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Prediction of heterosis using QTLs for yield traits in rapeseed (*Brassica napus* L.)

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Abstract Three double low (erucic acid and glucosinolates) self-incompatible lines and 22 varieties from different origins were selected to produce 66 hybrids according to a NC II mating design. Field experiments for identification of hybrid performance and heterosis were conducted in two successive rapeseed growing seasons in Wuhan, China. After heterosis identifications, SI-1300 and Eagle were chosen to construct an F_2 segregating population. One hundred and eighty four $F_{2:3}$ lines were planted at Wuhan and Jingmen to test yield traits. F_2 plants and the 25 parents were analyzed using simultaneously AFLP (amplified fragment length polymorphism) and SSR (simple sequence repeat) markers. A total of 270 and 718 polymorphic loci were detected in the F_2 population and among the 25 parental lines, respectively. Of the 718 polymorphic loci, 178 were significantly correlated to yield traits. With the use of one-way ANOVA, 84 common QTLs were detected for 12 traits at two trial locations. Although the genetic distances based on general/specific heterozygosities and single-locus QTLs showed significant correlations with hybrid performance and heterosis for some yield traits, the determination coefficients were low. The results suggested that neither heterozygosities nor QTLs for yield traits were suitable to predict hybrid performance and heterosis in *Brassica napus*.

Keywords *Brassica napus* · Heterosis · Heterozygosity · Prediction · Quantitative trait locus (QTL)

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Introduction

Heterosis is a common phenomenon in organisms. Utilization of heterosis has become a major strategy for increasing productivity of crops. Over a long period, hybrid cultivars with superior performance and strong heterosis have been developed through extensive field trials. This has involved high cost and considerable time. Therefore, it is very useful to find a simple and reliable method that could predict heterosis prior to expensive field testing.

Generally, hybrids from two parents with a distant genetic background (diverse in relatedness, ecotype, geographic origin etc) have high heterosis (Lefort-Buson et al. 1987; Brandle & McVetty, 1990; Ali et al. 1995), and therefore the genetic diversity between two parents has been proposed as a possible predictor of heterosis. Zhu and Zhang (1987) examined the correlation between heterosis and parental diversity for 8 isozymes

(esterase, peroxidase, cytochrome oxidase, polyphenol oxidase, glutamic-oxalacetic transaminase, amylyse, glucose-6-phosphate dehydrogenase, and malic dehydrogenase) in seedlings of hybrid rice, and found that diversity for esterase was more closely related with heterosis than diversity for the other isozymes. Chen (1996) demonstrated that the greater the difference between two parental isozymes spectra, the higher the heterosis of the F_1 hybrid. However, Peng et al. (1988) found no correlation between heterosis and isozymes variation in 75 F_1 hybrids derived from 18 parental lines. Yang et al. (1995) also demonstrated that it was difficult to predict heterosis using an index based on isozyme zymograms. The development of molecular markers provided a new technological base for selection of parental lines and prediction of hybrid performance and heterosis. At present, molecular markers are widely used in QTL mapping of quantitative traits (Butruille et al., 1999; Zhao & Meng, 2003) and exploration of genetic diversity, with the latter often employed in prediction of heterosis (Zhang et al., 1996; He et al., 2002; Liu et al. 2002). No attempt on prediction of heterosis using QTLs has been reported.

In this study, we investigated the correlation between SSR and AFLP markers and hybrid performance and heterosis. The objectives of this study were: (1) to analyze the correlation between hybrid performance and heterosis and genetic distances based on heterozygosities and QTL mapping for seed yield traits; (2) to explore the prediction of hybrid performance and heterosis using QTL mapping; and (3) to judge the predictive values of different methods.

Materials and methods

Plant materials

The materials used in this study consisted of three double low (erucic acid and glucosinolates) self-incompatible lines and twenty-two pure line varieties of *Brassica napus*. Three self-incompatible lines, SI-1300, SI-1310 and SI-1320, plus Zhongyou 821 and Huashuang 3 were from China; Dunkeld, Rainbow and Oscar were from Australia; Quantum-2, Sprint and Defender were from Canada; Sponsor, Impulse, Wildcat, Eagle, Senator, SW9475913, SW9372561, SW9473754, SW9375645, SW9475228, SW9474922, SW9376258, SW9474243 and SW9474933 were from Europe. The parental lines from China were semi-winter type, and the 20 other lines were spring types. All parents were selfed for more than 6 generations. With a North Carolina II crossing design, the 3 SI lines were hand pollinated by these inbred varieties to produce 66 hybrids. A total of 91 entries, including 66 hybrids and the 25 parental lines/varieties (Zhongyou 821 was the control), were tested for heterosis of seed yield per plant.

Based on the results of the field experiments and the geographical origins of the parental materials, SI-1300 and Eagle were chosen to produce a segregating population. One F_1 plant derived from the cross was self-pollinated to generate an F_2 population. One hundred and eighty four F_2 plants were selected at random and self-pollinated to produce 184 $F_{2:3}$ lines. Salt sodium spray, described by Fu et al. (1992), was employed to overcome self-incompatibility in the F_2 population. The $F_{2:3}$ lines, the two parents and the F_1 hybrid were planted to detect QTLs for yield traits.

Field experiment and data collection

Field experiments and data collection for heterosis determination were described by Shen et al. (2002).

Field experiments for QTL detection of seed yield traits were conducted at the Experimental Farm of Huazhong Agricultural University, Wuhan and the Institute of Agricultural Sciences of Jingmen, Hubei, China, in the rapeseed growing season from September, 2001 to May, 2002. The preceding crop was rice at Jingmen, while it was fallow at Wuhan. Each entry was grown in a one-row plot (3.50 m length and 0.27 m width) following a randomized complete block design with two replications. The field management followed the usual field practice.

Sowing time was in the early days of October at each location. At 40 days after emergency, 22 plants were left per row with a 0.15 m distance between plants. 15 plants were harvested randomly in the middle from each plot at maturity. Traits examined were the same as in the experiment for heterosis determination: total length of main inflorescence, effective length of main inflorescence, number of siliqua on main inflorescence, siliqua density of main inflorescence, number of primary branches, number of siliqua on primary branches, number of secondary branches, number of siliqua on secondary branches, number of siliqua per plant, number of seeds per siliqua, 1000-seed weight and yield

Table 1 Sequence of selective amplified primers for AFLP assays

+3 primer	*EcoR* I	*Pst* I	*Mse* I
1	AAA	AGA	CAA
2	AAT	AGT	CAT
3	AAC	CAC	CAC
4	AAG	CAG	CAG
5	ATA	CCA	CTA
6	ATT	CTG	CTT
7	ATC	GCA	CTC
8	ATG	GCT	CTG
9	ACA	GGA	CCA
10	ACT	GGT	CCT
11	ACC	GTT	CCC
12	ACG	TGA	CCG
13	AGA	TGT	CGA
14	AGT	TGC	CGT
15	AGC	CCA	CGC
16	AGG	GTT	CGG

per plant. The phenotypic mean of each $F_{2:3}$ line was used for trait assessment of the corresponding F_2 plant (Hayashi & Ukai, 1999).

DNA extraction

Leaf tissue was harvested from seedlings of the parental lines/varieties and the F_2 plants. Genomic DNA was extracted followed an SDS protocol modified by Li et al. (1994) and preserved at $-20\,^{\circ}$C. Before PCR (polymerase chain reaction), the DNA was diluted with double distilled H_2O (ddH_2O) at the concentration of 25 ng/μ1.

DNA markers and laboratory assays

SSR and AFLP were used to survey DNA polymorphism among the parental lines/varieties and the F_2 population.

The primer pairs for the SSR marker were synthesized by Shanghai Sangon according to the published sequences from http://ukcrop.net. The PCR procedure, electrophoresis and silver staining followed previously described methods (Lu et al., 2003).

For surveying with AFLP markers, DNA samples were digested with restriction enzymes in two combinations of *Pst* I, *Mse* I and *EcoR* I, *Mse* I (MBI). Primers were designed following Vos et al. (1995). The sequences of the adapters were as follows: for *Pst* I, the forward adapter was 5′-CTCGTAGACTGCGTACATGCA-3′ and the reverse adapter was 3′-CATCTGACGCATGT-5′; for *EcoR* I, the forward adapter was 5′-CTCGTAGA CTGCGTACC-3′ and the reverse adapter was 3′-CTGACGCATGGTTAA-5′; tor *Mse* I, the forward adapter was 5′-GACGATGAGTCCTGAG-3′ and the reverse adapter was 3′-TACTCAGGACTCAT-5′. Corresponding pre-amplification primers were GACTGCGTACCAATTCA for *EcoR* I, GACTGCGTACATGCAG. for *Pst* I and GATGAGTCCTGAGTAAC for *Mse* I. Primers for the selective amplification are listed in Table 1. All primers and adapters were synthesized by Shanghai Sangon. AFLP procedures were basically as described by Lu et al. (2001).

Data processing and statistical analysis

Each SSR and AFLP phenotype of a marker was scored as presence (1) or absence (0) for an allele variant/band. The genetic distance between parents was estimated from the marker data by Nei's distance equation (Nei & Li, 1979). Parental grouping was based on Nei's distance using an unweighted pair group method (UPGMA).

Two measurements of heterozygosity were used when calculating the correlations of molecular-marker distances with hybrid performance and heterosis: general heterozygosity and specific heterozygosity. General heterozygosity of an F_1 hybrid refers to the distance between the 2 parents based on all the polymorphic markers employed in the study, and specific heterozygosity for a particular trait of an F_1 hybrid refers to the distance between the 2 parents based only on the markers that detected significant effects on that trait (Zhang et al., 1996). In the determination of specific heterozygosity with additive and dominant effects, we used the single-locus model modified by Liu et al. (2002) at the 0.001 significance level.

QTLs for 12 seed yield traits were detected in the F_2 population at a 0.001 significance level when using one-way ANOVA from the statistical package STATISTICA 5.5 (Statsoft, 1999).

The relationships between genetic distance and heterosis/hybrid performance were estimated by regressing the heterosis or the trait values of the F_1 hybrids on the genetic distances using the REG procedure from the Statistical Analysis System programs (SAS

Institute, 1997). Mid-parent heterosis in percentage was calculated as MPH = [(hybrid-mid-parent)/mid-parent] × 100.

Results

Polymorphism of marker loci

Thirty-four AFLP primer sets and seventy-five SSR primer pairs which yielded clearly identifiable bands/allele variants within four randomly selected parents were used to amplify the 25 parents. A total number of 1528 bands were produced by AFLP marker across the 25 parents. Out of these, 582 were polymorphic. The primer set P16/M03 produced the highest number of bands, i.e. 87, while the primer sets P01/M02 and E13/M03 revealed the highest number of polymorphic bands with 24 for each set. The number of loci detected by SSR ranged from 1 to 5 with an average of 1.81. A total of 248 allele variants were generated among the 25 DNA samples at 136 loci. Of them, 210 were polymorphic.

Correlation of heterozygosities with hybrid performance and heterosis

Nei's genetic distances were computed for all 66 evaluated combinations involving the 25 parental lines based on 792 polymorphic bands/allele variants (582 from AFLPs, 210 from SSRs). As shown in Table 2, the genetic distance based on general heterozygosity was significantly positively correlated with hybrid performance for seed yield per plant, number of seeds per siliqua, siliqua density of main inflorescence, number of primary branches, but it was remarkably negatively correlated with that for 1000-seed weight, total length of main inflorescence, effective length of main inflorescence, number of secondary branches and number of siliqua on secondary branches. Correlations were very small between genetic distance and hybrid performance for the other characters, such as number of siliqua on main inflorescence, number of siliqua on primary branches, number of siliqua per plant and mid-parent heterosis for most the traits.

A total of 178 common active loci/alleles were detected for the 12 yield traits in the two growing seasons from September, 1999 to May, 2001. Of them, 38 were SSR allele variants and 140 were AFLP loci. The corre-

Table 2 Correlations of genetic distance based on molecular variants with hybrid performance and mid-parent heterosis

	General heterozygosity($n = 66$)		Specific heterozygosity ($n = 66$)		Single locus QTLs ($n = 65$)	
	Performance	MPH	Performance	MPH	Performance	MPH
Total length of main inflorescence	−0.61**	−0.24*	−0.60**	−0.28*	−0.61**	−0.44**
Effective length of main inflorescence	−0.60**	−0.27*	−0.57**	−0.32**	−0.56**	−0.46**
Number of siliqua on main inflorescence	−0.04	−0.26*	−0.02	−0.14	0.04	−0.23*
Siliqua density of main inflorescence	0.49**	0.18	0.49**	0.32**	0.52**	0.40**
Number of primary branches	0.49**	0.31*	0.42**	0.33**	0.57**	0.37**
Number of siliqua on primary branches	0.06	−0.00	0.18	0.14	0.28*	0.05
Number of secondary branches	−0.42**	−0.11	−0.32**	−0.09	−0.21*	−0.06
Number of siliqua on secondary branches	−0.36**	−0.14	−0.27*	−0.14	−0.19*	−0.08
Number of siliqua per plant	−0.04	−0.08	0.07	0.06	0.18*	−0.02
Number of seeds per siliqua	0.53**	−0.02	0.56**	0.03	0.38**	0.14
1000-seed weight	−0.31**	−0.06	−0.27*	−0.09	−0.33**	−0.10
Yield per plant	0.36**	−0.02	0.49**	0.09	0.40**	0.17

*and **represented significant relationships at 0.05 and 0.01 probability level, respectively

lation coefficients between the genetic distance based on the specific heterozygosity and hybrid performance and heterosis were similar to those based on the general heterozygosity for all 12 traits. As shown clearly in Table 2, the determination coefficients (R^2) calculated for the two heterozygosities were very low.

The results suggested that neither the general heterozygosity nor the specific heterozygosity indicated the performance of the yield traits clearly. Therefore, heterozygosities were considered not to be suitable to predict hybrid performance and heterosis.

Prediction of heterosis based on detection of QTLs for yield traits

Twenty-two AFLP primer sets and sixty-three SSR primer pairs which produced clearly polymorphic bands/allele variants within the two parents were used to judge the genotypes of the F_2 plants. A total of 201 dominant and 69 co-dominant marker loci were detected. For the 12 yield traits, 101 single-locus QTLs were detected in Wuhan, and 82 QTLs were resolved in Jingmen at the significance level of 0.001. Eighty four common single-locus QTLs (contributing from 2.3% to 15.9% to the phenotypic variation for the 12 yield traits) were identified at a 0.001 significance level at one location, and a 0.005 significance level at another. Among them, 18 were SSR loci (14 co-dominant loci, 4 dominant loci) and 66 were AFLP loci (all were dominant). Based on these marker loci, the genetic distances between SI-1300 and the male parental lines were larger than those based on general/specific heterozygosities, while the genetic distances between SI-1310, SI-1320 and the male parental lines were similar to those based on the heterozygosities. Although correlations between mid-parent heterosis and the genetic distance based on the QTLs were much higher/lower than those based on heterozygosities for most of the 12 traits, the determination coefficients (R^2) were still low. From Fig. 1, it is clear that the predictive value of yield per plant based on the genetic distance calculated from the 84 single-locus QTLs was very limited.

Discussion

Since molecular markers were used to predict hybrid performance and heterosis in crops, results have varied from one report to another. Stuber et al. (1992) used RFLP (restriction fragment length polymorphism) markers to quantify the genetic diversity of parents in maize and pointed out that genetic distance was highly correlated with the yield of F_1 hybrids and heterosis. He et al. (2002) investigated the relationships between yield and yield components and AFLP, RAPD (random amplification of polymorphic DNA) and SSR markers in rice. They assorted 931 marker loci surveyed in 15 parental lines into 4 types: positive loci, effect-increasing loci, effect-decreasing loci and non-environmental loci, and founded that effect-increasing and effect-decreasing loci could greatly improve the correlation coefficient and might be used to predict the yield and yield components. Liu et al. (2002) and Qian et al. (2003) reported that a larger genetic distance (GD) based on RFLPs resulted in a higher biomass yield for the interspecific hybrids between *Brassica napus* and *B. rapa*. In contrast, other studies showed that the correlations between hybrid performance and heterosis-and GD based on RFLP markers were too low to be of any predictive value in maize (Benchimol et al., 2000). Xiao et al., (1996), Liu et al., (1999) and Benchimol et al., (2000) reported that molecular markers could be applied to assign inbred lines to different heterotic groups in rice, wheat and maize, and generally intergroup heterosis was higher than intragroup heterosis. However, it was difficult to predict hybrid performance and heterosis using a genetic distance based on molecular markers.

Results from the previous studies indicated that molecular markers (heterozygosities) were not suitable for prediction of hybrid performance and heterosis in crops (Zhang et al., 1996; Liu et al., 1999). Molecular markers were even less powerful in predicting heterosis than methods based on combining ability of parents and genetic diversity revealed by isozymes (Brandle & McVetty, 1990; Yang et al., 1995; Diers et al., 1996). Our conclusion was consistent with the previous results. This may be due to the low correlations of molecular marker loci with genes for yield and yield related traits.

In QTL mapping, when the QTLs detected (based on a multi-loci model) are subjected to verification by one-way ANOVA results (based on the single-locus model) (Butruille et al., 1999), the two models have given identical results (Zhao & Meng, 2003). In fact, we used both one-way ANOVA and composite interval mapping (Zeng, 1994) in this study. All the single-locus

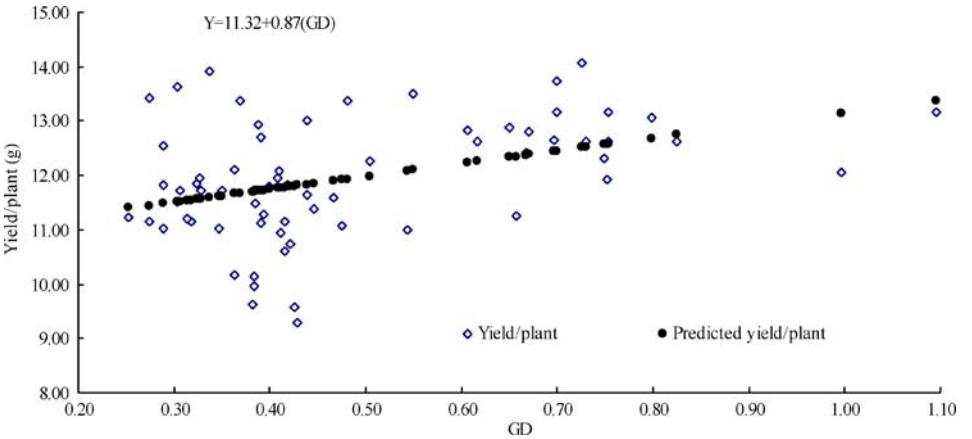

Fig. 1 Prediction of F_1 hybrid yield/plant based on genetic distance (GD) calculated from 84 QTLs for yield traits

QTLs were in the intervals detected by the latter. This means that our prediction using QTLs was reasonable. However, all QTLs for the 12 yield traits were based on the polymorphic loci in two parental lines: SI-1300 and Eagle. Thus, the genetic distances between SI-1300 and the male parents were enlarged in comparison with that based on heterozygosities, while GDs between SI-1310, SI-1320 and the male parents were consistent. This certainly influenced the predictive accuracy. In addition, QTLs detected in different materials might be different, and different materials also might contain different QTLs for the same trait (Pilet et al., 2001).

Performance of traits is controlled by genes. However, gene expression has spatio-temporal features, and is easily influenced by environmental conditions. Therefore, it is inaccurate to predict hybrid performance based on gene expression (isozymes, measurements of quantitative traits etc). Nevertheless, the DNA structure, especially that of functional genes, is very conservative along with plant species evolution (Takayama et al., 2000). That might mean that the stability of gene structure is much higher than that of gene expression. Thus, prediction from genetic diversity based on fragments of functional genes, especially the fragments related to genes controlling heterosis for yield traits, might be more powerful than that based on gene expression and that from genetic diversity based on the whole genome. The accomplishments of genome sequencing in model plants and fine mapping, together with isolation of QTLs for yield traits in some crops brings a bright prospect for studies on prediction of hybrid performance and heterosis.

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