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ORIGINAL PAPER

Stable progeny production of the amphidiploid resynthesized *Brassica napus* cv. Hanakkori, a newly bred vegetable

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Abstract Resynthesized *Brassica napus* cv. Hanakkori (AACC, $2n = 38$) was produced by cross-hybridization between *B. rapa* (AA, $2n = 20$) and *B. oleracea* (CC, $2n = 18$) as a new vegetative crop. Many studies have provided evidences for the instability and close relationship between A and C genome in the resynthesized *B. napus* cultivars. In fact, seed produced to obtain progeny in Hanakkori had unstable morphological characters and generated many off-type plants. In this study, we investigated the pollen fertility, chromosome number, structure, and behavior linked to various Hanakkori phenotypes to define factors of unstable phenotypic expression in the progeny. Hanakkori phenotypes were categorized into five types. The results of pollen fertility, chromosome number, and fluorescence in situ hybridization analysis for somatic mitosis cells indicated that the off-type plants had lower pollen fertility, aberrant chromosome number, and structures with small chromosome fragments. Observation of chromosomes at meiosis showed that the meiotic division in off-type plants led to appreciably higher abnormalities than in on-type plants. However, polyvalent chromosomes were observed frequently in both on- and off-type plants in diplotene stage of meiosis. We assume that the unstable morphological characters in resynthesized progeny were the result of abnormal division in meiosis. It results as important that the plants of normal phenotype, chromosome structure and minimized abnormal meiosis are selected to stabilize progeny.

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Introduction

It is possible relatively to produce artificially resynthesized *Brassica napus* from between A and C genomes. It is common knowledge that natural and resynthesized *B. napus* are cultured mainly as oil crops. The interspecific hybrid Hanakkori is a form of resynthesized *B. napus* (AACC, $2n = 38$), resulting from a cross between *B. rapa* var. *utilis*, Saishin (a Chinese vegetable; AA, $2n = 20$) and *B. oleracea* var. *italica*, Broccoli (CC, $2n = 18$) obtained in Yamaguchi prefecture, Japan, in 1996. Hanakkori is a new and original vegetable with edible flower buds and young stems. In Yamaguchi prefecture, Hanakkori has become an agriculturally important horticultural cultivar because of its easy cultivation, good taste, and high nutritive value. At present, Hanakkori yields are approximately 10 t/ha, and the crop is grown on roughly 20 ha and worth 100 million yen (US$ 1.1 million). The area of Hanakkori production is expected to increase in the future.

Hanakkori is a genetically amphidiploid plant produced by colchicine treatment after pollination and ovule culture in vitro. However, the morphological characters of Hanakkori are unstably expressed, and off-type plants frequently appear among progeny for seed production, as commonly observed in resynthesized *Brassica napus* plants (Nishi et al. 1959; Taguchi et al. 1993). In agricultural production, instability of morphological characters leads to decreased yield (2,000 kg/ha) and reduced supply of uniform plants for customers. Therefore, it is essential to resolve this problem through breeding. A reduction in off-

type Hanakkori cannot be achieved simply through mass selection based on phenotype characters. In addition, no genetic studies have examined the resynthesized *Brassica napus* vegetable Hanakkori. Thus, it is important to investigate the structure and behavior of Hanakkori chromosomes in detail.

Cytogenetic identification of *Brassica* chromosomes among different genomes, such as *B. rapa* (AA, $2n = 20$), *B. oleracea* (CC, $2n = 18$), and *B. nigra* (BB, $2n = 16$), and their allopolyploid hybrids *B. napus* (AACC, $2n = 38$), *B. carinata* (BBCC, $2n = 34$), and *B. juncea* (AABB, $2n = 36$), has been improved by recent advances in fluorescence in situ hybridization (FISH: Fukui et al. 1998; Kulak et al. 2002; Snowdon et al. 2002; Lim et al. 2005, 2007; Snowdon 2007; Xiong and Pires 2011) and genomic in situ hybridization (GISH: Snowdon et al. 1997; Benabdelmouna et al. 2003; Maluszynska and Hasterok 2005; Wang et al. 2005; Howell et al. 2008). Snowdon et al. (1997), using GISH, demonstrated that while the respective diploid donor genomes could be reliably distinguished in *B. juncea* and *B. carinata*, the A and C genome components in *B. napus* could not be clearly distinguished, confirming the considerable homoeology between these genomes. However, Snowdon et al. (2002) reported that FISH with the 5S and 25S ribosomal RNA gene (rDNA) probes to prometaphase chromosomes allowed more dependable identification of the A and C genomes in *Brassica* chromosomes. The morphology and molecular organization of heterochromatin domains in the mitotic chromosomes of *B. rapa* were described using 4′,6-diamidino-2-phenylindole (DAPI) staining and FISH on rDNA and pericentromere tandem repeats (Lim et al. 2005), allowing FISH mapping of the 5S and 45S rDNA, centromeric repeat in *B. rapa* (CentBrs), and bleached DAPI band (BDB) in mitotic metaphase chromosomes of *B. rapa*. Lim et al. (2007) also characterized the centromere and peri-centromere retrotransposons in *B. rapa* and their distribution in related *Brassica* species. Using FISH with element-specific probes, they localized centromere-specific retrotransposons of *Brassica* (CRB) CentBr, peri-centromere-specific retrotransposons of *Brassica* (PCRBr), 805 bp degenerate tandem repeats (TR805), and 238 bp degenerate tandem repeats (TR238) sequences in the genomes of diploid *Brassica* species (*B. rapa*, *B. nigra*, and *B. oleracea*). Xiong and Pires (2011) introduced a new chromosome nomenclature system that follows the international linkage group system for Brassica, and they reported that a complete karyotype analysis distinguished each chromosome in *B. rapa*, *B. oleracea,* and *B. napus* by using FISH for rDNA, CentBr, and BAC clone as a probe. Thus, in genomic study of *Brassica* species, the pairing of homoeologous chromosomes and identification of chromosomes has been advanced through molecular cytogenetics using FISH and GISH.

In this study, we examined mitotic cell metaphase chromosomes using FISH with 45S rDNA and specific probes and investigated the behavior of meiotic chromosomes in the resynthesized *B. napus*, Hanakkori, having various phenotypes. We discuss the factors leading to unstable phenotypic expression in progeny of Hanakkori, which are linked to pollen fertility and the number, structure, and behavior of mitotic and meiotic chromosomes. We also suggest ways to produce more stable progeny of Hanakkori through *Brassica* breeding.

Materials and methods

Plant material

Seeds of resynthesized *B. napus* cv. Hanakkori (AACC) were used. Hanakkori was produced in Yamaguchi prefecture by interspecies hybridization between *B. rapa* (AA, $2n = 20$) and *B. oleracea* ($2n = 18$) (Fig. 1). This plant variety was registered in 1999 to protect the rights of the breeder through the Seeds and Seedling Act of Japan. Seeds of Hanakkori were obtained after three generations by mass seed production. The seeds were sown on 136-hole cell trays containing commercial compost and germinated at ambient temperature. After 1 month, the seedlings were moved to a greenhouse, and the morphological characters of Hanakkori immediately before bolting were examined for each individual in 2007. Fifty individuals of Hanakkori were categorized according to differences in morphological characters, such as plant and leaf shape and shoot and bud characteristics to define phenotypes of Hanakkori.

Mitotic chromosome preparations and pollen fertility

Root tips were excised from the growing plantlets 3 weeks after germination, pretreated immediately in fresh water at 0°C for 24 h, and then fixed in fresh fixative (ethanol:acetic acid 3:1). The fixed root tips were preserved at −20°C until experiments were performed. The plantlets with excised root tips were then planted in a greenhouse to examine morphological characters of phenotypes in each individual Hanakkori plant. Mitotic chromosomes were prepared from 40 individuals of Hanakkori, and their phenotypes were defined. Chromosome samples were prepared by the enzymatic maceration/air-drying (EMA) method (Fukui and Iijima 1991) with minor modification, with the enzyme mixture of 4% cellulase Onozuka RS, 1% pectolyase Y-23, adjusted to pH 4.2. This was followed by maceration of root tips for 40 min at 37°C. Air-dried samples were stained with DAPI, and chromosome number was examined under a fluorescence microscope (BX-50, Olympus, Tokyo, Japan). In 2008, 100 plantlets of Hanakkori were cultured in a

Fig. 1 The appearances of resynthesized *B. napus* cv. Hanakkori and its parents. **a** Maternal parent of Hanakkori, Saishin (AA) **b** Paternal parent of Hanakkori, Broccori (CC) **c** Hanakkori (AACC) *Bar* 10 cm

greenhouse for 2 months, and 73 flowering individuals were examined for pollen fertility and chromosome number. Pollen samples were stained with 1% aceto-carmine, and fertility was examined under a microscope.

Fluorescence in situ hybridization (FISH)

The chromosomes prepared using the EMA method were subjected to FISH. The 45S rDNA from rice and CentBr2 (kindly provided by Dr. K. B. Lim; Lim et al. 2005) were directly labeled by nick translation with the fluorochromes Cy3 and fluorescein isothiocyanate (FITC), respectively. FISH methods followed those of Ohmido and Fukui (1997) with minor modification. After hybridization, slides were washed at 42°C for 5 min in 2× SSC and for 5 min in 0.1× SSC. Biotin-labeled rDNA and digoxigenin-labeled CentBr2 were detected with Cy3 streptavidin (Jackson Immuno Research Laboratories, West Grove, PA) and anti-digoxigenin-fluorescein (Roche, NJ), respectively. Slides were then washed for 5 min in 2× SSC at room temperature. At least five to ten well-spread prometaphase images were observed under a fluorescence microscope (BX50; Olympus, Tokyo, Japan) equipped with a sensitive cooled CCD camera (PXL1400; Photometrics, Tucson, AZ, USA) and captured through blue (B), green (G), and ultraviolet (UV) excitation and emission filter sets.

Anther cell preparation and chromosomes

Meiosis of Hanakkori was examined for high and low pollen fertility rates. The calyx and petals were excised from flower buds and treated with enzymes (2% cellulase Onozuka RS, 5% pectolyase Y-23) at 37°C for 3 h. The anthers were squashed on a slide, 5 µl of 60% acetic acid were added, and the slides were heated on a hotplate at 65°C for 50 s. After heating, 50 µl of fresh fixative (ethanol:acetic acid 3:1) were applied and the slides were air dried. Chromosomes in meiosis were stained with DAPI and their state and behavior were examined under the fluorescence microscope.

Results

Phenotype, chromosome number, and pollen fertility

Hanakkori was grouped into five types according to morphological characters (Fig. 2): one normal (on-type) and

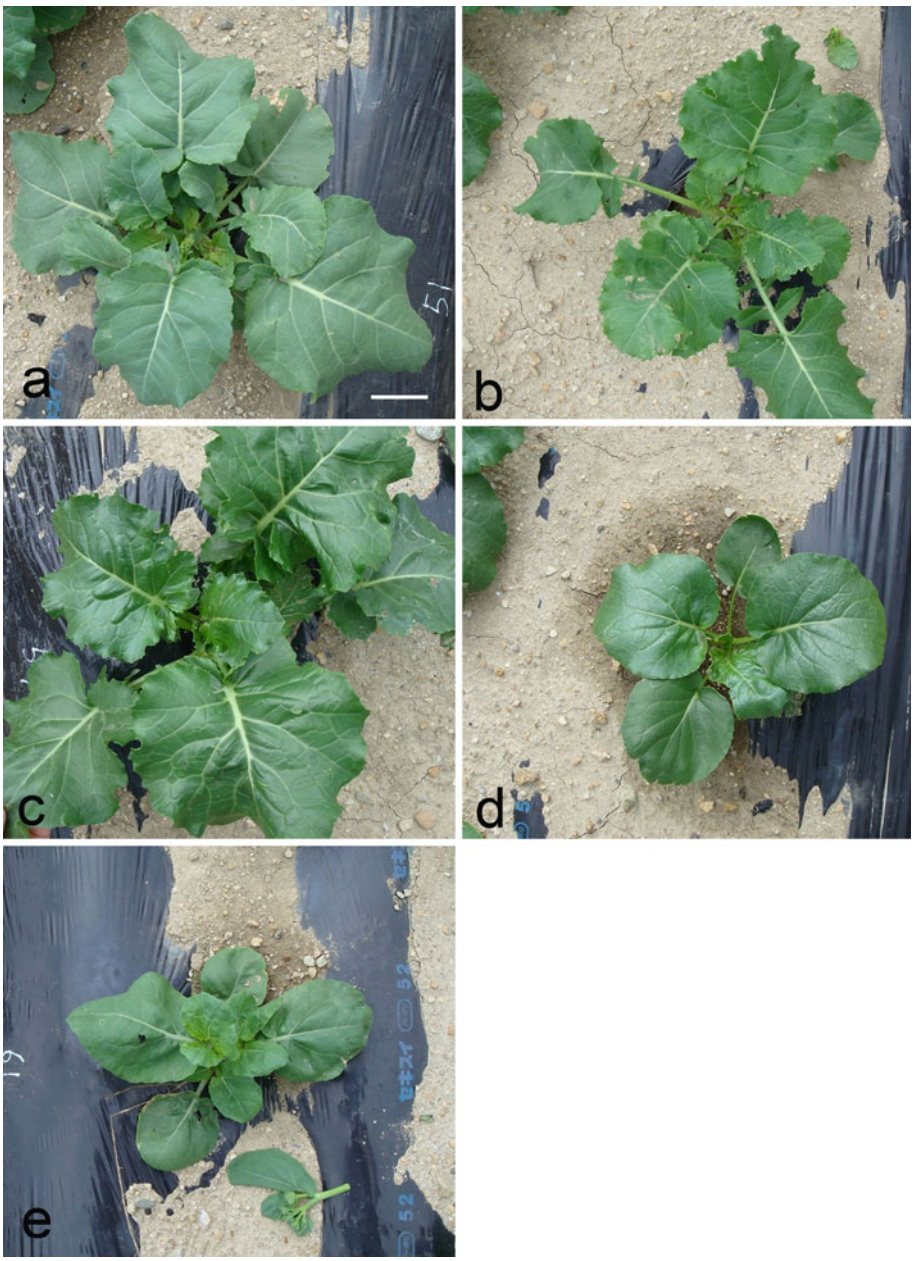

Fig. 2 Examples of phenotype variation in resynthesized *B. napus* cv. Hanakkori.
a On-type plant.
b Large-lobation off-type plant.
c Waxless off-type plant.
d No-lobation off-type plant.
e Anomaly off-type plant.
Bar 10 cm

four abnormal (off-types: waxless, large-lobation, no-lobation, and anomaly). The on-type of Hanakkori was similar to the Broccoli pollen parent, in terms of plant and leaf shape, and to the maternal parent Saishin, in terms of the high branching habit. The off-type waxless lacked wax on leaf surfaces (Fig. 2c), the large-lobation off-type leaves resembled those of radish (Fig. 2b), and the no-lobation off-type lacked lobation on leaf edges (Fig. 2d). The anomaly off-type was obviously different from the on-type and the three other off-types (Fig. 2e). Hanakkori is an amphidiploid resynthesized *B. napus* (AACC, $2n = 38$) produced by crossing Broccoli of *B. oleracea* (CC, $2n = 18$) and Saishin of *B. rapa* (AA, $2n = 20$). In 40 individual Hanakkori plants, chromosome number ranged from 36 to 42 (Table 1), with on-type plants mostly having 38 chromosomes, although a few on-type plants had different chromosome numbers. Chromosome numbers in the waxless and large-lobation off-types varied from plant to plant, but 38 chromosomes were never detected in the no-lobation and anomaly off-types. Hence, relationships between phenotype and chromosome number in Hanakkori were apparent.

Generally, the examined plants with 38 chromosomes showed high pollen fertility and the on-type phenotype, although the relationships among chromosome number, phenotype, and pollen fertility were poor (Table 2). Pollen

Table 1 Number of Hanakkori plants classified by phenotype and chromosome number

Phenotypes	Number of Chromosomes						
	36	37	38	39	40	41	42
On-type	3	0	11	1	0	0	0
Off-type (waxless)	2	2	4	1	0	0	0
Off-type (large-lobation)	0	4	4	2	1	0	0
Off-type (no-lobation)	1	1	0	0	0	0	0
Off-type (anomaly)	1	1	0	0	0	0	1

Table 2 Relationships of chromosome number to pollen fertility and phenotype

Phenotypes	Number of chromosomes	Percentage of samples (%)	Pollen fertility	
			Percentage (%)	SD[a]
On-type	38	35.5	87.4	4.6
On-type	36	13.2	62.4	13.7
Off-type (waxless)	38	18.4	80.6	10.8
Off-type (large-lobation)	37	23.7	75.9	9.8
Off-type (no-lobation)	37	7.9	73.1	9.5
Off-type (anomaly)	36	1.3	34.3	-[b]

[a] Standard deviation

[b] No calculation because too few samples

fertility for many on-type Hanakkori plants was over 80%, but low pollen fertility was also observed in some of these plants. Moreover, pollen fertility was high in waxless, large-lobation, and no-lobation types, but it was low in the anomaly off-type.

Chromosome structure

The somatic metaphase chromosomes of Hanakkori were examined using FISH. The red and green fluorescence signals (Figs. 3, 4) show 45S rDNA and CentBr2 with a centromere-specific tandem repeat on metaphase chromosomes of the resynthesized *Brassica napus* Hanakkori on-type and off-type, respectively. A chromosome image of the on-type with 38 chromosomes is shown in Fig. 3a. Metaphase somatic chromosomes in *B. napus* are too small to count; therefore, we counted at least ten cells of each line to ensure accurate numbers. We observed ten signals for 45S rDNA and CentBr2 probes, which hybridized with chromosome complements of on-type resynthesized *B. napus* Hanakkori (Fig. 3b). These chromosome complements of the on-type were normal without any atypical chromosomes, such as chromosome fragmentation and aberrations. We compared these signals for Hanakkori chromosomes with the ideogram of Xiong and Pires (2011). The ideogram presumed that these chromosomes possess signals derived from A and C genomes and that only one chromosome set carries both the CentBr2 locus on the centromere region and the 45S rDNA locus on the nucleolar organizer region (NOR) in Hanakkori, corresponding to chromosome A3 in *B. rapa*. Although Xiong and Pires (2011) reported that chromosome C8 in *B. oleracea* carried both the CentBr2 locus on centromere regions and the 45S rDNA locus on the terminal ends of chromosome short arms, the C8 chromosome of *B. oleracea* was not observed in complements of Hanakkori. However, two observed chromosome sets, corresponding to chromosomes C7 and C8 without CentBr2 signals, carried the 45S rDNA loci on the terminal ends of the short arms. It is thought that the four other signals of 45S rDNA on near-centromere regions of the two chromosome sets originate from chromosomes of *B. rapa*, and that the other eight signals of CentBr2 on centromere regions of the four chromosome sets originate from chromosomes of *B. oleracea*.

Figure 3c shows an on-type with 39 chromosomes. This plant had an aberrant chromosome fragment (Fig. 3c, arrowhead) which differed from other chromosomes in that it had a low concentration of chromatin, and the presence of centromere region was not clear. No signal for CentBr2 or 45S rDNA was detected on this fragment (Fig. 3d). An off-type large-lobation with 40 chromosomes is shown in Fig. 4a–b, in which the three chromosomes carry both the CentBr2 locus on centromere regions and the 45S rDNA locus on the nucleolar organizer region-bearing chromosome, possibly corresponding to chromosome A3 of *B. rapa*. This off-type may contain aberrant chromosome fragments. The waxless off-type with 36 chromosomes is shown in Fig. 4c; the missing chromosomes might be derived from C genome, because the 8 chromosomes with CentBr2 on the centromere were detected in this line (Fig. 4d). There was no

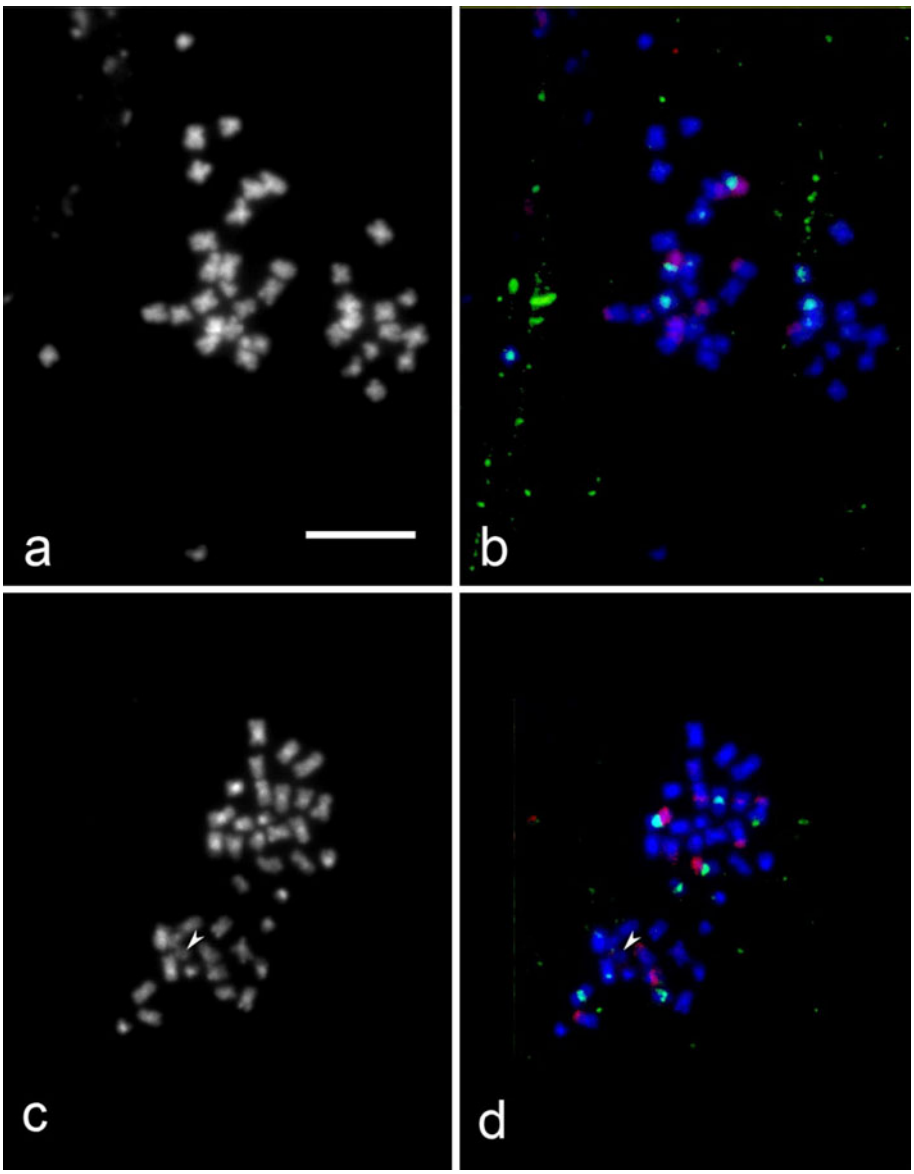

Fig. 3 FISH of somatic metaphase chromosomes for resynthesized *B. napus* cv. Hanakkori on-type, using 45S rDNA and CentBr2 as probes. Hybridization sites detected with Cy3 and FITC (**b**, **d**) and chromatin counterstained with DAPI (**a**, **c**). 45S rDNA and CentBr2 loci are indicated as *red* and *green* fluorescence, respectively. **a**, **b** Chromosome complement of 38 basic number in *B. napus*. **c**, **d** Chromosome complement of 39. *Arrowhead* indicates abnormal chromosome fragment. *Bar* 10 μm

aberrant chromosome fragment in this plant. The anomaly off-type with 42 chromosomes is shown in Fig. 4e; this off-type carries four excess and aberrant chromosome fragments without CentBr2 signals (Fig. 4f).

Fertility and chromosome behavior at meiosis

We determined the relationship between anther size and meiosis stage in Hanakkori. We then investigated chromosome structure and behavior at meiosis from the pachytene to telophase II stages in two types of Hanakkori: the on-type, with normal phenotype and chromosome number and high pollen fertility (87.4%), and the off-type with 36 chromosomes and low pollen fertility (34.3%). The meiosis stage of normal Hanakkori (pollen fertility is 87.4%) is shown in Fig. 5, with normal stages of pachytene to telophase II. However, chromosome pairing at the diplotene stage was not bivalent but polyvalent (arrowhead in Fig. 5b), and occasional aberrant chromosome fragments were produced at telophases I and II (Fig. 5j, k).

The meiosis stages of atypical Hanakkori with 36 chromosomes (pollen fertility 34.3%) are shown in Fig. 6. In this atypical Hanakkori, it was difficult to synchronize meiosis stages from pachytene to telophase II. The images in Fig. 6f–g are of the same anther and show various stages of meiosis; mainly pachytene was observed in the first division, followed by metaphase I, and a few stages of diplotene in polyvalent chromosomes, similar to in normal Hanakkori (Fig. 6b). Furthermore, abnormal meiosis of Hanakkori was more common than in the normal type. For

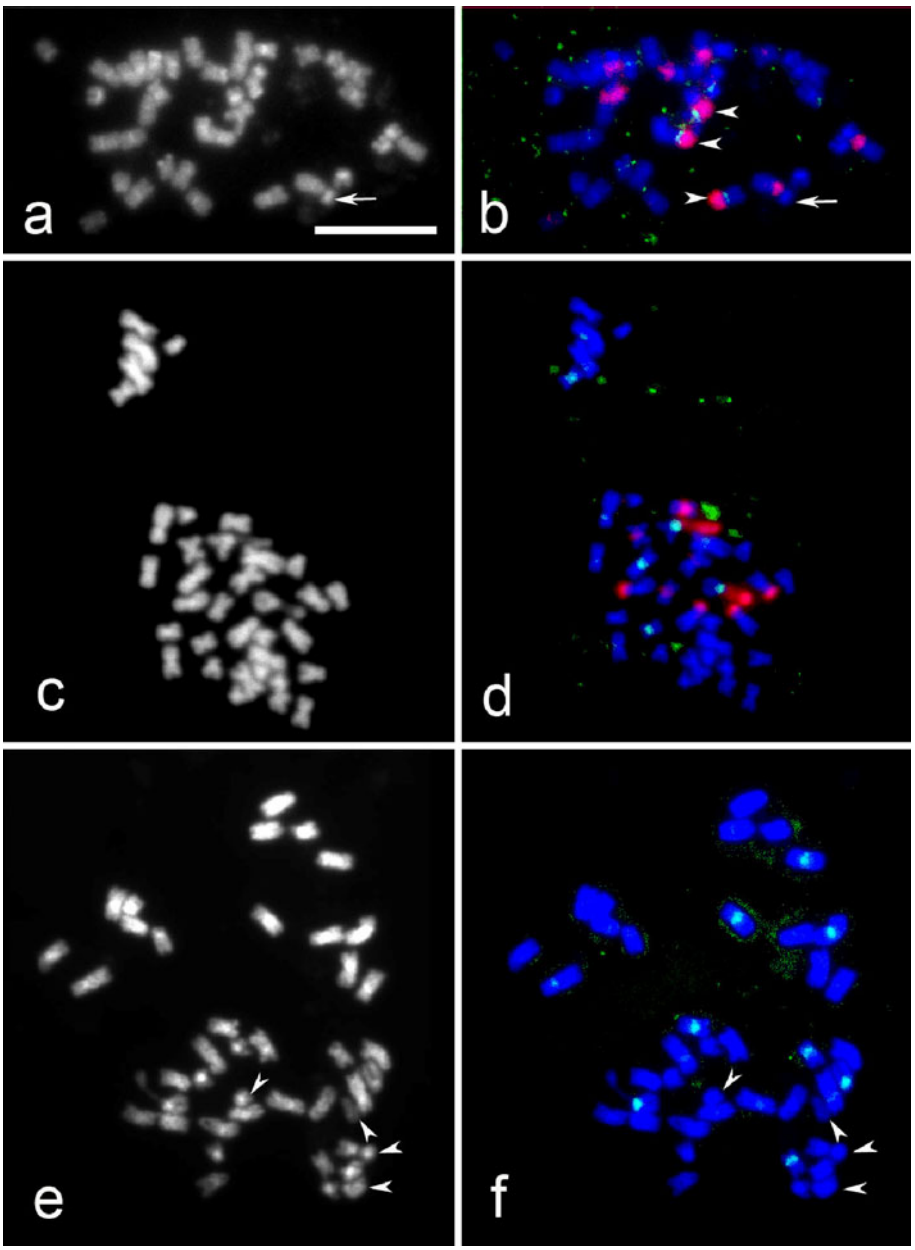

Fig. 4 FISH of somatic metaphase chromosomes for resynthesized *B. napus* cv. Hanakkori off-type using 45S rDNA and CentBr2 as probes. Hybridization sites detected with Cy3 and FITC (**b**, **d**, **f**) and chromatin counterstained with DAPI (**a**, **c**, **e**). 45S rDNA and CentBr2 loci indicated by *red* and *green* fluorescence, respectively. **a**, **b** Chromosome complement of 40 for Hanakkori large-lobation off-type. *Arrowheads* indicate A3 chromosome of *Brassica rapa* ($2n = 20$). *Arrows* indicate abnormal chromosome fragments. **c**, **d** Chromosome complement of 36 for Hanakkori waxless off-type. No abnormal chromosome fragment was observed, although chromosome number was less than 38. **e**, **f** Chromosome complements of 42 for Hanakkori anomaly off-type. *Arrowheads* indicate four abnormal chromosome fragments. CentBr2 was used as probe. *Bar* 10 μm

example, both no pairing chromosome fragments and univalent chromosomes were frequently observed in metaphase I, anaphase I, and anaphase II (Fig. 6c, d, e).

Discussion

It is difficult to fix the morphological characters of synthesized *Brassica* by polyploidization after crossing the A and C genomes. In resynthesized *Brassica napus*, such interspecific hybrids have unstable fertility for seed formation and fixation of morphogenesis (Zhang et al. 2001; Taguchi et al. 1993). Hanakkori bred as a new local specialty is also resynthesized *B. napus* in Yamaguchi prefecture. The morphogenetic characters were found to be unstable and segregated into various phenotypes such as waxless and aberrant lobation. In this study, the morphogenetic characters of progeny segregated into five types, and progeny often had atypical phenotypes for morphological characters.

We observed chromosomes to investigate factors leading to atypical phenotypes in Hanakkori. The common on-type normal phenotype had 38 normal chromosomes and high pollen fertility. In contrast, most off-types with an abnormal phenotype had other chromosome numbers and low pollen fertility. Xiong et al. (2011) also observed that the chromosome complement of individual plants of resynthesized *B. napus* ranged from 36 to 42, and aneuploidy

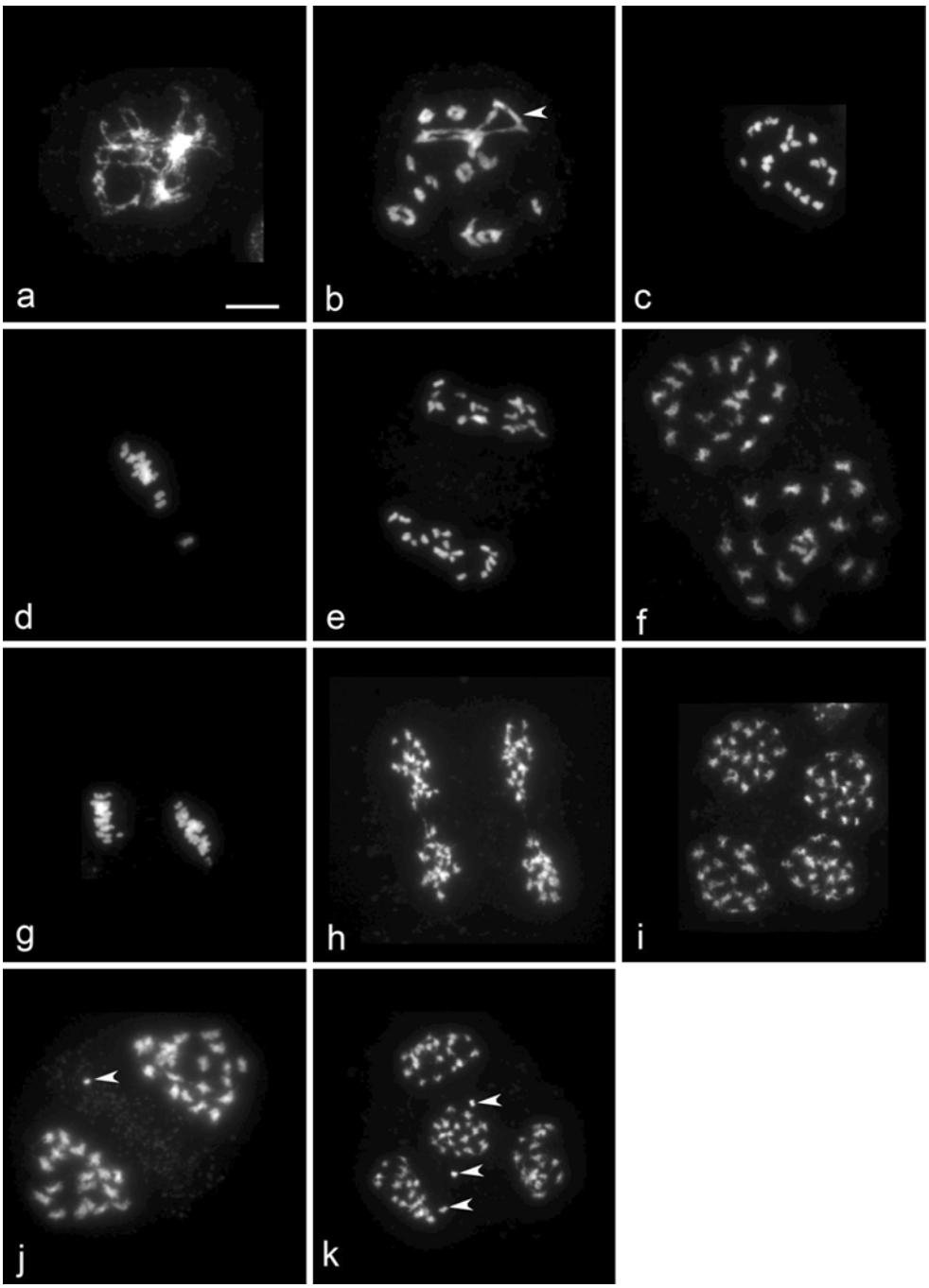

Fig. 5 Meiotic stages of normal resynthesized *B. napus* cv. Hanakkori. This Hanakkori is the normal phenotype, with 38 chromosomes and 87.4% pollen fertility. **j** and **k** Abnormal meiotic chromosomes. **a** Pachytene stage, **b** Diplotene stage, *arrowhead* indicates polyvalent chromosome. **c** Diakinesis stage, **d** Metaphese I, **e** Anaphese II, **f** Telophase I, **g** Metahphese II, **h** Anaphase II, **i** Telophase I, **j** Anaphese I, **k** Anaphese II, *arrowheads* indicate abnormal chromosome fragment. *Bar* 10 μm

was inversely correlated with seed yield and pollen viability. These results suggested that the atypical character for morphogenesis of Hanakkori is related to chromosome aberrations and then FISH was used to investigate chromosome structure of Hanakkori. In on-type Hanakkori, probes 45S rDNA and CentBr2 hybridized to mitotic metaphase complements were visualized in ten signals. Consequently, the FISH results differed from the observations of Xiong and Pires (2011) and Lim et al. (2007) in terms of signal number. Snowdon et al. (2002) reported that discrepancies in the rDNA locus and copy number could be expected. And also, Hasterok et al. (2006) reported that the number of rDNA sites can differ up to fivefold in species with the same chromosome number. For

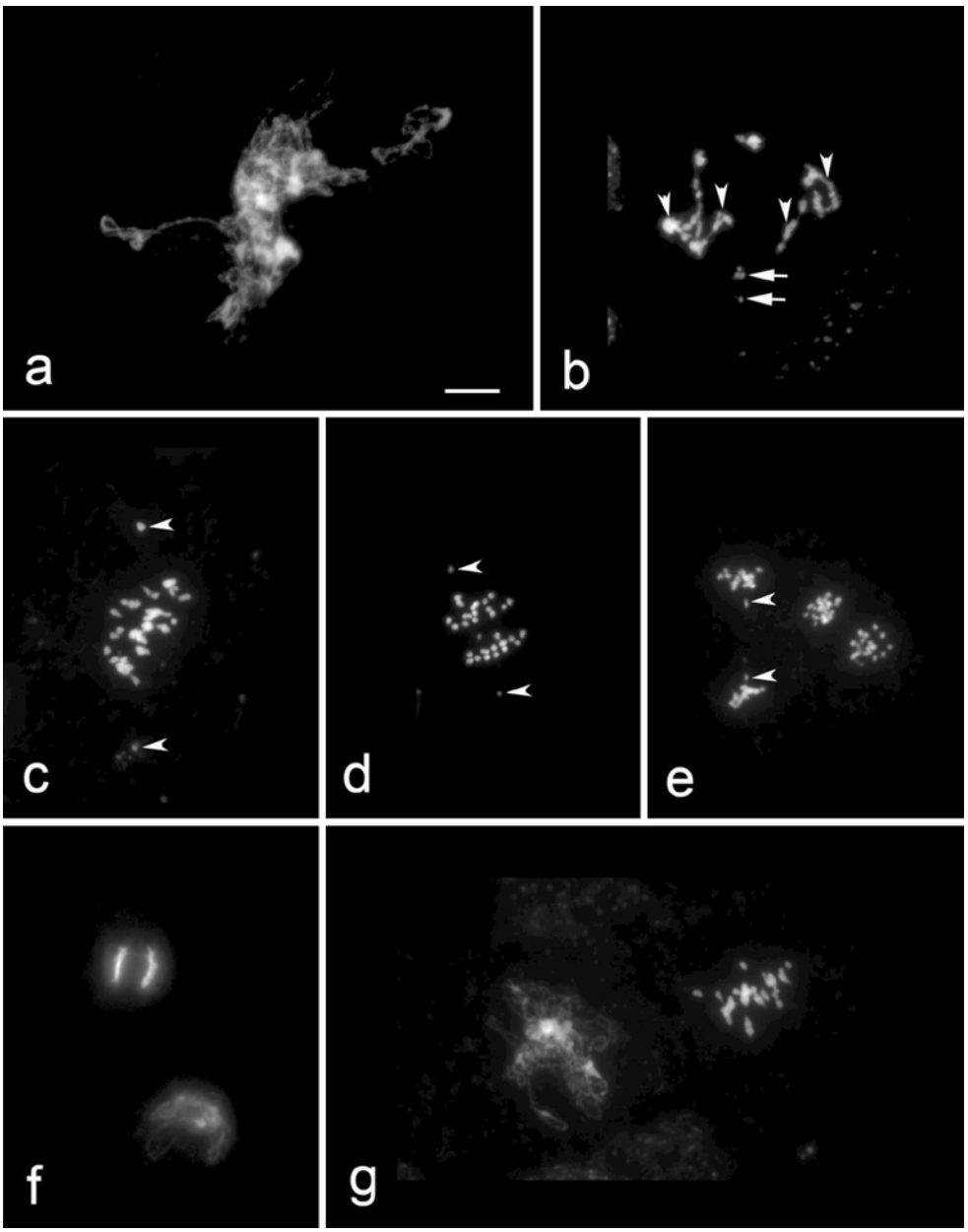

Fig. 6 Meiotic stages of abnormal resynthesized *B. napus* cv. Hanakkori. This phenotype is abnormal, with 36 chromosomes and 34.3% pollen fertility. **f** and **g** Division stage is not synchronized in various meiosis stages in one anther. **a** Pachytene stage. **b** Diplotene stage, *arrowheads* indicate polyvalent chromosomes. *Arrows* indicate univalent chromosomes. **c** Metaphase I, *arrowheads* indicate univalent chromosome. **d** Anaphase I, *arrowheads* indicate univalent chromosome. **e** Anaphase II, *arrowheads* indicate abnormal chromosome fragment. **f** Coexistence of pachytene and anaphase I. **g** Coexistence of pachytene and metaphase I. *Bar* 10 μm

example, losses or additions of rDNA loci and alterations in copy number are quite common in polyploid plant genomes such as *Brassica* amphidiploids (Dubcovsky and Dvořák 1995; Maluszynska and Heslop-Harrison 1993). Thus, as the discrepancies in the rDNA locus were common, in the case of Hanakkori, the difference in hybridization signal number might be caused by losses of rDNA and CentBr2 loci and alterations in their copy number. Moreover, the losses and alterations in copy number for loci might be related to homoeologous nonreciprocal translocation (Udall et al. 2005).

In off-type Hanakkori, the FISH results for mitotic metaphase complements showed that one atypical type had an extra A3 chromosome (Fig. 4b), and most of the atypical types had one to three small chromosome fragments that lacked a centromere and heterochromatin region. In somatic hybrids of *B. rapa* and *B. oleracea*, chromosome fragments appeared at high frequencies (data not shown).

The abnormal chromosome number and structure might be caused by aberrant meiosis, which we investigated in Hanakkori. Most chromosomes at diplotene were polyvalent in both the on-type and off-type Hanakkori. Thus, Hanakkori chromosomes tend to be polyvalent during meiosis, and unequal reduction in segregation of chromosomes causing polyvalent chromosomes produced some random chromosome fragments. Thus, we concluded that factors leading to atypical phenotypes of Hanakkori were caused by aberrant meiosis, possibly through a chromosome bridge breakage cycle, beginning with a dicentric chromosome forming a bridge as it is simultaneously pulled toward both poles during anaphase. The pairing aberration leads to random chromosome paring and breakage. It is well documented that the A and C genomes of *Brassica* have high homoeology (Snowdon et al. 1997; Snowdon 2007; Howell et al. 2008). Lim et al. (2005) also reported that the centromere repeats of *B. rapa*, *B. oleracea*, and *B. napus* had more than 90% sequence similarity in phylogenetic analysis. Gaeta et al. (2007) concluded also that exchanges among homoeologous chromosomes A1 and C1 were a major mechanism that creates novel combinations and phenotypic variations in newly formed *B. napus* polyploids. In fact, they observed that genetic changes were much frequent in the S5 generation. Udall et al. (2005) demonstrated that de novo homoeologous nonreciprocal translocations were more prevalent commonly in a resynthesized *B. napus*. Lu and Kato (2001) reported that sesqui-diploids from resynthesized *B. napus* had more frequently various segregation patterns in comparison with those in natural *B. napus*. Cifuentes et al. (2010) observed univalent and various polyvalent chromosomes in meiotic behavior of *B. napus* allohaploids and also reported that repeated polyploidy resulted in different levels of crossover suppression between homoeologs in *B. napus* allohaploids. Overall, we suggest that the polyvalent chromosomes and chromosome fragments observed in Hanakkori were specific to resynthesized *B. napus*, then that induced homoeologous reciprocal and/or nonreciprocal translocation.

Gaeta and Pires (2010) reported that homoeologous recombination in resynthesized *B. napus* could lead to aberrant meiotic behavior and reduced fertility. Most Hanakkori plants with low pollen fertility contained aberrant chromosome fragments in the somatic cells. Meiosis in these plants showed not only polyvalent chromosomes and aberrant chromosome fragments but also different stages of meiosis in an anther cell. It appears that meiosis of Hanakkori plants with low pollen fertility lost synchrony; few pollen mother cells progressed smoothly through the meiotic cycle and in some pollen mother cells, progress was unsuccessful, resulting in stopped or extremely slow meiotic cycles. Wu and Yang (2008) observed that no pollen mother cells of the dominant gene in male-sterile *B. napus* could pass the pachytene stage and concluded that male sterility was caused by meiotic abnormality. Our results were consistent with meiotic abnormalities.

We concluded that three processes leading to the atypical phenotypes in Hanakkori were aberrant meiosis. And we assumed these mechanisms as follows. First, when the polyvalent chromosomes in stage of diplotene were induced by higher homoeology between A and C genomes in resynthesized *B. napus* (Snowdon et al. 1997; Snowdon 2007; Howell et al. 2008), the chromosomal rearrangements derived from homoeologous recombination caused by homoeologous reciprocal and/or nonreciprocal translocation would produce atypical phenotypes (newly character) in their progenies (Szadknowski et al. 2010; Gaeta and Pires 2010; Gaeta et al. 2007; Udall et al. 2005). Second, the losses and alterations in copy number for loci (Dubcovsky and Dvorák 1995; Maluszynska and Heslop-Harrison 1993), the change of chromosome numbers and appearance of chromosome fragments followed by their chromosome rearrangements were unstable in Hanakkori progenies. Consequently, atypical phenotypes such as off-type morphological characters, lower pollen fertility, and lower seed production appeared.

Although Gaeta et al. (2007) observed that the mechanism of homoeologous exchanges in resynthesized *B. napus* could be particularly prevalent in the early generations after polyploid formation, they concluded the possibility in natural *B. napus* that homoeologous exchanges contributed to variation for pairing control and the evolution of more stabilized pairing. Xiong et al. (2011) discussed that early generations of resynthesized *B. napus* involved aneuploidy and gross chromosomal rearrangements and that dosage balance mechanisms enforced chromosome number stability. Jenczewski et al. (2003) reported that a major gene controlling homoeologous, named *PrBn,* presented in *B. napus* haploids and that *PrBn* could contribute to the regularity of chromosome pairing, but the allele present in genotypes with a high pairing behavior at the haploid stage could be ineffective at the hemizygous stage. Szadknowski et al. (2010) emphasized the role of the first meiosis in the genome instability of synthetic *B. napus*. They also reported the genome rearrangements in the first meiosis and the genome stabilization after several successive generations. Hence, we could presume that there were some possibilities of stabilizing phenotypes in Hanakkori, if homoeologous exchanges would develop for several generations. However, as the aberrant meiosis were induced such as polyvalent chromosomes and aberrant chromosome fragments in Hanakkori, the various changes of phenotype were originated until stabilizing chromosome rearrangement at meiosis. Therefore, we supposed that the more stable progenies could be maintained by repeated selection of on-type plants with the combination of such as normal

morphological characters, chromosome structures and lower aberrant meiosis with higher pollen fertility, in order to select progenies effectively without the loss of important agricultural characters, e.g., yield, flowering time, and quality for the vegetable.

Relationships of the A and C genomes to meiosis behavior have been reported, but this is the first clear demonstration of the relationship among atypical phenotypes, somatic chromosomes, pollen fertility, and meiosis behavior in resynthesized *B. napus* crop. The progeny selection procedure based on normal phenotypes, chromosome number and behavior, and pollen fertility successfully improved Hanakkori breeding for genetically stable stock seed production. These results are extremely useful for breeding of resynthesized *B. napus* and should lead to a reduction of breeding time and the new possibility of producing other progeny from resynthesized *B. napus* in the future.

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