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RESEARCH ARTICLE

Generation and identification of four new monosomic addition lines of flowering Chinese cabbage-Chinese kale

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Abstract **A selection test of four new flowering Chinese cabbage (*Brassica campestris* L. var. *utilis* Tsen et Lee)-Chinese kale (*Brassica alboglabra* Bailey) monosomic alien addition lines was conducted by karyotype analysis and morphological marker identification. The results mainly showed the following: 61'2*n* + 1' plants screened out from 655 progeny plants of the trigenomic hybrid (AAC) backcrossed with its parent flowering Chinese cabbage (AA), four new monosomic alien addition lines(AA + C_1, AA + C_5, AA + C_8 and AA + C_9) obtained from the 61 '2*n* + 1' plants, the transmission rates of the monosomic alien addition lines (AA + C_1, AA + C_5 and AA + C_8) of 19.7%, 23.44%, and 36.51% by female gametes and 7.84%, 8.89%, and 9.43% by male gametes, respectively, and the alien chromosome C-1 and C-5 had partial homology with at least one chromosome of A-genome.**

Keywords flowering Chinese cabbage (*Brassica campestris* L. var. *utilis* Tsen et Lee), Chinese kale (*Brassica alboglabra* Bailey), monosnomic alien addition lines, transmission rate

Introduction

Interspecific alien chromosome addition lines play a significant role in crop genetic studies and variety improvement. The studies of alien addition lines have been shown to be an efficient method to research chromosomal homeology relationship between closely related species, assign linkage groups to corresponding chromosomes, transfer genes between species, and identify chromosomes carrying different agronomic trait genes (Zhu et al., 1993; Biyashev et al., 1997; Chetelat et al., 1998; Barthes and Ricroh, 2001).

Brassica is an important genus of which many species are widely cultivated vegetable and oil crops. *B. campestris* (AA, $2n = 2x = 20$), *B. oleracea* (CC, $2n = 2x = 18$), and *B. nigra* (BB, $2n = 2x = 16$) represent three basic diploid species, and *B. juncea* (AABB, $2n = 4x = 36$), *B. napus* (AACC, $2n = 4x = 38$), and *B. carinata* (BBCC, $2n = 4x = 34$) represent three natural spontaneous amphidiploid species within the genus. In recent years, a set (or part) of alien addition lines of *B. campestris-B. oleracea*, *B. campestris-B. alboglabra*, *B. rapa-B. alboglabra*, and *B. napus-B. nigra* have been obtained (Quiros et al., 1987; Jahier et al., 1989; Chen et al., 1992; Cheng et al., 1994; Hasterok et al., 2005; Liu et al., 2008), which greatly promote the studies on gene mapping, variety improvement, and chromosome homeology in this genus. For instance, the genes controlling white flower color, erucic acid, and faster migrating band of leucine amino peptidase were located on the special chromosomes by cytogenetic analysis of the *B. campestris-B. alboglabra* monosomic addition line (Chen et al., 1992). The elementary homologous relationships between A-genome and C-genome were revealed by molecular marker analysis and meiotic studies on the *B. campestris-B. alboglabra* monosomic addition line (McGrath et al., 1990; Chen et al., 1992; Chen et al., 1997). However, the studies on alien addition lines in *Brassica* were mainly focused on some oilseed crops, and a set of alien chromosome addition lines in vegetables have not been generated so far.

Flowering Chinese cabbage and Chinese kale are important vegetables in *Brassica*. In recent years, we have synthesized the amphidiploid (AACC) and allotriploid (AAC) by hybridization between the flowering Chinese cabbage (AA) and the Chinese kale (CC) and by immature embryo culture in vitro (Man et al., 2007; Yuan et al., 2008). We have obtained the five flowering Chinese cabbage-Chinese kale monosomic

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alien addition lines (AA + C_2, AA + C_3, AA + C_4, AA + C_6, AA + C_7) from the backcross progeny of the AAC × AA by morphological marker identification and karyotype analysis (Wang et al., 2008). In this paper, we reported on the generation and identification of the other four new flowering Chinese cabbage-Chinese kale monosomic alien addition lines (AA + C_1, AA + C_5, AA + C_8, and AA + C_9).

Materials and methods

Materials

Diploid and tetraploid flowering Chinese cabbage 'Qinglu 9601' and Chinese kale 'Baihua 9705' were used as the basic materials. The amphidiploid (AACC) was synthesized by hybridization between the tetraploid flowering Chinese cabbage (AAAA) and tetraploid Chinese kale (CCCC), and the allotriploid hybrids (AAC) were synthesized by hybridization between the amphidiploid (AACC) and diploid flowering Chinese cabbage (AA) under the condition of immature embryo culture *in vitro* (Man et al., 2007; Yuan et al., 2008).

Methods

For screening the monosomic alien addition lines, the hybrid seeds of AAC × AA were cultured on the 1/2 MS medium under 25°C. When the length of the roots of seedlings was about 1cm, the root tip and shoot apex were cut, respectively. The root tip was immerged in 0.002 mol/L 8-hydroxide quinoline solution for 2 h, then fixated in a Carnoys solution (95% ethanol: glacial acetic acid = 3:1) for 24 h and kept in a refrigerator for chromosome number examination; and the corresponding shoot apex was transferred to the MS medium to culture temporarily. The chromosome preparation of root tip cells was made by squash method and examined under the Olympus BH-2 microscope. The roots identified with $2n + 1 = 21$ chromosomes and their corresponding shoot apexes cultured on the MS medium were kept on propagating. These '$2n + 1$' clones were directly used for the selection of the monosomic alien addition lines by morphological identification and karyotype analysis.

Morphological identification was carried out in a greenhouse during the growth stage. The parents (flowering Chinese cabbage and Chinese kale) were used as controls. By careful observation and comparison, if a '$2n + 1$' plant carrying a particular character was derived from the parent Chinese kale (CC), it was preliminarily determined as a monosomic alien addition line (AA added a chromosome from C-genome). Karyotype analysis was conducted according to the method of Li and Zhang (1996).

For meiotic studies, the floral buds of each monosomic alien addition line were fixed in a Carnoy solution (95% ethanol : glacial acetic acid = 3:1) at room temperature for 24 h and then stored in a 70% ethanol solution at 4°C. Meiotic preparations were made by smear method and staining with PIHCH solution (propinoic acid–iron–hematoxyllin–chloral hydrate) for 5 min and then observed under an Olympus BH-2 microscope.

The pollen fertility of the monosomic alien addition lines was tested by the triphenyl tetrazolium chloride (TTC) method. At least 1000 pollen grains were observed for each monosomic alien addition line.

The pollen amount of the monosomic alien addition lines was assessed by counting pollen grains of the anther with haemacytometer under the Olympus BH-2 microscope. Twelve anthers were examined for each monosomic alien addition line.

Transmission rate of each monosomic alien addition line was determined by examining chromosome number of the backcross progenies. The percentage of '$2n + 1$' plants in the population of each monosomic alien addition line backcrossed with its diploid parent flowering Chinese cabbage was denoted as the transmission rate by female gametes; and the percentage in the reciprocal cross was denoted by male gametes.

Results and analysis

Screening of $2n + 1$ plants from the progeny of AAC × AA

Chromosome numbers of 655 progeny seedlings (AAC × AA) ranging from $2n = 20$ to $2n = 28$ at different frequencies (Table 1), of which 61 individuals had $2n + 1 = 21$ chromosomes and occupied 9.31%. These $2n + 1 = 21$ seedlings were propagated in the MS medium to form 61 individual clones, which were directly used for identifying different monosomic alien addition lines.

Morphological identification of the $2n + 1$ plant

The plantlets with $2n + 1$chromosomes and their parents were transplanted to greenhouse and investigated during the growth stage. The results showed that there were nine groups classified among the total 61 '$2n + 1$' plantlet lines. Each group carried at least a special morphological marker inherited from the parent Chinese kale. Four new types were distinguished from the nine groups, and other five types

Table 1 Chromosome numbers in the progeny plants of AAC × AA

No. of chromosomes in plants	20	21	22	23	24	25	26	27	28	29
No. of plants	73	61	157	151	98	45	39	27	4	0
Percentage(%)	11.15	9.31	23.97	23.05	14.96	6.87	5.95	4.12	0.61	0

were similar to the monosomic alien addition lines reported previously (Wang et al., 2008). The four new types and the special markers were shown in Table 2.

Karyotype analysis of the four new types

The karyotypes of the parents (flowering Chinese cabbage and Chinese kale) were used as the control, through karyotype analysis of the four new types; the added alien chromosome in Type-1, Type-2, Type-3, and Type-4 was determined as AA + C_1, AA + C_5, AA + C_8, and AA + C_9 (Figs. 1 A–D), respectively. Thus, with the other five monosomic addition lines (AA + C_2, AA + C_3, AA + C_4, AA + C_6, and AA + C_7) we reported previously, a set of flowering Chinese cabbage-Chinese kale monosomic alien addition lines (AA + C_1–AA + C_9) were preliminarily generated.

Meiotic behavior of the monosomic alien addition lines

The meiotic observation of the new monosomic alien addition lines showed that the alien chromosome in monosomic addition line AA + C_5 mainly stayed as a univalent (10II + 1I) at diakinesis and metaphase I, but the alien chromosome of the monosomic addition lines (AA + C_8 and AA + C_1) was usually paired with one and two pairs of A chromosomes (Figs. 1 E and F); when the alien chromosome was used as a univalent, it usually stayed outside the equator plane (Fig. 1 G) at metaphase I and freely moved to either of the two poles (Fig. 1 H); once in a while, it lagged behind or divided equably (Figs. 1 I and J) at anaphase I. The rate of the three behaviors at anaphase I was shown in Table 3. The results revealed that the rate of alien chromosome moved to a pole, ranging from 78.13% to 80.81%, without obvious difference in the three monosomic alien addition lines.

Pollen amount and viability of the monosomic alien addition lines

The pollen amount and viability of the new monosomic alien addition lines were tested (Table 4). The results showed that the pollen amount in the three monosomic alien addition lines ranged from 4375 to 7190 grains per anther, and the pollen viability ranged from 82.93% to 96.07%. Although the pollen amount of AA + C_1 was only about half of parent (AA), its pollen viability was almost the same as the parent (Table 4). This indicated that there was no close relationship between pollen amount and pollen viability.

Transmission of the monosomic alien addition lines

Transmission rates of the monosomic alien addition lines were determined by examining the chromosome number of the progeny plants of each mosomic alien addition line that backcrossed with its parent flowering Chinese cabbage. The result showed that the alien chromosome in the three monosomic alien addition lines could be transmitted by female and male gametes, and the transmission rates ranged from 19.7% to 36.51% for female and from 7.84% to 9.43% for male, respectively (Table 5).

Discussion

The progeny of interspecies allotriploid hybrid backcrossed with its diploid parent are generally used to produce monosomic alien addition lines. These backcross populations theoretically included a series of hyperploid individuals, such as $2n + 1$, $2n + 2$, $2n + 3$, and so on, but only the '$2n + 1$' individuals were directly used for the production of monosomic alien addition lines. However, the frequency of '$2n + 1$' individuals in a backcross population is very low. Thus, the selection of a set of monosomic alien addition lines usually needs to carry out backcross repeatedly and takes a lot of time. In the present study, the seed setting of the trigenomic hybrid (AAC) backcrossed with its parent flowering Chinese cabbage was as high as 25.79% (Wang et al. 2008), and the rate of '$2n + 1$' plants in the backcross population was 9.31%. The high seed setting and high rate of '$2n + 1$' individuals are favorable to obtain a set of flowering Chinese cabbage-Chinese kale monosomic alien addition lines.

It is important for selection of monosomic alien addition lines to identify alien chromosome. Some special genetic markers, such as morphological markers, chromosome markers, and various molecular markers, have been successfully used for identifying the different alien addition lines. Based on the special isozymes and rDNA markers, Quiros et al. (1987) identified some monosomic and disomic addition plants of *B. campestris-B. oleracea*. Using morphological, isozyme, and restriction fragment length polymorphism (RFLP) markers, McGrath et al. (1990) characterized 25 monosomic addition plants of *B. campestris-B. oleracea*. By random amplified polymorphic DNA (RAPD) marker

Table 2 The four new types with special traits

Special traits in the four new types	Relevant traits in parent AA	Relevant traits in parent CC
Long and round silique in type-1	Long and flat silique	Long and round silique
Thick wax layer on stalk in type-2	Thin wax layer on stalk	Thick wax layer on stalk
Flowering duration obviously long in type-3	Flowering duration short	Flowering duration long
Deep green leaves, flowering early in type-4	Light green leaves, flowering early	Deep green leaves, flowering late

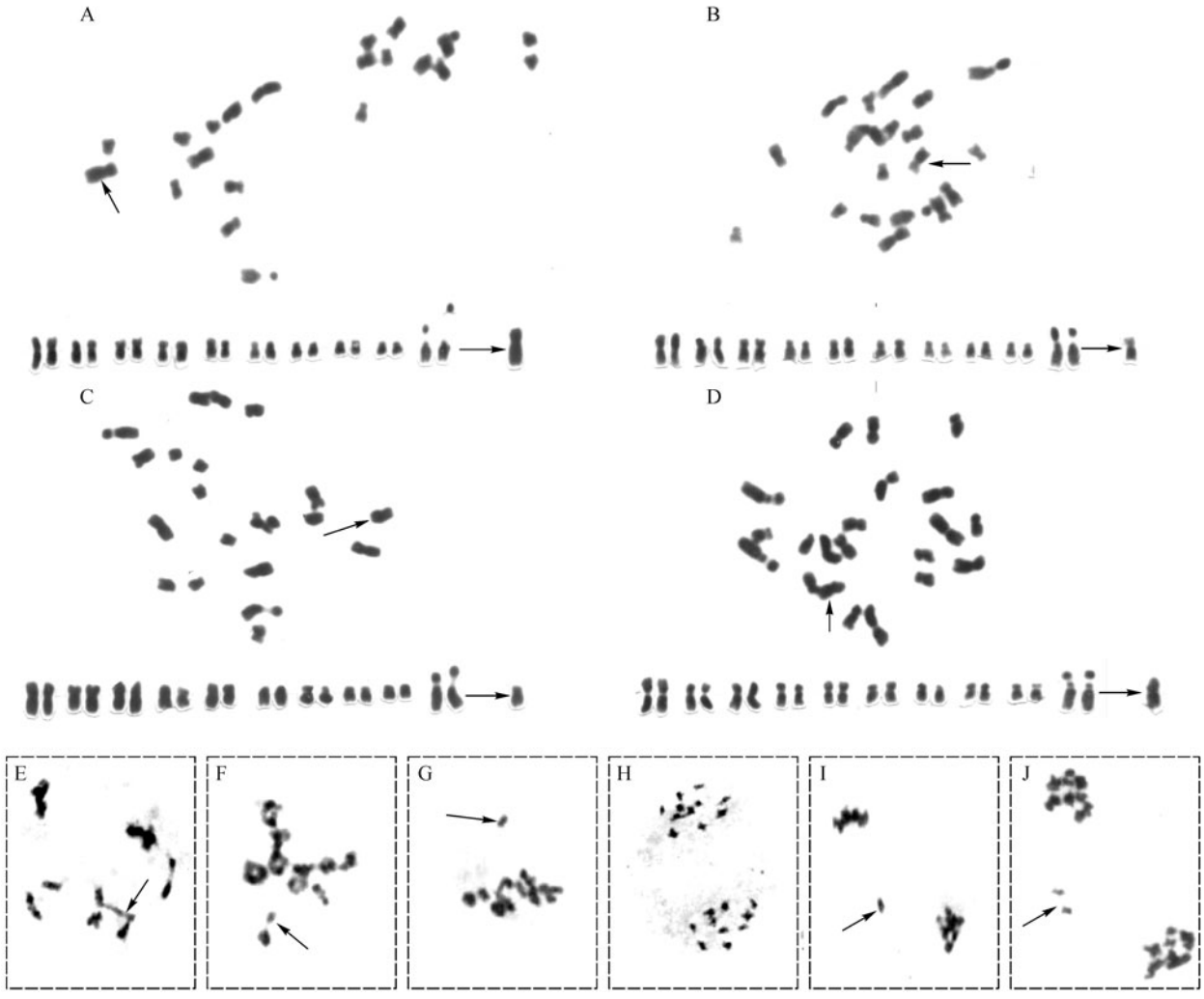

Figure 1 Karyotypes and meiotic behavior of the monosomic addition lines. A–D represent the karyotype of monosomic addition lines AA + C_1, AA + C_5, AA + C_8, and AA + C_9. E is the diakinesis of monosomic addition line AA + C_1, with the arrow showing that C1 chromosome has partial homology with two pairs of A chromosomes. F is a diakinesis of monosomic addition line AA + C_5, with the arrow showing C5 chromosome paired with a pair of A chromosome. G is metaphase I of monosomic addition line AA + C_5, with the arrow showing C5 chromosome. H is 10/11 segregation at anaphase I of monosomic addition line AA + C_5. I is the lagged C5 chromosome at anaphase I of monosomic addition line AA + C_5. J is C5 chromosome divided equationally at anaphase I of monosomic addition line AA + C_5.

analysis and mitotic chromosome identification, Chen et al. (1997) obtained four different monosomic alien addition lines of *B. campestris-B. alboglabra*. Moreover, by genomic in situ hybridization (GISH), Wang et al. (2006) obtained two different *Brassic napus-Crammbe abyssinica* disomic addition lines. In our studies, 61 '$2n + 1$' plants were identified from the 655 progeny plants of the trigenomic hybrid (AAC) backcrossed with its parent flowering Chinese cabbage (AA), and four new monosomic alien addition lines AA + C_1, AA + C_5, AA + C_8 and AA + C_9) were characterized based on

Table 3 Behaviors of the alien chromosome at anaphase I in the monosomic addition lines

Addition lines	No. and rates of PMCs in which the alien chromosome moved to either of two poles at anaphase I		No. and rates of PMCs in which the alien chromosome lagged behind at anaphase I		No. and rates of PMCs in which the alien chromosome is divided at anaphase I	
	PMCs	Rates (%)	PMCs	Rates (%)	PMCs	Rates (%)
AA + C_1	42	79.25	11	20.75	0	0
AA + C_5	80	80.81	15	15.15	4	4.04
AA + C_8	50	78.13	14	21.87	0	0
AA + C_9	–	–	–	–	–	–

Table 4 Pollen amount and viability of flowering Chinese cabbage×Chinese kale monosomic alien addition lines

Materials	Pollen amount per anther	Viability of pollen (%)
AA + C_1	4375 a	96.07 b
AA + C_5	6940 b	82.93 a
AA + C_8	7190 b	88.43 a
AA + C_9	–	–
Parent(AA)	8630 c	95.50 b

Note: Different letters in the same column indicate significant differences at 0.05 probability level.

Table 5 Transmission rates of flowering Chinese cabbage-Chinese kale alien monosomic addition lines

Combinations	Total	$2n + 1$	Transmission	Combinations	Total	$2n + 1$	Transmission
(AA + C_1) × AA	66	13	19.70	AA × (AA + C_1)	53	5	9.43
(AA + C_5) × AA	63	23	36.51	AA × (AA + C_5)	51	4	7.84
(AA + C_8) × AA	64	15	23.44	AA × (AA + C_8)	45	4	8.89
(AA + C_9) × AA	0	–	–	AA × (AA + C_9)	0	–	–

the morphological identification and karyotype analysis. Thus, with the other five monosomic alien addition lines (AA + C_2, AA + C_3, AA + C_4, AA + C_6 and AA + C_7) reported previously (Wang et al., 2008), a set of flowering Chinese cabbage-Chinese kale monosomic alien addition lines were preliminarily generated.

The determination of alien chromosome transmission rate is significant in the utilization of monosomic alien addition lines that it can be used for estimating the frequencies of disomic addition plants in the selfed populations of monosomic alien addition lines. There were some reports on transmission of monosomic alien addition lines. Using the special isozyme markers, Chèvre et al. (1997) assessed the transmission of five different monosomic alien addition lines of *B. oleracea-B. nigra* and observed the transmission rates ranging from 1.3% to 28.8% for male and 14.1% to 23.8% for female. In the present study, the alien chromosome in each monosomic addition line was also able to be transmitted by female and male gametes, but the frequencies for female were generally higher than that for male. It indicated that the impact of an alien chromosome on the fertilization was more serious for male gametophyte (pollen) than that for female gametophyte (embryo sac).

Meiotic studies on monosomic alien addition lines are helpful to reveal the homeology relationships among chromosomes of two different genomes, thus providing a deeper insight into the evolution of the genomes. The meiotic studies on the three *B. campestris-B. alboglabra* monosomic alien addition lines, for instance, revealing that C-4, C-8, and C-9 of *B. alboglabra* all had more than one pair partner in the A-genome, and more specially, the long arm of A-9 was homeologous with the long arm C-4, while the short arm of A-9 was homeologous with the short arms of both C-8 and C-9 (Chen et al., 1997). In our present and previous studies (Yuan et al., 2008), we observed that the alien chromosomes paired with A-genome of the flowering Chinese cabbage at least involved C-1, C-2, C-4, C-6, C-7, and C-8 of Chinese kale. These homeologous relationships once again revealed that the chromosome translocation and recombination occurred between the C-genome and A-genome.

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References

Barthes L, Ricroh A (2001). Interspecific chromosomal rearrangements in monosomic addition lines of *Allium*. Genome, 44(5): 929–935

Biyashev R M, Ragab R A, Maughan P J (1997). Molecular mapping, chromosomal assignment and genetic diversity analysis of phytochrome loci in barley. Heredity, 88: 21–26

Chen B Y, Cheng B F, Jorgensen R B, Heneen W K (1997). Production and cytogenetics of *Brassica campestris-alboglabra* chromosome addition lines. Theor Appl Genet, 94(5): 633–640

Chen B Y, Simonsen V, Lanner-Herrera C, Heneen W K (1992). A *Brassica campestris-alboglabra* addition line and its use for gene mapping, intergenomic gene transfer and generation of trisomics. Theor Appl Genet, 84: 592–599

Cheng B F, Chen B Y, Heneen W K (1994). Addition of *Brassica alboglabra* Bailey chromosomes to *B. campestris* L. with special emphasis on seed color. Heredity, 73(2): 185–189

Chetelat R T, Rick C M, Cisneros P, Alpert K B, DeVerna J W (1998). Identification, transmission, and cytological behavior of *Solanum lycopersicoides* Dun. monosomic alien addition lines in tomato (*Lycopersicon esculentum* Mill.). Genome, 41(1): 40–50

Chèvre A M, Eber F, Barret P, Dupuy P, Brace J (1997). Identification of the different *Brassica nigra* chromosome from both sets of *B. oleracea-B. nigra* and *B. napus-B. nigra addition lines* with a special emphasis on chromosome transmission and self-incompathibility. Theor Appl Genet, 94(5): 603–611

Hasterok R, Wolny E, Kulak S, Zdziechiewicz A, Maluszynska J, Heneen W K (2005). Molecular cytogenetic analysis of *Brassica*

rapa×*Brassica oleracea* var. *alboglabra* monosomic addition lines. Theor Appl Genet, 111(2): 196–205

Jahier J, Chevre A M, Tanguy A M, Eber F (1989). Extraction of disomic addition lines of *Brassica napus-B. nigra*. Genome, 32: 408–413

Li M X, Zhang Z P (1996). Crop Chromosome And Manipulation Technique. Beijing: China Agricultural Press, 17–19 (in Chinese)

Liu W, Shen S X, Wang Y H, Zhang C H, Xuan S X (2008). A study on obtaining and identification of Chinese cabbage-cabbage addition lines. Acta Horticulturae Sinica, 35(2): 207–212 (in Chinese)

Man H, Zhang C H, Wang X E, Zhang G H (2007). Obtaining and identification of the allotetraploid from 4*x* Caitai 9601×4*x* Jielan 9705. Acta Horticulturae Sinica, 34(5): 1163–1168 (in Chinese)

McGrath J M, Quiros C F, Harada J J, Landry B S (1990). Identification of *Brassica oleracea* monosomic alien chromosome addition lines with molecular markers reveals extensive gene duplication. Mol Gen Genet, 223(2): 198–204

Quiros C F, Ochoa O, Kianian S F, Douches D (1987). Analysis of the *Brassica. alboglabra* genome by the generation of *B. campestris-B. alercea* addition lines: characterization by isozymes and rDNA genes. Theor Appl Genet, 74(6): 758–766

Wang X E, Zhang C H, Xuan S X, Man H, Liu H H, Shen S (2008). Monosomic addition lines of flowering Chinese cabbage (*B. campestris* Lssp. *Chinensis* var. *parachinensis* L. H. Bailey) - Chinese kale (*B.oleracea* L. var. *alboglabra* L. H. Bailey). Agric Sci China, 7(6): 656–663

Wang Y P, Sonntag K, Rudloff E, Wihling P, Snowdon R J (2006). GISH analysis of disomic *Brassica napus-Crambe abyssinica* chromosome addition lines produced by microspore culture from monosomic addition lines. Plant Cell Rep, 25(1): 35–40

Yuan H, Zhang C H, Man H, Wang X E (2008). Embryo development observation of 4x flowering Chinese cabbage-2x Chinese kale and obtaining of the allotriploid by ovary culture. Journal of Plant Genetic Resources, 9(3): 297–301 (in Chinese)

Zhu J S, Struss D, Robbelen G (1993). Studies on resistance to *phoma lingam* in *Brassica napus-Brassica nigra* addition lines. Plant Breed, 111(3): 192–197