Theor Appl Genet (2010) 121:1141–1150
DOI 10.1007/s00122-010-1378-6

ORIGINAL PAPER

Development of a population for substantial new type *Brassica napus* diversified at both A/C genomes

Yong Xiao · Lunlin Chen · Jun Zou · Entang Tian · Wei Xia · Jinling Meng

Received: 26 September 2009 / Accepted: 3 June 2010 / Published online: 17 June 2010

Abstract Intersubgenomic heterosis in rapeseed has been revealed in previous studies by using traditional *Brassica napus* ($A^nA^nC^nC^n$) to cross partial new type *B. napus* with A^r/C^c introgression from the genomes of *B. rapa* and *B. carinata*, respectively. To further enlarge the genetic basis of *B. napus* and to facilitate a sustained heterosis breeding in rapeseed, it is crucial to create a population for substantial new type *B. napus* diversified at both A/C genomes. In this experiment, hundreds of artificial hexaploid plants ($A^rA^rB^cB^cC^cC^c$) involving hundreds of *B. carinata*/*B. rapa* combinations were first crossed with elite lines of partial new type *B. napus*. The pentaploid plants (AABCC) were open-pollinated in isolated conditions, and their offspring were successively self-pollinated and intensively selected for two generations. Thereafter, a population of substantial new type *B. napus* mainly with a genomic composition of $A^rA^rC^cC^c$ harbouring genetic diversity from 25 original cultivars of *B. rapa* and 72 accessions of *B. carinata* was constructed. The population was cytologically verified to have the correct chromosome constitution of AACC and differed genetically from traditional *B. napus,* in terms of the genome components of A^r/C^c and B^c as well as the novel genetic variations induced by the interspecific hybridisation process. Synchronously, rich phenotypic variation with plenty of novel valuable traits was observed in the population. The origin of the novel variations and the value of the population are discussed.

Communicated by H. Becker.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-010-1378-6) contains supplementary material, which is available to authorized users.

Y. Xiao · L. Chen · J. Zou · E. Tian · W. Xia · J. Meng (✉)
National Key Laboratory of Crop Genetic Improvement, Huazhong Agriculture University, Wuhan 430070, Hubei, China
e-mail: jmeng@mail.hzau.edu.cn

Introduction

Allopolyploid *Brassica napus* was derived from a natural cross between *B. rapa* and *B. oleracea* along the Mediterranean coast with uncertain evolutionary origin time approximate ranging from 0.12 to 1.37 million years ago (UN 1935; Morinaga 1928; Cheung et al. 2009). The first domesticated oilseed rape may have been the semi-winter type because of the mild climate in the area about 400 years ago (Diers and Osborn 1994; Gómez-Campo and Prakash 1999). The winter and spring type rapeseeds were developed by selecting for cold hardness or early flowering mutants when European farmers were growing their rapeseed northward early in the last century. *B. napus* has seldom exchanged genetic material with other *Brassica* species, with the exception of some specific circumstances in the later part of the twentieth century, e.g., crosses with oilseed *B. rapa* to introduce early maturing characteristics into winter type *B. napus* in order to adapt the species to the cropping system in China (Liu 2000) and crosses with three *Brassica* B genome species and *B. rapa* to transfer *phoma*-resistant genes that protect against the serious disease in Australia and Canada (Delourme et al. 2006). The short domestication history and traditional breeding schedule of *B. napus* has led to a narrow genetic range in the population. As a whole, although the allopolyploid species has been rapidly and widely cultivated globally as an oilseed due to the advantages of high yield and wide adaptation, rapeseed breeding and heterosis utilisation have undergone genetic bottlenecks due to exhaustion of the

genetic variation (Prakash and Hinata 1980; Becker et al. 1995).

There are at least two approaches to widening the genetic diversity of *B. napus.* One is to increase genetic diversity by creating mutations in traditional agricultural *B. napus* (we refer to this as traditional *B. napus* for short). The other approach is to create new type *B. napus* by integrating A/C genomes from different *Brassica* oil crop species into the traditional *B. napus* (Inomata 1980). The second approach would also facilitate the usage of rapeseed heterosis if plant breeders select their super-combinations from crosses between the new type *B. napus* and traditional *B. napus*. Pioneering research was conducted on developing partial new type *B. napus,* in part with an A^r genome from seven cultivars of *B. rapa* and a C^c genome from two cultivars of *B. carinata* (Li et al. 2004), which were named as the first generation of the new type *B. napus* (new rapeseed^{1st}). Strong heterosis on seed yield was observed in the intersubgenomic hybrids between the new rapeseed^{1st} and traditional *B. napus*. It was noteworthy that stronger heterosis in seed yield was observed when increasing the introgression rate of A^r/C^c in the parental new rapeseed^{1st} (Li et al. 2006). To enhance intersubgenomic heterosis, the second generation of *B. napus* (new rapeseed^{2nd}, with 60% of exotic subgenomic introgression on average) were developed by pyramiding more exotic subgenomic components through intercrossing and recombination between the new rapeseed^{1st} (with 40% of exotic subgenomic introgression on average) followed by marker-assisted selection (Zou et al. 2010). It is obvious that further developing a gene pool for substantial (up to 90% of A^r/C^c introgression) new type *B. napus* constituted of $A^rA^rC^cC^c$ and plenty of genetic variation in the A^r/C^c genome would have a positive impact on enlarging the genetic variation of *B. napus* and ensuring a sustainable heterosis for future breeding.

Two hundred and seventy-six combinations consisting of about 10,000 hexaploid plants ($A^rA^rB^cB^cC^cC^c$) were obtained through a massive interspecific crossing between 29 accessions of *B. rapa* and 107 accessions of *B. carinata* (Jiang et al. 2007). Plants from the hexaploid population were crossed to selected lines of new rapeseed^{2nd} (with 75% of exotic subgenome introgression) of creating the gene pool of substantial new type *B. napus*. Here we reported the latest progress of such a research.

Materials and methods

Plant material and field experiment

Approximate 300 hexaploid plants ($A^rA^rB^cB^cC^cC^c$) synthesised by Jiang et al. (2007), originated from crosses between 110 *B. carinata* and 29 *B. rapa* cultivars, were used as female plants, and 8 new rapeseed^{2nd} lines with a common ancestor, HS3 (a Chinese elite DH cultivar, Zou et al. 2010), were used as male parent to produce pentaploid hybrids and self-pollinated generations. The eight new rapeseed^{2nd} possess higher introgressed rate from A^r, C^c and $A^r + C^c$ (Table 1) and excellent quality traits (with low erucic acid and low glucosinolate content in seeds).

All plants, from F_1 (pentaploid) to F_4 generations, were grown in the field in three and a half successive years. Self-pollinated seeds harvested from each pentaploid plant were grown as a single row, and those from selected plants in other generations were grown as one or two rows depending on the number of seeds, and each row with about ten plants. Three to four matured plants for each F_4 line were harvested to evaluate their agronomic traits (seed number per pod, seed weight, etc.) and seed quality (oil content, glucosinolate content, etc.).

Cytology analysis

Pollen fertility was evaluated by staining the pollen grains with aceto-carmine and then counting the number of viable pollen grains. Styles were picked out from buds and treated with 2 mmol/L 8-hydroxyquinoline for 4 h at 22–25°C. The tissue was transferred into Carnoy's

Table 1 Pedigree of the 8 selected lines from the partial new type *B. napus* (new rapeseed^{2nd})

Lines selected		Female parent of the selected lines[a,b]		
Code	ISG[c] (%)	Code	ISG (%)	Accessions of *B. rapa*
5R304	64	3R011	62	XiShui
5R298	75	3R013	62	XiShui
5R306	69	3R014	41	XiShui
5R326	81	3R016	62	XiShui
5R327	86	3R017	64	XiShui
5R311	75	3R015	52	Yangyou 2
5R300	78	3R047	62	ShiQian
5R305	72	3R052	60	ShiQian

[a] Female parents of the selected lines were developed from interspecific crosses of (*B. carinata* × *B. rapa*) × *B. napus* in which 101.67 and Huashuang 3 were the common parents of *B. carinata* and *B. napus*, respectively; the parents of *B. rapa* are shown in the right-hand side of the table

[b] Male parent of the selected lines was one line of the first generation with 45% ISG used; it was selected from the interspecific cross of (*B. rapa* × *B. napus*) × *B. rapa* with the combination of (Tian-Men × HuaShuang 3) × HuaShuang 3

[c] Ratio of the introgressed exotic genomic components (ISG)

solution for storing at 4°C. Anthers were separated from the buds and fixed in Carnoy's solution for meiosis observation (Li and Heneen 1999). For the genome in situ hybridisation test, the total genomic DNA was extracted from the young leaves of *B. nigra* and then labelled with biotin by nick translation. Slide preparation of chromosome was carried out following the method of Song and Gustafson (1993). Genomic in situ hybridisation was performed according to the protocols of Snowdon et al. (1997) and Li et al. (2002).

Genotype assay

One hundred F_4 lines of new typed *B. napus* and their original parental accessions (55 accessions of *B. carinata* and 10 accessions of *B. rapa*) were selected for the genotype assay (Supplementary Table 1). DNA samples from leaves of four individual plants were bulked to represent the genome of each selected F_4 line or parental accession.

Forty-eight primers for SSR markers were provided by: Agriculture and Agri-Food Canada, the National Institute of Agricultural Biotechnology (NIAB), Chungnam National University (CNU), Celera AgGen Brassica Consortium and JIC (http://www.brassica.bbsrc.ac.uk/BrassicaDB/) (Table 2).

Data analysis

The SSR bands were described by absence (0) or presence (1). The ratio of the introgressed exotic genome component to the receptor parent of *B. napus* HS3 was described by the index of subgenomic components (ISG) for A^r, C^c or $A^r + C^c$, which was calculated based on Li et al. (2006). Additionally, the considerable newly appearing bands (n^n) observed in the new type *B. napus* that did not appear in the original parents for resynthesis of the new type *B. napus* were added into the ISG in this study. The formula was modified as follows:

$$\mathrm{ISG} = \left(n_{\mathrm{A}}^{\mathrm{r}} + n_{\mathrm{C}}^{\mathrm{c}} + n^{\mathrm{n}}\right)/N$$

(superscripts r, c, n represent the bands from *B. rapa*, *B. carinata* and the new band, respectively; subscripts A, C represent the A and C genomes, respectively) where $n_{\mathrm{A}}^{\mathrm{r}}$ and $n_{\mathrm{C}}^{\mathrm{c}}$ represent the number of specific bands appearing in new type *B. napus* and its *B. rapa* (A^r) or *B. carinata* (C^c) parents, respectively. N represents the total number of bands appearing in the new type *B. napus*, excluding those bands appearing between the *B. rapa*/*B. carinata* parents and the receptor parent of *B. napus* without polymorphism. Specific bands from *B. nigra* were used to examine the bands from the B^c genome of *B. carinata*. Principal component analysis was performed with the NTSYS-PC program.

Table 2 The information of SSR primers used in the experiment

Code	Linkage group of detected allele[a]	Code	Linkage group of detected allele
CB10081[b]	A1	NIAB037	A6
BRAS078[c]	A1	CNU044	A7
NIAB096[d]	A1	CNU167	A7
Niab097	A1	CNU168	A7
SN11641[e]	A1	NIAB043	A7
CB10355	A2	CNU208	A8
NIAB071	A1	CNU090	A8
SN3761	A2	CNU008	A9
CNU250[f]	A3	NiAB004	A9
CNU288	A3	CNU280	A9
CNU270	A3	NIAB009	A10
CNU215	A3	NIAB015	A10
CNU002	A3	NIAB034	A10
CNU321	A3	CB10277	C1
SR12015	A3	CB10208	C1
SN3514f	A4	CB10530	C2, C4
NIAB048	A4	CNU099	C3
CNU029	A5	CB10124	C5
CNU286	A5	CB10234	C6
CNU257	A5	CNU400	C7
NIAB017	A5	CB10028	C8
CB10006	A6	NIAB022	C9
CNU149	A6	CNU203	Unknown
CNU219	A6	CB10314	Unknown

[a] A single primer pair usually amplifies multiple fragments and one or two alleles were mapped on the TN DH map (Qiu et al. 2006)

[b] Primers with prefix CB were from Celera AgGen Brassica Consortium and JIC (http://www.brassica.bbsrc.ac.uk/BrassicaDB/)

[c] From Suwabe et al. (2003)

[d] Primers with prefix NIAB were from The National Institute of Agricultural Biotechnology

[e] Primers with prefix SN were from Agriculture and Agri-Food Canada (http://www.brassica.agr.gc.ca/index_e.shtml)

[f] Primers with prefix CNU were from Chungnam National University

Results

Constructing the diverse population of substantial new type *B. napus* in the early generations

Six hundred and twenty pentaploid plants ($A^rA^rB^cC^cC^c$, $2n = 46$, which we regard as the F_1 generation) were obtained from crosses between 171 different hexaploid ($A^rA^rB^cB^cC^cC^c$), involving 78 accessions of *B. carinata* and 25 accessions of *B. rapa*, as female parent and eight lines of partial new type *B. napus* (novel rapeseed^{2nd}) as male parent (Fig. 1). Approximately 84% of the pollen mother cells (average 45 pollen mother cells per plant)

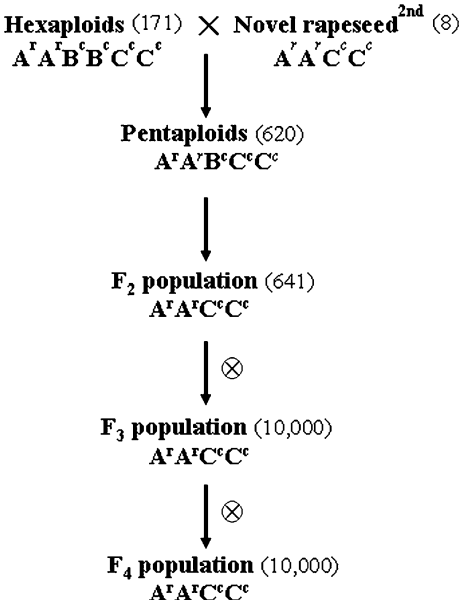

Fig. 1 A diagram for developing the diverse population of the substantial new type *B. napus*. The *numbers shown in brackets* are the amount of plants involved in the crosses or produced in each generation. Only 38-chromosomed plants in F_2 generation were used to create the F_3 generation. A *superscript in italics* indicates that the genome component was partially A^r or C^c

from 49 observed pentaploid plants showed various abnormalities associated with the chromosomes lost at different stages of meiosis (Fig. 2b–d). GISH analysis showed that most of the lost chromosomes were likely from the mono B genome (Fig. 2e–f). Occasionally (8.3%), cells with no any hybridization signal from B genome could be found in tetrad cells (Fig. 2g) which imply that normal A/C gametes should be produced by the complete loss of chromosomes from the B genome origin.

Consistent with abnormal meiosis, the pentaploid plants exhibited a relatively low rate of seed setting, with an average of 3.31 seeds per pod; each produced approximately ten F_2 plants (Fig. 2h). The F_2 population consisted of about 6,000 plants that showed great variation in both morphology and fertility. Nearly half of the plants appeared to have returned to their original maternal species morphologically, and one-third of the plants (most likely aneuploid) had very low fertility. After ruling out these two types of inferior plants, 1,137 F_2 plants were subjected to chromosome counting. The number of chromosomes in the selected F_2 population still varied from 27 to 46; the most abundant class contained the plants with 38 chromosomes that were expected from meiosis observation of the pentaploid (Fig. 3). The plants with 38 chromosomes also showed the highest fertility (although only 1–2 seeds developed to maturity in each pod) in the F_2 population. Consequently, 641 plants with 38 chromosomes were self-pollinated to yield the F_3 generation.

Establishing a population of substantial new type *B. napus* with 38 chromosomes at the F_4 generation

After surveying the quality (erucic acid and glucosinolate content) of seeds produced from F_2 plants, the F_3 population contained approximately 10,000 plants evaluated in the field. The F_3 generation was much better for the fertility than the F_2 population, although segregation on fertility still existed within and between families. The 958 F_3 plants with the best fertility were selected (without chromosome checking) to produce an F_4 population with the same size as the F_3 generation. The F_4 population involved 72 accessions of *B. carinata* and 25 accession of *B. rapa* and the morphology, fertility and chromosome status were further investigated.

The majority of the plants in the F_4 generation were similar to traditional *B. napus* in both morphology and fertility. The plant stem was half-encircled by cauline leaves at the base (a typical characteristic of *B. napus*), while the stem was not encircled by cauline leaves in *B. carinata* and was totally encircled in *B. rapa*. The plant architecture and the shape of the inflorescence were also shaped in the same way as traditional *B. napus* (Fig. 4a–d). There is no *B. carinata*/*B. rapa*-typed plants appeared in the population. The fertility of the F_4 plants was much increased (19.54 ± 7.0 seeds per pod, Fig. 4e) and was very close to that of commercial cultivars of *B. napus* (20.97 ± 3.2). To improve the population, one F_4 plant from each line was selected to produce subsequent generation based on agronomic traits and seed quality.

One hundred individuals were randomly selected from the F_4 population to evaluate their chromosome constitution. All of the checked somatic cells contained 38 chromosomes, which suggested the stability of the genome from selected F_3 plants to the F_4 generation (Fig. 4f). It was observed that 99.5% of the pollen mother cells (2,741 in total) exhibited normal meiosis behaviour almost without chromosome losing at different stages of meiosis, suggesting that the chromosomes of the A^r genome from *B. rapa* and the chromosomes of the C^c genome from *B. carinata* worked harmoniously in a common cell. Consistent with the cytology results, the F_4 plants bear pollen grains with relatively high fertility, ranging from 79 to 100%. To test whether there were any B genome-original chromosomes remaining, ten plants in the F_4 generation were randomly selected for GISH staining. The results showed that the chromosomes of the B genome had been completely eliminated from the cell (Fig. 4g). The results obtained from the morphological and cytological studies suggested that, after introduction via massive interspecific crosses with the A^r genome from *B. rapa* and the C^c genome from *B. carinata*, a population of substantial new type *B. napus* was established in the F_4 generation.

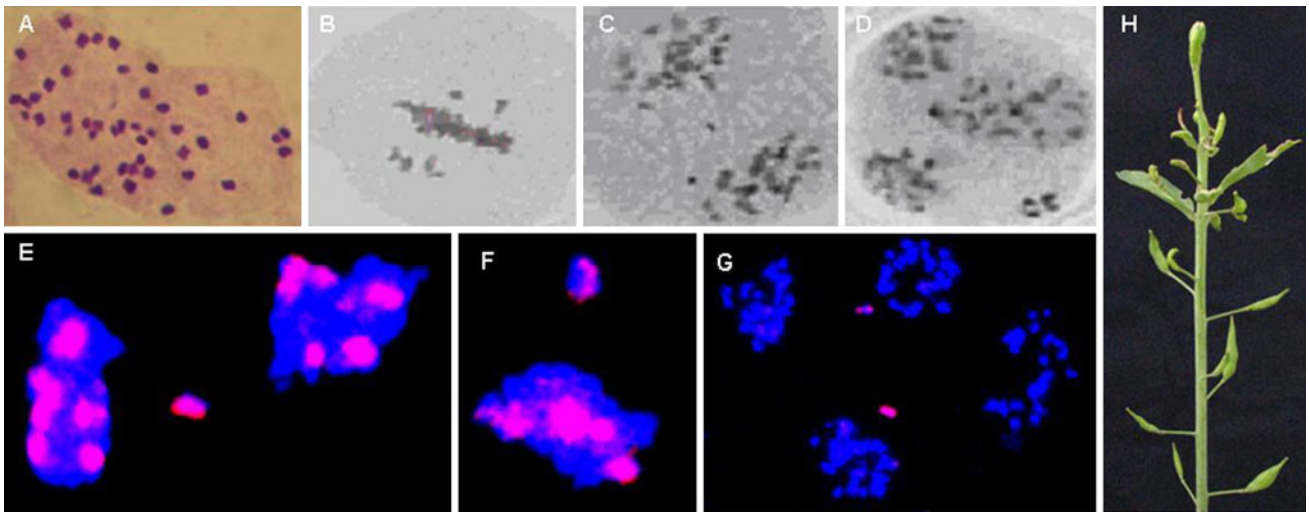

Fig. 2 The cytological characteristics and seed setting in pentaploid plants of the F_1 generation. **a** A pentaploid plant with the expected 46 chromosomes. **b–d** Various events of chromosome loss at meiotic stages MI (**b**), AI (**c**) and TII (**d** forming a micronucleus and then lost). **e–g** Using GISH to identify statues of the chromosome from the B genome that would be lost as laggards at equatorial in AI (**e**), away from equatorial in MI (**f**), or be a complete rule-out in some union of the tetrad (**g**). **h** A pentaploid plant bears siliques each with a few seeds. Pollen mother cells were used for all of the cytological studies except **a**, in which somatic cells from style tissue were used

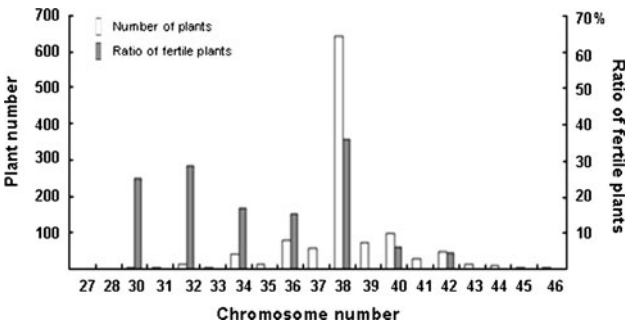

Fig. 3 Distribution of plants with different chromosome numbers and fertility in the selected F_2 population. The 38-chromosome plants comprised the largest fraction with the highest fertility

Genetic diversity in the population of substantial new type *B. napus*

Because the F_4 plants of new type *B. napus* were created with genetic introgression from 25 cultivars of *B. rapa* and 72 accessions of *B. carinata* into 8 lines of new rapeseed[2nd], it is worthwhile to estimate how large the genetic diversity is in the population. The genetic constitution of the F_4 population was analysed with the 100 sampled lines, along with their original parental species, 10 cultivars of *B. rapa* (AA), 55 accessions of *B. carinata* (BBCC), and 26 accessions of traditional *B. napus* with worldwide origins as determined by SSR markers. The two parental species, *B. rapa* (AA) and *B. carinata* (BBCC), although not possessing a common genome, shared 23% of common markers (59), which suggests that the A-, B- and C-genomes had the same evolutionary origins (Fig. 5a). The *B. rapa* that showed the most abundant genetic diversity and all of the identified 43 A^r-specific alleles were introduced into the newly established population of *B. napus*. The *B. carinata* with less abundant variation showed the all of the specific allele in C genome and some of the allele in B genome was introgressed to the population of new type *B. napus* (Fig. 5b). Both the marker analysis and the GISH staining also indicated that a few B^c-specific DNA sequences were integrated into the A/C chromosome of the new type *B. napus* (Fig. 5c). Interestingly, more than 10% of the alleles in the new type *B. napus* population appeared to be novel since such alleles were not detected not only from parental accessions but also from neither of parental pools (55 accessions of *B. carinata* and 10 accessions of *B. rapa*). In total, compared to the traditional *B. napus* population, one-third of the genetic constitution of the population of the new type *B. napus* was renewed due to interspecific introgression and genome alteration during the hybridisation process. Thereafter, significant genetic variation was expected in the population; this was validated by principal component analysis (Fig. 6).

For every line, the ratio of the exotic genomic component was evaluated by SSR markers. It was shown that the introgression rate from *B. carinata* and *B. rapa* into the receptor parent of *B. napus* ranged from 79.4 to 96%, with an average of 87.2%. Thereafter, the genome of the new type *B. napus* for the F_4 population was substantially constituted by $A^rA^rC^cC^c$ plus a few DNA fragments from B genome and de novo appeared; and differed significantly from the traditional *B. napus,* as well as the two parental species (Fig. 6).

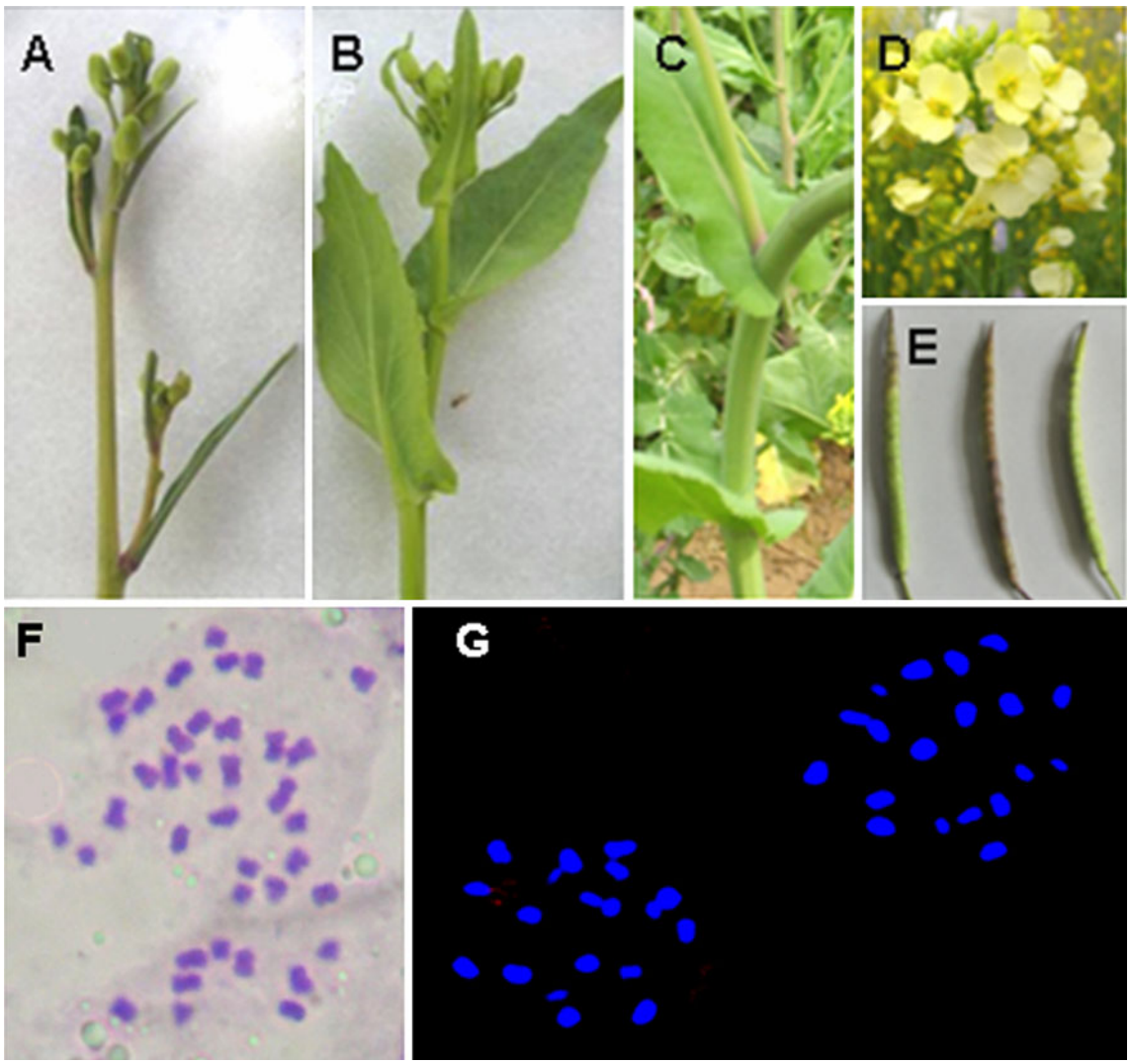

Fig. 4 Morphological and cytological characteristics of individual plants in the F_4 generation. **a–c** The cauline leaves in *B. carinata* were not encircling the plant stem at the base (**a**), while they encircled the stem completely in *B. rapa* (**b**), and half-encircled the stem in the F_4 plants, exhibiting a typical trait of *B. napus* (**c**). **d**, **e** These indicate that the F_4 plants were the same as traditional *B. napus* on a range of morphological characteristics such as flowers (**d**) and siliques (**e**). **f** The somatic cell of the F_4 plant has the expected 38 chromosomes. **g** This pollen mother cell at AI shows two sets of 19 chromosomes without any B-genome-related chromosomes, which was confirmed by GISH staining

The population diversity of the substantial new type *B. napus* on agronomic traits

Plant samples (about 3,000) from the substantial new type *B. napus* (F_4) population were investigated for agronomical traits and seed quality. Some valuable agronomic characteristics, such as yellow seeds and being vernalisation free, were transmitted from *B. rapa*/*B. carinata* into the population. In accordance with the large genetic variation revealed by molecular markers in the population (Fig. 6), the population was also shown to have great phenotypic variation for important agronomic traits (Fig. 7). Compared with the parental species (including the lines of novel rapeseed^{2nd}), the novel population had greater variation in seed number and seed weight, with the highest value (40 seeds per pod) for the former and the heaviest weight (6.98 g/1,000 seeds) for the latter.

The oil content in the seeds and the components of the fatty acid for the oil varied very much. The highest oil content in the novel population reached 51.2%, an unusually high value in the germplasm of *B. napus* in the semi-winter rapeseed growing conditions in China. The erucic acid varied from 0 to 57.6% and glucosinolate from 0 to 328 μmol/g in the novel population, which provides a large room to select lines with canola quality (low erucic acid and low glucosinolate in seeds) and high erucic acid for industry usage, e.g. for lubricating oil.

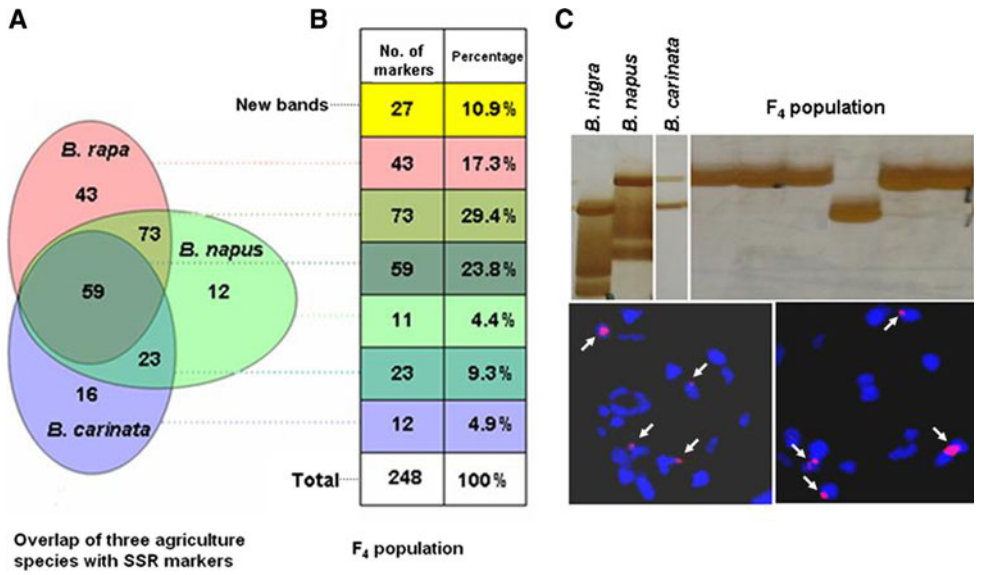

Fig. 5 Genetic variation analysis of the F_4 population. **a**, **b** A set of SSR markers was assessed for three agriculture species in which the 16 markers in *B. carinata* were identified to be half from B genome and half from C genome respectively (**a**) and the F_4 population of the substantial new type *B. napus* (**b**). The F_4 population harboured nearly all of the specific markers of the three agriculture species, except four in *B. carinata* and one in *B. napus*. Twenty-seven new SSR alleles (10.9%) were found in the population of substantial new type *B. napus*. **c** DNA fragments of B genome specific were integrated into the genome of substantial new type *B. napus*, identified either by the B genome-specific molecular marker (Plieske and Sturss 2001) (*above*) or by GISH staining in two pollen mother cells at diakinesis (*below*, shown by *arrows*)

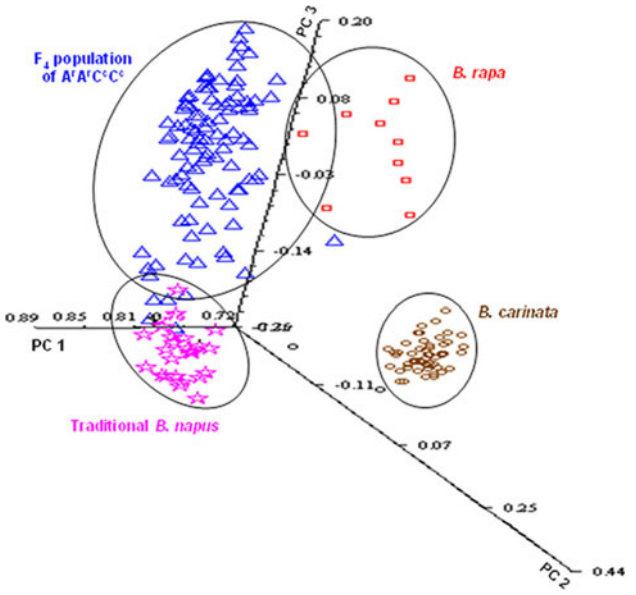

Fig. 6 Diagram of association among substantial new type *B. napus* and its relative species revealed by principal component analysis. The plot show that the first principal component (*PC1*, explaining 71.64% variance) separated *B. carinata* and *B. rapa* from B. napus, and PC3 (1.18%) and PC2 (6.56%) jointly separated substantial new type *B. napus* consisting of F_4 plants from traditional *B. napus*. The genotype data were the same as that shown in Fig. 5

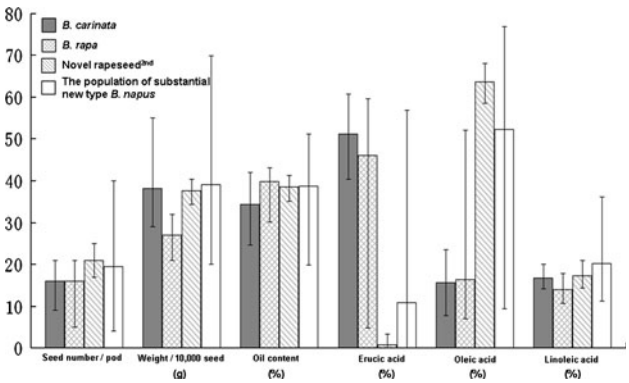

Fig. 7 Phenotype variation in the population of substantial new type *B. napus*. The sample size was as follows: the population of substantial new type *B. napus*, 3,225; *B. carinata,* 72; *B. rapa*, 25; novel rapeseed^{2nd}, 8. Except for erucic acid (due to artificial selection), the plants in the F_4 population showed increasing quality and agronomical trait variation compared with their progenitors. *Columns* represent trait average value. ⊥ represents variation range

Discussion

After two rounds of interspecific hybridisation and intensive selection, a population of substantial new type *B. napus* was established in the F_4 generation. The population, with traces of the A^n/C^n specific sequence, is distinguished from traditional *B. napus* by its genome, which was nearly 90% substituted by the A^r genome from *B. rapa* and the C^c genome from *B. carinata*. The population harboured genetic resources from 25 cultivars of *B. rapa* and 72 cultivars of *B. carinata,* plus considerable novel genetic variation caused by various potential origins which will be discussed late on.

Benefits of breeding *Brassica* with exotic genetic libraries from relatives of cultivated crops

In *Brassica* breeding, different approaches could be used to create the artificial resynthesized *B. napus*, i.e. direct hybridisation between the two diploid progenies (*B. rapa* and *B. oleracea*) with emphasis on valuable characteristics introgression (Akbar 1989; Chen et al. 1988; Morgan et al. 1998; Ren et al. 2000). The other type of resynthesised *B. napus* was descended from a triploid hybrid ($A^rA^nC^n$) of oil crop, *B. rapa* and *B. napus*, which was obtained only by hybridisation and possessed high fertility and good seed setting (Lu and Masahiro 2001; Mikkelsen et al. 1996; Shiga 1970). These hybrids were successfully used for the improvement of seed yield in *B. napus*, especially in China (Liu 2000). However, only partial A^r alleles were introgressed into the resynthesised *B. napus*, which limited their further application for the improvement of seed yield.

In this experiment, substantial new type *B. napus* lines with good fertility were synthesised by the substitution of the A^n and C^n genome of traditional *B. napus* with the A^r genome from *B. rapa* and the C^c genome from *B. carinata*, respectively. Meanwhile, *B. rapa* and *B. carinata*, as oil crops with thousands of cultivation, possess many valuable agronomic traits that are beneficial to the improvement of the seed yield of *B. napus* (Li et al. 2004). Unlike previous work, this study attached more importance to increasing the content of $A^r + C^c$ and the introgression of genetic variation from the exotic genetic libraries contained within hundreds of cultivars of *B. rapa* and *B. carinata*. This approach to synthesised substantial new type *B. napus* will provide rich genetic variation and phenotypic variation for the genetic improvement of *B. napus*. As a valuable breeding population for oilseed *Brassica*, genetic divergence in the population is expected to be further enlarged by the introduction of more exotic resources and by cross pollination, which will be further improved by recurrent selection. Improving plant breeding with exotic genetic libraries provides plant breeders with an important opportunity to improve the agricultural performance of modern crop varieties (Zamir 2001). Introgressing genomic components from widely related cultivated crops into the receptor crop were an efficient way in which to enlarge the receptor crop's genetic basis rapidly and to explore heterosis for yield production.

Great genetic and phenotypic variation in the substantial new type *B. napus* population

Brassica rapa, an old oil crop, was widely grown in Asia and Europe (Downey and Röbbelen 1989) and possessed rich diversity in terms of agronomic characteristics and DNA sequences (Prakash and Hinata 1980; Song et al. 1988a). Although cultivation of *B. carinata* was limited to several districts in Africa, such as Ethiopia (Pearson 1972), it possessed many valuable agronomic characteristics such as resistance to biotic and abiotic stress and yellow-seed germplasm (Rashid et al. 2006). The genetic basis between *B. rapa* and *B. napus* and between *B. carinata* and *B. napus* is believed to be rather different (Song et al. 1988b). In this study, the genome divergence between the A^r/A^n and the C^c/C^n subgenomes was revealed with species-specific molecular markers. Consequently, the newly established population accounted for all of the allele variation in the A^r subgenome with 25 accessions of *B. rapa* and in the C^c subgenome with 72 accessions of *B. carinata*.

In *Brassica*, homoeologous exchange is considered to be an important mechanism responsible for genome rearrangement and chromosome organisation induced by interspecific hybridization (Gaeta et al. 2007). Allosyndesis pairing between the B- and A/C-genome chromosomes occurred frequently in the meiosis of *Brassica* triploid (ABC) (Ge and Li 2007), which may allow illegitimate recombination between them. The de novo homoeologous nonreciprocal transposition (HNRT) between homoeologous regions of A and C genome had been well documented in resynthesized allotetraploid *B. napus* in resynthesized allotetraploid *B. napus* (Gaeta et al. 2007; Pires et al. 2004). For the population of substantial new type *B. napus*, the introgression of genetic components from the B genome was validated by the identification of B genomic specific markers and GISH staining, which implicate the homoeologous exchange between B and A/C genome chromosomes had occurred in the meiosis stage of hexaploid or pentaploid, which would provide de novo genetic variation, positive or negative, to the new population. Although genetic recombination between A and C genome was not traced, in this experiment because of the difficulties for distinguishing them due to high homology. On the other hand, this high homology would permit a higher frequency of homoeologous recombinations between A and C genome at the meiosis of hexaploid, pentaploid and even following generation. Therefore, the genetic diversity for the novel population should be obviously beyond the introduction of A^r and C^c resources from *B. rapa* and *B. carinata*.

Because of the introgression of the allele variation from A^r-, C^c- and B^c-genome chromosomes, profitable agronomic characteristics from *B. carinata* and *B. rapa* should be expected to appear in the population. Yellow-seed germplasm has been found in the population, and some individuals with resistance to *L. maculans* and tolerance for low nutrient elements (nitrogen/phosphorous) have been identified (unpublished data).

Interspecific hybridisation is an important path for the formation of allopolyploidy and might be a driving force

for biodiversity in flowering plants (Moore 2002; Leitch and Leitch 2008). Accumulating evidence showed that genetic changes such as the deletion, insertion, translocation of DNA sequences, chromosome rearrangement, and functional divergence of duplicate genes, frequently occur in the early generation after allopolyploidy offspring have formed (Pontes et al. 2004; Gaeta et al. 2007; Ma and Gustafson 2006). These genetic changes may lead to extensive novel genetic variation. Examination of the SSR allele variation shows that new bands also occurred in the population, which could be attributed to various genetic changes although we cannot totally rule out the effect of heterozygous from parents. Moreover, epigenetic alterations in interspecific hybridisation, such as DNA methylation, histone modification and RNA interference, have been documented (Adams and Wendel 2005; Wang et al. 2004; Lee and Chen 2001). These alterations may lead to gene expression changes in the offspring derived from interspecific hybridisation. Genetic and epigenetic changes may confer novel phenotypic variation to allopolyploid offspring derived from interspecific hybridisation (Gaeta et al. 2007; Pires et al. 2004). For the population, novel morphological and agronomic characteristics also occur frequently, including flowering with 12 stamens, flowers without petals, male sterile flowers, large seeds, high oil content and high linolenic acid, etc. The novel genetic and phenotypic gains in the population suggest that interspecific hybridisation is an excellent approach for the creation of novel *B. napus* germplasm.

In conclusion, genetic variation in the population of substantial new type *B. napus* was detailed and characterised into the following genotype origins: A^r-, C^c- and B^c-genotype and novel genetic variation produced by the interspecific hybridisation. It should be noted that as seen in principal component analysis, *B. rapa* cultivars show very wide genetic diversity compared to *B. carinata* cultivars, which suggests that genetic variation for the population may be increased by the introduction of A^r from more *B. rapa* cultivars.

Breeding potential of the population of substantial new type *B. napus* for intersubgenomic heterosis exploring in rapeseed

Previous studies revealed that the increasing genomic proportion of $A^r + C^c$ in partial new type *B. napus* can contribute to the improvement of seed yield for hybrids between partial new type *B. napus* and traditional *B. napus*. Some efforts have been made to screen out partial new type *B. napus* with a high genome proportion of $A^r + C^c$ (Qian et al. 2005; Li et al. 2006). For the population of substantial new type *B. napus*, the introgression rate of the genomic proportion for all of the individuals may be more than 80%, which suggests that individuals from the population should have promising *B. napus* germplasm for exploiting intersubgenome heterosis.

Recurrent selection using the male sterile gene is a population breeding system for increasing the frequency of favourable alleles and maintaining population heterogeneity (Doerksen et al. 2003). The system allows us to accumulate favourable alleles from A^r, C^c, B^c and genetic changes so as to produce a series of recombination individuals with not only high exotic genomic content but also excellent agronomy and quality traits. The seed yield of the hybrid between the recombination individuals and traditional *B. napus* is expected to be further enhanced.

Acknowledgments The authors are grateful to Professor Dr Zaiyun Li of Huazhong Agricultural University for his valuable help with the GISH technique, Dr Xingliang Liu for critical reading the manuscript. This study was supported by the National Natural Science Foundation of China (project code: 30830073) National 863 High Technology Program, P. R. China (2008AA10Z147).

References

Adams KL, Wendel JF (2005) Novel patterns of gene expression in polyploid plants. Trends Genet 21:539–543

Akbar M (1989) Chromosomal stability and performance of resynthesized *Brassica napus* produced for gain in earliness and short-day response. Hereditas 111:247–253

Becker HC, Engqvist GM, Karlsson B (1995) Comparison of rapeseed cultivars and resynthesized lines based on allozyme and RFLP markers. Theor Appl Genet 91:62–67

Chen BY, Heneen WK, Jonsson R (1988) Resynthesis of *Brassica napus L.* through interspecific hybridization between *B. alboglabra* Bailey and *B. campestris* L. with special emphasis on seed colour. Plant Breed 101:52–59

Cheung F, Trick M, Drou N, Lim YP, Park JY, Kwon SJ, Kim JA, Scott R, Pires JC, Paterson AH, Town C, Bancroft L (2009) Comparative analysis between homoeologous genome segments of *Brassica napus* and its progenitor species reveals extensive sequence-level divergence. Plant Cell 21:1912–1928

Delourme R et al (2006) Major gene and polygenic resistance to *Leptosphaerie maculans* in oilseed rape (*Brassica napus*). Eur J Plant Pathol 114:41–52

Diers BW, Osborn TC (1994) Genetic diversity of oilseed *Brassica napus* germplasm based on restriction fragment length polymorphisms. Theor Appl Genet 87:697–704

Doerksen TK, Kannenberg LW, Lee EA (2003) Effect of recurrent selection on combining ability in maize breeding populations. Crop Sci 43:1652–1658

Downey RK, Röbbelen G (1989) *Brassica* species. In: Röbbelen G, Downey RK, Ashri A (eds) Oil crops of the world. McGraw–Hill, New York, pp 167–169

Gaeta RT, Pires JC, Iniguez-Luy F, Leon E, Osborn TC (2007) Genomic change in resynthesized *Brassica napus* and their effect on gene expression and phenotype. Plant Cell 19:3403–3417

Ge XH, Li ZY (2007) Intra- and intergenomic homology of B-genome chromosomes in trigenomic combinations of the cultivated *Brassica* species revealed by GISH analysis. Chromosome Res 15:849–861

Gómez-Campo C, Prakash S (1999) Origin and domestication. In: Gómez-Campo C (ed) Biology of *Brassica* genospecies. Elsevier, Amsterdam, pp 33–58

Inomata N (1980) Hybrid progenies of the cross, *Brassica campestris* × *B. oleracea*. I. cytogenetical studies on F1 hybrids. Jpn J Genetics 3:1189–1202

Jiang Y, Tian E, Li R, Chen L, Meng J (2007) Genetic diversity of *Brassica carinata* with emphasis on the interspecific crossability with *B. rapa*. Plant Breed 126:487–491

Lee HS, Chen ZJ (2001) Protein-coding genes are epigenetically regulated in Arabidopsis polyploids. Proc Natl Acad Sci USA 98:6753–6758

Leitch AR, Leitch IJ (2008) Genomic plasticity and diversity of polyploid plants. Science 320:481–483

Li Z, Heneen WK (1999) Production and cytogenetics of intergenetic hybrids between the three *Brassica* diploids and *Orychphragmus violaceus*. Theor Appl Genet 99:694–704

Li ZY, Li MT, Huang RG, Wu XM, Song YC (2002) Genomic in situ hybridization (GISH) discriminates the A, B and C genomes in *Brassica* allotetraploid species. Chin J Oil Crop Sci 24:10–14

Li M, Qian W, Meng J, Li Z (2004) Construction of novel *Brassica napus* genotypes through chromosomal substitution and elimination using interploid species hybridization. Chromosome Res 12:417–426

Li M, Chen X, Meng J (2006) Intersubgenomic heterosis in rapeseed production with a partial new-typed *Brassica napus* containing subgenome A^r from *B. rapa* and C^c from *Brassica carinata*. Crop Sci 46:234–242

Liu H (2000) Genetic and breeding in rapeseed. Chinese Agricultural Universitatis, Beijing, pp 182–188

Lu C, Masahiro K (2001) Fertilization fitness and relation to chromosome number in interspecific progeny between *Brassica napus* and *B. campestris* study using current and resynthesized *B. napus*. Breed Sci 51:73–81

Ma XF, Gustafson JP (2006) Timing and rate of genome variation in Triticale following allopolyploidization. Genome 49:950–958

Mikkelsen TR, Jensen J, Jørgensen RB (1996) Inheritance of oilseed rape (*Brassica napus*) RAPD markers in a backcross progeny with *Brassica campestris*. Theor Appl Genet 92:492–497

Moore G (2002) Meiosis in allopolyploids—the importance of 'Teflon' chromosomes. Trends Genet 18:456–463

Morgan CL, Bruce DM, Child R, Ladbrooke ZL, Arthur AE (1998) Genetic variation for pod shatter resistance among lines of oilseed rape developed from synthetic *B. napus*. Field Crops Res 58:153–165

Morinaga T (1928) Preliminary note on interspecific hybridization in *Brassica*. Proc Imp Acad 4:620–622

Pearson OH (1972) Cytoplasmically inheritable male sterility characteristics and flavour components from a species cross *B. nigra* Koch × *B. oleracea* L. J Am Soc Hortic Sci 97:397–402

Pires JC, Zhao J, Schranz ME, Leon EJ, Quijada P, Lukens LN, Osborn TC (2004) Flowering time divergence and genomic rearrangements in resynthesized *Brassica* polyploids (Brassicaceae). Biol J Linn Soc 82:675–688

Plieske J, Sturss D (2001) STS markers linked to *Phoma* resistance genes of the *Brassica* B-genome revealed sequence homology between *Brassica nigra* and *Brassica napus*. Theor Appl Genet 102:483–488

Pontes O et al (2004) Chromosomal locus rearrangements are a rapid response to formation of the allotetraploid *Arabidopsis suecica* genome. Proc Natl Acad USA 101:18240–18245

Prakash S, Hinata K (1980) Taxonomy, cytogenetics and origin of crop Brassicas, a review. Opera Bot 55:3–57

Qian W, Chen X, Fu D, Zou J, Meng J (2005) Intersubgenomic heterosis in seed yield potential observed in a new type of *Brassica napus* introgressed with partial *Brassica rapa* genome. Theor Appl Genet 110:1187–1194

Qiu D, Morgan C, Shi J, Long Y, Liu J, Li R, Zhuang X, Wang Y, Tan X, Dietrich E, Weihmann T, Everett C, Vanstraelen S, Beckett P, Fraser F, Trick M, Barnes S, Wilmer J, Schmidt R, Li J, Li D, Meng J, Bancroft I (2006) A comparative linkage map of oilseed rape and its use for QTL analysis of seed oil and erucic acid content. Theor Appl Genet 114:67–80

Rashid A, Rakow G, Downey RK (2006) Development of yellow seeded *Brassica napus* through interspecific crosses. Plant Breed 2:127–134

Ren JP, Dickson MH, Earle ED (2000) Improved resistance to bacterial soft rot by protoplast fusion between *Brassica rapa* and *B. oleracea*. Theor Appl Genet 100:810–819

Shiga T (1970) Rape breeding by interspecific crossing between *Brassica napus* and *Brassica campestris* in Japan. Jpn Agric Res Q 5:5–10

Snowdon RJ, Köhler W, Friedt W, Köhler A (1997) Genomic in situ hybridization in *Brassica* amphidiploids and interspecific hybrids. Theor Appl Genet 95:1320–1324

Song YC, Gustafson JP (1993) Physical mapping of 5S rDNA gene complex in rice (*Oryza sativa*). Genome 36:658–661

Song KM, Osborn TC, Williams PH (1988a) *Brassica* taxonomy based on nuclear restriction fragment length polymorphism (RFLP) 2. Preliminary analysis of subspecies within *B. campestris* (syn. *rapa*) and *B. oleracea*. Theor Appl Genet 76:593–600

Song KM, Osborn TC, Williams PH (1988b) *Brassica* taxonomy based on nuclear restriction fragment length polymorphism (RFLP) 1. Genome evolution of diploid and amphidiploid species. Theor Appl Genet 75:784–794

Suwabe K, Tsukazaki H, Iketani H, Hatakeama K, Fujimura M, Nunome T, Fukuoka H, Matsumoto S, Hirai M (2003) Identification of two loci for resistance to clubroot (Plasmodiophora Brassicae Woronin) in *Brassica rapa* L. Theor Appl Genet 107:997–1002

UN (1935) Genomic analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jpn J Bot 7:389–452

Wang J et al (2004) Stochastic and epigenetic changes of gene expression in Arabidopsis polyploids. Genetics 167:1961–1973

Zamir D (2001) Improving plant breeding with exotic genetic libraries. Nat Rev Genetics 2:983–989

Zou J, Zhu J, Huang S, Tian E, Xiao Y, Fu D, Tu J, Fu T, Meng J (2010) Broadening the avenue of intersubgenomic heterosis in oilseed *Brassica*. Theor Appl Genet 120:283–290