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ORIGINAL PAPER

Gene expression profiles associated with intersubgenomic heterosis in *Brassica napus*

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Abstract In order to understand the genetic mechanism of heterosis that has been observed in hybrids between *Brassica napus* and partial new-type *B. napus* which had exotic genome components from relative species, this study focused on the difference in gene expression patterns among partial new-typed *B. napus* lines, *B. napus* cultivars and their hybrids using the cDNA amplified fragment length polymorphism technique (cDNA-AFLP) technique. First, three partial new-type *B. napus* lines were compared with their original parents. One new line contained the exotic genomic components from *B. rapa*, and the other two new lines were obtained by the introgression of genomic components from *B. rapa* and *B. carinata*. The experimental results showed that the introgression of A^r and C^c genome components from *B. rapa* and *B. carinata* led to considerable differences in the gene expression profiles of the partial new-type lines when compared with their parents. Secondly, the gene expression profiles of nine cross-combinations between three partial new-type lines and three *B. napus* cultivars were compared. Twenty transcript-derived fragments (TDFs) associated with intersubgenomic heterosis were randomly selected and converted into PCR-based molecular markers. Some of them were mapped in the confidence intervals of quantitative trait loci (QTLs) for yield and yield-related traits in three segregative populations of *B. napus*. These results suggested that a proportion of the heterosis-associated TDFs were really responsible for fluctuating seed yield in rapeseed.

Xin Chen and Maoteng Li contributed equally to this paper.

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Introduction

Brassica napus (AACC, $2n = 38$) is an amphidiploid species that originated from spontaneous hybridization between *B. rapa* (AA) and *B. oleracea* (CC) (U 1935; Olsson 1960; Prakash and Hinata 1980). The role of cultivated *B. napus* as a commercial oil crop in Asia, Europe, North America and Australia has progressively increased due to better production potential and improvement in seed quality (Baranyk and Fábry 1999; Fu et al. 2003; McVetty et al. 2007). However, the short cultivation history and intensive breeding of this species has led to a comparatively narrow genetic base (Becker et al. 1995; Cowling 2007). Considerable efforts have been made to enrich the genetic diversity of *B. napus* by the introgression of genomic components of its related species or by developing synthetic *B. napus* lines by hybridization between *B. rapa* and *B. oleracea* (Heath and

Earle 1995; Schranz and Osborn 2000; Qian et al. 2005; Li et al. 2006). Several interesting changes have been observed in synthetic *B. napus*, such as chromosomal rearrangements, deletions of DNA sequences, variations at DNA methylation loci and the alteration of gene expression patterns (Song et al. 1993, 1995; Osborn et al. 2003; Lukens et al. 2006).

Heterosis has been widely used in agriculture to increase the seed yield, and it has been applied to a large number of crops (Meyer et al. 2004). Research has revealed that analysis of the differences in gene expression profiles between the hybrids and their parents is an important method for elucidating the molecular basis of heterosis in crops such as wheat (Sun et al. 2004), rice (Xiong et al. 1998; Huang et al. 2006) and maize (Guo et al. 2006; Swanson-Wagner et al. 2006). These results are consistent with the hypothesis that multiple molecular mechanisms contribute to heterosis. In *Brassica*, heterosis was first reported in biomass for Sun (1943) in China. Subsequently many studies have estimated the extent of heterosis for seed yield (Liu 2000). Divergent evolution and isolation have led to differentiation within the same genome among different species. To distinguish different subgenomes in *Brassica*, the genomes of the three diploid species *B. rapa*, *B. nigra* and *B. oleracea* were designated as A^r, B^n and C^o while the genomes of the three amphidiploid species *B. napus*, *B. juncea* and *B. carinata* were designated as A^nC^n, A^jC^j and B^cC^c, respectively (Li et al. 2004). A partial new-type *B. napus*, $A^{r/n}A^{r/n}C^nC^n$ or $A^{r/n}A^{r/n}C^{c/n}C^{c/n}$, was created by interspecific hybridization and artificial selection. A hybrid ($A^{r/n}A^nC^nC^n$ or $A^{r/n}A^nC^{c/n}C^n$) was developed by crossing the partial new-type *B. napus* to *B. napus* and strong heterosis was observed in the hybrids (Qian et al. 2005; Li et al. 2006). This type of heterosis was considered as intersubgenomic heterosis and its existence showed that some alleles that were derived from related species could favorably contribute to increasing the seed yield of rapeseed (Liu et al. 2002; Qian et al. 2005). But, it remains to be known whether these introgressed alleles expressed or not, and how they contributed to increasing the seed yield of the intersubgenomic hybrid.

In this study, we compared the gene expression profiles of partial new-type *B. napus* lines with those of hybrids derived from crosses between partial new-type *B. napus* lines and *B. napus* cultivars Two objectives were addressed: (1) Unique transcript-derived fragments (TDFs) and display patterns were revealed from the comparison of gene expression profiles between a partial new-type *B. napus* line and its parents, and between an intersubgenomic hybrid and its parents using the cDNA-AFLP technique; (2) Some TDFs were mapped to positions that were coincident with the confidence intervals of QTLs for yield-related traits in other segregative populations.

Materials and methods

Plant materials and field trials

Huashuang 3 (HS_3) and Xiangyou 15 (XY_{15}) were elite *B. napus* cultivars in China and the seeds were provided by their breeders (Professor Jiangsheng Wu, Huazhong Agricultural University, Wuhan, China, and Chunyun Guan, Hunan Agricultural University, Changsha, China). Tianmen Youcaibai (TY) and Xinhua Youcai (XY) were Chinese *B. rapa*, and were brother–sister mating for over four generations. 10167 and Gz-1 were Ethiopian *B. carinata*, also were self-pollinated for four generations before being used as parents. Three lines of partial new-type *B. napus*, which comprised one line derived from two species (2SL, derived from *B. napus* and *B. rapa*) and two lines derived from three species (3SL-1 and 3SL-2, derived from *B. napus*, *B. rapa, B. carinata*) were developed from interspecific crosses (Qian et al. 2005; Li et al. 2006). After the characteristics of *B. napus* (38 chromosomes with normal meiotic behavior and good fertility) had been confirmed in each line, the F_2 lines were self-pollinated for five successive generations (Table 1).

Nine intersubgenomic hybrids were developed between the three partial new-type *B. napus* lines as female parents and three *B. napus* cultivars as male parents. The hybrids and their parents were planted in 3-row plots (the first year) and 6-row plots (the second year), with a randomized block design and three replications in Wuhan, China. Each row was 1.9 m long with a gap of 25.6 cm between adjacent rows. Ten representative plants in the first year and 60 plants in the second year were harvested from each plot to measure the seed yield and its components (branch number per plant, pod number per plant, seed number per pod, kilo-seed weight and flowering time). The homogeneity test for the field trial was performed after 2 years according to the method of Bartlett (1937).

TN population (Qiu et al. 2006; Long et al. 2007) was planted in 3-row plots with a randomized block design with three replications in seven environments in China. HT RIL population which was derived from a cross between a Chinese semi-winter *B. napus* cultivar var. Huashuang 3 and a Chinese semi-winter *B. rapa* cultivar var. Tianmen Youcaibai, and HT RIL-BC_1 population that derived from backcrossing the lines of HT RIL population to parental Huashuang 3, were also planted with the same field design in two environments. For these three populations, ten representative plants and all 30 plants from

Table 1 Plant materials used in the study

Name of line	Pedigree
Partial new-type *B. napus*	
2SL	(*B. napus* var. Huashuang 3/*B. rapa* var. Tianmen Youcaibai), F_7
3SL-1	(*B. carinata* var. 10167/*B. rapa* var. Tianmen Youcaibai)/*B. napus* var. Huashuang 3, F_7
3SL-2	(*B. carinata* var. Gz-1/*B. rapa* var. Xinhua Youcaibai)/*B. napus* var. Xiangyou 15, F_7
B. napus	
Huashuang 3 (HS_3)	{[Huayou 821/(Huayou 3/Marnoo)]/Zhongyou 821}/Zhongyou 821,Chinese cultivar
Xiangyou 15 (XY_{15})	Xiangyou11 × Xiangyou 10, Chinese cultivar
Grouse	BLN584/BLN602[Haya//Zephyr/Bronowski/3/Chisaya//Zephyr/Bronowski/7/Mutu/3/Chikuzen//Zephyr/Bronowski/5/Sv62.371/Zephyr//Norin20/3/Erglu/4/BJ168/Cresus-o-Precose/6/Haya//Zephyr/Bronowski/3/Chisaya//Zephyr/Bronowski], Australian Cultivar
B. rapa	
Tianmen Youcaibai (TY)	Chinese landrace
Xinhua Youcaibai (XY)	Chinese landrace
B. carinata	
10167	Ethiopian landrace
Gz-1	Ethiopian landrace

each plot were used to measure seed yield and its related traits.

Analysis of the index of subgenomic components in partial new-type *B. napus*

Genomic DNA was extracted from the young leaves of partial new-type *B. napus* and their parents as described by Kidwell and Osborn (2001). The genomic DNA of three individual plants from three replicates was pooled to give one biological sample, which was digested with two restriction enzymes, *Eco*RI and *Mse*I. Adapter ligation and the two successive PCR reactions for AFLP analysis were performed according to the method described by Vos et al. (1995). The PCR products were analyzed on a 6% denaturing polyacrylamide gel, and were silver stained following the manufacturer's instructions for sequencing kit Q4310 (Promega Corporation, USA).

The presence or absence of AFLP bands was scored as 1 or 0, respectively. Only bands that were detected or undetected in two independent technical replicates were recorded; otherwise, the AFLP band was disregarded. The ratio of introgressed subgenomic components of *B. rapa* and *B. carinata* in partial new-type *B. napus* was described by the index of subgenomic components (ISG) for A^r, C^c or $A^r + C^c$, which was calculated according to the following formula (Qian et al. 2005; Li et al. 2006):

$$\mathrm{ISG}(A^r) = n_A^r/N \times 100$$
$$\mathrm{ISG}(C^c) = n_C^c/N \times 100$$
$$\mathrm{ISG}(A^r + C^c) = (n_A^r + n_C^c)/N \times 100$$

Where $n_A{}^r$ and n_C^c represent the number of subgenomic specific bands that were present in both the partial new-type *B. napus* and the parents of *B. rapa* (A^r) or *B. carinata* (C^c), respectively, but were not present in the parental *B. napus*. *N* represents the total number of bands that appeared in the partial new-type *B. napus*, excluding the bands that were presented in all three parental species of *B. napus*, *B. rapa* and *B. carinata*.

Gene expression profile analysis

Total RNA was extracted from the leaves of approximately 1-month-old seedlings with 6–7 leaves as described in the TRI*zol* reagent manual (Invitrogen, CA, USA). The total RNA from three individual plants was pooled to give one biological sample. Single- and double-stranded cDNA was synthesized using the SMART PCR cDNA synthesis kit (BD Biosciences, Oxford, UK). The cDNA-AFLP analysis, which used 32 primer pairs, followed the method of Bachem et al. (1996). The presence or absence of a TDF was scored as 1 or 0, respectively. Only TDFs that were consistently detected or undetected in two independent technical replications were recorded; otherwise, the TDF

was disregarded. The degree of differential gene expression between two different self-crossing lines was calculated as follows:

$$D_e = N_d/(N_d + N_c) \times 100$$

Where N_d and N_c represent the number of differentially displayed TDFs between any two different self-crossing lines and the number of TDFs in common between the lines, respectively.

Cloning and sequencing of TDFs and development of TDF-derived markers

Twenty TDFs were excised from the gel and eluted in 50 μl ddH_2O overnight. The eluted DNA was amplified using cDNA-AFLP pre-amplified primer pairs. The PCR products were cloned into the pGEM-T Easy vector (Promega) and then sequenced by Augct Company (Beijing, China) using an ABI 377 automated DNA sequencer (Perkin-Elmer corporation, MA, USA). The TDF sequences were all deposited in the GenBank dbEST database (TDF01–20: the dbEST_Ids are 46371782 to 46371801, and the GenBank_Accns are ES315663 to ES315682).

Two kinds of TDF-derived molecular markers were developed using either the original sequence or the homologous sequence of the TDF. Two SSR primer pairs were designed using the WEBTROLL software (Martins et al. 2006) and the original sequences of two TDFs. Target region amplified polymorphism (TRAP) primers were designed following the protocol of Hu and Vick (2003) with minor modification. The fixed primers were the forward or reverse primers of the primer pairs, which were designed with the software Primer Premier 5.0 software using the original sequence of the TDF or the homologous sequence (similarity ≥ 97%), and the primer pairs were verified by RT-PCR. The arbitrary primers had an AT- or GC-rich core that annealed with an intron or exon, and were obtained from Dr. Jinguo Hu (USDA-ARS, Northern Crop Science Laboratory). The primers used in this study are listed in Supplementary Materials Table S1.

Statistical analysis

The presence or absence of 1,210 TDFs were considered as various attributes, and which were used to calculate Pearson's simple correlations with hybrid performance, middle parent heterosis (MPH) and high-parent heterosis (HPH) of six tested traits with SAS8.0 (SAS Institute 1999). Differences between the TDFs means of seven different display types were evaluated with Duncan's multiple range test (Duncan 1955).

Seven hundred and forty five molecular markers (including 12 TDF-derived markers) were constructed using JoinMap 3.0 (Stam 1993) with DH population (Qiu et al. 2006; Long et al. 2007), the threshold of fit set to ≤5.0 with LOD scores >1.0 and a recombination frequency <0.4. The QTL analysis was performed composite interval mapping analysis by using WinQTLCart2.5 (Wang et al. 2005) and the significant LOD scores were determined by permutation analysis (using 1,000 assortments) for each trait. Single marker analysis was also conducted with a significance threshold of $P < 0.01$ in HT RIL and HT RIL-BC1 populations.

Results

Evaluation of introgression of exotic genomic components and comparison of gene expression profiles between partial new-type *B. napus* and parental *B. napus.*

The ratio of introgressed exotic genomic components in partial new-type *B. napus* was evaluated using AFLP molecular markers. Six hundred and seventy-three polymorphic bands were produced from three partial new-type lines and their parents. The ISG (A^r) in the 2SL was 37%, and the ISG ($A^r + C^c$) in 3SL-1 and 3SL-2 were 58 and 50%, respectively. This revealed that the genome of the partial new-type *B. napus* changed considerably after the introgression of genome components of *B. rapa* or/and *B. carinata*.

The difference in gene expression between the partial new-type *B. napus* lines and their parents were detected by cDNA-AFLP (Fig. 1a). In total, 1,167 TDFs ranging from 50 to 600 base pairs in size were detected using 32 primer pairs with, on average, 36.5 TDFs/primer pair. There were

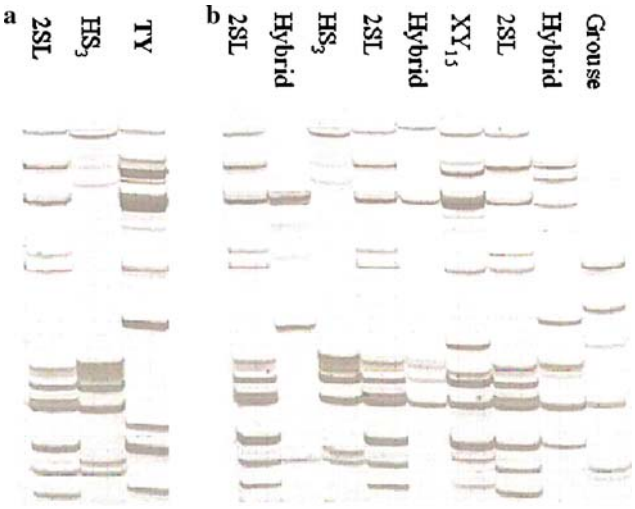

Fig. 1 A typical cDNA-AFLP display gel. **a** Shows the results from a partial new-type *B. napus* line (2SL) and its parents (HS_3 and TY); **b** shows the bands obtained with different intersubgenomic combinations

substantial changes in gene expression between the three partial new-type lines and their parental *B. napus* cultivars (HS_3 or XY_{15}). The degree of differential gene expression among the three partial new-type *B. napus* lines was larger than that of between two elite varieties of *B. napus* that do not share a direct common ancestor (Fig. 2, Supplementary Materials Table S2). These results indicate that the introgression of A^r and/or C^c genomic components could increase the degree of differential gene expression.

Genetic components of *B. rapa* or *B. carinata* introgressed into the partial new-type *B. napus* lines, and led to high heterozygosity in the hybrids of the partial new-type *B. napus* lines and *B. napus* cultivars. Altogether, 1,210 differential TDFs were produced from a comparison of the nine hybrids and their parents using the above-mentioned 32 primer pairs (Fig. 1b). Nevertheless, the number of detected TDFs changed considerably in the different primer pairs. The TDF display patterns could be divided into seven types according to whether the TDF appeared or disappeared between the hybrid and its parents (Fig. 3, Supplementary Materials Table S3). The largest category (40%) of TDF belonged to type $N_t^+H^-N^-$ and type $N_t^-H^-N^+$ (where N_t represents partial new-type *B. napus*, H is hybrid and N is *B. napus*), in which the TDF appeared in one of the parents but was absent from the hybrids, and exhibited the characteristic of dominance (negative). Interestingly, the combination 2SL × XY_{15}, which had the largest number of TDFs (257) of type $N_t^+H^-N^-$, also had the highest seed yield of all nine hybrids (Supplementary Materials Table S3). Types $N_t^-H^+N^-$ and $N_t^+H^-N^+$ were the second largest category (21.7%), might exhibit characteristic of overdominance or underdominace. Types $N_t^-H^+N^+$ and $N_t^+H^+N^-$ were the third largest category (20.5%) and they also exhibited the characteristic of dominance (positive). The remaining of 17.7% fell into type $N_t^+H^+N^+$ (Table 2).

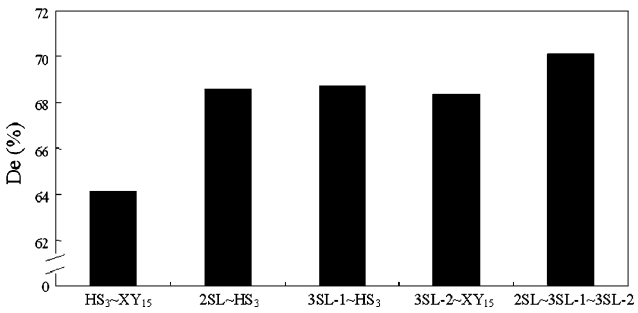

Fig. 2 The degree of differential gene expression detected with differentially displayed TDF between different lines. D_e value means that the degree of differential gene expression between two different lines. $HS_3 \sim XY_{15}$ represents the D_e value between two cultivars of *B. napus* (HS_3 and XY_{15}); 2SL ~ HS_3, 3SL-1 ~ HS_3 and 3SL-2 ~ XY_{15} represent the D_e values between lines of partial new-type *B. napus* and their parental lines; 2SL ~ 3SL-1 ~ 3SL-2 represents the average D_e value of any two of three partial new-typed *B. napus* lines (for example: 2SL and 3SL-1, 2SL and 3SL-2, 3SL-1 and 3SL-2)

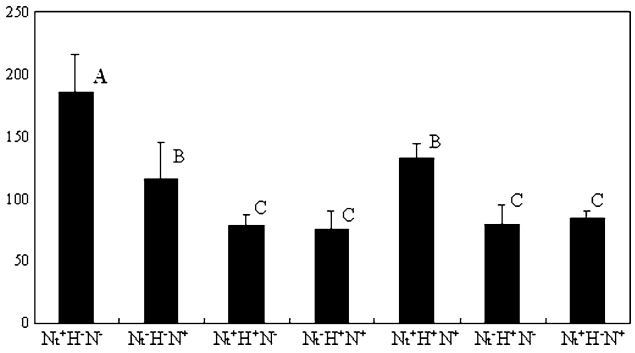

Fig. 3 The number of transcript-derived fragments in each of the seven display types from nine combinations (mean ± standard deviation). N_t, H and N represent partial new-type *B. napus*, the hybrid and *B. napus*, respectively. The superscript "+" or "−" indicates whether the TDF is present or absent. For example, $N_t^+H^+N^-$ means that the TDFs are present in both the partial new-type *B. napus* parent and the hybrid but absent in the *B. napus* parent. Different letters on the *bars* indicate a significant difference at $P < 0.05$ level by multiple comparison among seven display types

Correlation analysis between a single differential TDF and hybrid performance

Nine hybrids and their parents were planted in 2003 and 2004. Most of the hybrids exhibited strong heterosis for seed yield and its related traits over two successive years (Supplementary Materials Table S4). The mid-parent heterosis (MPH) value for seed yield exceeded 40% in four hybrids, and the high-parent heterosis (HPH) value for seed yield was over 50% in two hybrids. The highest MPH and HPH values of seed yield were achieved when three partial new-type lines were crossed to the Australian cultivar "Grouse". Eight out of nine tested hybrids showed significant higher seed yield than that of their parents (Fig. 4), and this finding suggests that the intersubgenomic hybrids exhibited strong heterosis. Furthermore, the seed yield of intersubgenomic hybrids exhibited higher than or equivalent to that of the respective normal hybrid in two years (Supplementary Table S5).

The TDFs that were differentially expressed between the hybrids and their parents were analyzed in order to examine whether they correlated with hybrid performance and MPH or HPH for six tested traits. Of the 1,210 TDFs, about 15.04% showed significant correlation with at least one of the analyzed traits and 0.66% of the 1210 TDFs showed a significant correlation with two or three of the analyzed traits. TDFs that showed correlations with at least one trait were designated heterosis-associated TDFs (H-TDFs).

Table 2 Summary of transcript-derived fragment differential display types between parents and hybrid in different crops

Display type	Item	Rice (Xiong et al. 1998)	Wheat S1 (Sun et al. 2004)	Wheat S2 (Sun et al. 2004)	Maize (Tian and Dai 2004)	Rapeseed (current work) (Total TDFs)
$P_1^+H^+P_2^-$	Dominance (positive)	8.5[a]	12.7	10.7	21.3	10.4[b]
$P_1^-H^+P_2^+$		7.7				10.1
$P_1^-H^+P_2^-$	Overdominance	4.1	3.5	4.1	14.9	10.5
$P_1^+H^-P_2^-$	Dominance (negative)	3.4	8.4	9.0	37.0	24.7
$P_1^-H^-P_2^+$		3.7				15.4
$P_1^+H^-P_2^+$	Underdominance	3.6	6.0	2.9	7.6	11.2
$P_1^+H^+P_2^+$	Monotype	69.0	70.0	73.3	19.3	17.7

[a] P_1, H and P_2 indicate parent 1 (or partial new-type *B. napus* line), hybrid and parent 2 (or *B. napus* cultivar)

[b] Indicates the percentage of each display type in cross-combinations of rice, wheat, maize and rapeseed

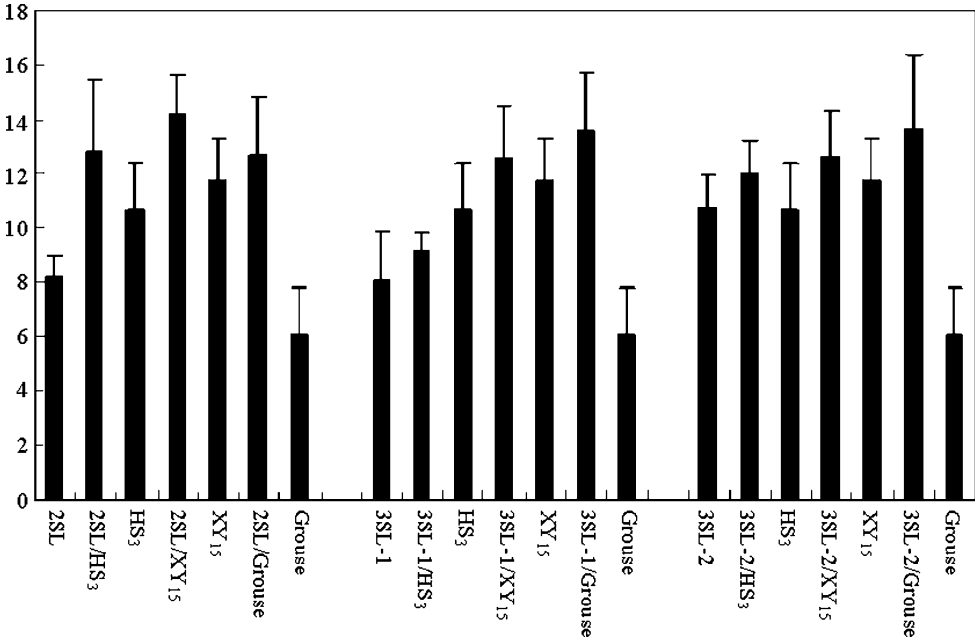

Fig. 4 Seed yield of parents and intersubgenomic hybrids (mean ± standard deviation). 2SL, 3SL-1 and 3SL-2 are partial new-type *B. napus* lines; HS_3, XY_{15} and Grouse are tester cultivars, respectively

Cloning, sequencing and mapping of heterosis-associated TDFs

Twenty H-TDFs, which correlated significantly with at least one analyzed trait, were cloned and sequenced. The results of BLAST analysis showed that: eight of them were homologous to known functional genes or proteins, two of them belonged to the *Copia*-like retrotransposon family and the others had no known homologues. Further analysis demonstrated that the *Copia*-like TDFs frequently appeared as type $N_t^+H^-N^-$ (Supplementary Materials Table S6). In other words, they were mainly activated in the partial new-type *B. napus* lines and suppressed in the hybrids. In addition, two cross-combinations, 2SL-1 × HS_3 and 3SL-2 × XY_{15}, were chosen to validate the differential expression of these 20 H-TDFs. Fifteen of the twenty H-TDFs could be reproduced by RT-PCR, and five of these fifteen H-TDFs were further confirmed by semi-quantitative RT-PCR with the same samples as cDNA-AFLP assay and two technical replicates in at least one cross-combination (Supplementary Materials Fig S1).

Twelve TDF-markers derived from seven of the sequenced H-TDFs showed polymorphism in the TN DH mapping population (Qiu et al. 2006) and a number of them were polymorphic in the partial new-type HT RIL *B. napus* population (an unpublished population). Single marker analysis revealed that markers derived from TDF14, which showed homology to Phytochrome C, correlated with three seed yield-related traits (branch number, seed number per pod and kilo-seed weight) in five environments, and the markers derived from TDF20 correlated with flowering time in three environments in the TN DH population

(unpublished data). In contrast, TDF20-derived markers were significantly correlated with kilo-seed weight in the HT RIL population and the HT RIL-BC_1 population in the DY05 environment (Supplementary Materials Table S6).

The 12 TDF-markers were mapped to 12 different linkage groups within the TN population (Supplementary Materials Table S5). Four of them (derived from two TDFs) were located within the confidence intervals of eight QTLs for yield-related traits, which could explain the phenotype variation from 4.41 to 13.45% in the TN DH population (Supplementary Materials Table S7; Fig. 5).

Although we failed to map any TDF-markers on the genetic map with the HT RIL population, comparative mapping revealed that the regions surrounding three TDF-markers located in the N4, N6 and N7 linkage groups of TN genetic map were homologous to the other three corresponding regions of the HT RIL genetic map (Fig. 5). Three TDF marker regions located within or near the confidence intervals of yield-related QTLs, which indicates that those QTL may harbor some functional genes that are homologous to the heterosis-associated TDFs.

Discussion

Alterations in the TDFs expressed in partial new-type *B. napus*

Because of the plasticity of paleopolyploids, the *Brassica* genomes are prone to frequent structural change (Quiros 1999). Owing to long-term isolation and evolution, the A^r genome of *B. rapa* and the C^c genome of *B. carinata* show considerable variation when compared with the A^n and C^n genomes of *B. napus*. In this study, the differences in gene expression between a partial new-type *B. napus* lines and its parental cultivars were larger than those of between two Chinese *B. napus* cultivars that did not have any direct kin. This suggested that genetic rearrangement might occur when different genomes combined during the process of interspecific hybridization. In fact, various events accompany with interspecific crosses: (1) Genomic rearrangements resulting from homoeologous recombination (Feldman and Levy 2005) and parental DNA sequence elimination (Ozkan et al. 2001). Leflon et al. (2006)

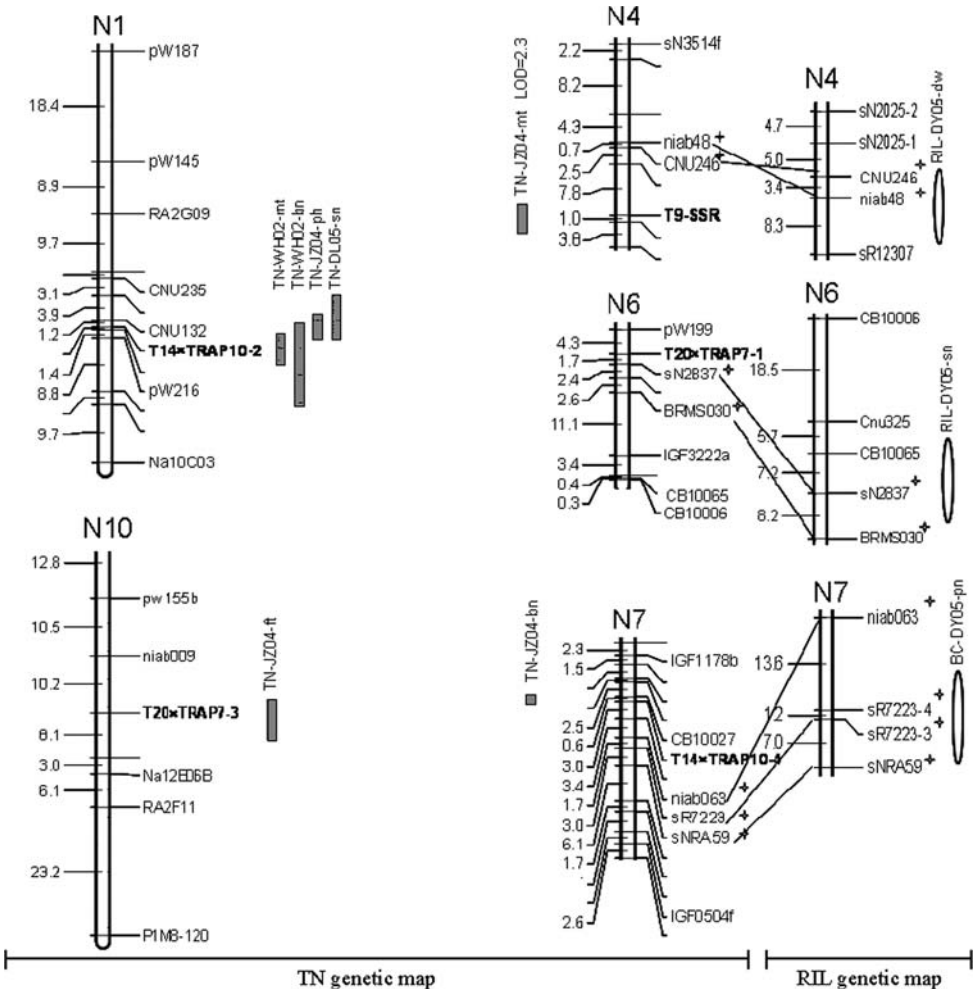

Fig. 5 The TDF-markers map to yield-related QTL regions on linkage maps. The markers and the corresponding genetic distances in cM are shown on the *right* and *left* sides, respectively. Markers showed in *bold* are the TDF-derived markers. TN, RIL and BC represent the TN DH population, the HT RIL population and its derived RIL-BC_1 population, respectively. The *bars* (or *ellipses*) and their label indicate the QTL and their corresponding confidence intervals in different population and environments. "*DL*" signifies Dali county of Shanxi province, "*DY*" corresponds to Daye City, "*JZ*" Jingzhou City, "*WH*" Wuhan City of Hubei Province in China, and the numbers following these abbreviations show the seeding year. *bn* first branch number per plant; *ft* flowering time; *ph* plant height; *mt* mature time; *sn* seed number per pod

observed gene transfer between the A^r genome of *B. rapa* and the A^n/C^n genomes of *B. napus* in triploid hybrids. Homologous pairing and chromosome exchange between different subgenomes also happened in the pentaploid hybrids that were derived from the hybridization between hexaploid plants ($A^rA^rB^cB^cC^cC^c$) and *B. napus* (Li et al. 2004). (2) Retrotransposon reactivation and retrotransposon-induced inserted mutagenesis occurs (Kashkush et al. 2003). In fact, two TDFs homologous to a retrotransposon were detected and one of them was activated in two of the three partial new-type *B. napus* lines. (3) Epigenetic modifications, such as DNA or histone methylation and deacetylation, small RNA or RNA interference, can induce the alteration of gene expression (Wang et al. 2004; Adams and Wendel 2005).

The technique of cDNA-AFLP is an efficient method to examine gene expression at a genome-wide level. Guo et al. (2006) reported that a substantial proportion of differentially expressed cDNA fragments were attributed to the differential regulation of parental alleles in the hybrid. Although DNA polymorphisms that existed between parents might be accounted as differential gene expression, the proportion of miscounted would not be crucial. In our RT-PCR experiment, two out of fifteen primer pairs products (13.3%) produced fragment length polymorphism between the three tested species. Thereafter, most of differentially expressed TDFs scored from this study (about 86.7%) would be resulted from the differential regulation of parental alleles.

The possible mechanism of intersubgenomic heterosis

Allelic combinations present in hybrids might result in the alteration of allele expression profiles, production of novel allelic interactions and genesis of beneficial adaptations in the hybrids, and give rise to heterotic phenotypes (Springer and Stupar 2007). This study found that the intersubgenomic hybrids exhibited higher seed yield heterosis compared to that of their parents, it made us infer that allelic variation introduced from A^r/C^c genome in the lines of partial new-type *B. napus* may lead to many positive allelic combinations in the intersubgenomic hybrids.

Conversely, the negative allelic variation resulting from "genome shock" may disturb the biological process in partial new-type *B. napus* lines and result in negative effects in the hybrids. The number of TDFs in type $N_t^+H^-N^-$ was considerably more than that in type $N_t^+H^+N^-$, and the number of TDFs in type $N_t^+H^+N^+$ significantly exceeded that in type $N_t^+H^-N^+$ in the nine cross-combinations (Fig. 3). This suggests that the specific (or negative?) TDFs of partial new-type *B. napus* lines tended to be absent in the hybrids, and the TDFs common to the partial new-type *B. napus* and *B. napus* tended to be present in the hybrids. In addition, the number of TDFs in type $N_t^+H^-N^-$ was significantly more than that in type $N_t^-H^-N^+$, which indicated that the TDFs arising from the partial new-type lines were more easily repressed than those derived from *B. napus* in the hybrids. DNA methylation and chromatin remodeling are known to alter gene expression (Hirochika et al. 2000; Zilberman et al. 2007). This was supported by the fact that TDF12, a *Copia*-like TDF, was activated in the partial new-type *B. napus* lines and absented in most hybrids. Combined with the observation that most hybrids showed strong heterosis, this could imply that the silencing of unfavorable alleles or retrotransposons in the hybrids might contribute to heterosis.

In contrast to the additive effects that play a major role in the hybrids of rice and wheat (self-pollinated crops), dominance and overdominance effects provided a significant contribution to hybrid performance in maize (a cross-pollinated crop) in terms of TDF display patterns (Xiong et al. 1998; Tian and Dai 2004; Sun et al. 2004; see Table 2). Rapeseed is a mainly self-pollinated crop, and the partial new-type *B. napus* lines contained many novel alleles due to the introgression of exotic genomic components and genome rearrangement. Genomic heterozygosity in the intersubgenomic hybrids of *B. napus* cultivars and partial new-type *B. napus* lines, as in the hybrids of maize, would be expected to be much higher than that in hybrids of rice and wheat. This suggests that the dominance and overdominance effects were prominent in the intersubgenomic hybrids.

Differentially displayed TDFs have been used as genetic markers in segregative population to find further correlations between the TDFs and the target traits. Some genes or ESTs (TDFs) were mapped within the confidence intervals of QTLs for the target traits in rice, rapeseed and maize (Mao et al. 2004; Liu et al. 2005; Huang et al. 2006; Ju et al. 2006). In this study, TDF-markers derived from a number of the H-TDFs were correlated with yield-related traits among three segregative populations, and some were even detected in multiple populations and multiple environments. Furthermore, four TDF-markers (derived from two H-TDFs) were mapped within the confidence intervals of eight QTLs for yield-related traits. This further revealed that the H-TDFs, or at least a proportion of them, were really responsible for fluctuating seed yield in rapeseed.

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