Relationship between heterosis and genetic distance based on AFLPs in *Brassica napus*

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Abstract: Three double low self-incompatible lines and 22 male parent varieties of *Brassica napus* from different origins, together with their 66 hybrids produced in a North Carolina II (NCII) mating design, were used to estimate genetic distance (GD) and analyze the relationship between hybrid performance and GD in *Brassica napus*. GD was estimated using amplified fragment length polymorphism (AFLP). The results indicated that 3 self-incompatible lines and 22 male parents might be divided into two and five groups, respectively. There were deviations among different group hybrids from 2 groups of SI lines and different male parental groups for most yield components. Higher mid-parent heterosis for seed yield per plant was observed among hybrids produced by foreign male parents. Hybrid yield per plant showed significant positive correlations with genetic distance, but the determination coefficients were low. Results indicated that it might not be suitable to use AFLP-based genetic distance to predict hybrid performance in *Brassica napus*.

Key Words AFLP; Genetic distance; Heterosis; *Brassica napu*

Exploiting heterosis is the major way to improve crop yield, so scientists make every effort to study heterosis of crops and attempt to predict it by a mathematic model. Previously, the relationships between GDs of parents and their hybrids performance have been studied on the basis of combining abilities or the geographical origin or iso-enzyme technique (Lefort-Buson et al, 1987; Zhang, 1987; Ali et al, 1995; Brandle and McVetty, 1990). But it would spend much more time to estimate the parental combining abilities, and addition to, the geographical distance and the limited number of iso-enzyme loci could not reflect accurately the genetic distance of parents. In present, molecular marker techniques have been improved and utilized to reveal the differences among parents in molecular level, such as the retriction fragment length polymorphism (RFLP), simple sequence repeat (SSR) and random amplified polymorhic DNA (RAPD). These techniques have been used to reveal the relationship between GDs and heterosis in rice, maize, wheat and etc (Zhang et al, 1994; Bernardo, 1992; Benchimol et al, 2000; Liu et al, 1999; Diers et al, 1995). AFLP was developed a few years ago (Vos et al, 1995), and is only used in maize to predict heterosis (Wu and Dai, 2000). Rapeseed is one of the main edible oil crops worldwide, and its heterosis has been utilized in the production. The purpose of this study was to detect the difference of parents with the use of AFLPs, and used it to find out the possibility to predict hybrid performance in *Brassica napus.*

1 Materials and Methods

1.1 Materials

The materials used in this study included 3 double low self-incompatible (SI) lines and 22 inbred pure line varieties (Table 1). 3 SI lines, Zhongyou 821 and Huashuang No. 3 were semi-winter type, and the others were spring type. With a North Carolina II (NCII) crossing design, 3 SI lines were crossed with these inbred varieties by hand pollination to produce 66 hybrids. Total 91 entries, including 66 hybrids and their parental lines/varieties (Zhongyou 821 was as the control), were tested for their yield.

1.2 Field experiment

Field experiments were conducted at the Experimental Farm of Huazhong Agricultural University during two growing seasons from 1999 to 2001. Both the preceding crops were rice in the two years. Field planting followed a random block design with three replications, and sowing time was in the early of October each year. Plot area was 3.78 m^2 (4 rows with 3.50m length and 0.27m apart). After emerging 40 days, 22 plants were left per row with 15 cm distance between plants. The field management followed usual field practice. 22 plants were harvested randomly in the middle 2 rows from each plot at maturity and examined for yield traits.

Table 1 Lines (varieties) tested and their origins[1)]

Self-incompatible line			Male parent variety					
Line	Origin	SQ	Variety	Origin	SQ	Variety	Origin	SQ
			Zhongyou 821	China	DH	Eagle	Europe	DL
SI-1300	China	DL	Huashuang No.3	China	DL	SW9475913	Europe	DL
			Dunkecl	Australia	DL	SW9372561	Europe	DL
			Rainbow	Australlia	DL	SW9473754	Europe	DL
SI-1310	China	DL	Oscar	Australia	DL	SW9375645	Europe	DL
			Quantum-2	Canada	DL	SW9475228	Europe	DL
			Sprint	Canada	DL	SW9474922	Europe	DL
			Defendor	Canada	DL	SW9376258	Europe	DL
SI-1320	China	DL	Sponsor	Europe	DL	SW9474243	Europe	DL
			Impulse	Europe	DL	SW9474933	Europe	DL
			Wildcat	Europe	DL	Senator	Europe	DL

[1)] SQ: Seed quality (erucic acid and glucosinolates,); DH: double high; DL: double low

1.3 DNA extraction and AFLP analysis

Total DNA extraction and AFLP analysis were performed as described in Li Jia et al, (1994) and Lu (2001), respectively. Two restriction enzymes, *Pst* I and *Mse* I, were used in AFLP analysis.

1.4 Data analysis

Twenty plants were used to calculate the average of yield and its components in a plot. All trait value was the average in the two years. Mid-parent heterosis (MH)%=(F_1-MP)/MP×100, where MP represented the average of two parents. The 0,1 data were used to estimate genetic similarity (GS) among parents followed Nei and Li (1979). The genetic distance (GD)=-ln(GS). Unweighted pair-group method (UPGM) was employed in cluster analysis based on GD.

2 Results and analysis

2.1 Polymorphism of marker loci

Sixteen pairs of primers amplified 799 bands, of which 277 were polymorphism. The average of bands amplified by one pair of primers was 49.9, with 17.3 polymorphic bands.

2.2 Genetic distance of parents and grouping

GDs between 3 self-incompatibility lines and 22 male parent varieties ranged from 0.1401 to 0.4205 with an overall mean of 0.2427. The range of GDs among 22 male parents was from 0.0636 to 0.5469. Based on GDs, 3 SI lines and 22 male parents were divided into 2 and 5 groups, respectively. In 3 SI lines, SI-1300 and SI-1310 were closely related, so they were clustered into one group. In 5 male parent groups, the first one, including all 2 varieties from China, had higher plant height, silique density on main inflorescence (SDMI) and yield per plant, but seed oil content was lowest; group two, consisting of one from Canada and another from Australia, had highest 1000-seed weight and least seeds per silique and lowest height of primary effective branch in all male parent groups; group three, involving two varieties from Australia, had most second branches and less SDMI and siliques/plant; group four, including all from Europe and one from Canada, had most siliques/plant, with the middle level of plant height, SDMI and 1000-seed weight; and group five, only one from Canada, had least siliques/plant. This result was similar to grouping based on geographical origins.

2.3 Hybrid performance for yield and seed oil content

Hybrid performance was analyzed based on parent grouping. The result indicated that significant difference existed in plant height, silique/plant and yield/plant (Table 2). Significant difference was obtained for plant height, SDMI, siliques on primary branches, siliques/plant and yield/plant in hybrids produced by SI-1300 and SI-1310 with male parents from foreign countries compared to those with Chinese varieties. Heterosis of seed oil content appeared the same tendency as the characters mentioned above except the hybrids of SI-1300 and SI-1310 with male parent group III and group V.

2.4 Relationship between hybrid performance and genetic distance

Results of correlation analysis showed that F_1 yield/plant was highly significant related to GD (r=0.3288**), but the determination coefficient (R^2) was only 0.1084, while MHYP was negative (r=-0.1841) related to GD. Relationships between siliques/plant, seeds/silique, 1000-seed weight and GD were negative (r=-0.0303), positive (r=0.2845*), and significant positive (r=0.3192**), respectively. For seed oil content and MHOP, GDs were both negative (r=-0.3901**, R^2=0.1522 and r=-0.1843, respectively) related to them.

Table 2 Performance of hybrids produced by SI lines for yielding traits and seed oil content[1)]

SI line	Trait	Male parent variety				
		Group I	Group II	Group III	Group IV	Group V
Group I	Plant height	152.20±4.12	135.92±8.17	132.53±6.98	143.80±6.46	140.23±0.06
	SDMI	1.40±0.04	1.28±0.05	1.20±0.17	1.36±0.07	1.32±0.13
	Seeds/silique	20.05±0.30	18.54±1.65	20.70±2.29	19.68±1.97	20.82±1.25
	Siliques/plant	251.56±12.98	255.01±21.65	233.50±37.07	260.72±23.95	230.00±20.02
	1000-seed weight	3.33±0.07	3.40±0.15	3.11±0.27	2.96±0.17	3.08±0.13
	Yield/plant	13.31±0.21	12.26±0.43	12.11±1.21	12.39±0.89	11.83±1.15
	MHYP	24.47±4.01	29.65±1.65	32.01±12.77	34.55±10.14	28.18±4.81
	Seed oil content	41.44±1.17	43.29±1.23	41.65±0.10	42.64±0.93	42.51±0.53
	MHOP	2.63±2.13	4.41±1.78	2.10±1.26	2.90±1.99	2.31±0.87
Group II	Plant height	149.26±3.61	134.61±6.48	129.34±0.49	138.16±3.19	140.22
	SDMI	1.43±0.07	1.21±0.00	1.04±0.19	1.28±0.05	1.23
	Seeds/silique	17.61±0.30	15.53±2.34	18.26±3.39	16.76±1.65	18.87
	Siliques/plant	264.75±12.14	265.14±31.93	239.73±27.70	246.35±19.75	193.35
	1000-seed weight	3.38±0.14	3.50±0.13	2.99±0.16	3.14±0.16	3.08
	Yield/plant	12.18±0.14	11.09±0.09	11.75±0.51	10.87±0.82	9.30
	MHYP	18.12±1.10	22.36±0.61	33.74±0.84	23.35±9.86	5.50

Seed oil content	39.69±1.71	41.64±0.25	40.15±0.42	41.48±0.85	41.25
MHOP	2.08±3.04	4.26±2.44	2.19±2.43	3.87±1.47	3.00

1) MHYP: mid-parent heterosis for seed yield/plant (%); MHOP: mid-parent heterosis for seed oil content

3 Conclusion

Together with the results above, AFLP was useful molecular technique for genetic diversity detection, but it might not be suitable in prediction of hybrid performance for yield traits and seed oil content.

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