Theor Appl Genet (2005) 110: 1187–1194
DOI 10.1007/s00122-005-1932-9

ORIGINAL PAPER

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Intersubgenomic heterosis in seed yield potential observed in a new type of *Brassica napus* introgressed with partial *Brassica rapa* genome

Received: 18 March 2004 / Accepted: 10 January 2005 / Published online: 2 April 2005

Abstract This paper reports the observation on the intersubgenomic heterosis for seed yield among hybrids between natural *Brassica napus* ($A^nA^nC^nC^n$) and a new type of *B. napus* with introgressions of genomic components of *Brassica rapa* (A^rA^r). This *B. napus* was selected from the progeny of *B. napus* × *B. rapa* and (*B. napus* × *B. rapa*) × *B. rapa* based on extensive phenotypic and cytological observation. Among the 129 studied partial intersubgenomic hybrids, which were obtained by randomly crossing 13 lines of the new type of *B. napus* in F_3 or BC_1F_3 to 27 cultivars of *B. napus* from different regions as tester lines, about 90% of combinations exceeded the yield of their respective tester lines, whereas about 75% and 25% of combinations surpassed two elite Chinese cultivars, respectively. This strong heterosis was further confirmed by reevaluating 2 out of the 129 combinations in a successive year and by surveying hybrids between 20 lines of the new type of *B. napus* in BC_1F_5 and its parental *B. napus* in two locations. Some DNA segments from *B. rapa* were identified with significant effects on seed yield and yield components of the new type of *B. napus* in BC_1F_5 and intersubgenomic hybrids in positive or negative direction. It seems that the genomic components introgressed from *B. rapa* contributed to improvement of seed yield of rapeseed.

Keywords *Brassica napus* · *Brassica rapa* · Intersubgenomic heterosis · Introgression · Seed yield

Introduction

Brassica napus (AACC, $2n=38$) originated from a spontaneous hybridization between *B. rapa* (AA, $2n=20$) and *B. oleracea* (CC, $2n=18$) (UN 1935), containing the entire chromosome sets of both parental genomes (Olsson 1960; Schiemann 1932; Sinskaya 1928; Tsunoda 1980). Although this amphidiploid species was domesticated only about 400 years ago, it became the most important oilseed *Brassica* crop in the world due to good production potential and resistances (Gómez-Campo 1999; Liu 2000). In China, *B. napus* accounts for about 85% of oilseed *Brassica* (Fu 2000). However, modern *B. napus* cultivars have a narrow genetic basis, limiting its potential for improving seed yield (Becker et al. 1995).

B. rapa, an old oilseed crop, exhibits wide genetic diversity and interesting agronomic traits (Downey and Röbbelen 1989; Prakash and Hinata 1980) and is rather different from *B. napus* with regard to its genome structure (Hoenecke and Chyi 1991; Song et al. 1988, 1995). To depict those differences, the concept of subgenomes was proposed where "A^r" was suggested to represent the genome of *B. rapa* (A^rA^r) and "A^n" and "C^n" were defined as the genomes of *B. napus* ($A^nA^nC^nC^n$) (Qian et al. 2003).

Intervarietal heterosis has been wildly utilized, and positive correlations between genetic distance between parents of hybrid and mid-parent heterosis has been demonstrated for seed yield in *B. napus* (Ali et al. 1995; Diers et al. 1996; Grant and Beversdorf 1985; Lefort-Buson et al. 1987; Riaz et al. 2001). Due to the large genetic differences between *B. rapa* and *B. napus* and the strong heterosis for biomass yield among hybrids between *B. napus* and *B. rapa* (Liu 2000; Liu et al. 2002; Qian et al. 2003; Sun 1943; Zhao and Becker 1998), a way to utilize intersubgenomic heterosis was proposed

Communicated by H.C. Becker

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for seed production by crossing natural *B. napus* with a new type of *B. napus* with introgression of the *B. rapa* genome (Liu et al. 2002).

The objectives of this study were (1) to describe the selection of new type of *B. napus* by screening the progeny of interspecific hybrids between *B. napus* and *B. rapa* based on morphological and cytological observations, (2) to exploit the seed yield potential of intersubgenomic hybrids between natural and new type of *B. napus*, and (3) to identify DNA segments introgressed from *B. rapa* with significant effects on seed yield and yield components in the new type of *B. napus* and hybrids derived from them.

Materials and methods

Development of plant material

Fifty of 120 triploid combinations ($A^rA^nC^n$) between *B. napus* and *B. rapa* with high biomass yield (Liu et al. 2002; Qian et al. 2003) were employed to develop the new type of *B. napus* by two breeding programs: successive selfing them, and backcrossing them to parental *B. rapa* followed by successive selfing (Fig. 1).

The seed yield potential of the new type of *B. napus* was evaluated by developing hybrids between natural and new type of *B. napus* in the different generations (Fig. 1), which were defined as partial intersubgenomic hybrids (PIGH) in order to distinguish the A^r subgenome-contained hybrids from the conventional intervarietal hybrids. Individual plants from 13 lines of the new type of *B. napus* in F_3 and BC_1F_3, as donors of pollen (Table 1), were randomly crossed with 27 cultivars of *B. napus* from Australia, China, and Europe as tester lines to produce 129 PIGH to test seed yield. In a successive year, the heterosis of PIGH was reevaluated with two PIGH from the new type of *B. napus* in F_4, 971 × XD and 77101 × XD (971 and 77101 as test lines and XD as the new type of *B. napus* derived from a natural *B. napus*, ‘Xiangyou 13’). Moreover, respective intervarietal hybrids (971 × ‘Xiangyou 13’, 77101 × ‘Xiangyou 13’) were also produced as additional controls. Twenty inbred lines with a high degree of uniformity but large variation among the lines were chosen from a population of BC_1F_5 derived from an individual of the new type of *B. napus* in BC_1F_2, H3T1 [(‘Huashuang 3’ × ‘Tianmen Youcai’) × ‘Tianmen Youcai’], to investigate seed yield and yield components as well as the PIGH between them and ‘Huashuang 3’. PIGH produced with the new type of *B. napus* lines at F_3 or BC_1F_3, F_4, and BC_1F_5 generations were named as PIGH-3, PIGH-4, and PIGH-5, respectively.

Field experiments

Randomized complete block design was used for all three field experiments. Plant density was according to farmers’ practice in the region of the Yangtze River, i.e., row spacing of 25.6 cm and 12 plants grown in 1.9-m short rows or 25 plants grown in 4-m long rows. As checks, ‘Zhongyou 821’ (an elite open pollination cultivar with high erucic acid and high glucosinolates in seed) as CK_1 in all field experiments and ‘Huaza 4’ (an elite commercial canola hybrid) as CK_2 in the field experiment evaluating PIGH-3 and PIGH-4 were chosen, because they have been widely planted in China for several years. Another elite commercial canola hybrid, ‘Huaza 6’, which has won the first place in the region of Yangtze River in recent years, was used as CK_3 in the field experiment evaluating PIGH-5.

The PIGH-3 were grown with two replications together with tester lines in Wuhan in 2001. CK_1 and CK_2 were planted every ten plots. Due to the limited seeds, plots consisted of single short rows. Ten plants in the

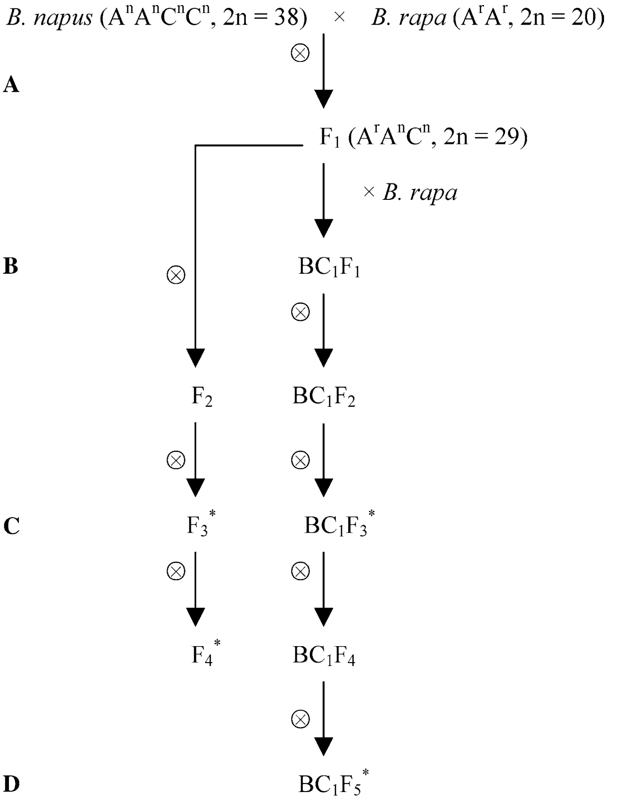

Fig. 1 Breeding diagram of producing a new type of *Brassica napus* for evaluating intersubgenomic heterosis. **A** Evaluation of intersubgenomic heterosis among 120 triploid hybrids between *B. napus* and *B. rapa* for biomass yield (Liu et al. 2002; Qian et al. 2003). **B** Selection for the new type of *B. napus* with 38 chromosomes in somatic cells, high fertility, and A subgenome recombined between A^n and A^r from the progenies of the 50 triploid hybrids with high biomass yield. **C** Evaluation of heterosis for seed yield among 129 partial intersubgenomic hybrids (PIGH-3) generated with the new type *B. napus*, and reevaluation of two combinations out of 129 PIGH-3 by developing PIGH-4. **D** Survey on heterosis among the hybrids between the 20 inbred lines of $BC_1F_5$and their parental *B. napus* in two locations and identification of DNA segments from A^rsignificantly affecting seed yield and seed-related traits by amplified fragment length polymorphisms. *Asterisks* indicate the donors of pollen to produce respective PIGH

Table 1 Characteristics of 29 individual plants of the new type of *Brassica napus* in BC$_1$F$_2$ or F$_2$ and seed yield potential among 129 partial intersubgenomic hybrids (*PIGH*)-3 derived from the random crosses between 13 lines of the new type of *B. napus* in F$_3$ or BC$_1$F$_3$ and 27 cultivars of natural *B. napus*. *I(A^r)* Index of A^r subgenome components

New type of *B. napus*				PIGH-3		
Code	Pedigree	I(A^r) (%)	Seeds set (seeds/pod)	Number of tester lines	Seed yield (g/plant)	
					Means	Range
BZ2	BC$_1$F$_2$ [('Bullet' × 'Zhejiang 1') × 'Zhejiang 1']	60.2	12.8			
H3M1	BC$_1$F$_2$ [('Huashuang 3' × 'Maverick') × 'Maverick']	68.2	12.6	17	13.4^{a}	8.2–21.2
H3M2	BC$_1$F$_2$ [('Huashuang 3' × 'Maverick') × 'Maverick']	43.6	11.2			
H3M3	BC$_1$F$_2$ [('Huashuang 3' × 'Maverick') × 'Maverick']	61.8	9.6			
H3M4	BC$_1$F$_2$ [('Huashuang 3' × 'Maverick') × 'Maverick']	55.4	11.6			
H3T1	BC$_1$F$_2$ [('Huashuang 3' × 'Tianmen Youcai') × 'Tianmen Youcai']	69.6	13.0	9	10.2	7.3–12.7
H3T2	BC$_1$F$_2$ [('Huashuang 3' × 'Tianmen Youcai') × 'Tianmen Youcai']	63.4	9.0			
H2T1	BC$_1$F$_2$ [('Huashuang 2' × 'Tianmen Daye Youcai') × 'Tianmen Daye Youcai']	67.7	14.0	9	10.9	4.9–14.0
H2T2	BC$_1$F$_2$ [('Huashuang 2' × 'Tianmen Daye Youcai') × 'Tianmen Daye Youcai']	54.2	9.8			
Mean		60.5	11.5			
6D1	F$_2$ (6203 × 'Denglong Zhong')	56.0	15.8	13	10.6	4.8–16.3
6D2	F$_2$ (6203 × 'Denglong Zhong')	52.0	13.4	9	11.1	6.3–13.8
6Y1	F$_2$ (6203 × 'Yanyou 2')	61.5	18.0	11	14.7^{a,b}	12.7–17.5
6Y2	F$_2$ (6203 × 'Yanyou 2')	45.2	9.0			
6Z	F$_2$ (6203 × 'Zhejiang1')	28.2	7.0			
BD	F$_2$ ('Bullet' × 'Denglong Zhong')	56.7	8.0	11	10.4	3.7–15.0
BX1	F$_2$ ('Bullet' × 'Xishui Youcai Bai')	54.6	9.0	9	10.0	6.7–12.8
BX2	F$_2$ ('Bullet' × 'Xishui Youcai Bai')	65.7	7.6			
BX3	F$_2$ ('Bullet' × 'Xishui Youcai Bai')	33.3	6.6	8	7.9	5.2–11.8
BX4	F$_2$ ('Bullet' × 'Xishui Youcai Bai')	54.5	11.2			
BZ1	F$_2$ ('Bullet' × 'Zhejiang 1')	56.7	13.0			
BT	F$_2$ ('Bullet' × 'Tianmen Daye Youcai')	–	9.0			
H2X	F$_2$ ('Huashuang 2' × 'Xishui Youcai Bai')	56.1	7.2	8	11.4	5.7–15.8
H2X	F$_2$ ('Huashuang 2' × 'Xishui Youcai Bai')	–	7.0			
H3Y	F$_2$ ('Huashuang 3' × 'Yanyou 2')	53.2	14.0			
SZ	F$_2$ (S2501 × 'Zhejiang1')	48.6	16.0	5	12.9^{a}	10.6–15.2
ST	F$_2$ (S2501× 'Tianmen Youcai')	50.0	12.6			
SY	F$_2$ (S2501× 'Yaanhuang')	35.2	14.0			
XC	F$_2$ ('Xianyou 13' × 'Chengdu Ai Youcai')	52.3	9.0	11	9.2	2.8–12.4
XD	F$_2$ ('Xianyou 13' × 'Denglong Zhong')	35.7	10.4	6	11.9	10.1–14.9
Mean		49.3	10.9			

[a] Significant exceeding check cultivar 1 (CK$_1$), 'Zhongyou 821', at $P = 0.05$

[b] Significant exceeding check cultivar 2 (CK$_2$), 'Huaza 4', at $P = 0.05$

center of each plot were harvested to calculate the seed yield per plant.

Seeds of PIGH-4 were sown together with respective intervarietal hybrids and tester lines with three replications in Wuhan in 2002. One hundred and twenty-five plants were grown per plot with five long rows, and all plants in the plot were harvested to measure seed yield.

Seeds of PIGH-5 and their parents were sown with three replications in two locations, Wuhan and Jingzhou, in 2002. Every plot was composed of 36 individual plants in three short rows. Ten plants in the center of each plot were harvested to calculate the seed yield per plant and yield components.

Cytological examination

The number of chromosomes in somatic cells of candidate individuals of the new type of *B. napus* was counted according to the method of Li et al. (1995). Ten to thirty metaphase cells from young buds were evaluated in each plant. Chromosomes were stained with 10% of carbol fuchsin.

Molecular marker assay

Amplified fragment length polymorphism (AFLP) markers were developed from DNA samples of the new type of *B. napus* and their parents, following the method of Vos et al. (1995) and Xu et al. (2001). Five and 19 pairs of primers were used to detect the DNA introgression from *B. rapa* among the new type of *B. napus* in F$_2$–BC$_1$F$_2$ and BC$_1$F$_5$ populations, respectively. The presence or absence of an AFLP band was scored with 1 or 0, respectively. The degree of introgression from *B. rapa* was described with the index of A^r subgenome components in the new type of *B. napus*, symbolized as $I(\mathrm{A}^r)$, which was calculated as follows:

$$I(A^r) = n_A^r/N \times 100$$

where n_A^r represents the number of the AFLP bands that were present in both the new type of *B. napus* and parental *B. rapa*, but absent in parental *B. napus*, and N represents the total number of polymorphic AFLP bands in its parental *B. napus* and *B. rapa*. The genetic distance (GD) between line X and Y was calculated on the basis of their Dice genetic similar coefficient (Nei and Li 1979) as follows:

$$GD_{X}y = 1 - 2N_{X}y/(N_X + N_Y)$$

where N_{XY} is the number of common bands shared by lines X and Y, and N_X and N_Y are the total number of bands in line X and Y, respectively.

The effects of polymorphic AFLP molecular marker loci on seed yield and yield components were assessed by one-way analysis of variance (ANOVA) in the inbred lines of $\mathrm{BC_1F_5}$ and PIGH-5, respectively (Zhang et al. 1994; Liu et al. 2002). If a molecular marker locus had significant effects on a trait, we denoted it as an active marker locus. If the allele from $\mathrm{A^r}$ in an active marker locus had significant positively or negative effect, the locus was defined as a favorable active marker locus (FAM) or an unfavorable active marker locus (UAM), respectively. The effect value of an active marker locus was estimated as the phenotype difference between the average of inbred lines or hybrids from inbred lines that had the same band as the *B. rapa* cultivar 'Tianmen Youcai' and the average of the other inbred lines or hybrids from inbred lines that had the same band as 'Huashuang 3' at an active marker locus.

The ANOVA was done with the Statistical Analysis System (SAS Institute 1992). Pearson's simple correlation coefficients were calculated among variables of interest.

Results

Introgression of the genetic components of *B. rapa*

The triploid $\mathrm{F_1}$ plants ($\mathrm{A^rA^nC^n}$, $2n=29$) exhibited low fertility, with an average of 4.4 seeds/pod among the 120 interspecific combinations between *B. napus* and *B. rapa* (data not shown). On the other hand, some individuals from $\mathrm{F_2}$ and $\mathrm{BC_1F_2}$ restored the fertility to some extent. There were 29 individual plants of the new type of *B. napus* with 38 chromosomes and high fecundity selected from the population of $\mathrm{F_2}$ and $\mathrm{BC_1F_2}$ (Table 1). Out of these, 20 plants were identified from 1,872 individuals in $\mathrm{F_2}$, and nine plants were identified from 860 individuals in $\mathrm{BC_1F_2}$, which were the descendants of four individuals with 29 chromosomes selected from 482 $\mathrm{BC_1F_1}$ plants. It seems that recombined euploid gametes (AC, $n=19$) were highly abundant in the generations of $\mathrm{F_1}$, $\mathrm{BC_1F_1}$, and $\mathrm{BC_1F_2}$.

Introgression from $\mathrm{A^r}$ in the new type of *B. napus* was identified with AFLP markers. The $I(\mathrm{A^r})$ of 29 individuals in $\mathrm{F_2}$ and $\mathrm{BC_1F_2}$ varied from 28.2% to 69.6%, with the average of 60.5% for $\mathrm{BC_1F_2}$ and 49.3% for $\mathrm{F_2}$ estimated with about 100 polymorphic markers (Table 1). When identified 20 inbred lines of $\mathrm{BC_1F_5}$ using 344 polymorphic markers, the $I(\mathrm{A^r})$ of this population was 42.3% on average, ranging from 38.8% to 45.0%, which was obviously lower than that of its ancestor, H3T1, with the $I(\mathrm{A^r})$ of 69.6% (Tables 1, 2). Moreover, recombination might happen in the process of the introgression, because we detected about 6% of new bands differed from their parents' band in the 20 inbred lines of $\mathrm{BC_1F_5}$. However, the genetic distance between the inbred line of $\mathrm{BC_1F_5}$ and 'Huashuang 3' significantly and positively related with the $I(\mathrm{A^r})$ ($r=0.67$, $P \le 0.01$). In other words, the larger the introgression from $\mathrm{A^r}$ in the new type of *B. napus*, the greater the genetic distance between it and its parental *B. napus*.

Partial intersubgenomic heterosis potential for seed yield

Strong seed yield heterosis was observed among PIGH in all three field experiments. Among 129 PIGH-3, about 90% of combinations exceeded their respective tester lines whereas about 75% and 25% of combinations surpassed $\mathrm{CK_1}$ and $\mathrm{CK_2}$, respectively (Fig.2). It should be noted that some of new type of *B. napus* lines, for example, 6Y1, H3M1, and SZ, exhibited high combining ability for seed yield, because the average seed yield of combinations derived from them significantly exceeded that of the controls ($P \le 0.05$) (Table 1).

Two combinations, 971 × XD and 77101 × XD, which slightly exceeded $\mathrm{CK_1}$ and $\mathrm{CK_2}$, respectively, in the field trial evaluating PIGH-3, still exhibited obvious heterosis in PIGH-4 in the enlarged area of plot in a successive year. They exceeded the respective tester line, controls, and intervarietal hybrid in seed yield, and the combination, 77101 × XD, even significantly exceeded the controls ($P \le 0.05$) (Fig.3).

The mean value of accessions over two locations in the field trial evaluating PIGH-5 is shown in Table 2. Seed yield showed the highest level of heterosis, followed by pods per plant and seeds per pod; very little heterosis was detected for seed weight. All PIGH-5 exceeded 'Huashuang 3', with heterosis from 29.17% to 95.83%, and the amount of mid-parental heterosis varied from 21.73% to 86.50%, with an average of 43.15% for seed yield. Some inbred lines and PIGH-5 significantly exceeded controls on seed yield and yield components ($P \le 0.05$). Joint ANOVA between two locations showed significant differences for seed yield and yield components ($P \le 0.01$) and no significant interactions between accessions and locations (Table 3).

Table 2 Performances of seed yield and yield components (means ± SD) among PIGH-5 derived from the inbred lines of BC_1F_5, their parents, and the controls over two locations

Code	$I(A^r)$	Pods/plant		Seeds/pod		Weight of 1,000 seeds (g)		Seed yield (g/plant)	
		Inbred lines	Hybrids	Inbred lines	Hybrids	Inbred lines	Hybrids	Inbred lines	Hybrids
1	42.6	221.3 ± 32.3	276.9 ± 131.8[a]	16.0 ± 2.8	17.2 ± 3.7	3.8 ± 0.2[a,b]	3.4 ± 0.4	8.3 ± 2.1	10.2 ± 4.1
2	43.4	174.5 ± 22.1	218.8 ± 32.3	16.0 ± 2.5	18.1 ± 4.0	3.7 ± 0.3[a,b]	3.6 ± 0.2[a,b]	6.6 ± 0.8	9.8 ± 1.0
3	41.9	184.1 ± 47.1	247.4 ± 51.5	16.4 ± 3.1	18.0 ± 2.7	3.8 ± 0.3[a,b]	4.0 ± 0.2[a,b]	8.1 ± 2.6	10.3 ± 2.9
4	43.0	192.9 ± 20.4	204.2 ± 33.5	18.4 ± 4.1	19.2 ± 2.9	3.3 ± 0.2	3.6 ± 0.2[a,b]	9.0 ± 1.8	10.1 ± 1.7
5	42.6	260.2 ± 40.3	263.0 ± 39.5[a]	14.3 ± 3.3	16.8 ± 1.2	3.7 ± 0.4[a,b]	3.5 ± 0.2[b]	8.5 ± 1.8	9.7 ± 3.9
6	43.8	204.6 ± 22.7	272.2 ± 48.9[a]	15.2 ± 2.9	18.3 ± 2.8	3.7 ± 0.2[a,b]	3.8 ± 0.2[a,b]	7.7 ± 2.5	14.1 ± 2.3[b]
7	43.0	231.4 ± 16.3	246.5 ± 53.9	19.2 ± 1.5	17.4 ± 1.5	3.7 ± 0.2[a,b]	3.9 ± 0.2[a,b]	10.6 ± 0.6[a,b]	10.9 ± 2.6[a,b]
8	42.2	207.6 ± 35.1	292.3 ± 56.8[a,b]	17.5 ± 3.0	17.5 ± 1.3	3.9 ± 0.3[a,b]	3.9 ± 0.2[a,b]	8.5 ± 2.0	13.6 ± 2.4[b]
9	41.9	182.8 ± 34.9	209.6 ± 15.0	17.8 ± 0.9	19.0 ± 2.5	3.9 ± 0.2[a,b]	4.0 ± 0.2[a,b]	6.9 ± 1.6	10.3 ± 2.1
10	38.8	229.4 ± 80.5	282.3 ± 31.3[a,b]	16.0 ± 1.2	16.5 ± 2.8	3.5 ± 0.3[b]	3.7 ± 0.2[a,b]	7.3 ± 1.9	11.6 ± 1.5[a,b]
11	44.2	213.2 ± 46.1	282.3 ± 37.6[a,b]	17.0 ± 4.2	17.2 ± 2.7	3.6 ± 0.4[a,b]	3.3 ± 0.1	7.5 ± 1.2	12.3 ± 2.6[b]
12	44.6	240.6 ± 51.3	212.0 ± 26.5	11.3 ± 4.0	19.1 ± 3.2	4.1 ± 0.3[a,b]	3.9 ± 0.3[a,b]	5.3 ± 1.5	9.7 ± 1.3
13	40.3	156.1 ± 28.7	188.5 ± 35.2	19.8 ± 4.0	19.0 ± 1.4	3.4 ± 0.1	3.8 ± 0.3[a,b]	8.1 ± 2.3	10.0 ± 2.6
14	42.2	208.0 ± 28.0	225.3 ± 12.1	15.3 ± 4.2	18.2 ± 1.5	3.9 ± 0.3[a,b]	3.7 ± 0.2[a,b]	8.4 ± 1.1	11.1 ± 1.0[a,b]
15	39.5	222.5 ± 57.8	263.8 ± 51.5[a]	15.1 ± 2.7	16.0 ± 0.8	4.1 ± 0.2[a,b]	3.7 ± 0.2[a,b]	7.8 ± 1.7	9.9 ± 1.8
16	38.8	185.3 ± 32.2	222.7 ± 32.1	16.9 ± 1.9	19.7 ± 4.0	3.8 ± 0.2[a,b]	3.8 ± 0.2[a,b]	7.0 ± 0.9	9.7 ± 1.5
17	39.5	203.3 ± 24.3	245.1 ± 42.2	11.5 ± 1.8	15.3 ± 1.4	3.8 ± 0.1[a,b]	3.9 ± 0.2[a,b]	6.5 ± 1.5	9.6 ± 1.9
18	45.0	161.7 ± 32.1	194.8 ± 34.5	19.2 ± 4.2	21.1 ± 3.9	3.3 ± 0.2	3.8 ± 0.1[a,b]	8.0 ± 1.3	10.0 ± 1.7
19	45.0	162.6 ± 62.1	238.5 ± 48.9	15.4 ± 2.4	15.1 ± 2.3	4.0 ± 0.1[a,b]	4.0 ± 0.3[a,b]	6.7 ± 2.0	9.3 ± 2.3
20	43.4	196.3 ± 43.7	247.2 ± 30.9	14.7 ± 2.6	17.4 ± 2.8	3.5 ± 0.1[b]	3.5 ± 0.1[b]	6.9 ± 2.6	11.1 ± 2.2[a,b]
Means	42.3	201.9	241.7	16.1	17.8	3.7	3.7	7.7	10.7
Huashuang 3		217.1 ± 47.1		16.7 ± 1.0		3.4 ± 0.1		7.2 ± 0.9	
CK_1		205.2 ± 33.6		19.4 ± 3.6		3.3 ± 0.1		8.3 ± 1.7	
CK_3			237.2 ± 28.6		18.1 ± 1.8		3.2 ± 0.3		10.6 ± 1.0

[a] Significant, exceeding Ck_1 at $P = 0.05$
[b] Significant, exceeding Ck_2 at $P = 0.05$

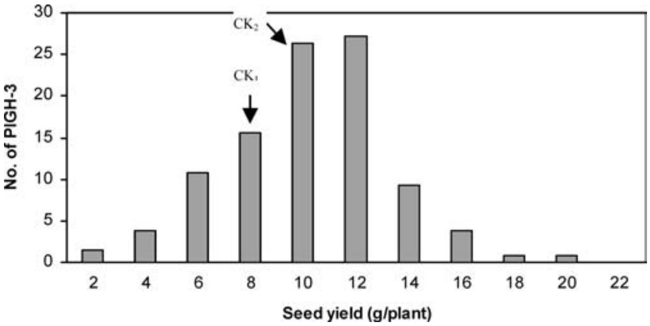

Fig. 2 Frequency distributions of the average of seed yield (g/plant) of 129 PIGH-3. The *arrows* indicate the positions of the two check cultivars, 'Zhongyou 821' (*CK_1*), and 'Huaza 4' (*CK_2*).

Identification of DNA segments from A^r with significant effects on seed yield and yield components

Among 344 polymorphic AFLP marker loci detected in the 20 inbred lines of BC_1F_5, there were 41 active marker loci that had significant effects on seed yield or yield components in the inbred lines and hybrids ($P \leq 0.05$), 24 FAMs, and 27 UAMs (Table 4). In addition, six marker loci contributed to two or more of the traits. The total of effects of FAMs exceeded those of UAMs for seed yield and yield components except for pods per plant in the inbred lines and hybrids and seeds per pod in inbred lines.

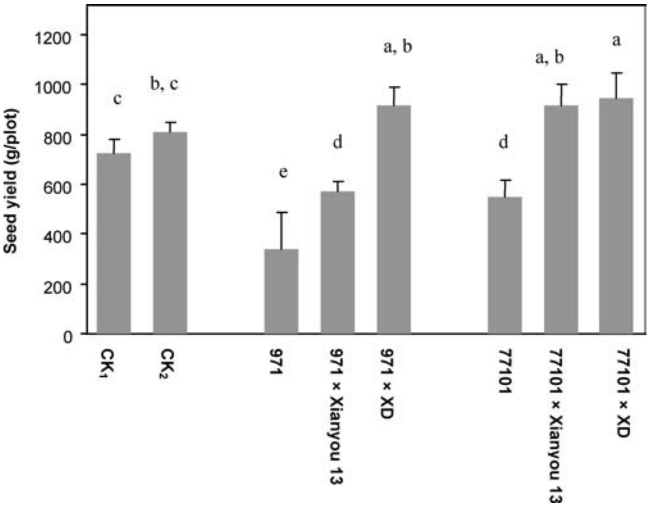

Fig. 3 Comparison of two PIGH-4 with respective tester lines and intervarietal hybrids for seed yield shown as mean values (*columns*) and half the standard deviation (*bars*). Columns with the same letter do not different significantly

Discussion

Introgression of A^r subgenome

Generally, there are two strategies to introgress genetic components of *B. rapa* into *B. napus*. One strategy is to

Table 3 Joint one-way analysis of variance for yield and yield components of the PIGH-5, their parents, and the controls over two locations

Items	*df*	Mean squares		Seed weight (g/1,000)	Seed yield (g)
		Pods/ plant	Seeds/ pod		
Replications (locations)	4	13,857.3*	1.48	0.012	17.29*
Locations	1	48,459.2*	447.22*	0.268*	167.53*
Accesions	42	7,076.2*	22.26*	0.330*	21.66*
Accesion[a] locations	42	1,351.2	7.12	0.062	2.31
Error	168	1,565.3	5.61	0.052	3.29

[a] Significant at $P=0.01$

Table 4 Active molecular marker loci with significant effects on seed yield and yield components and the effects of the alleles from A^r at those loci on these traits in the 20 inbred lines of BC_1F_5 and PIGH-5

Item	Inbred lines	Hybrids	Overlap
Pods/plant			
UAM[a]	2 (−73.5)[b]	8 (−255.7)	1
FAM[c]	2 (59.7)	3 (124.8)	0
Sum	4 (−13.8)	11 (−130.9)	1
Seeds/pod			
UAM	4 (−12.1)	2 (−3.4)	1
FAM	0	3 (5.2)	0
Sum	4 (−12.1)	5 (1.8)	1
Seed weight (g/1,000 seeds)			
UAM	4 (−1.0)	1 (−0.5)	0
FAM	4 (1.1)	3 (5.6)	0
Sum	8 (0.1)	4 (5.1)	0
Seed yield (g/plant)			
UAM	2 (−5.6)	4 (−6.3)	0
FAM	6 (11.8)	3 (6.4)	0
Sum	8 (6.2)	7 (0.1)	0

[a] *UAM* Unfavorable active marker locus
[b] Number of active marker loci, the sum of effects of the alleles from the introgressed A^r
[c] *FAM* Favorable active marker locus

artificially resynthesized *B. napus* ($A^rA^rC^oC^o$) by sexual or somatic hybridization between *B. rapa* and *B. oleracea* (C^oC^o). There were some successes transferring gene(s) (Akbar 1989; Chen et al. 1988; Heath and Earle 1997; Mackay 1977; Morgan et al. 1998; Olsson and Ellerström 1980; Prakash and Raut 1983; Ren et al. 2000; Schranz and Osborn 2000). However, multivalent or univalent formation at meiosis result in low fertility and low seed set in these resynthesized lines, especially in low generations, which were less direct utilized in seed production (Olsson 1960; Schenck and Röbbelen 1982; Prakash and Raut 1983; Sundberg et al. 1987; Rosén et al. 1988; Heath and Earle 1996, 1997). Namai et al. (1980) suggested a way to reduce chromosomal irregularities and to enhance the seeding fertility for the artificially resynthesized *B. napus* by crossing it with natural *B. napus*. In the practical breeding program, resynthesized *B. napus* was usually backcrossed to natural *B. napus* for several times as bridging materials or raw materials, and only a few favorable genetic components from *B. rapa* were transferred into *B. napus* (Kräling 1987; Becker et al. 1995; Prakash et al. 1999). The other strategy is to develop the new type of *B. napus* from the progeny of hybrid between *B. napus* and *B. rapa*. Easy development to hybrids between *B. napus* and *B. rapa* and high frequencies of euploid gametes in the F_1 have enabled this strategy to be widely utilized in Asia (Liu 2000; Lu and Masahiro 2001; Mikkelsen et al. 1996; Shiga 1970; Zhou and Scarth 1995). Namai (1976) observed higher frequency of multivalents in the meiosis of triploid with the genomic constitute of A^r A^r C^o than that of $A^rA^nC^n$. In this study, the observation of high fertility among the new type of *B. napus* in low generations and of high seed yield potential of PIGH also suggests that the A^r/A^n genome pair is more compatible or harmonious with the C^n genome.

Although genomic components of A^r were transferred into the new type of *B. napus*, it seemed to exist a decreasing tendency to $I(A^r)$ from low to high generations. An important reason was that the candidate individuals in high generations possessed some basic traits partially or completely controlled by the gene(s) in A^n, not in A^r. For example, low erucic acid and low glucosinolates in seed, which were present in parental *B. napus* ‘Huashuang 3’ as a canola cultivar, but not in parental *B. rapa* ‘Tianmen Youcai’ as an old cultivar. In other words, the direction of artificial selection for these traits in high generations was negative against the introgression of A^r. Moreover, a relatively high degree of heterozygosity in low generations might lead to overestimation of the $I(A^r)$ of low generations, because AFLPs as dominant markers were used.

Intersubgenomic heterosis in *B. napus*

Intergenomic heterosis is a universal phenomenon in nature, which is extensively and directly utilized in forest tree and grass (Allard 1960; Brewbaker and Sun 1999). Recent advances in heterosis have generated considerable interest in intersubspecific heterosis for seed yield in rice (Yuan 1994, 1997, 1998; Peng et al. 1999). In this study, we observed that some PIGH derived from the new type of *B. napus* in different generations exhibited strong vigor, and detected that the DNA segments introgressed from A^r had positive effects on seed yield on the whole. It suggests that the utilization of intersubgenomic heterosis be an accessible breeding strategy to increase seed yield in rapeseed.

It should be noted that genomic components of A^r were partially transferred into the new type of *B. napus*, and that some DNA segments introgressed from A^r unfavorably took effects on seed yield and yield components in this study. The potential of intersubgenomic heterosis may be enhanced by pyramiding more favorable genomic components of A^r.

Acknowledgements This work was supported by National 863 High Technology Program in China and Special Research Fund for the Doctoral Program of Higher Education. The authors gratefully acknowledge Professor J. Wu for assistance in plant selection. We also thank Dr. Christian Jung, Dr. Martin Frauen, Dr. Jianwei Zhao, and Dr. Jinguo Hu for critically reading the manuscript.

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