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The genetic diversity and heterotic groups of 169 Chinese semi-winter rapeseed (*Brassica napus*) cultivars and inbred lines

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Abstract Until now, no distinct heterotic groups have been established in many rapeseed-growing countries. Numerous modern breeding lines with desirable seed quality have been developed but the loss of genetic diversity would be problematic. Simple sequence repeat markers covering all 19 *B. napus* linkage groups were used to evaluate the genetic diversity and to classify the heterotic groups of 169 Chinese semi-winter cultivars or inbred lines. The results indicate moderate genetic differentiation among the 169 accessions, which distributed in 10 major groups according to their pedigrees or origins. The Chinese accessions could be divided into two genotypes: northern restorers and southern maintainers for Polima cytoplasm male sterility. The maintainers consisted of nine clades originating from 10 major rapeseed-growing provinces in the upper, middle and lower reaches of the Yangtze River. The mid-parent heterosis on plant height and yield ability of the intragroup crosses was inferior to that of the intergroup hybrids. When the intragroup parental combinations with short genetic distances (GD) were included in all regressing data, GD is significantly correlated to heterosis level. Three major heterotic patterns were identified: (1) northern restorer × southern maintainer, (2) maintainer from the upper reaches of the Yangtze River × maintainer from the lower reaches of the Yangtze River and (3) maintainer from the middle Yangtze River × maintainer from the lower reaches of the Yangtze River. For the first time, the results showed the outline of heterotic groups in Chinese rapeseed under the conditions of the Huang-Huai River region.

Keywords Rapeseed · Genetic diversity · Heterotic group · Heterosis · Simple sequence repeat

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Introduction

Enhancing genetic diversity is the premise for improving yield ability and environmental adaptation of a crop, especially rapeseed (*Brassica napus*, AACC = 38) in which hybrid varieties are widely adopted. Currently, more than 85% (Brewin and Malla 2012) and 70% (Fu and Zhou 2013) of commercial varieties are hybrid types in the two largest rapeseed-growing countries Canada and China, respectively. Investigating genomic variations in breeding materials is essential to understanding of the molecular mechanisms underlying plant heterosis (Yang et al. 2017), to estimation of hybrid phenotypes (Leckband et al. 2018; Werner et al. 2018) and to development of commercial hybrids. Although a positive correlation did exist between parental GD and heterosis (Ahmad et al. 2010; Leckband et al. 2018; Qian et al. 2007, 2009; Yu et al. 2005), hybrid performance cannot be exactly predicted.

A compromised and practical method for designing parental combinations is to classify the heterotic group/population (Melchinger and Gumber 1998). A heterotic group contains some genotypes that display a similar combining ability when crossed with the same testers (Melchinger and Gumber 1998). Germplasms can be assigned into different heterotic groups using pedigree information, quantitative genetic analysis or cluster methods based on more reliable molecular markers (Melchinger and Gumber 1998). The superiority of intergroup over intragroup crosses in terms of hybrid performance, and heterosis has been well-documented (Melchinger and Gumber 1998). In the case of rapeseed, significant heterosis has been observed in crosses between indigenous genotypes and geographically distant cultivars (Qian et al. 2007, 2009; Tian et al. 2017; Yu et al. 2005). However, no distinct heterotic groups comparable with maize have been identified in many rapeseed-growing countries such as Germany (Leckband et al. 2018) and China until now. Seventy years cultivation in the Huang-Huai River region (temperate zone) and the Yangtze River region (subtropical zone) in China have led to the evolution of a large cluster of semi-winter rapeseed germplasms, with fewer requirements for low temperature for vernalization, weak winter hardiness and shorter life span, compared with the European winter rapeseed. Under the quality standard of double-low (low content of erucic acid and glucosinolates) for 30 years and an additional requirement of high oil content since 2010, numerous new varieties and breeding materials of improved seed quality have been developed in China by using directional selection or intervarietal hybridization. However, the loss of genetic diversity would be problematic when breeding bottlenecks are encountered during the introgression of the genes manipulating seed quality. The genetic diversity of some traditional Chinese cultivars has been investigated (Luo et al., 2016; Wu et al. 2019; Zhou et al. 2017), but the differences that exist among the new breeding lines and heterotic pattern among diverse groups are largely unknown.

In addition, selection of well-paired parents by combining ability requires the testing of large quantities of hybrid combinations in field trials (Leckband et al. 2018). However, only limited parents are allowed in a study (Channa et al. 2018; Leckband et al. 2018; Yu et al. 2005; Tian et al. 2017) because the evaluation of dozens of parents will need hundreds of hybrids when combining ability is tested through the use of a diallel mating design. Thus, many randomly or deliberately selected parental combinations, without number limitation by diallel mating design, need to be considered to study the relationship between parental GD and heterosis, as well as the pattern of heterotic grouping, because more representative genotypes, including traditional cultivars and new elite inbred lines, need to be included in the plan. In this study, the genetic diversity of various elite cultivars, backbone inbred lines and breeding materials was analysed by detecting simple sequence repeat (SSR) molecular markers, revealing their genetic relationships at the molecular level, and providing a reference to classify heterotic group of these accessions. Dozens of intergroup and intragroup combinations were deliberately designed to allow analysis of the correlation between GD and heterosis and most importantly, the hybrid performance among heterotic groups.

Materials and methods

Plant materials

The plants used in this study included 179 *B. napus* accessions (introduced cultivars originated from other institutes and elite inbred lines of Northwest A&F University), as well as five accessions of reference species, including *Brassica juncea*, *Brassica oleracea* and

Brassica rapa. These *B. napus* materials contained three ecotypes: six spring accessions (Armada, Profit, Polo, Boomer, Sapphire and A35), four European winter-type cultivars (Solida, Ramiro, Mohican and CDH) and 169 Chinese semi-winter accessions. These Chinese semi-winter accessions included 36 registered cultivars and 133 breeding lines, representing a broad range of agronomic and phenological traits from the Chinese semi-winter rapeseed gene pool. The originations and pedigrees of these plant materials are shown in Table S1. All materials were sown in the experimental field of Northwest A&F University. Each entry was grown in one or two rows, 2 m in length and 0.40 m in row space.

Detection of SSR markers

The leaves of at least five plants of each accession were mixed, and the total DNA was extracted using the well-known CTAB method. The SSR markers covering each of the *B. napus* linkage groups, namely, BoGMS, cnu_m and nia_m, which denoted primer pairs, were developed in previous works (Kim et al. 2009, Li et al., 2011). In total, 160 pairs of primers were preselected across twelve represented genotypes, and 48 pairs of primers with polymorphic and clear bands were selected (Table S2). Among them, 25 pairs of primers were designed from the ten linkage groups of genome A and 23 pairs from the nine linkage groups of genome C (Kim et al. 2009, Li et al., 2011).

The PCR amplification was carried out in a final volume of 25 μl, which contained 1 μl DNA template (50–l00 ng); 0.5 μl forward-primer (10 ng/ul); 0.5 μl reverse-primer (10 ng/ul); and 5 μl PCR Mix (Tiangen, Peking, China) containing dNTPs, PCR buffer, MgCl2 and Taq polymerase. The procedure of PCR was as follows: pre-denaturation at 94 °C for 4 min; 30 cycles of 45 s at 95 °C, 45 s at 51–58 °C and 1 min at 72 °C; and a final extension for 7 min at 72 °C. The PCR products were electrophoresed on polyacrylamide gel and stained with silver.

Evaluation of the heterosis level among the different heterotic groups of *B. napus*

To validate the hybrid performances of crosses among the different groups, some elite inbred lines or cultivars in each clustering group or subgroup was selected for the crossing. The designed experiment included two type of hybrids: (type I) large seed lots, which included 29 hybrids between several elite maintainers for Polima cytoplasmic male sterile (CMS) lines and several representative restorer lines, which served as the major heterotic groups available in northern China, and four hybrids produced by temperature-sensitive male sterility (TMS) “SP2S” (Yu et al., 2015), and (type II) smaller seed lots of 47 hybrids from artificial pollination, whose seeds were obtained by using artificial emasculation and pollination. The restorer lines for the type I hybrids, including “QXD”, “YC4” and “FuGreen”, were developed by NWAFU and represented the indigenous restorer and the other restorers—“D636”, “H9958” and “Zayou105”—which were second-cycle restorer groups derived from commercial hybrid varieties. Another intermediate restorer, “Shengguang77”, which had a close relationship with the maintainers, was also used to detect its combining ability. The selected maintainers represented several groups with southern China origins. The hybrid seeds produced by these pairwise CMS lines and restorers in isolated cages were enough for repeat experiment in large plot. The abovementioned 29 CMS and four TMS hybrids, as well as a control hybrid variety “Qinyou7”, were grown in the experimental field of Northwest A&F University. Each plot contained 300 plants of a type I hybrid, which were grown in six rows, with row length = 4 m and row space = 0.4 m. The seed lots for the 46 parental combinations in type II were obtained by using artificial emasculation hybridization, and thus, the seed amounts were less than the CMS/TMS hybrids. Each genotype of type II was grown in a plot with the same seedling density but half the size as type I. All the parental lines of the 79 hybrids were also grown in the same field, with the same plot as the type II hybrids. The CMS lines were substituted by their maintainers and SP2S was replaced by its nearly isogenic line SP2F to estimate the seed yield. The field experiments were conducted following local agronomic procedures in the growing season of 2017/2018 and 2018/2019 in Yangling, China. The plant height of ten random selected plants and average seed yield per plant were investigated. The parents and hybrids in types I and II were grown with two replications in 2 years, and the obtained four datasets were treated as four replications in analysis of variance and correlation using the software DPS 7.5 (Tang and Zhang 2013).

There are high degree of correlations among the four datasets and thus, the average values for plant height and seed yield in 2 years were used for heterosis estimation.

Data statistics

With DNA ladder as the molecular weight reference, clear and easily distinguishable electrophoretic bands were recorded. A band in the same position is marked as present (1) or absent (0) to establish a binary matrix. The paired GDs (or dissimilarity coefficients) were calculated by using the software DPS 7.5 (Tang and Zhang 2013): GD = 1–2 M/(Mi + Mj), where Mi and MJ are the total bands of the two accessions, respectively, and M is the number of shared band by the two accessions. The GD matrix of all genotypes was used to analyse principal coordinates analysis (PCoA) by using NTSYS2.1e (Rohlf 2000) and constructing dendrograms by using unweighted pair-group methods with arithmetic averages (UPGMA) and neighbour-joining (NJ) methods in the computer program MEGA-X (Kumar et al., 2018). Since SSR is not a high-throughput marker technology when handling too many samples, cluster results using all markers and reduced markers (removal of one or two bands for each primer pairs) were compared. If similar cluster results were obtained, it was indicated enough representative ability of the SSR markers for genetic diversity evaluation. The dendrograms were visualized in a graphical viewer FigTree 1.4.3 (http://tree.bio.ed.ac.uk/software/). The genetic components were analysed by using the Structure 2.3.4 program (Pritchard et al. 2000).

Hybrids between genetically distant rapeseed populations can display both biomass superiority and yield heterosis (Yang et al. 2017). In the hybrid evaluation experiment, both the average plant height (representing a rough estimation of the biological yield/biomass) and seed yield of 2 years were investigated, and the percentage deviation was estimated based on the mid-parent heterosis (MPH) and the high-parent heterosis (HPH), which were computed using the formulas MPH = $100 \times (F_1 - \mathrm{MP})/\mathrm{MP}$ and HPH = $100 \times (F_1 - \mathrm{HP})/\mathrm{HP}$, respectively, where F_1 refers to the plant height/seed yield of the F_1 hybrid, MP is the mean of the parents population and HP is the plant height/seed yield of the parent with the higher performance. For hybrid breeding, the goal is to find better hybrids with high yielding ability other than heterosis per se. Thus, the gap between the F_1 and the leading hybrid variety “Qinyou7” was calculated to find the best combinations with over-control heterosis (OCH = $100 \times (F_1 - \mathrm{Qinyou7})/\mathrm{Qinyou7}$). A linear regression between parental GDs and the values of MPH, HPH and OCH, and analysis of correlation among these values in the 2-year experiments were conducted by using DPS 7.5 (Tang and Zhang 2013).

Results

Polymorphism of SSR markers

The statistics of the PCR products in all rapeseed accessions are shown in Table S3. A total of 186 clear bands and 132 polymorphic bands among them were amplified by 25 pairs of genome A primers, with an average of 5.3 polymorphic bands per prime pair. A total of 150 bands were amplified by 23 pairs of genome C primers, and 93 bands were polymorphic, with an average of 4.0 polymorphic bands per prime pair. After the paired similarity coefficients of 184 materials had been calculated, the dissimilarity coefficients (=GD) of all materials ranged from 0.04 to 0.41. The materials that had similar origins or pedigrees showed the highest similarity. The distribution of all accessions along two principal coordinates is shown in Fig. 1, and the restorers developed by several institutes in northern China were separate from the southern and foreign germplasms. Furthermore, the differences in their genetic structure can be shown by the representative components in the different genotypes (Fig. 2). Five main groups of accessions were showed: (i) accessions from southern China (maintainer), constructed mainly by blue blocks (cluster K5) or red blocks (K2); (ii) restorer lines from northern China, mainly constructed by K3 (pale blue blocks); and (iii) restorers of a second cycle line (SCI) that shared more green blocks (K1) with another group (iv) of southern maintainers and distant species (v) that contained more yellow blocks (K4). The observed genetic structure in this study implied higher differentiation between geographically contrasting germplasm, and it established a reference to the exploitation of heterosis.

Clustering of genotypes based on SSR markers

Compared with PCoA and genetic structure analysis, a hierarchical clustering dendrogram (Fig. 3) can clearly

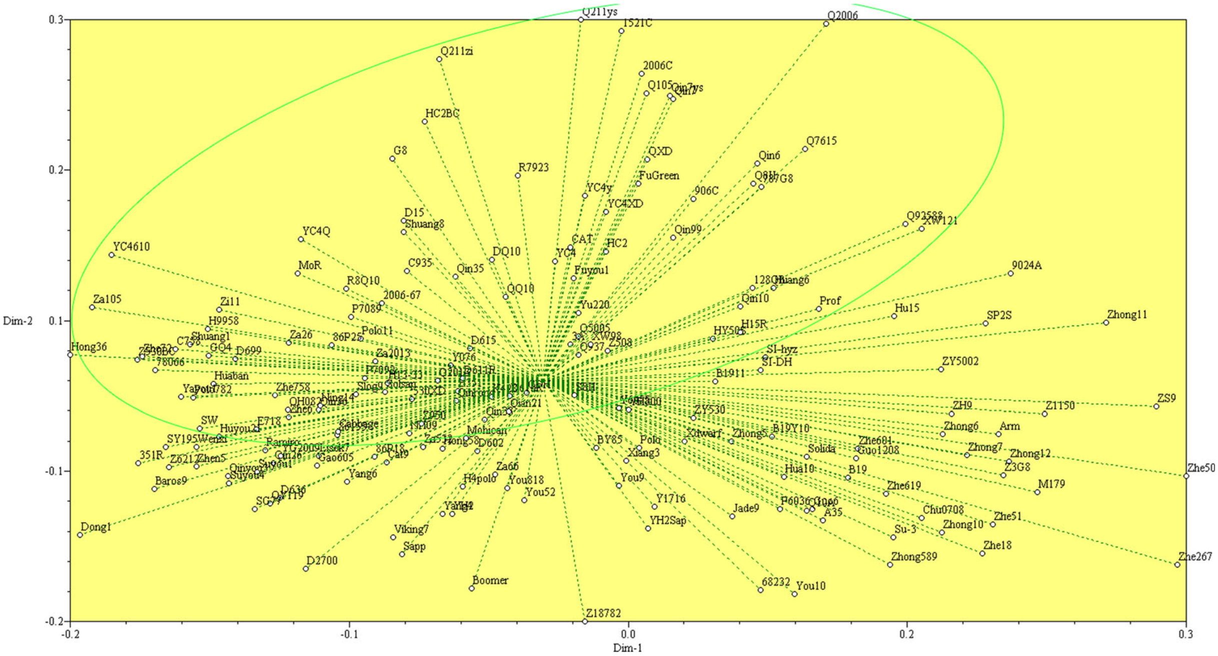

Fig. 1 Principal coordinates analysis of the 184 genotypes based on genetic distance

but simply show the distribution of different accessions. The clustering results using primers for genome A and C of rapeseed were quite different because they represented different genetic components in the genome A/C. Therefore, we combined all SSR markers together to get an overall result. The dendrogram created by using the NJ method was similar but somewhat different from the UPGMA clustering (Figs. 3 and 4). *Brassica napus* and three reference species, including *B. juncea*, *B. oleracea* and *B. rapa*, were separated, except that in the NJ dendrogram, the cabbage was placed near three European cultivars—Ramiro, Mohican and Solida. The accessions were largely clustered according to geographical origin, for instance, three European cultivars, Ramiro, Mohican and Solida; two Australian spring-type cultivars, Sapphire and Boomer and two Canadian cultivars, Profit and Amanda. The accessions originated from the same breeder were often distributed together or in neighbouring subgroups, for example, the Zhongshuang serials from OCR of CAAS and the Zheyou serials from Zhejiang AAS. In one word, the clustering pattern mostly supported the respective pedigrees and origins.

The 179 *B. napus* can be clustered into ten or eleven major groups according to their locations in the two dendrograms (Figs. 3 and 4, Tables S4 and S5**)**. In the UPGMA results, group I was the largest group and contained mainly 26 maintainers and 31 restorers for Polima CMS. The majority of the 31 group I restorers, except for Qinyou3, Shengguang77 and Dadi699, are SCIs that were developed from some commercial hybrid varieties, and thus, they belong to the intermediate type between restorers and maintainers. Several inbred lines from European × Chinese crosses, including Cat7, Cat9, Viking7, Lisek7 and Baros9, are located in group I separately from other accessions, indicating the usefulness in expanding the genetic diversity of Chinese germplasm by introducing new genes from geographically distant genotypes. Group II had 34 accessions that mainly originated from several institutes located in North China, including NWAFU, the Hybrid Rapeseed Research Centre (HRRC) of Shaanxi Province and Henan AAS. This group was denoted as the northern China restorers for Polima CMS, with the exception of two maintainer lines A3 and Huang6. Small group III only contained H15R, B351, ZY530 and 530XD, and group IV contained only Zheshuang72, Zheyou758 and Zheshuang6, which are all maintainers from Zhejiang AAS. Group V contained 15 yellow-seeded or spring-type maintainers. Group VI contained eight yellow-seeded maintainers derived from

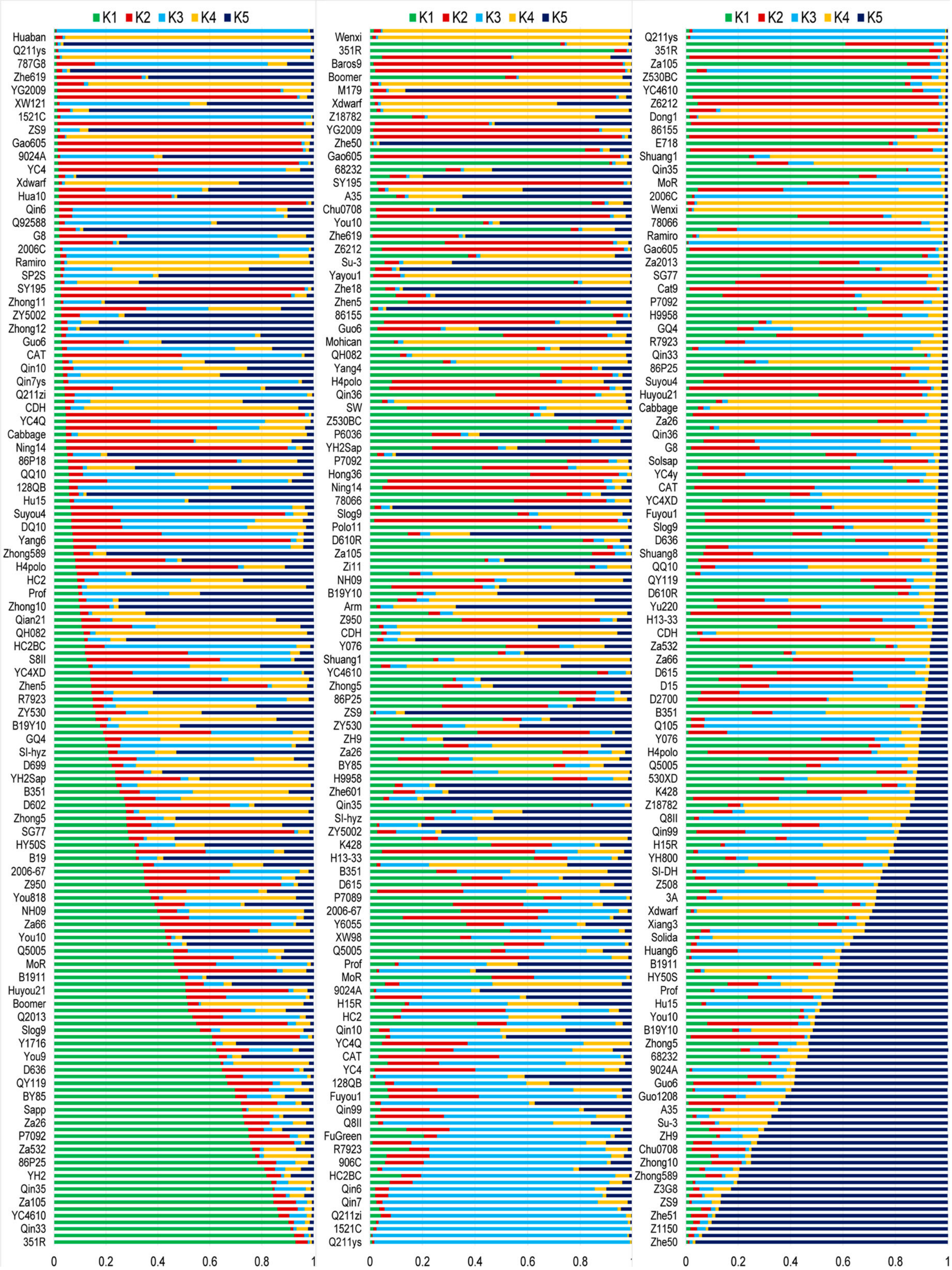

Fig. 2 Schematic diagram of genetic structure of the 184 genotypes based on SSR markers. The components (K, number of clusters) were calculated in the Structure program (http://web.stanford.edu/group/pritchardlab/structure_software/release_versions/v2.3.4/html/structure.html). The order of all accessions was rearranged as the values of K1, K3 and K5

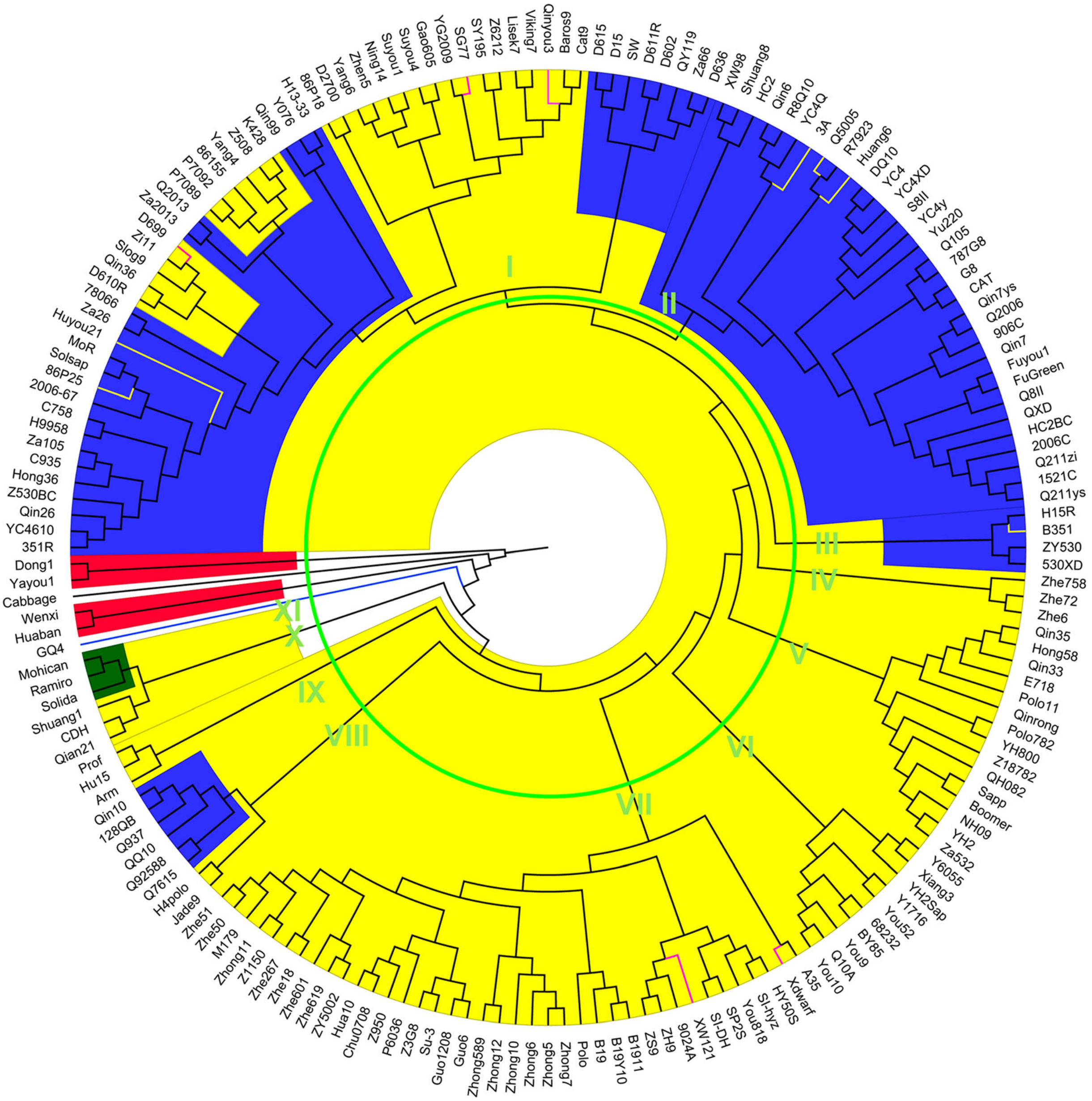

Fig. 3 Dendrogram of UPGMA clustering based on polymorphism revealed by SSR markers in the 184 genotypes. The tree was visualized in Figtree (v1.4.3), and the branches were transformed to clearly show the nodes. The restorer lines for Polima CMS are indicated by blue, and the maintainer lines are indicated by yellow. The red block shows the reference species other than *B. napus*. The position of the three European cultivars is shown by the green block. Distinct clusters by the UPGMA method have been numbered for ease of viewing

the hybrids based on recessive genic male sterility (GMS) from Guizhou AAS and two brown-seeded maintainers Q10A and YH2sap. Group VII was mainly constituted by nine Zhongshuang and eight Zheyou series and their derivations, which came from the Institute of Oil Crops of Chinese AAS (Academy of Agricultural Sciences) and Zhejiang AAS, respectively. These can be denoted as the maintainer group because all of these except Xdwarf and XW121 were second-cycle-line restorers. Group VIII contained two spring × semi-winter intermediates (Jade9 and H4polo) and six restorers (Q926, Q92588, 128QBC, Qinyou10, Q937 and QQ10) from Xianyang AAS and NWAFU. Group IX consisted of three spring rapeseed Prof, Arm and Hu15. The European strong

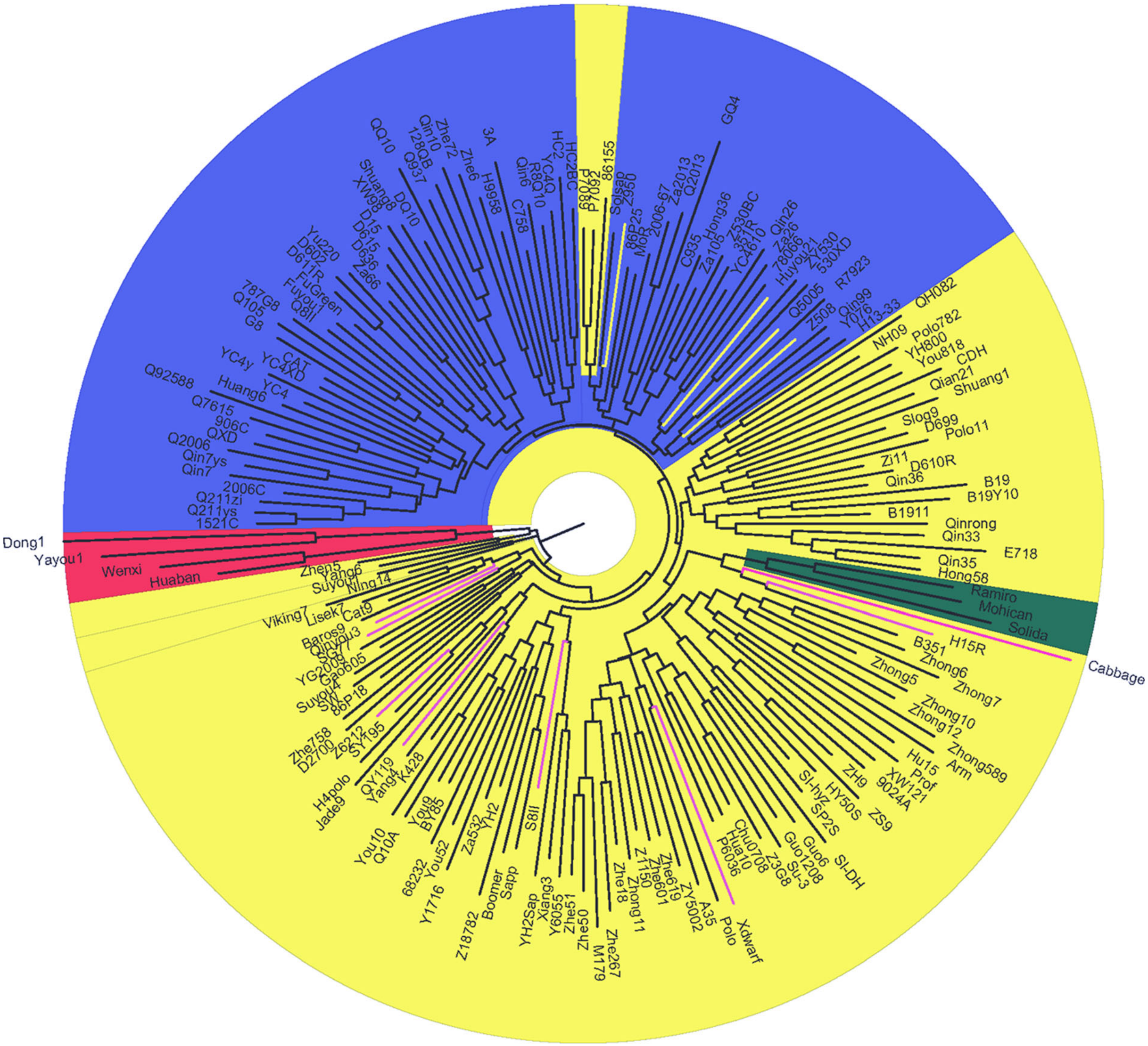

Fig. 4 Dendrogram of neighbour-joining clustering based on polymorphism revealed by SSR markers in the 184 genotypes

winter-type cultivars, including Ramiro, Mohican, Solida and CDH, and two Chinese accessions, Qian21 and Shuangyou1, constituted group X. Restorer GQ4 was located alone as group XI.

Generally, the restorer groups were distant geographically and genetically from several samples of European winter-type or foreign spring-type rapeseed. The maintainer groups, which consisted of more than nine clades originating from 10 major rapeseed-growing provinces in the Huang-Huai River region and the upper, middle and lower reaches of the Yangtze River, displayed wide variation accordingly to the geographical diversity.

Comparison of intrapopulation and interpopulation hybrid performance and genetic distances computed from SSR markers

The data from the 2 years revealed similar trends of hybrid performances over their parents for plant height and seed yield (Table S6) and thus, the average values of 2 years (Table S7) were used for heterosis estimation. The MPH for plant height ranged from 0.01 to 27.92%, with an average of 13.74% and an MPH for seed yield ranging from − 0.04 to 75.04%, with a mean of 30.55% (Table 1). Most hybrids surpassed their respective parental lines for the two traits, except for a cross

Table 1 The GD, MPH, OBH and OCH for plant height and seed yield of the hybrids from various parental combinations

Parent1	Parent2	GD	MPH-height	MPH-yield	BPH-height	BPH-yield	OCH-height	OCH-yield
Intra-group hybrids								
2006C	Q211ys	0.104	0.016	0.003	0.013	0.016	− 0.112	− 0.075
ZH9	ZS9	0.122	0.058	0.056	0.028	0.072	− 0.180	− 0.338
Boomer	Sapphire	0.141	0.060	0.146	0.028	0.205	− 0.163	− 0.475
D636	Zayou105	0.155	0.094	0.144	0.023	− 0.042	− 0.017	− 0.023
CMS Q5005	R7923	0.166	0.131	0.123	0.030	0.109	− 0.028	− 0.129
CMS Huyou21	Zayou105	0.167	0.166	0.126	0.035	− 0.067	− 0.006	− 0.048
Huyou21	Yangguang2009	0.173	0.092	0.125	0.066	0.081	− 0.096	− 0.274
Zheyou267	Zheyou50	0.174	0.060	0.132	0.037	0.027	− 0.056	− 0.077
QXD	2006C	0.174	0.023	− 0.027	0.026	− 0.057	− 0.112	− 0.085
Baoyou85	Y1716	0.174	0.066	0.001	0.081	0.043	− 0.096	− 0.424
SP2S	ZH9	0.177	0.169	0.193	0.291	− 0.007	− 0.028	− 0.077
Zhongshuang11	Zheyou50	0.179	0.009	0.293	0.006	0.140	− 0.084	− 0.165
Zheyou50	Chu0708	0.192	0.085	0.181	0.203	0.194	− 0.101	− 0.144
YC4	Zayou105	0.197	0.055	0.091	0.058	0.047	0.017	0.068
CMS Q5005	Zayou105	0.201	0.139	0.137	0.029	− 0.005	− 0.011	0.015
QXD	YC4	0.203	0.123	0.182	0.064	0.162	0.028	*0.128*
Zheyou267	Chu0708	0.205	0.153	0.066	0.248	− 0.042	− 0.067	− 0.140
CMS Huyou21	D636	0.210	0.154	0.207	0.134	0.192	− 0.051	− 0.179
Zheyou267	Zhongshuang11	0.211	0.089	0.179	0.068	− 0.043	− 0.034	− 0.141
Baoyou85	Youyan10	0.219	0.058	0.239	0.040	0.207	− 0.129	− 0.333
Huyou21	Q5005	0.220	0.128	0.007	0.152	− 0.105	− 0.107	− 0.227
ZH9	Zhongshuang11	0.223	0.105	0.260	0.216	0.200	− 0.084	− 0.259
SP2S	Zhongshuang11	0.229	0.111	0.362	0.082	0.090	0.039	0.014
QXD	G8	0.241	0.065	− 0.026	0.058	0.081	− 0.084	− 0.140
YH800	NH09	0.251	0.066	− 0.118	0.045	− 0.251	− 0.096	− 0.288
Ramiro	Solida	0.258	0.091	0.278	0.082	0.253	0.039	− 0.499
ZH9	Zheyou50	0.254	0.149	0.255	0.049	0.157	− 0.045	− 0.153
CMS Q5005	YC4	0.214	0.174	0.292	0.058	0.173	0.022	0.101
Huyou21	H9958	0.216	0.125	0.268	0.041	0.175	− 0.011	− 0.075
CMS Q5005	D636	0.214	0.178	0.449	0.134	0.377	− 0.051	0.054
QXD	FuGreen	0.220	0.100	0.336	0.034	0.183	0.017	0.149
Inter-group hybrids								
SP2S	2006C	0.213	0.060	0.317	0.037	0.303	− 0.056	*0.212*
CMS Zhongshuang11	FuGreen	0.213	0.143	0.463	0.119	0.191	0.056	0.059
Zayou105	Shengguang77	0.214	0.082	0.210	0.076	0.000	0.034	0.020
Baoyou85	ZH9	0.218	0.166	0.280	0.107	0.212	− 0.073	− 0.251
CMS ZH9	YC4	0.234	0.183	0.471	0.052	0.220	0.017	*0.145*
CMS 9024	1521C	0.235	0.158	0.484	0.110	0.136	− 0.034	0.082
QXD	Z66	0.237	0.127	0.317	0.156	0.519	0.000	*0.129*
CMS Z18	YC4	0.237	0.204	0.288	0.134	0.192	0.096	*0.118*
SP2S	YC4	0.224	0.156	0.206	0.122	0.201	0.084	*0.127*
YC4	Qin10	0.228	0.099	0.347	0.093	0.468	0.056	*0.169*
CMS Huyou21	Shengguang77	0.244	0.105	0.371	0.024	0.376	− 0.028	− 0.083

Table 1 (continued)

Parent1	Parent2	GD	MPH-height	MPH-yield	BPH-height	BPH-yield	OCH-height	OCH-yield
CMS Huyou21	YC4	0.244	0.158	0.393	0.064	0.195	0.028	*0.121*
CMS Ramiro	Zayou105	0.246	0.109	0.314	0.099	− 0.095	0.056	− 0.077
Arm	ZH9	0.247	0.154	0.288	0.110	0.216	− 0.096	− 0.155
CMS Huyou21	QXD	0.248	0.181	0.335	0.143	0.129	− 0.011	0.096
YH800	Q5005	0.252	0.281	0.141	0.355	0.198	0.051	0.035
CMS ZH9	FuGreen	0.252	0.199	0.531	0.077	0.298	0.017	*0.153*
QXD	H9958	0.252	0.164	0.287	0.112	0.166	0.056	*0.132*
QXD	D636	0.252	0.182	0.326	0.201	0.134	0.006	*0.101*
CMS ZH9	D636	0.260	0.279	0.665	0.215	0.579	0.017	0.087
CMS Q5005	Shengguang77	0.260	0.166	0.181	0.059	0.105	0.006	− 0.155
Zheyou267	ZH9	0.261	0.225	0.247	0.321	0.052	− 0.006	− 0.055
CMS Zhongshuang11	Zayou105	0.262	0.157	0.313	0.123	0.016	0.079	0.036
YC4	Shengguang77	0.262	0.097	0.409	0.087	0.204	0.051	*0.130*
QXD	Zayou105	0.266	0.163	0.108	0.105	0.081	0.062	*0.103*
Zhongshuang11	Solida	0.267	0.193	0.607	0.158	0.926	0.112	− 0.229
CMS Zhongshuang11	YC4	0.268	0.189	0.491	0.151	0.190	0.112	*0.116*
CMS ZH9	Shengguang77	0.269	0.155	0.600	0.036	0.542	− 0.017	0.027
Zhongshuang11	Zheshuang6	0.269	0.177	0.638	0.156	0.535	0.084	− 0.019
CMS ZH9	QXD	0.270	0.153	0.416	0.078	0.159	− 0.067	*0.125*
CMS ZH9	Zayou105	0.274	0.167	0.172	0.041	− 0.059	0.000	− 0.040
CMS YH800	YC4	0.276	0.209	0.257	0.145	0.265	0.107	*0.187*
Arm	Zhongshuang11	0.278	0.163	0.459	0.106	0.316	0.000	− 0.085
Zhongshuang11	Ramiro	0.279	0.191	0.750	0.167	0.477	0.101	− 0.175
CMS Zheyou50	YC4	0.280	0.186	0.397	0.151	0.243	0.112	*0.167*
CMS Zheyou50	QXD	0.280	0.147	0.376	0.166	0.206	0.028	*0.171*
YH800	Zhongshuang11	0.288	0.156	0.458	0.130	0.157	0.022	*0.101*
Zheyou50	Shengguang77	0.290	0.106	0.492	0.083	0.425	0.028	0.043
CMS Ramiro	2006C	0.291	0.102	0.620	0.148	0.152	0.000	0.049
QXD	Shengguang77	0.292	0.133	0.409	0.083	0.188	0.028	*0.153*
Huyou21	ZH9	0.295	0.178	0.477	0.162	0.485	− 0.034	− 0.014
CMS Q5005	QXD	0.295	0.205	0.278	0.143	0.143	− 0.011	*0.110*
CMS Ramiro	YC4	0.308	0.241	0.572	0.227	0.108	0.185	0.040
Baoyou85	Zheyou50	0.309	0.094	0.537	0.248	0.348	0.045	− 0.013
Y1716	Zheyou51	0.318	0.094	0.560	0.216	0.306	0.045	0.017
CMS Zheyou50	Zayou105	0.331	0.189	0.259	0.158	0.082	0.112	*0.103*
Zhongshuang11	Zheyou758	0.336	0.145	0.496	0.118	0.366	0.062	− 0.076
Y1716	M179	0.354	0.094	0.609	0.229	0.437	0.056	− 0.040
Average		0.236	0.137	0.305	0.112	0.185	− 0.001	− 0.030

The OCH-yield values >0.10 are in italic type

combination among two related lines QXD and 2006C. In general, crosses of lines located in different groups had higher heterosis level than those from the same subgroups.

When both parental lines came from the same group and had very narrow GD, for example, 2006C × Q211ys, ZH9 × ZS9, Boomer × Sapphire, Huyou21 × Yangguang2009, Zheyou267 × Zheyou50 and

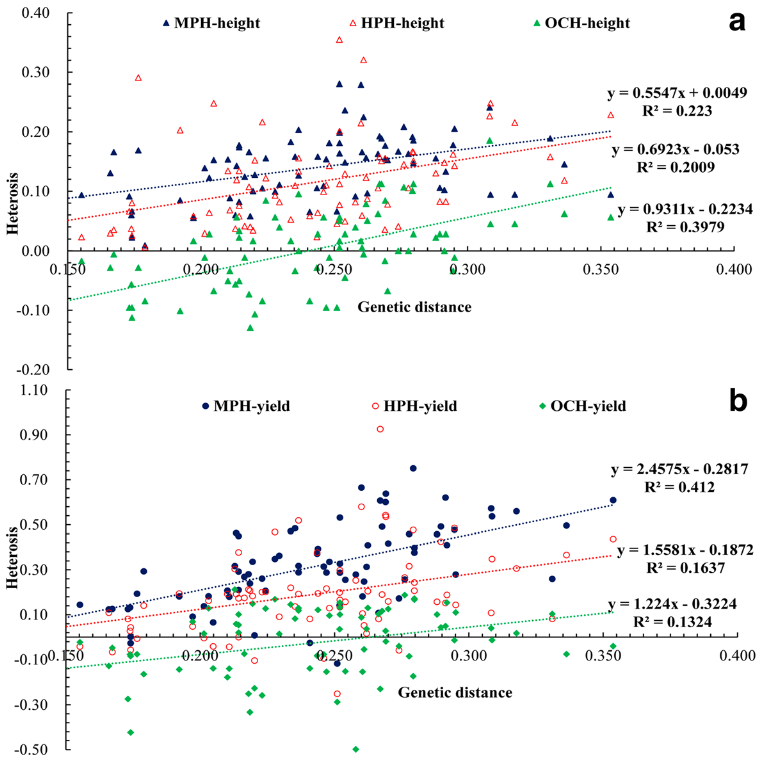

Fig. 5 Correlations of genetic distance with MPH, HPH and OCH of plant height and seed yield of cross combinations

Baoyou85 × YH1716, their hybrid combination always showed much lower hybrid vigour than those hybrids with parental combinations that originated from the different groups (Table 1). These intragroup crosses would not be evaluated for heterosis in a practical breeding programme, but the addition of these crosses to the regression analysis did result in a significant correlation coefficient between GD with MPH for plant height and seed yield (Fig. 5 and Table 2). However, the distribution of the MPH/HPH of the plant height and seed yield cannot be accurately predicted from these mathematical functions, and many combinations stood aloof from the regression lines (Fig. 5).

If the two parental lines came from different heterotic groups and they have considerable GD, their F_1 hybrid combination showed very strong biomass heterosis, and often, but not always, high yielding ability. For example, all registered hybrids developed by NWAFU came from intergroup parental combinations: Shaanyou28 (9024A × 1521C), Xiyou1 (CMS ZH9 × YC4) (Fig. 6), Xiyou2 (CMS ZH9 × FuGreen) and Xiyou3 (CMS Zhe18 × YC4). Some other major patterns among intergroup parental combinations also showed significant heterosis: (i) the maintainer from the upper reaches of the Yangtze River × the maintainer from the lower reaches of the Yangtze River, for example, Baoyou85 × Zheyou50, Y1716 × M179 and Y1716 × Zheyou51, and (ii) the maintainer from the middle Yangtze River × the maintainer from the lower reaches of the Yangtze River, for example, Zheyou267 × ZH9, Zhongshuang11 ×

Table 2 Correlation of GD with heterosis for plant height and seed yield

Item	MPH-height	MPH-yield	BPH-height	BPH-yield	OCH-height	OCH-yield
All crosses	0.472**	0.642**	0.448**	0.405**	0.631**	0.364**
Intra-group	0.346	0.300	0.221	0.159	0.428*	0.045
Inter-group	0.015	0.386**	0.354*	0.137	0.305*	− 0.097

* and ** indicate significance at the confidence level of 95% and 99%

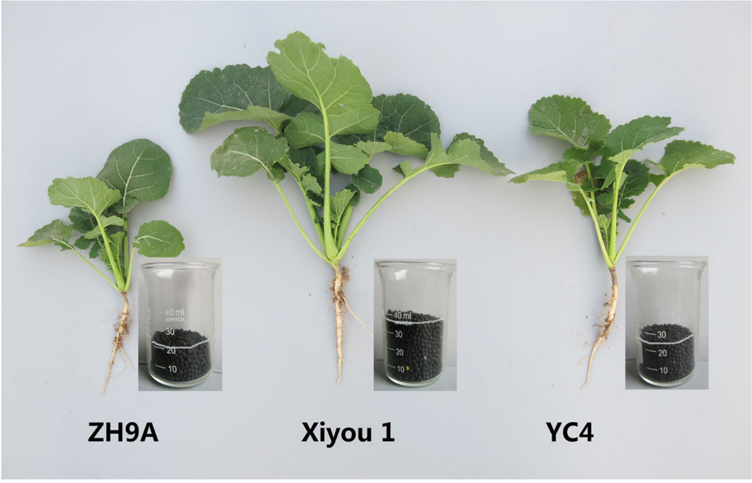

Fig. 6 Heterosis of seedling biomass and seed yield of Xiyou1, which is a registered hybrid variety from the cross between CMS ZH9 and restorer YC4

Zheshuang6, Zhongshuang11 × Zheyou758 and Huyou21 × ZH9. However, the OCH for yielding ability of these inter-maintainer crosses are not very high in this study (Table 1) because these parents have weaker winter hardiness than northern restorers, and the vigorous growth of these hybrids in the autumn would lead to freezing injuries in the winter.

Our study also showed that most crosses between geographically distant groups, semi-winter × winter or semi-winter × spring-type, had high levels of MPH or HPH regarding plant height but lower heterosis on seed yield. Some crosses among these would be useful when developing hybrid combinations with high heterosis level, such as Zhongshuang11 × Solida, Zhongshuang11 × Ramiro, CMS Ramiro × YC4, CMS Ramiro × 2006C, SP2S × 2006C and Arm × Zhongshuang11 (Table 1). However, the seed yield of most those combinations were still lower than the control "Qinyou7" because the growth rhythm is too slow, except that the hybrid of 2006C with a European original line SP2S, which had merits of strong winter hardiness and mid-maturity, topped the trials with a 21.2% yield increase over Qinyou7.

The seed yield of 21 intergroup hybrids and only four intragroup hybrids had positive OCH over Qinyou7. The top five hybrids were CMS Zheyou50 × YC4, CMS YH800 × YC4, QXD × YC4, CMS ZH9 × YC4 (Fig. 6) and CMS Zheyou50 × QXD, which were followed by Xiyou3 (Zhe18A × YC4), QXD × Fuyou1, Xiyou2 (CMS ZH9 × FuGreen), SP2S × 2006C and Xiyou7 (CMS ZH9 × QXD), which had ≥ 10% yield increase over the control (Table 1). Some intergroup combinations with earlier maturity and shorter plant height, which are now desirable traits in semi-winter rapeseed breeding, including Xiyou8 (CMS ZH9A × D636), Xiyou9 (CMS Q5005 × QXD), Q5005 × Zhongshuang11, CMS H21 × QXD and Sapphire × Zhongshuang11, also had > 5% seed yield increase compared with the control (Table 1).

Discussion

The genetic diversity in Chinese *B. napus* breeding materials

Our present data suggest that the pairwise GDs of 184 materials in this study ranged from 0.06 to 0.50, but the largest GDs were mainly contributed by three distant species, and the pairwise GDs among most Chinese accessions of double low quality were less than 0.35. Diversity reduction can be expected since introgression was performed to obtain genes for zero erucic acid content and low glucosinolate content (Zou et al. 2019), which are donated mainly by several spring cultivars such as Oro, Zephyr, Midas, Tower, Westar, Regent, Marnoo, Wesroona and Wesbrook. The modern Chinese inbred lines were also selected through high-intensity inter-variety hybridization from several imported materials for the purpose of increasing the oil content. Fortunately, the 169 modern breeding materials in *B. napus* have divided into 10 major groups by GD measures and cluster analysis, in accordance with their pedigrees or origins. These results of genetic diversity are important for crop breeding because this fundamentally reflects the evolutionary history of crops and coincided with the pedigrees of most accessions.

A dilemma of whether or not to perform an inter-variety cross

For the past few decades, breeders have been accustomed to using fewer core germplasms in their pedigree-based breeding programs, and thus, the genetic relationship of the lines from the same ancestry will inevitably lead to narrow diversity. For example, our clustering results show that the materials from some breeders, such as NWAFU, Shaanxi HRRC and Zhejiang AAS, are highly concentrated. If the heterotic groups and patterns among them are not identified, extensive intergroup hybridization and wide exchange of genetic resources among the different breeders would create a problem by reducing the genetic differences among the different groups and reducing the combining ability among them. For example, the genetic similarity between some SCI restorers and the maintainer populations was expected because most SCI restorers were the progenitors of hybrids who shared a parent with some maintainers. Thus, most of the SCI germplasms are intermediate types between the southern maintainers and northern restorers. Thus, a dilemma whether or not to perform inter-variety crossing exists. The right way would be to establish two or few heterotic groups and then to cautiously select parents for intragroup hybridization and to avoid formation of too many SCIs.

Addition of crosses between close-related inbred lines elevates the theoretic correlation between GD and heterosis

Some previous study showed that parental GD was positive correlated with hybrid yield/yield heterosis (Ahmad et al. 2010; Qian et al. 2007, 2009; Xu et al. 2004; Yu et al. 2005) but it was difficult to exactly predict the yield heterosis by GD. In this study, we found that the correlation changed with the difference in number and pedigree relationship among parents, similar to the results in maize (Xu et al., 2004). Therefore, the crosses among close relatives and parental combinations with short GD should be added in the theoretic study of the correlation between GD and heterosis. Unfortunately, no such plan was designed in all the previous studies (Channa et al. 2018; Qian et al. 2007, 2009; Tian et al. 2017; Xu et al. 2004; Yu et al. 2005), because such crosses among close-related parents were useless for developing commercial hybrid. We deliberately designed the crosses among close-related parents with short GD in the experiment, and the results showed the correlation between GD and MPH or HPH is higher than that in some other studies (Channa et al. 2018; Qian et al. 2007, 2009; Tian et al. 2017; Xu et al. 2004; Yu et al. 2005). Although GD is not an exact indicator for predicting hybrid performances, the genetic diversity revealed by the PCoA and the hierarchical cluster would be useful to classify the inbred groups. The identified heterotic groups can be used to select parental combinations with suitable GD and to avoid producing a large number of useless intragroup F_1 hybrids.

Three major patterns when selecting parental combinations with significant heterosis

Until now, no distinct heterotic groups comparable with maize have been established in many rapeseed-growing countries because this allotetraploid has rich genomic variations (Wu et al. 2019; Zou et al. 2019). Leckband et al. (2018) found that, in Germany rapeseed, no significant difference existed between the performance and MPH of within-group and between-group hybrids. Germany rapeseed cultivars were produced using standard pedigree-based methods; thus, parental components were crossed without consideration of their combining abilities (Leckband et al. 2018). When conversion to hybrid breeding recently, GMS NPZ-lembke is employed, which would use any normal cultivar as the restorer, and the restorer did not diverge from the few maintainers of the GMS. As a consequence, distinct heterotic groups with representative female testers are not yet established in their breeding pools (Leckband et al., 2018).

In contrast, the results of the present study showed that Chinese cultivars can be divided into some distinct groups, and significant difference existed between the heterosis of intergroup and intragroup hybrids. Firstly, China has even played a leading role in hybrid rapeseed breeding since Polima CMS was discovered in 1972 (Fu and Zhou 2013) and the first CMS hybrid Qinyou2 was registered in 1986 (Li and Tian 2015). The restorer gene *Rfp* mainly exists in several inbred lines derived from a northern "Yuejin youcai" population that was introduced from Italy in 1957. Most southern maintainer lines were developed from a Japan original landrace "Shengli youcai" after it had been crossed with Canadian, Australian or European double low cultivars (Zou et al. 2019). Thus, the two heterotic groups of the southern maintainer and northern restorer have been established in the 30 years hybrid breeding history. Secondly, China had several different rapeseed-growing regions, and the breeders in the different institutes have employed Polima CMS, recessive GMS, dominant GMS and CIMS in different regions since 1976 (Fu and Zhou 2013), 10 years earlier than Germany. This history and situation would force Chinese rapeseed inbred lines into divergent groups. In the present study, the clustering result showed that most germplasms were clustered largely according to geographic origin, restorer lines and maintainer lines, which was consistent with previous results (Ahmad et al. 2010, Lees et al. 2016, Wu et al. 2019, Zhou et al. 2017). The result also suggests different domestication routes for rapeseed in southern and northern China. The high diversity of the southern maintainer population agrees with their provincial origins, whereas the relative low diversity of the northern population might be the result of lower number and less exchanges in the landraces derived from their ancestry—"Yuejin youcai".

Based on the results of this study and experience accumulated in 30 years of hybrid breeding, we proposed three major patterns for selecting parental combinations with significant heterosis: (1) southern maintainer × northern restorer, (2) maintainer from the upper reaches of the Yangtze River (most are yellow-seeded) × maintainer from the lower reaches of the Yangtze River and (3) maintainer from the middle reaches of Yangtze River × maintainer from the lower reaches of the Yangtze River. Pattern I has been widely used in China successfully, for example, the famous hybrid varieties Qinyou7, Zhongyouza2 and Zhongyouza11. The other two patterns can be achieved by using recessive GMS or CIMS in the female parents.

In addition, genetic divergence was present between European winter and Chinese semi-winter populations because of their extreme geographic distance. Some intergroup crosses among winter, semi-winter and spring rapeseed are productive (Brandle and McVetty 1990; Kebede et al. 2010; Qian et al. 2007; Werner et al. 2018), just as Ramiro × YC4 and SP2S × 2006C did in this study. Notwithstanding, environmental adaptation will be a critical criterion for selecting these combinations and a limited factor for good hybrid performances, other than MPH or HPH heterosis, because most European winter populations do not carry sufficiently favourable alleles, especially earlier maturity, for adaptation to the climate conditions in China.

Conclusion

The 169 Chinese modern rapeseed breeding materials had moderate genetic diversity and they were clustered into ten or eleven major groups by geographical origin or by ecological type, restorer/maintainer lines and intermediate type. The intergroup crosses showed higher heterosis than intragroup crosses. GD measure is significantly correlated to heterosis level when the intragroup parental combinations with short GD were included in all regressing data. The major heterotic patterns among northern restorers and southern maintainers from the upper, middle and lower reaches of the Yangtze River have been identified. The results are instructive for modern rapeseed breeding by provision of a reference for selecting parental combination and exploiting the heterosis potential.

Availability of supporting data All supporting data are included within the article and its additional files.

Authors' contributions CYY conceived and designed the experiments and prepared the manuscript; QG, JLL and XRL carried out the experiments and ZH, AXX and JGD coordinated the study. All authors drafted and approved the final manuscript.

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Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Abbreviations *AAS,* Academy of Agriculture Science; *CMS,* cytoplasmic male sterility; *GD,* genetic distance; *GMS,* genic male sterility; *HAU,* Huazhong Agriculture University; *HRRC,* Hybrid Rapeseed Research Centre; *NWAFU,* Northwest A&F University; *OCH,* over-control heterosis; *ORI,* Oil Crop Research Institute; *MPH,* mid-parent heterosis; *PCoA,* principal coordinates analysis; *HPH,* high-parent heterosis; *PH,* plant height; *SCA,* specific combining ability; *SCI,* second cycle line; *SSR,* simple sequence repeat

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