

Relationship between genetic distance of parent molecular maker and trait performance of hybrid in *Brassica napus* L.

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Abstract

Four traits, plant seed yield, 1000-seed weight, protein and oil content of seed, were investigated in hybrids produced by 9 yellow-seeded lines \times 10 black-seeded lines and their parents in rapeseed (*Brassica napus* L.). The relations between the genetic distances of SSR, AFLP and SRAP molecular makers from parents, the hybrid's maker-type value which was estimated using two parents' maker effect values and the hybrid performances in 4 traits were analyzed and the mathematic models for prediction of hybrid performance by parent maker effect value were set up by means of stepwise regression analysis. Total 910 polymorphic loci were obtained from parents by use of 283 SSR, 64 AFLP and 81 SRAP pairs of primers respectively and these polymorphic loci were used to calculate general genetic distance (GGD) of molecular maker in parent. The results of cluster analysis by GGDs were almost consistent with trait performances in parents. 81 and 178 loci linkaged to the 4 traits were screened from 910 loci based on two-group method and three-group method respectively; some loci had only additive or dominant effect, while others had both of them. In each trait, dominant loci were more than additive loci, indicating that dominant effect was more important in hybrid heterosis. The correlations of quadratic polynomial between GGDs in parents and trait performances in hybrid were slightly stronger than those of line and exponent in the 4 traits, but none of them reached significant level ($P > 0.05$). Most of special genetic distances (SGDs) computed with the loci screened by two-group method and by three-group method had significant ($P < 0.05$) or high significant ($P < 0.01$) correlations with hybrid performances, however both of correlation coefficients and determination coefficients were small. The maker-type values of hybrid showed significantly positive correlations to hybrid performance with correlation coefficients from 0.5473 to 0.6433 in 4 traits. The correlation coefficients between marker effect value of parent and average performance of all hybrids concerning this parent varied from 0.7686 to 0.9249 in 4 traits, reaching high significant level ($P < 0.01$). The prediction models for hybrid performance of plant seed yield and 1000-seed weight had no practicability because of low precision and low stability. Those of protein and oil content had some values in practice.

Key words: *Brassica napus* L., genetic distance, molecular maker, hybrid performance, correlation analysis

Introduction

Molecular marker techniques, such as RFLP, RAPD, SSR and AFLP etc, had widely been used to analyze the genetic diversity and its relationship to heterosis of hybrids in rice (Zhang et al., 1995, 1996; Xiao et al., 1996; Zhang et al., 1999, 2001), maize (Melchinger et al., 1990; Stuber et al., 1992; Boppenmaier et al., 1993; Wang et al., 2001; Fei and Gao, 2002), wheat (Liu et al., 1999), cotton (Wu et al., 2002) and soybean (Cerna et al., 1997). The researches of relationship between genetic distance of molecular marker and trait performance and heterosis of hybrid in rapeseed (*Brassica napus* L.) were lag in comparison with in rice and maize. Only Diers et al. (1996), Riaz et al. (2001), Hu et al. (2003) and Shen et al. (2002, 2004) had some reports. The objective of present study was to analyze the relationship between genetic distance of molecular maker in parent and the trait performance of hybrid using SSR, AFLP and SRAP markers and to establish the prediction model of hybrid performance.

Materials and methods

Plant materials and Field trial: 9 yellow-seeded lines of rapeseed (*Brassica napus* L.) as female parents were crossed with 10 black-seeded lines as male parents according to NC II mating design. 90 hybrids and 19 parent lines were planted at experimental field of Southwest University farm in the year 2004/2005 using randomized complete block design with two repetitions. The plot was 2.33m \times 2.00m and planted 5 rows with 10 plants each row. When seed was fully maturity, 10 plants were selected randomly from 3 rows at middle of each plot to investigate seed yield per plant and all the seeds of 10 plants were mixed to test 1000-seed weight, protein content and oil content of seed. The contents of protein and oil in seed were determined by using Soxhlet extraction method and Kjeldahl method respectively.

Molecular marker analysis: Tender leaves of 10 plants of each parent were mixed to extract total DNA using modified CTAB method. Primer sequences of SSR were searched from the *Brassica* database on <http://ukcrop.net> and amplification system was employed from Saal et al. (2001). Adapter and primer of AFLP were designed by referring to Vos et al. (1995) and amplification system and procedure were referred to Zhao and Meng (2003). Primer sequences, amplification system and procedure of SRAP were designed according to Li and Quiros (2001) and 3' end of primer contained three different selective nucleotides.

Marker screening and genetic distance calculation: Marker of every parent at each marker locus was recorded as 1 or 0 according to the polymorphic band existed or not. Screening of the marker loci related to trait was performed by analysis of

variance using two-group method and three-group method. In the two-group method, all the hybrids were classified into homogeneity group and heterogeneity group, the markers of two parents were 0 or 1 in the former and the marker of one parent was 1 and the other was 0 in the latter (He et al., 2002). In the three-group method, the hybrids of the homogeneity group were again divided into homogeneity group 0 and homogeneity group 1 according to the markers of two parents were 1 or 0. The method of Nei and Li (1979) was used to calculate general genetic distance (GGD) of molecular maker using all the polymorphic loci obtained in parents and special genetic distance (SGD) using the polymorphic loci screened trait-related. Cluster analysis of parents was carried out using UPGMA method.

Estimating of marker effect value of parent and marker-type value of hybrid: The additive effect (a) and dominant effect (d) of marker locus were computed using following formula:

$$a = \frac{1}{2}(\bar{x}_{11} - \bar{x}_{00}) / se \quad d = (\bar{x}_{10} - \frac{1}{2}(\bar{x}_{11} + \bar{x}_{00})) / se$$

where \bar{x}_{11} , \bar{x}_{00} and \bar{x}_{10} are the trait averages of hybrids in homogeneity group 1, homogeneity group 0 and heterogeneity group respectively. se is the standard error of the trait. At the marker locus k , the marker-type of hybrid was composed of the markers of two parents of this hybrid, the marker effect value of parent i (p_{ik}) and marker-type value of hybrid crossed with parent i and parent j (F_{ijk}) could be calculated using

$$p_{ik} = a_k(2M_{ik} - 1) \quad F_{ijk} = a_k(M_{ik} + M_{jk} - 1) + d_k |M_{ik} - M_{jk}|$$

where M_{ik} and M_{jk} are the markers of parent i and parent j at marker locus k respectively, with value 1 or 0. When the interactions of different marker locus were be ignored, the total of marker effect value of parent i (p_i) and the total of marker-type value of hybrid crossed with parent i and parent j (F_{ij}) could be $p_i = \sum_k p_{ik}$ and $F_{ij} = \sum_k F_{ijk}$.

Establishing of prediction model of hybrid performance: Prediction model of hybrid performance were established using stepwise regression analysis approach. The trait performance of hybrid was treated as an independent variable and the marker-type values of hybrid at different marker locus as dependent variables. The precision and stability of model was evaluated by sum of square of model residua and Jackknife sample technique.

Results

Genetic relationships of parents: 260, 447 and 203 polymorphic loci were detected through the amplification of genomic DNA templates of 19 parents using 283 pairs of SSR primers, 64 pairs of AFLP primers and 81 pairs of primers respectively. It is obvious that AFLP has the largest capacity to detect the polymorphic loci; SRAP takes second place and then is SSR. The result of cluster analysis by GGDs of all the 910 polymorphic loci was almost consistent with trait performances in parents. At the level of coefficient of similarity 0.43, all the yellow-seeded lines except m113 were gathered together and all the black-seeded lines except m105 divided into 2 groups. In general, genetic differences between yellow-seeded lines and black-seeded lines were greater than those within each type. Dendrograms of cluster analysis based alone on SSR, AFLP and SRAP were similar to that based on them together.

Screening of trait-related loci and effect estimation: 81 loci related to the 4 traits were found at the significant level 0.01 based on the two-group method. 7, 12, 31 and 31 loci were associated with seed yield per plant, 1000-seed weight, protein content and oil content of seed respectively. Except protein content of seed, the effect-increasing loci of the other 3 traits were more than the effect-decreasing loci. Based on the three-group method, 178 loci related to the 4 traits were screened from 910 loci at the significant level 0.01. For each trait, the number of loci screened by two-group method was more than that by three-group method. Some loci had only additive or dominant effect, while others had both of them. These marker loci had different effects on size and direction to trait. In each trait, dominant loci were more than additive loci, indicating that dominant effect was more important than additive effect in hybrid heterosis.

Relationships between genetic distances and hybrid performance: All the linear correlation coefficients between parents GGDs and hybrids performances of the 4 traits using one of SSR, AFLP and SRAP markers were too small to up to the significant level, with no obvious differences comparing to those using all of SSR, AFLP and SRAP markers (Table 1). The correlations of quadratic polynomial between GGDs of parents and trait performance of hybrids were slightly stronger than those of line and exponent in the 4 traits using all 910 marker loci, but none of them reached significant level 0.05.

Table 1. Correlation coefficients between GGD of parent and performance of hybrid

Marker		Seed yield	1000-seed weight	Protein content	Oil content
SSR	Line	-0.1387	-0.0577	0.0034	0.0706
AFLP	Line	0.0170	0.1333	-0.0282	0.0603
SRAP	Line	-0.0241	0.0936	-0.1231	0.1404
	Line	-0.1153	0.0624	-0.0374	0.0837
All marker	Exponent	-0.1082	0.0686	-0.0458	0.0735
	Quadratic polynomial	-0.1323	0.2152*	-0.1432	0.1109

Note: ** $r_{0.01} = 0.267$, * $r_{0.05} = 0.205$

The linear correlation coefficients between the parents SGDs calculated with the loci screened by two-group method and the hybrids performances were greater than those between GGDs and the hybrids performances, all of them reached significant level 0.05 or 0.01 for the 4 traits (Table 2), which indicated that linear correlation coefficients could be efficiently increased by screening positive marker loci. The effect-increasing loci and the effect-decreasing loci had more close linear correlation with the hybrids performances than positive loci for all traits except protein content of seed. The linear correlations between the parents SGDs calculated with the loci screened by three-group method and the hybrids performances were similar to those by two-group method, but only one of them was significant. However, both of correlation coefficients and determination coefficients were small.

Table 2. Correlation coefficients between SGD of parent and performance of hybrid

Method	Locus	Seed yield	1000-seed weight	Protein content	Oil content
Two-group	Positive	0.3599**	0.3206**	-0.5474**	0.2466*
	Effect-increasing	0.5095**	0.4372**	0.3643**	0.5291**
	Effect-decreasing	-0.4215**	-0.3351**	-0.6217**	-0.5490**
Three-group		-0.2143*	0.1963	-0.1033	-0.0017

Note: ** $r_{0.01} = 0.267$, * $r_{0.05} = 0.205$

Relationships between marker values and hybrid performance: The marker-type values of hybrid had significantly positive correlations to hybrid performance, with correlation coefficient were 0.5473, 0.6295, 0.6433 and 0.6332 for seed yield per plant, 1000-seed weight, seed protein content and seed oil content respectively. No significant difference was detected between the correlation coefficients estimated by using only effects of dominant loci and by the combination of both effects of dominant and additive loci. The correlation coefficients between marker effect value of parent and average performance of all hybrids concerning this parent reached significant level 0.01 and varied from 0.7686 to 0.9249 in 4 traits.

Prediction model of hybrid performance: The mathematic models of 4 traits for prediction of hybrid performance by parent maker effect value were established by means of stepwise regression analysis. There were 9, 9, 17 and 13 marker loci including in the prediction model of seed yield per plant, 1000-seed weight, protein content and oil content of seed respectively. The multiple correlation coefficients of the 4 prediction models were up to 0.647, 0.700, 0.803 and 0.811. The prediction models of seed yield per plant and 1000-seed weight had no practicability because of low precision and low stability. Those of protein content and oil content of seed had both high precision and stability and so they had reliable prediction result.

Discussion

In present study, 81 positive loci screened by two-group method was only a small part of 910 marker loci. The correlation coefficients between SGDs based on these positive loci and the trait performance were much higher than those based on all loci, which accorded with Bernardo (1992). When positive loci were distinguished as effect-increasing loci and effect-decreasing loci, the correlations could be improved; all the correlation coefficients of effect-increasing loci were positive and all the correlation coefficients of effect-decreasing loci were negative (see Table 2), which indicated that this distinguishing of positive loci was effective in estimating the correlation between SGD of parent and trait performance of hybrid.

The results of studies on the correlation between genetic distance of molecular marker and hybrid performance and/or heterosis were different with different researchers. Melchinger et al. (1990) and Stuber et al. (1992) reported there were strongly correlations between hybrid performance and genetic distance of parents. On the contrary, Godshalk et al. (1990) and Dudley et al. (1991) deemed a little correlation existed between hybrid yield and genetic distance based on RFLP. Melchinger et al. (1990) and Boppenmaier et al. (1993) pointed out that this correlation depended on experimental material.

The method of estimating marker effect value of parent and marker-type value of hybrid was a newly try. The prediction model of trait performance of hybrid, established using marker-type values of hybrid that was calculated by marker effect value of parent, awaited more proof in breeding practice.

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