACTCAAATGCATCGGAGCGGTCCATGGAATGGAGTCAAATTTAGTGGCA 697
SLG9    604:TTCTATACATTTAAAGACAACACTCTAGTGCATCGGACTGGTCCATGGAATGGAATCCGATTTAGTGGCA 673
            ** **** **** * *    ****** * *********  *************** **  ***********

SLG17   698:TACCAGAGGACCAAAAGTTGAATTACATGGTGTACAATTTCACAGAGAATAGTGAAGAGGTCGCTTATAC 767
SLG9    674:TACCAGAGGAACAACAGTTGAGTTACATGGTTTACAATTTCACAGAGAATAGTGAGGAGGTCGCTTATAC 743
            ********** *** ****** ********* ********************** **************

SLG17   768:ATTCCTAGTTACCAACAACAGCATCTACTCGAGATTGAGAATAAGTACCTCAGGGTATTTTCAGCGACTG 837
SLG9    744:ATTTCTAGTGACCAACAACAGCATCTACTCAAGATTGACAATAAATTTCTCAGGGTTTTTTGAGCGACTG 813
            *** ***** ******************** ******* ***** *  ******** **** ********

SLG17   838:ACGTGGAGTCCATCATCAGAGATATGGAACCTGTTCTGGTCTTCTCCAGTGAACCTCCAGTGCGATATGT 907
SLG9    814:ACATGGACTCCGTCATTAGTGATATGGAACCCAATCTGGTCTTCTCCAGCGAGCTTCCAGTGCGATCCGT 883
            ** **** *** **** ** ***********    *************** ** * ***********  **

SLG17   908:ACAGGGTTTGTGGGCCTAACGCTTACTGTGACGTGAACACATCACCGGTGTGTAACTGTATCCAAGGGTT 977
SLG9    884:ACATGATTTGTGGGCCTGGCTCTTACTGTGACGTGAACACATTACCGTTGTGTAATTGTATCCAAGGGTT 953
            *** * ***********  * ******************** **** ******* **************

SLG17   978:CATTCCCTTGAATGTGCAGCAGTGGGATCTGAGAAACGGGTCCAGTGGGTGTATAAGGAGGACGCGGCTT 1047
SLG9    954:CAAGCCCTTGAATGTGCAAGAGTGGGATATGAGAGACAGGTCCAGTGGGTGTATAAGGAGGACGCGGCTG 1023
            **  **************  ******** ***** ** ********************************

SLG17  1048:AGCTGCAGTGGTGATGGTTTTACCAGGATGAGGAGGATGAAGTTGCCAGAGACTACGAAGGCGATTGTGG 1117
SLG9   1024:AGCTGCAGAGGAGATGGTTTTACCAGGATGAAGAATATGAAGTTGCCAGAAACTACGATGGCTACTGTCG 1093
            ******** ** ****************** **  ************* ******* *** * *** *

SLG17  1118:ACAGGAGTATTGGTGTGAAAGAATGTGAGAAGAGGTGTCTTAGCGATTGTAATTGTACCGCGTTTGCAAA 1187
SLG9   1094:ACCGCAGTATTGGTGTGAAAGAATGTGAGAAGAAGTGCCTTAGCGACTGTAATTGTACCGCGTTTGCAAA 1163
            ** * **************************** *** ******** **********************

SLG17  1188:TGCTGATATCCGGAATGGTGGGACGGGTTGTGTGATTTGGACCGGAGAGCTTGAGGATATCCGGACTTAC 1257
SLG9   1164:TGCGGATATCCGGGATGGTGGGACAGGTTGTGTGATTTGGACCGGACGGCTTGACGATATGCGGAATTAC 1233
            *** ********* ********** *********************  ****** ***** **** ****

SLG17  1258:TT---TGATGACGGTCAAGATCTTTATGTCAGATTGGCTCCTGCTGACCTTGTTAGCTCTTTCTCTTAAA 1324
SLG9   1234:GTTGCTGATCACGGTCAAGATCTTTATGTCAGATTGGCTCCTGCTGACCTTGTTAGCTCTTTCTCTTAAA 1303
             *     **** **********************************************************

SLG17  1325:ATAAAACACGG 1335
SLG9   1304:ATAAAACACGG 1314
            ***********
            PS15 primer
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Cloning of the PCR product and determination of the nucleotide sequence

PCR products were cloned into the pCRII vector with the TA cloning system (Invitrogen). *SLG*-positive clones were selected by colony hybridization. The plasmids having the insert of the expected size were isolated, and the nucleotide sequences of the inserted DNA were determined with a DNA sequencer (Perkin-Elmer ABI, Prism 377).

FISH

The chromosome sample was prepared by the enzyme maceration/air-drying (EMA) method previously described (Fukui and Iijima 1991; Fukui 1996).

FISH for detection of *SLG-17* locus was carried out by the method reported previously (Ohmido and Fukui 1997). Briefly, the full length (1.3 kb) of a cloned PCR product from T-17 genomic DNA was biotin-labeled by PCR. The biotin-labeled probe was hybridized and detected by avidin-FITC (fluorescein isothiocyanate) conjugate. The FITC signal was enhanced by a secondary immunological reaction of the biotinylated anti-avidin and fluorescein-avidin. The chromosomes were counterstained with DAPI (4,6,-diamidino-2-phenylindole).

A 4.4 kb DNA fragment containing the *SLG-9* region (ca. 1.3 kb) has already been subcloned from S9 homozygote (Suzuki et al. 1995). Since *SLG-17* and *SLG-9* have approximately 85% sequence homology, a 4.4-kb DNA fragment from S9 could be used as the probe for detection of the *SLG-17* locus in ‘Kukai’ by FISH. McFISH was performed using *SLG-17* and *SLG-9* as probes. *SLG-17* was biotin-labeled by PCR, while the *SLG9* fragment (4.4 kb) was digoxigenin-labeled by the random primer labeling method. Two kinds of probes, the biotin-labeled *SLG-17* and the digoxigenin-labeled *SLG-9*, were simultaneously hybridized to the chromosome by the multi-color FISH method. The biotin-labeled probe was detected following the same method described above. The digoxigenin-labeled probe was detected with anti-digoxigenin-rhodamine (Boehringer), and the signal was enhanced by anti-Sheep-Texas red (Vector). The chromosomes were counterstained with DAPI.

Three kinds of fluorescent signals, FITC (green), rhodamine (red) and DAPI (blue), were detected by fluorescence microscopy (Leica) with Micro Mover-W (Photometrics). Three images were acquired separately in the IP Lab-PVCAM system through a cooled CCD camera (Photometrics). These were then integrated into a single composite image and were pseudo-colored based on the original fluorescent colors. The composite images were printed out with Pictrography (Fuji).

Results and discussion

DNA fragments 1.3 kb in length were amplified by PCR from the genomic DNA of the T-17 parental line using the class-I *SLG* specific primers. These fragments were of the same size as those amplified previously from several kinds of S-homozygotes with class-I *SLG* specific primers (Brace et al. 1993; Nishio et al. 1996). From the genomic DNA of another parental line, T-18, no DNA fragment was amplified using these class-I *SLG* specific primers. This result clearly suggests that the *SLG* of T-17 is class I and the *SLG* of T-18 is class II.

In order to characterize the amplified DNA with class-I *SLG* specific primers, we Southern-hybridized the cloned PCR product to the total genomic DNAs of T-17, T-18 and the F_1 hybrid digested with *Eco*RI. Two fragments from T-17 and the F_1 hybrid hybridized with the PCR product (Fig. 1). As there is no *Eco*RI site in the amplified probe, one of the fragments contains the *SLG* gene while the other is expected to be a fragment containing the S-domain of an *SRK* gene, because the sequence homology between the S domain of *SRK* and *SLG* is 76–97% (Yamakawa et al. 1995; Watanabe et al. 1994). The hybridization signal of T-17 was more intense than that of the F_1 hybrid as described previously (Nishio et al. 1994). For the genomic DNA of T-18, no fragment hybridized with the probe. Therefore, the sequence homology between the *SLG*s of T-17 and T-18 should be low.

The fragment amplified by the class-I *SLG* specific primers was subcloned into pCRII and sequenced (Fig. 2). The nucleotide sequence homology between the amplified fragment and *SLG-9*, *SLG-8* and *SLG-12*, respectively, was more than 85% (Yamakawa et al. 1995; Watanabe et al. 1994). From the results of the genomic Southern hybridization and sequencing, it was revealed that the DNA fragment amplified from the T-17 genomic DNA is a new gene belonging to class-I *SLG*; it was therefore designated as *SLG-17*.

In the case of the T-18 genomic DNA, a DNA fragment was amplified using the class-II specific primers, PS3 and PS21. This result suggests that T-18 has a class II *SLG* gene. However, the amplified DNA fragment hybridized with several fragments in digested genomic DNA of T-17, T-18 and the F_1 hybrid in Southern hybridization (data is not shown). *S*-like genes other than the *SLR2* genes have been isolated (Kumar and Trick 1994; Suzuki et al. 1995) that are not linked to the *S*-locus and have high sequence homology to class-II *SLG*s. The DNA fragment would hybridize with *S*-like genes in addition to *SLG-18*. Therefore, the DNA fragment amplified from the genomic DNA of T-18 with class-II specific primers was not used as the probe for FISH in this study.

In order to reveal the position of *SLG* locus in the chromosomes of *B. campestris* L. ‘pekinensis’ cv ‘Kukai’, we performed FISH using biotin-labeled *SLG-17* (Fig. 3). Figure 3a shows metaphase chromosomes ($2n = 20$) stained with DAPI. All 20 chromosomes were clearly observed. The *SLG-17* locus was detected as green fluorescent signals in Fig. 3b, which has been composed with the DAPI-stained chromosome image, and localized as a doublet signal at the interstitial region close to the end of a single small chromosome. The doublet signal indicated the existence of the *SLG-17* locus on each replicated sister chromatid. Since *SLG-17* was amplified from T-17 and did not hybridize with T-18 genomic DNA in genomic Southern hybridization, the chromosome in which the signal was detected should be derived from the parental line, T-17. There was no signal on any other homologous chromosome from T-18.

The *SLG-17* locus was detected on the metaphase chromosome of an F_1 hybrid by FISH. However, it was difficult to identify every metaphase chromosome of

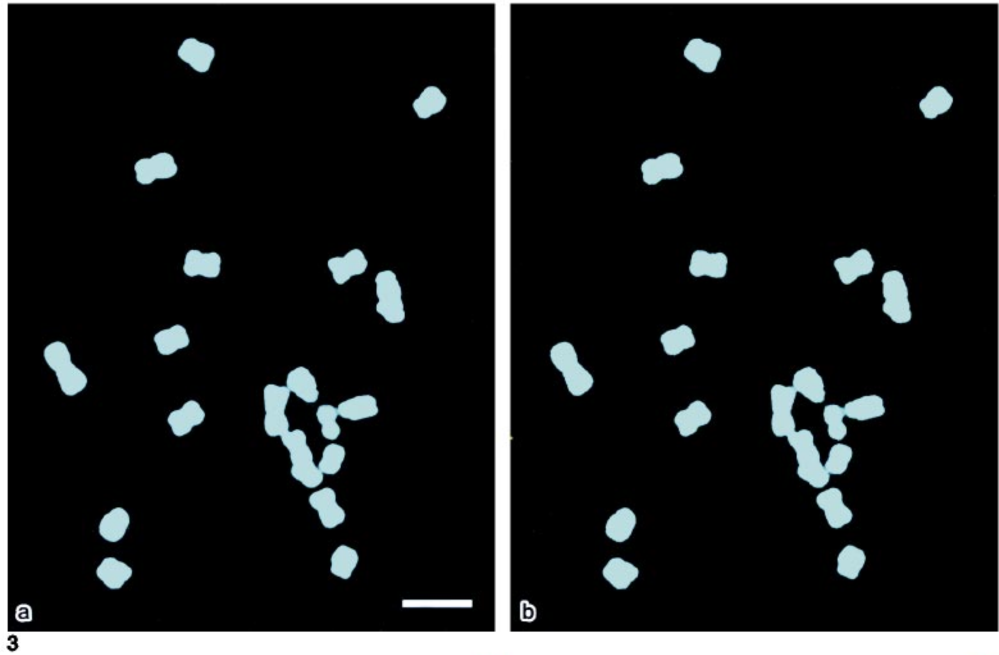

Fig. 3a, b The hybridization signals of metaphase chromosomes of *B. campestris* obtained by FISH using *SLG-17* as the probe. **a** DAPI-stained chromosome, **b** signals of *SLG-17* after FISH. *Bar*: 5 μm

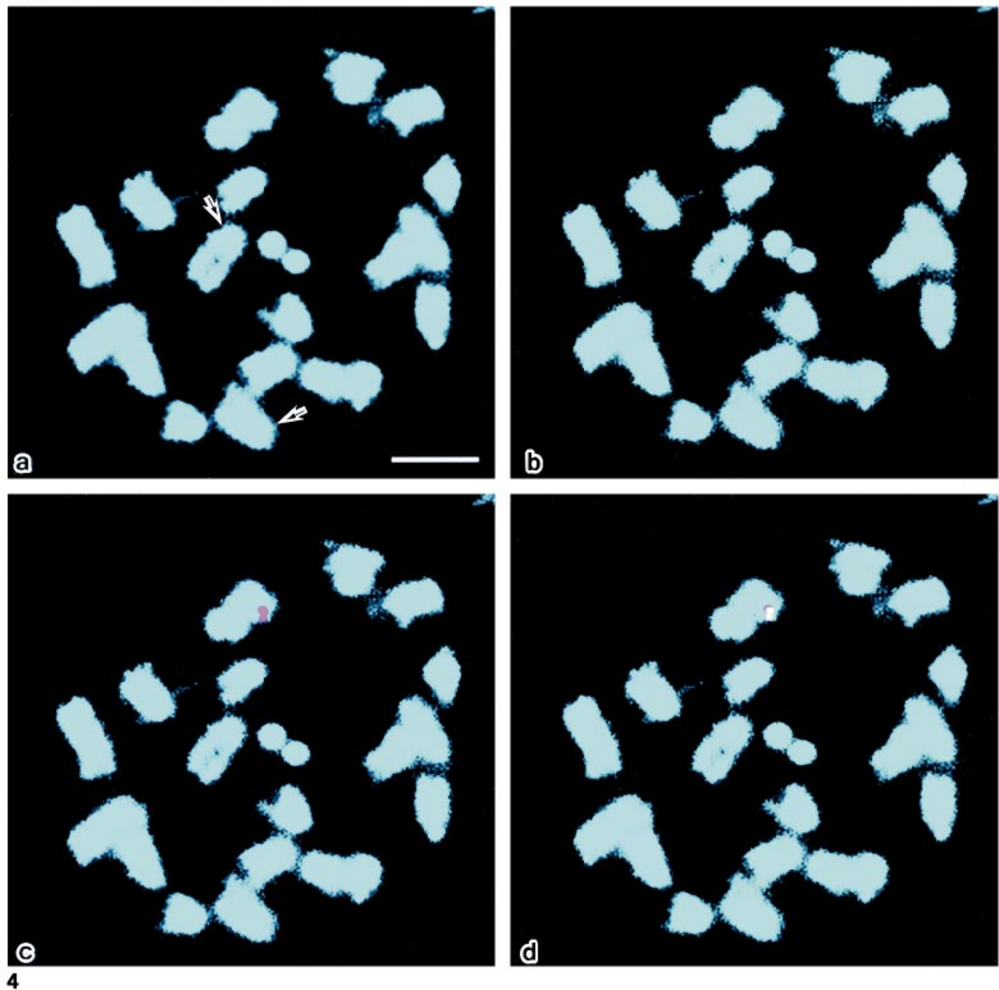

Fig. 4a–d McFISH of *B. campestris* with simultaneous hybridization of the *SLG17* and *SLG-9* probes. **a** DAPI-stained chromosome; two satellite chromosomes are indicated by *arrows*, **b** signal of *SLG-17* (*green fluorescent signal*), **c** signal of *SLG-9* (*reddish fluorescent signal*), **d** the composite image of the DAPI-stained chromosome image, *SLG-17* and *SLG-9* signal images. *Bar*: 5 μm

Brassica because of their smallness in size and the similarity of the karyotype. Conversely, prometaphase chromosomes of *Brassica* individually show characteristic condensation patterns. Based on these condensation patterns, idiograms of the three *Brassica* diploid spp. has been developed (Fukui et al. 1998). Thus there is a great possibility that the chromosome with the *SLG-17* locus can be identified when FISH is applied to the chromosomes at the prometaphase stage.

The detection of a single-copy gene by FISH often depends on the probe size. When the probe is longer, the fluorescent signal becomes more intense. In the S9 homozygote of *B. campestris*, a 4.4-kb DNA fragment containing the *SLG-9* region has been subcloned (Suzuki et al. 1995), while *SLG-9* gene is aprroximately 1.3 kb. Since the *SLG-17* and *SLG-9* genes have approximately 85% sequence homology, the 4.4-kb DNA fragment from S9 could also be useful as the probe for detection of the *SLG-17* locus in 'Kukai' by FISH. McFISH was therefore performed using *SLG-17* and the 4.4-kb DNA fragment with *SLG-9* as probes on the prometaphase chromosome (Fig. 4). Figure 4a shows the image of prometaphase chromosomes stained with DAPI. Although the satellites exist apart from their harboring chromosomes, 20 chromosomes could easily be discriminated. Figure 4b shows the *SLG-17* locus as a green fluorescent signal on the DAPI-stained chromosome, localized as the doublet signal at the interstitial region close to the end of a small chromosome as in the metaphase chromosome. The reddish signal of *SLG-9* was stronger than that of *SLG-17* and also observed at the same site as in the case of the *SLG-17* probe (Fig. 4c). Figure 4d is the composite image of the image of the DAPI-stained chromosomes, the green *SLG-17* signal image and the reddish *SLG-9* signal image. The two signals of *SLG-17* and *SLG-9* overlapped each other close to the end of the chromosome. Therefore, we concluded that *SLG* in *B. campestris* L. 'pekinensis' cv 'Kukai', was localized close to the end of the chromosome.

It has been difficult to physically detect a single-copy gene in a chromosome by FISH. In this study, the intensity of the signals was relatively strong, although the size of the probe was small. It was shown that the *SLG* gene links to the *SRK* gene and that the sequence homology between the S domain of *SRK* and *SLG* is 76–97% (Yamakawa et al. 1995; Watanabe et al. 1994). It was confirmed that the *SLG* probe hybridized with two fragments, the *SLG* and *SRK* fragments, in genomic Southern hybridization. It was recently reported that the physical distance between *SRK* and *SLG* is 25 kb in *B. napus* (Yu et al. 1996). Therefore, the more distinct than expected signals in the chromosome sample could mean that the *SLG* probe would hybridize with both the *SLG* and the S-domain site of *SRK*.

In this study, the *SLG-17* gene was isolated from the parental line, T-17, of 'Kukai', and the character of the *SLG-17* gene was examined with genomic Southern hybridization and sequence analysis. FISH using the *SLG-17* gene as the probe resulted in a doublet signal being detected on a single chromosome derived from the parental line, T-17. In *B. campestris*, the *SLG* locus was visualized for the first time. In a future investigation the *SLG* locus in *B. campestris* will be compared with the *SLG* locus in *B. oleracea* reported previously (Fukui et al. 1998) by quantitative analysis of the chromosomes. In the future, it will be feasible to reveal the entire localization of *S*-like genes of *Brassica* by combining the quantitative chromosome maps and the efficient McFISH that was developed by the current research.

Acknowledgements This work was supported in a part by Grants-in-Aid for Special Research on Priority Areas (nos. 07281102 and 17281103; Genetic Dissection of Sexual Differentiation and Pollination Process in Higher Plants) from the Ministry of Education, Science, Culture and Sports, Japan.

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