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R. Liu · W. Qian · J. Meng

Association of RFLP markers and biomass heterosis in trigenomic hybrids of oilseed rape (*Brassica napus* × *B. campestris*)

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Abstract Laboratory screening with DNA-based markers and field measurements of biomass production were carried out on each of the 120 trigenomic hybrids, obtained by interspecific hybridization between *Brassica napus* (AACC) and *Brassica campestris* (A′A′). The objective of this study was to elucidate the relationship between molecular markers and biomass heterosis of the interspecific hybrid between *B. napus* and *B. campestris*, which has been explored practically in rapeseed production for many years. The experiment was first carried out on 65 trigenomic hybrids in 1999. The average over-mid-parent heterosis of biomass production was around 30%, and the highest value was 175.4%. In the following year, the observation was expanded to 120 trigenomic hybrids and the best average over-mid-parent heterosis was 93%. A total of 1,477 DNA fragments, generated by Southern hybridization with 50 *Brassica* cDNA clones and 25 *Arabidopsis* EST clones, was scored across their parental lines. One hundred and twenty six and 215 fragments were identified as significantly associated with biomass production respectively in the 2 successive years. Using these active markers, a statistical model to resolve the heterosis is proposed and a new way to make use of the subgenomic heterosis is also discussed.

Keywords Biomass heterosis · Genetic heterozygosity · *Brassica napus* · *Brassica campestris*

Introduction

Heterosis is a common term used to describe the prevailing phenomenon in the organic world, in which the performance of a crossed hybrid is greater than either parent in different aspects. It exists in higher plants for many measurable characters such as plant height, stem diameter, leaf size, plant biomass production and seed yield. Hybrid cultivars of oilseed rape (*Brassica napus* L.) have been successfully used in agriculture for many years due to their significant yield increase of 30 to 60% over the mid-parents (Sernyk and Stefansson 1983; Grant and Beversdorf 1985). Even stronger heterosis (biological heterosis) has been observed in the interspecific hybrids among some *Brassica* species (Sun 1943; Liu 1985). Since there were significant correlations between biomass production and seed yield in rapeseed and other crops (O'Callaghan et al. 1994; Cabelguenne et al. 1999; Yuan et al. 1999), to explore the interspecific or intergenomic heterosis might provide a new way to increase the crop yield production. In hybrids between *B. napus* (AACC, 2n = 38) and *Brassica campestris* (AA, 2n = 20), not only is the heterosis of plant biomass production even stronger but also it is not difficult to obtain hybrid seeds and offspring especially when *B. napus* is used as the female parent. However, it is impossible to make direct use of the triploid F_1 in spite of its dramatic heterosis of biomass production. The major hurdle is the poor fecundity of the trigenomic hybrids seeds. Under general circumstances, only 3 to 15 F_2 seeds could result from each F_1 plant (Meng 1987). After intensive selection in the progenies derived from interspecific hybrids between *B. napus* and *B. campestris*, many rapeseed (*B. napus*) varieties had been released in Japan during the period of 1930 to 1960, and a range of diverse elite cultivars of *B. napus*, such as Chuannong Changjiao, Huayou 3 and Huahuang 1, had also been released in many provinces of China in the period of 1960 to 1980. As a result, desirable characteristics, such as early maturity and better local adaptation, were introduced from *B. campestris*, and the seed yield was increased dramatically as well (Liu 1985; Meng et al. 1998). However, it was not known which DNA segments had been transferred from *B. campestris* into the new varieties of *B. napus*. If we could selectively accumulate the DNA segments that

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R. Liu · W. Qian · J. Meng (✉)
National Key Laboratory of Crop Genetic Improvement,
National Center of Crop Molecular Breeding,
Huazhong Agricultural University, Wuhan 430070,
People's Republic of China
e-mail: jmeng@public.wh.hb.cn

have a significant contribution to yield, our breeding procedure would be more efficient.

To explore the complex basis of such heterosis, molecular markers, especially restriction fragment length polymorphic markers (RFLPs), were used in the diallel analysis of agronomic traits, which had been successfully employed in the identification of powdery mildew resistance segments in barley (Saghai Maroof et al. 1994) and markers contributing to rice heterosis and its components (Zhang et al. 1994; Liu and Wu 1998). Since Smith et al. (1990) and Lee et al. (1990) announced a significant correlation between the heterosis of F_1 performance and the heterozygosity of the RFLP marker-loci in corn, this relationship has been studied again and again, not only in this crop (Dudley et al. 1991; Bernardo 1992) but also in rice (Zhang et al. 1994; Saghai Maroof et al. 1997; Liu and Wu 1998), rapeseed (Ali et al. 1995; Diers et al. 1996) and cotton (Xu and Zhu 1999). However, the results of these studies showed that the extent of the correlation between hybrid performance and molecular marker heterozygosity was variable, depending on what genetic materials were used in their studies. Zhang et al. (1994) posed the idea of specific heterozygosity to assess the level of correlation of heterozygosity with heterosis and found a good correlation between specific heterozygosity and the grain yield heterosis of rice. However, later on, further studies showed that the extent of correlation also varied widely from one trait to another and from one dataset to another (Zhang et al. 1995, 1996).

In this study, we investigate the relationship between RFLP markers and the biological heterosis of 120 trigenomic hybrids based on diallel analysis (Saghai Maroof et al. 1994; Zhang et al. 1994). The objectives of this study were: (1) to analyze the correlation between these markers and hybrid performance; and (2) to identify specific DNA segments that significantly contribute to heterosis in either direction, favorable or unfavorable, in *Brassica* oilseeds.

Materials and methods

Parents and combinations

The 26 accessions used in this study including winter and spring ecotypes of *B. napus* (six accessions) and *B. campestris* (20 accessions) originated from Europe, Canada and various provinces of China (Table 1). Each of these lines had been selfed for at least three generations in our field, prior to making hybrids for the current study. All possible interspecific crosses, NC design II crosses (Comstock and Robinson 1952), were manually made between *B. napus* (as female) and *B. campestris* (as male) lines in order to obtain a full picture of the biomass heterosis between them. In the spring of 1999, 65 F_1s obtained with 5 *B. napus* and 13 *B. campestris* lines were observed in the fields. And in the spring of 2000, the observations were extended to 120 F_1s generated with 6 *B. napus* and 20 *B. campestris* lines.

Field experiment

All the F_1 hybrids, together with their parental lines, were grown in the agricultural experimental station of Huazhong Agricultural University, Wuhan, China. A randomized complete block design was used with three and four replications in the fall of 1998 and 1999, respectively. Each block comprised 30 plants. The field management was essentially the same as in normal agricultural production fields.

Table 1 A list of the rapeseed lines used in this study

ID Code	Name	Origin	Species
1	Baiguotian Youcai	Landrace from Hubei, China	*B. campestris*
2	Baijian 13	Cultivar from Jiangshu, China	*B. campestris*
3	Chengdu Ai Youcai	Landrace from Sichuan, China	*B. campestris*
4	Denglong Zhong	Landrace from Zhejiang, China	*B. campestris*
5	Fenyang Youcai	Landrace from Shanxi, China	*B. campestris*
6	Gold Rush	Released by Svalof weibull, Sweden	*B. campestris*
7	Horizon	Released by Svalof weibull, Sweden	*B. campestris*
8	Maverick	Released by Svalof weibull, Sweden	*B. campestris*
9	Qixingjian	Landrace from Sichuan, China	*B. campestris*
10	Reward	Cultivar from University of Manitoba, Canada	*B. campestris*
11	Shixian Bai Youcai	Landrace from Guizhou, China	*B. campestris*
12	Sv. Tyko	Sweden	*B. campestris*
13	Tianmen Daye Youcai	Landrace from Hubei, China	*B. campestris*
14	Tianmen Youcai Bai	Landrace from Hubei, China	*B. campestris*
15	Tobin	Canada	*B. campestris*
16	Xinghua Youcai	Landrace from Zhejiang, China	*B. campestris*
17	Xishui Youcai Bai	Landrace from Hubei, China	*B. campestris*
18	Yaanhuang	Landrace from Sichuan, China	*B. campestris*
19	Yangyou 2	Cultivar from Jiangshu, China	*B. campestris*
20	Zhejiang 1	Cultivar from Zhejiang, China	*B. campestris*
A	6203	Canola restore line from Hubei, China	*B. napus*
B	Huashuang 2	Canola cultivar from Huibei, China	*B. napus*
C	Bullet	Released by Svalof Weibull, Sweden	*B. napus*
D	S2501	Restore line from Jiangshu, China	*B. napus*
E	Huashuang 3	Canola cultivar from Hubei, China.	*B. napus*
F	Xiangyou 13	Cultivar released by Hunan, China	*B. napus*

Weight measurement

The above ground parts of ten plants in each block were harvested and mixed together to determine the fresh weight and dry weight when the plants reached the flowering telophase in the spring of 1999 and 2000 respectively. The fresh plants were weighed immediately after harvest about 8:30 am–11:00 am. Then the fresh samples were cut into pieces with a crush machine and kept in an oven at 105 °C for 30 min and then at 80 °C until they were completely dried for dry weight measurement. To minimize the experimental error, fresh weight measures were used to check the dry weight measures. If the ratios of fresh weight to dry weight were significantly different from others, the data of this block was discarded and data for this combination was only collected from the other two replications. The biomass production was based on plant dry weight in this study.

Molecular marker assay

Total DNA was isolated by a SDS method (Horn and Rafalski 1992) from the 26 parental lines. Restriction enzyme digestion, gel electrophoresis, alkaline transfer and Southern hybridization were carried out as described by Sharpe et al. (1995). A total of 50 *Brassica* clones (provided by Professor T.C. Osborn of Wisconsin University, USA) and 25 *Arabidopsis* EST clones (obtained from Dr. R. Scholl of Ohio State University, USA) were employed in the experiment.

Statistical analysis

Each RFLP genotype of a marker was scored across the 26 samples as '1' (presence) or '0' (absence) for a band. In the identification of the markers contributing to biomass production of F_1 plants, one-way analysis of variance was used with a marker genotype as a group effect, and entries within each group as an error term. In order to reduce false positive markers, only those markers that had a significant effect at the 0.01 significance level with at least three F_1s in each genotype group for one-way ANOVA were considered as active markers. When an active marker was identified, two genetic parameters, additive effect and dominant effect, were estimated based on a single-locus model (Zhang et al. 1994). The additive effect (A) and the dominant effect (D) of a marker were respectively calculated as:

$$A = (\bar{X}_{11} - \bar{X}_{00})/2,$$
$$D = \bar{X}_{10} - (\bar{X}_{11} + \bar{X}_{00})/2.$$

Here, $\bar{X}_{11}$ and $\bar{X}_{00}$ mean the average performance of the F_1s whose parents have the same marker genotype '1' and '0' respectively on this marker. $\bar{X}_{10}$ means the average performance of the F_1s whose genotype is heterozygous for this marker. So, if two parents were only polymorphic at one marker, their F_1 performance (Y) could be rewritten as:

$$Y = \alpha + A + D.$$

The variable α is the biomass production of the parent with an '0' genotype on the polymorphic marker, the variable A is the additive effect of the marker, and the variable D is its dominant effect. If there were i markers showing polymorphism between two parents, without consideration of epistasis, the equation was:

$$Y = \alpha + \sum_i (\beta Ai + \gamma Di).$$

Here, α means the expected performance of a parent with a '0' genotype on every polymorphic marker. A_i represents the additive effect of the '1' genotype on the ith active marker and D_i represents the dominant effect of the '1' genotype on the ith active marker contributing to the F_1 performance. However, if we estimate A_i and D_i by the single-locus-model equations, which assume that A and D on each locus are independent against each other, they cannot be used in this multi-locus equation directly. In order to estimate the additive and the dominant effect of an active marker in the multi-locus model by that in a single-locus model, we assume that there was a significant correlation between A and D in the single-locus model and those in the multi-locus model. Then, the values of A_i and D_i in this equation could be modified by the coefficients of β and γ, which represent the corresponding correlation coefficients. Therefore, if there were a positive value for the sum of the additive and dominant effect on an active marker, this marker genotype would probably have a favorable effect on the F_1 performance. We call this kind of marker as the Favorable Active Marker (FAM). By contrast, if the value was negative, we call it an Unfavorable Active Marker (UAM).

According to the previous description, we propose the following model to resolve the F_1 performance:

$$Y = a + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4,$$

where Y means the biomass production of each cross; a represents the contribution that has not been explained by the active markers; X_1, X_2, X_3 and X_4 mean the count of the active markers (with a significant effect on biomass production in the F_1s) with a favorable additive effect, an unfavorable additive effect, a favorable dominant effect and an unfavorable dominant effect respectively; and b_1, b_2, b_3 and b_4 represent the corresponding coefficients for X_1, X_2, X_3 and X_4.

Results

Biomass production of the F_1s performance

As shown in Table 2, most of the interspecific F_1 hybrids between *B. napus* and *B. campestris* showed striking heterosis on the plant biological yield. This agrees with the previous observation of Sun (1943). Highly significant differences (α = 0.01) among all the crosses and their parents for biomass production were consistent in 2 successive years. The values of the mid-parent heterosis of biomass production varied on a large scale, from –26.5% to 175.4% (in the combination of A × 20), with an average of 36.8% in 1999, and from –31.2% to 93.0% (in the combination of E × 20) and with an average of 32.6% in 2000. The percentage of combinations with negative mid-parent heterosis was 9.2% and 10.0% in 1999 and 2000 respectively. In other words, 90% of the interspecific hybrids showed superiority to mid-parents in 2 successive years. A good correlation between dry weight and fresh weight, r = 0.9510 in 1999 and r = 0.9853 in 2000, was found. It appeared that most superior combinations came from the crosses between Chinese *B. napus* and Chinese *B. campestris* in the 2 year experiments.

Identification of molecular markers contributing to heterosis in plant biomass production

A total of 1,477 polymorphic RFLP markers was scored across the 26 DNA samples with 166 probe/enzyme combinations. The number of active markers (associated with biomass production) was 126 in the year 1999, and 215 in 2000. The effects of 93 of these markers were consistent in both years. And all the active markers could also be divided into various groups (Table 3).

Table 2 Average biomass production[a] of F_1 plants and their parents measured in 2 successive years

ID code	Male	A	B	C	D	E	F
Female		243.00/144.00	240.33/160.25	160.00/143.75	237.67/200.50	207.00/215.00	–/190.50
1	290.00/251.50	375.67/291.00	351.00/263.75	355.00/175.25	413.00/322.00	351.00/251.50	–/334.00
2	–/248.25	–/266.25	–/297.00	–/185.00	–/341.75	–/262.00	–/353.00
3	–/356.50	–/299.00	–/310.75	–/253.25	–/292.75	–/349.50	–/413.50
4	301.67/260.25	430.33/285.75	406.67/321.75	346.00/222.00	427.00/274.75	305.00/396.00	–/351.25
5	230.00/239.50	413.00/305.00	350.33/261.75	211.33/230.00	363.33/289.50	247.67/243.00	–/278.00
6	361.07/232.50	445.00/259.00	360.33/306.50	210.00/165.50	245.33/356.00	340.00/271.25	–/279.25
7	287.73/261.00	271.33/334.75	408.00/304.50	279.33/198.25	297.67/295.00	330.33/297.75	–/357.00
8	301.60/233.50	437.00/245.50	339.67/298.25	224.67/209.00	260.33/241.75	373.33/242.25	–/315.00
9	–/266.50	–/298.75	–/319.25	–/216.75	–/296.00	–/295.75	–/302.50
10	–/241.25	–/223.75	–/281.00	–/131.00	–/201.25	–/172.25	–/267.50
11	–/282.00	–/349.00	–/360.50	–/208.50	–/304.75	–/325.25	–/349.75
12	–/222.00	–/267.25	–/244.75	–/133.25	–/189.25	–/264.50	–/249.00
13	313.33/308.25	379.00/301.25	375.33/323.50	174.00/274.25	420.33/338.50	373.67/368.50	–/332.50
14	318.33/331.25	447.67/423.25	331.00/281.00	252.00/249.75	373.33/342.00	318.00/361.25	–/394.75
15	–/237.25	–/255.25	–/272.50	–/135.25	–/239.75	–/198.25	–/284.75
16	300.00/216.75	322.67/315.00	366.67/283.75	260.33/204.50	318.67/304.25	306.67/280.75	–/296.75
17	367.73/278.50	436.50/346.00	464.33/288.00	283.33/214.00	377.33/318.50	413.33/375.00	–/311.75
18	301.67/269.25	381.67/357.25	285.33/408.75	280.67/240.50	494.00/398.50	455.00/381.75	–/346.75
19	256.67/241.50	438.00/331.00	335.33/266.75	180.33/254.25	299.33/372.25	341.00/291.75	–/330.75
20	291.00/289.75	735.33/344.75	366.00/344.75	317.33/311.75	658.33/270.75	418.67/487.00	–/365.50

[a] Average dry weight (grams per ten plants) in 1999, average dry weight in 2000

Table 3 Counts of the active markers we identified in this study. +A means the active markers with favorable additive effects; +D means the active markers with favorable dominant effects; –A means the active markers with unfavorable additive effects; and –D means the active markers with unfavorable dominant effects

Item	1999	2000	Overlapped in 2 years
Total number of active markers	126	215	93
FAM	51	104	31
UAM	75	111	60
Overdominace in FAM	16	29	9
Overdominace in UAM	6	9	0
+A, +D (SFAM)	26	69	15
+A, –D	17	23	0
–A, +D	40	88	25
–A, –D (SUAM)	43	35	15

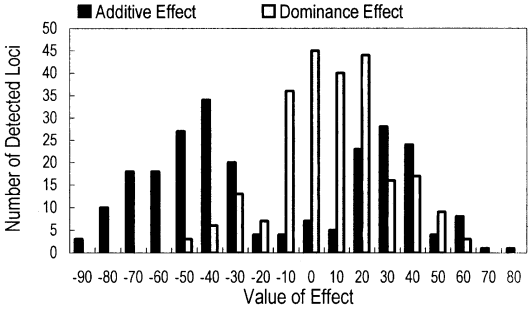

Fig. 1 Distribution of the additive and the dominant effects of the active markers detected in the year 2000

Interestingly, the distribution of the additive effect showed a completely different figure from the dominant effect of these active markers (Fig. 1). The average additive effect value was –10.30 in 1999 and –14.46 in 2000. This indicated that a large portion of the DNA segments of the hybrids anchored by these markers had an unfavorable effect on heterosis. However, there should be a considerable additive effect of active markers contributing a favorable effect to heterosis since the absolute value of the average additive effect of active markers was large; 40.79 in 1999 and 59.05 in 2000. If the average additive value of active markers changed from negative to positive by genetic manipulation, e.g. replacing the unfavorable markers with favorable markers, the heterosis of rapeseed might be remarkably increased. However, the mean of the dominant effect for the active markers was 12.65 in 1999 and 12.18 in 2000. This positive feature showed that the dominant effect generally was favorable to heterosis although the average of the absolute value was small, 20.16 in 1999 and 32.15 in 2000, about half of the average additive effect (absolute value).

Relationship between marker heterozygosity and the biomass production of the F_1 plants

The correlation between overall heterozygosity or general heterozygosity based on RFLP markers and the F_1s biomass production was significant at the 0.01 probability level, although the correlation coefficient was small (Model 1 in Table 4). However, the correlation of specific heterozygosity with F_1 biomass production was not significant at all in both years (Model 2 in Table 4). In order to find a better way to analyse the heterosis, an alternative model (Model 3) was proposed. The general consideration of the new model is that some markers have a favorable effect on the heterosis and some have

Table 4 Analysis of the relationship between RFLP and biomass production of F_1s using different models

Model no.	Equation[a]	Significance	*R* square	Parameter/its significance[b]	Prediction result *r*/*P*[c]
		Year 1999			
1	$Y = a + bX_5$	0.0001	0.2262	20.36/0.7973, 0.16/0.0001	0.01/0.9131
2	$Y = a + bX_6$	0.0668	0.0524	445.34/0.0001, –1.59/0.0668	
3	$Y = a + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4$	0.0001	0.4503	262.58/0.0001, 5.37/0.0031 – 0.91/0.4453, 2.00/0.1190 – 1.74/0.0850	0.55/0.0001
		Year 2000			
1	$Y = a + bX_5$	0.0001	0.1646	83.90/0.0552, 0.42/0.0001	0.23/0.0619
2	$Y = a + bX_6$	0.7372	0.0010	300.19/0.0001, 0.62/0.7372	
3	$Y = a + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4$	0.0001	0.5423	242.28/0.0001, 2.14/0.0001, –2.13/0.0001, 0.52/0.1732, –0.01/0.9712	0.58/0.0001

[a] X_5 and X_6 in Model 1 and Model 2 mean general heterozygosity and special heterozygosity respectively (Zhang et al. 1994, 1995, 1996). Model 3 was interpreted in the Materials and methods section

[b] Corresponding parameters and their statistical significance, which were calculated by SAS software package Version 6.12, were estimated on the basis of the dry weight of ten F_1 plants

[c] Data of one year were applied to the equation to predict the plant biomass performance in the other year. The coefficient and its statistical significance (*r*/*P*) of the correlations between real performance and predicted performance were listed

Table 5 The contribution (g) of the SFAMs and the SUAMs to the dry weight of ten F_1 plants in 2 successive years based on the single-locus model

Item	Marker[a]	Year 2000		Year 1999	
		Additive	Dominant	Additive	Dominant
SFAM	36H9T7B4.8	66.3429	34.0679	65.0952	22.6148
	36H9T7E2.5	69.9649	28.0375	71.5625	16.5895
	36D9T7E3.2	44.1333	53.5822	58.8239	63.9489
	WG7B6B10.5	43.1092	54.0847	34.7917	87.7713
	35D5T7E9	58.3458	38.6793	70.3523	61.0801
	WG2D5B4.1	59.2342	36.9050	82.6250	34.6709
	WG3H6E5.5	41.3050	53.8231	55.9375	66.0408
	9C1T7B5	28.8833	63.3431	34.4583	90.2594
	38A6T7B6	29.1656	60.6216	49.6333	80.9202
	WG7B6E8.5	42.1625	29.3750	74.3214	41.0567
	EC3B4H3	43.4306	8.4472	65.6333	30.0161
	36D9T7B8	38.4479	13.3462	53.1825	29.1118
	104C15T7E19	44.8312	4.4021	57.3095	8.9459
	WG3G11E4.8	44.1903	2.3254	50.5635	31.4928
	WG6C1H13	37.3627	4.8715	49.5476	20.5325
Mean		47.8304	32.4240	57.9628	47.3552
SUAM	WG1G4E13	–39.6211	–47.1419	–86.1562	–13.6562
	108D6T7B9	–71.3724	–7.7578	–86.4896	–11.9271
	EC3F12H3.3	–71.3724	–7.7578	–86.4896	–11.9271
	WG5A6B18	–71.3724	–7.7578	–86.4896	–11.9271
	WG6A11H2.2	–71.3724	–7.7578	–86.4896	–11.9271
	WG6B4H1.5	–71.3724	–7.7578	–86.4896	–11.9271
	35D5T7H21	–54.3816	–20.0066	–52.8542	–56.7917
	WG3G9H2	–53.7750	–19.7393	–50.6364	–35.1100
	38A6T7E19	–27.5092	–37.8946	–64.6562	–29.7188
	WG4A1B3.4	–38.7028	–22.4171	–72.7159	–7.2159
	35D5T7B3	–31.6566	–28.6253	–48.6354	–35.3854
	EC2D9B4.7	–31.6566	–28.6253	–48.6354	–35.3854
	TG5B2H5.7	–48.8597	–4.6544	–76.6932	–3.9563
	33G5T7E6.5	–41.4892	–8.2288	–78.7812	–18.9062
	33G5T7E19	–25.8472	–21.7401	–64.6562	–29.7188
Mean		–50.0241	–18.5242	–71.7912	–21.6987

[a] The first part of the marker name, before the last alphabetic character, is the name of the probe that we used, e.g. 36H9T7, is *Arabidopsis* EST clone ID, and WG7B6 is the code of probe obtained from T. Osborn; the last alphabetic character, the second part of the marker name, i.e. B, H or E, means that the detected active band was digested by *Bam*HI, *Hin*dIII or *Eco*RI endonuclease, respectively; the last Arabic numerals, e.g. 4.8, indicate the size of the detected band size in kb

an unfavorable effect. The situation also applies to the same markers. Sometimes, homogenic patterns have effects in one direction while the heterogenic patterns have effects in the other direction. For instance, the additive effect of WG3A2B9.5 (the 9.5-kb *Bam*HI-digested band probed with the WG3A2) was –48.54 and –47.42, while its dominant effect was 47.71 and 47.79 in 1999 and 2000, respectively. Therefore, we can divide the favorable active markers (FAM) into two types, active markers with favorable additive effects (X_1) and active markers with favorable dominant effects (X_3). The unfavorable active markers (UAM) can also be divided into two corresponding types, X_2 and X_4. Compared with Models 1 and 2 (Table 4), a much higher correlation was achieved from Model 3, $R^2 = 0.4503$ in 1999 and $R^2 = 0.5423$ in 2000. In order to test the models, equations obtained from one year were used to predict the biomass of F_1s in the other year. Results showed that the correlation between the predicted values and the real values of the F_1s performance was quite high compared with the correlation of real biomass values ($r = 0.36$, $P = 0.0031$) between the two growing seasons. However, all the present models excluding epistasis also play an important role in heterosis (Yu et al. 1997).

The significance of Super Active Markers (SAMs) in breeding

Heterosis is a phenomenon with a very complex genetic basis and is controlled by a number of genes (Zhang et al. 1996; Yu et al. 1997). This set of positive markers identified in this experiment is probably just a part of the total. However, these markers are difficult to be used in molecular breeding because the application of RFLP markers in marker-assisted selection is labor and money consuming. Hence, it is necessary to determine the positive markers that have more contributions to the F_1s performance. From the 93 active markers that overlapped in 2 successive years, we found 15 markers with positive additive effects and positive dominant effects, and 15 markers with negative effects (Table 3). We paid more interest to these markers, and called them Super Favorable Active Makers (SFAMs) and Super Unfavorable Active Markers (SUAMs), respectively, because not only did these markers show the same effect (favorable or unfavorable) in both homozygotes and heterozygotes, but also the total absolute values of the effects of A and D were larger than most of the others (Table 5).

In order to check whether these SAMs were effective enough in marker-assistant selection, we applied the 30 SAMs in the Model 3 of Table 4 based on the F_1s performance in 2000. The results showed that the model was still very significant ($R^2 = 0.4436$, $P = 0.0001$) and the parameters and the significance of this model was: a/P = 98.44/0.0184, b_1/P = 16.52/0.0001, b_2/P = 10.75/0.0001, b_3/P = 12.17/0.0468, and b_4/P = 3.11/0.1325. Compared with the results based on the total active markers (Table 4), these SAMs provided most of the information that the active markers do. So these SAMs were very effective to be applied in breeding.

Discussion

It has been accepted that *B. napus* (AACC) is an amphidiploid species that originated in Western Europe from a cross between ancient *B. campestris* (AA) and *B. oleracea* (CC) (U 1935). However, cytogenetic and molecular genetic studies showed that divergent evolution has made the genomes of *B. napus* quite different from its modern diploid relatives (Inomata 1985; Song et al. 1988). And also there are distinct differences between the Asian-ecotype of *B. campestris* ssp. *chinensis* and its European contemporary *B. campestris* ssp. *oleifera* on morphological and cytological characteristics (Sun 1943; Inomata 1985). We use A′ for the genome of *B. campestris* ssp. *chinensis* hereafter to distinguish it from the genome A in the amphidiploid species *B. napus*. The striking biological heterosis in the interspecific F_1 hybrids (A′AC) may reflect a strong interaction among the three genomes, A′, A and C. If this were true, introgenomic hybrids of *B. napus* (A′ACC) between common *B. napus* (AACC) and synthetic *B. napus* (A′A′CC) would express very strong heterosis and would be useful in oilseed production. The synthetic *B. napus* (A′A′CC) could be developed by selfing the F_1 (A′AC) plants and screening the offspring for A′A′CC plants with the aid of field observation, cytological testing and marker-assistant selection based on DNA finger-printing. Meanwhile, the A′A′CC plants with more FAMs and fewer UAMs, especially the SFAMs and SUAMs, identified from this experiment, will be given priority to be selected out for rapeseed breeding. Actually, a large breeding procedure for developing A′A′CC lines is currently being carried out in our laboratory.

From the results in Table 4, we showed that the dominant effects of the active markers did not significantly contribute to the F_1s performance. Actually, different markers have different values of contribution to heterosis (Fig. 1), besides the differences of working directions and working manner (additive or dominant). If we took for granted that each marker has the same contribution, some error would happen. In fact, we detected that most of the additive effects were favorable to the F_1 performance. This meant that most of the heterozygosity was of benefit for the F_1s performance. However, most of the values of their contribution were not so large as the additive contributions (Fig. 1). That is, on average the contribution of the dominant effects was not as significant as that of the additive effects. This was also confirmed by the significance and *R* square of Model 1 in Table 4. We can also obtain a clue from this that markers with a dominant effect are easier to make use of, and those with an additive effect are more effective in molecular breeding.

In many QTL mapping researches (Butruille et al. 1999; Zimnoch-Guzowska et al. 2000), the QTLs they detected (based on a multi-locus model) were subjected

to verification by the one-way ANOVA results (based on the single-locus model) and the results were well consistent with the QTL mapping results. This meant that our assumption about the correlation between the values of A and D in the single-locus model and those in the multi-locus model was based on some truth. However, the extent of the correlation needs further confirmation.

In this study, we used three endonuclease digestions to detect more multiple alleles of a locus. Some of these multiple alleles were possibly redundant, but most of them, we think, stood for different information because their band patterns were different. For example, the probe 33G5T7 could detect polymorphism between materials 1 and 2 with *Eco*RI digestion. However, when *Hin*dIII was used, the polymorphism existed among other materials. Moreover, *B. napus* is amphidiploid. Not only are the A and C genomes highly homologous to each other, but also there are many repeats of some segments in one genome (Song et al. 1990; Ferreira et al. 1994). Without genetic study in a segregating population, we cannot even decide whether these markers are from the same loci or not even if these markers were detected by the same probe. To map these markers can help us in selecting the nonredundant information out of these models. However, given that there are many multicopy loci throughout the genomes, it is hardly possible to map all these markers without enough polymorphism among a map population. So we did not consider the redundant information in this research, although it was important to confirm the validity of these models. However, some of these probes that had been mapped showed that they were randomly distributed on different positions of different chromosomes without the case of tightly linked markers. We are not sure whether other probes are evenly distributed among the chromosomes. If we could remove all the redundant information in later research, the models would be more valid.

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