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Efficient marker-assisted breeding for clubroot resistance in elite Pol-CMS rapeseed varieties by updating the *PbBa8.1* locus

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Abstract Clubroot disease poses a severe threat to rapeseed (*Brassica napus*) production worldwide and has recently been spreading across China at an unprecedented pace. Breeding and cultivation of resistant varieties constitute a promising and environment-friendly approach to mitigating this threat. In this study, the clubroot resistance locus *PbBa8.1* was successfully transferred into SC4, a shared paternal line of three elite varieties in five generations by marker-assisted backcross breeding. Kompetitive allele specific PCR (KASP) markers of clubroot resistance gene *PbBa8.1* and its linked high erucic acid gene (*FAE1*) were designed and applied for foreground selection, and 1,000 single-nucleotide polymorphisms (SNPs) were selected and used for the background selection. This breeding strategy produced recombinants with the highest recovery ratio of the recurrent parent genome (> 95%) at BC_2F_2 while breaking the linkage with *FAE1* during the selection. An updated version of the paternal line (SC4R) was generated at BC_2F_3, showing significantly improved clubroot resistance at the seedling stage via artificial inoculation, and was comparable to that of the donor parent. Field trials of the three elite varieties and their updated versions in five environments indicated similar agronomic appearance and final yield. The introduced breeding strategy precisely pyramids the *PbBa8.1* and *FAE1* loci with the assistance of technical markers in a shorter period and could be applied to other desirable traits for directional improvement in the future.

Keywords *Brassica napus* · Clubroot resistance · Backcross breeding · Marker-assisted selection · KASP platform

Yiming Guo and Bao Li contributed equally to this work.

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Introduction

Rapeseed is a globally cultivated commercial crop, primarily grown for its oil. Clubroot disease (*Plasmodiophora brassicae*) is an increasing pathogenic threat to rapeseed (*Brassica napus*) plantations

worldwide. Outbreaks of this disease have been frequently reported in *Brassica* growing areas. Recently, more than 20% total yield loss has been reported in highly infected fields of Canada, China, India, Europe, and Australia (Bhattacharya et al. 2014; Chai et al. 2014; Donald and Porter 2014; Rahman et al. 2014; Wallenhammar et al. 2014). In China, mechanized agricultural production across rapeseed growing regions has accelerated the spread of clubroot. Affected areas have expanded to Sichuan, Hubei, Hunan, Yunnan, and Anhui provinces, increasing by approximately 6.67 Mhm^2 in a short period of time and causing heavy yield loss or no harvest at all in growing regions (Chai et al. 2014; Wang et al. 2021).

Seedling transplantation, crop rotation, late seed sowing, biological prevention, and application of resistant varieties are technical approaches to clubroot disease prevention used by the rapeseed industry (Niwa et al. 2007; Pang et al. 2014). However, the resting spores of *P. brassicae* can survive for over 20 years in the soil, making it difficult to control the infection using cultural or chemical approaches (Donald et al. 2001). Cultivation of resistant cultivars is a more reliable, low-cost, environmentally sustainable, and effective approach to limiting disease spread (Pang et al. 2018). Further, the utilization of clubroot resistance (CR) genes is the premise of disease resistance breeding. More than 26 CR genes have been identified and located on chromosomes A01, A02, A03, A06, and A08 of the A genome in European turnips and Chinese cabbage (Chen et al. 2013; Hirani et al. 2018; Pang et al. 2018; Huang et al. 2019; Karim et al. 2020). The resistance genes *CRa* and *Crr1a* have been cloned, are known to encode TIR-NBS-LRR (Toll-interleukin-1 receptor-like domain-nucleotide binding site-leucine-rich repeat) proteins, and share the same locus as *CRb* (Zhang et al. 2014; Hatakeyama et al. 2017; Shah et al. 2019). In *B. rapa*, the resistance genes *CRk*, *Crr3*, *PbBa3.3*, and *PbBa3.2* are clustered in the same proximal region of chromosome A03 as *CRa*; however, it remains unclear if they represent a single or multiple genes (Hirai et al. 2004; Piao et al. 2004; Sakamoto et al. 2008; Chen et al. 2013). Additionally, *Crr1*, *Rcr3*, and *PbBa8.1* have been identified in chromosome A08 from European folder turnips (Matsumoto et al. 1998; Suwabe et al. 2003, 2006; Pang et al. 2014).

Most of these genes were from different genetic resources and were associated with distinct *P. brassicae* pathotypes. *PbBa8.1* was highly resistant to race 4, the most widespread group of *P. brassicae*, especially in China's main rapeseed production area (Chen et al. 2013). When crossed with the bridging line ECD04, the clubroot resistant (CR) locus *PbBa8.1* was successfully transferred into the elite conventional variety Huashuang5 (HS5), locating the gene within a 2.9 Mb region on chromosome A08, resulting in development of the ZHE226 line (Zhan et al. 2017, 2020). However, the seeds of the improved CR homozygous line contained high levels of erucic acid (approximately 18%) which is associated with potential health risks when ingested by humans and some animals. Therefore, selecting CR materials with low erucic acid content is crucial for rapeseed breeding programs (Tian et al. 2018). The erucic acid content determining gene *FAE1* (fatty acid elongase 1) extends fatty acid chain length from C18 to C20 and C22 in *B. napus* (Han et al. 2001). It has been identified as the main gene that catalyzes the condensation step in the elongation of very long chain fatty acids, resulting in the accumulation of erucic acid (Sun et al. 2013). A close linkage between *PbBa8.1* and *FAE1* was revealed, and a co-segregating SSR marker CAP-134 has been designed which has the potential to assist in breaking the linkage drag between *PbBa8.1* and *FAE1* (Zhan et al. 2020).

These CR genes and their closely linked markers have greatly improved CR breeding through marker-assisted selection (MAS) in *Brassica* crops. Molecular markers have been developed from AFLP, RFLP, RAPD, SSR, InDel, and single-nucleotide polymorphism (SNP) markers (Lander and Botstein 1989; Lynch and Milligan 1994; Vos et al. 1995; Rafalski 2002; Qu and Liu 2013; Liu et al. 2015). Based on advanced SNP genotyping, Kompetitive Allele-Specific Polymerase Chain Reaction (KASP) has emerged as an accurate, cost-effective, and high-throughput SNP genotyping method (Pabinger et al. 2014) which has already been applied to MAS in wheat (Rasheed et al. 2016; Kaur et al. 2020), lentils (Wang et al. 2020a, b, c), maize (Chen et al. 2021), and rice (Feng et al. 2019; Cheon et al. 2020). However, MAS using DNA markers may result in the loss of desirable traits, or a gain in unwanted genes; therefore, clear genetic background selection is required during MAS to preserve original traits. Recently, the Bnapus50K array was developed specifically for *B. napus*. It provides more accurate and high-density

SNPs and can be used for genomic background selection for *B. napus* breeding (Xiao et al. 2021).

In this study, the CR gene *PbBa8.1* from the ZHE226 line was successfully transferred into a polima cytoplasmic male sterility (Pol-CMS) paternal line (SC4) through backcross breeding combining KASP markers and a 1,000 SNP panel. The breeding goal was achieved within five generations and produced an improved paternal line (SC4R), as well as three updated hybrid accessions (Fengyou 306R (FY306R); Fengyou 737R (FY737R); and Fengyou 792R (FY792R)) by crossing corresponding maternal lines. The CR level of SC4R was validated, and the agronomic performance of previous and updated varieties were evaluated and compared to provide resistant varieties for rapeseed production in disease affected areas. Additionally, a modified MAS-based breeding strategy was presented as a reference for selecting other traits in the future.

Materials and methods

Plant materials

The CR gene donor parent ZHE226 (provided by Prof. Chunyu Zhang, Huazhong Agricultural University) contained the resistance locus *PbBa8.1*, which had been transferred from an ECD04 donor into the elite rapeseed conventional variety HS5 following the method described by Zhan et al. (2020).

F1 plants were generated by crossing ZHE226 and SC4, the recurrent parent, which is a restoring line for Pol-CMS. A series of selfing or segregating populations were produced in the fields of Changsha (28.48 N, 113.36E) and Xining (36.57 N, 101.75E), as well as in a controlled environmental room from 2019 to 2021. Field evaluations of agronomic traits and yield were conducted at five different locations in Hunan Province, China (Fig. 1).

Genotyping and design of specific KASP markers

Seedlings from BC_1F_1 populations were subjected to foreground and background selection before further backcrossing, while seedlings from BC_2F_1 were subjected to foreground and recombinant selection (Fig. 1). The SNP panel used for background selection was designed based on the SNP results for SC4 and ZHE226 from the Bnapus50K array (Xiao et al. 2021). First, 5,374 out of 42,090 SNPs were initially selected according to their chromosomal distribution, which were then designed as markers for amplification, and a total of 2,426 SNPs could be designed and validated for markers. Second, 1,000 pairs of SNP markers were manually selected for background screening for even distribution in the chromosomes as much as possible. Finally, a total of 330 SNP markers showing polymorphisms between two parents were

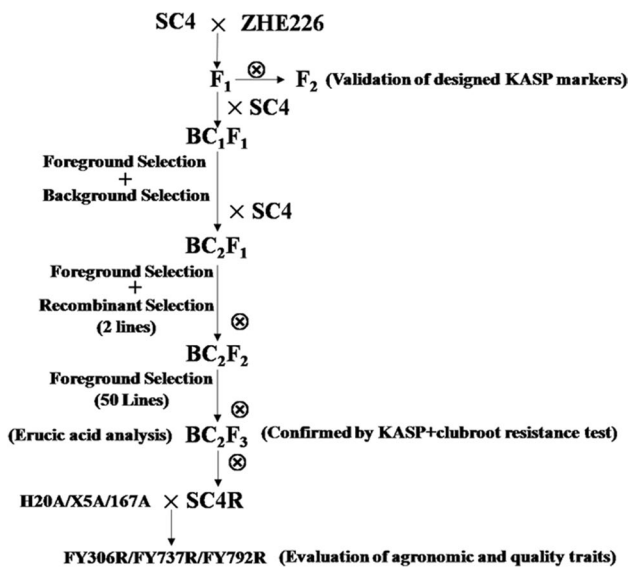

Fig. 1 Procedure for population development and selection scheme

chosen for background screening, with an average density of 1 SNP marker per 3 Mb (Fig. S1).

The ratio of the recurrent parent genome was calculated based on the screening results of the 330 SNP loci at BC_1F_1 population. The recovery ratio (%) = No. of SNPs from recurrent parent (SC4)/ Total screened SNPs × 100%.

Foreground selection included a fertility gene (restorer gene of Pol-CMS, *Rfp*), CR gene (*PbBa8.1* locus), and *FAE1*. Specific KASP markers for each gene were designed for SNPs around the coding region. KASP markers for *Rfp* were designed around the flanking sequences of *Rfp* with 1953 bp coding region about 29.2 K of A9 from *B. rapa* genome (Liu et al. 2012, 2016). They were designed and validated in Pol-CMS hybrid purity identification and published as technical regulations in Hunan Province Wang et al. 2020a, c).

KASP markers for *PbBa8.1* and *FAE1* were designed based on the polymorphic SNPs between HS5R and SC4, which were co-segregated with the previously reported closely linked marker CAP-134 (Zhan et al. 2020). In total, 14 SNP polymorphic markers around the position of CAP-134 (physical location: chrA08, 9.5 Mb) were obtained and used to design KASP markers for CR and *FAE1* selection (Table S1). Three SNPs were selected and designed for KASP markers based on the sequences and applicable conditions and are highlighted in Table S1.

Plasmodiophora brassicae inoculation and resistance tests

The clubroot pathogen *P. brassicae* was isolated from a seriously infected *B. napus* plant collected from Xupu, Hunan Province, and was identified as race 4 using the Williams classification (Williams 1966). For the CR test, four steps were followed:

a) Seedling preparation for inoculation: The seeds of SC4, BC_2F_3-L49, BC_2F_3-L59, and ZHE226 (control) were sown in nursery trays (2 cm × 2 cm per hole) using sterile soil, and the resulting seedlings were transferred into 10 cm × 10 cm × 9 cm plastic pots in a controlled environment room, at 20–25 °C room temperature and 70% relative humility, with an 18 h light period between 06:00 and 24:00. The plants were watered regularly to maintain soil moisture.

b) *Preparation of conidial suspension:* Approximately 50 g of disease-affected clubroots were smashed then filtered with four-layer gauze, centrifuged for 15 min at 2,500 g, precipitated twice using 30 ml sterile water after discarding the upper layer, then precipitated using 50% sucrose solution, and further centrifuged twice using sterile water before counting on a blood cell counting plate (Pang et al. 2014, 2018).

c) Inoculation to roots using conidial suspension: Prepared seedlings from step (a) were inoculated at the 4–5 leaf stage on July 8, 2021, and again on July 15, 2021, with a spore concentration of 1.59×10^8 and 2.12×10^8 per ml, respectively. Seedlings were in separate pots to avoid cross infection, and 20 ml of conidial suspension was dripped onto roots using a pipette.

d) Infection investigation: The seedling infection investigation was conducted 50 days post-inoculation (dpi). The roots were washed carefully and examined for *P. brassicae* infection; the number of plants at different infection levels was recorded; and the disease rate and index are calculated using the following equations:

$$\text{Disease rate} = \text{No of infected plants}/\text{No of total plants} \times 100\%$$

$$\text{Disease index (DI)} = \frac{\sum(\text{No of infected plants} \times \text{Identified level})}{\text{No of the highest level} \times \text{No of total plants}} \times 100\%$$

The classification of different levels of *P. brassicae* disease at seedling stage followed the criteria established by the Ministry of Agriculture for Brassicaceae crops as follows:

Level 0: no tumor at roots
Level 1: small tumor at lateral roots
Level 3: enlarged tumor at the main root with diameter less than 2 times that of the basal part of stem
Level 5: enlarged tumor at the main root with diameter 2–3 times that of the basal part of stem
Level 7: enlarged tumor at the main root with diameter 3–4 times that of the basal part of stem
Level 9: enlarged tumor at the main root with diameter more than 4 times that of the basal part of stem or turning black at the enlarged root

Evaluation of agronomic traits and erucic acid in seeds

The improved restorer SC4R was crossed with the corresponding maternal lines of FY306, FY737, and FY792 (H20A, X5A, and 167A) to produce the updated hybrid varieties FY306R, FY737R, and FY792R, respectively (Fig. 1).

Six accessions (FY792, FY792R, FY737, FY737R, FY306, and FY306R) were used to evaluate agronomic traits in fields from five environments in Hunan Province: Changsha (28.48 N, 113.36E), Changde (29.05 N, 111.69E), Hengyang (26.98 N, 112.39E,), Huaihua (27.52 N, 109.95E), and Jishou (28.3 N, 109.71E). The field experiments followed a randomized complete block design with three replicates (10 m long × 2 m wide for each plot). Between 25 and 27 plants were kept in each row with 33 cm between rows. Field management followed the regular methods of the China National Field Trials.

At harvest time, 10 representative plants from the middle of each plot were selected randomly for evaluation of agronomic traits, ensuring that each plant had normal growth vigor. Plant height, branch number, main inflorescence length, plant silique number, seed number per pod, 1000-seed weight, and total seed yield were measured and calculated for comparison. Quality traits such as oil, glucosinolate, and erucic acid content were tested using a near-infrared spectrometer (DS 2500F, Foss NIRSystems Inc., Denmark) with dried clean seeds (water content < 9%).

Microscopic investigation and data analysis

Following the examination of the *P. brassicae* infection during the resistance test, the roots from SC4, BC_2F_3-L49, and BC_2F_3-L59 were kept for microscopic investigation. Epidermal tissues from the healthy or enlarged tumor roots were repeatedly scraped into a drop of water on a clean microscope slide using a sharp blade. The tissue samples were covered with a coverslip and then examined under a microscope (DS-Ril, Nikon, Japan).

Agronomic trait data from 10 sample plants from five environments and quality trait data from five environments were analyzed using Microsoft Excel.

Results

Validation of specific KASP markers for PbBa8.1 and FAE1

Based on the genomic information of the previously reported SSR marker, CAP-134, which was co-segregated with *PbBa8.1* and *FAE1*, three KASP markers were designed for *PbBa8.1* and *FAE1*. They were SNP1 (seq-new-rs36374), SNP2 (seq-new-rs42497), and SNP3 (BN-A08-p11380260), which were approximately 130, 50, and 280 Kb away from CAP-134 and named BN900051, BN900052, and BN900053, respectively. BN900051 and BN900053 were used for selection of the CR gene *PbBa8.1*, and BN900052 was used for the selection of the *FAE1* gene. The sequences of all the applied KASP markers in this study are presented in Table S2.

A total of 188 plants from the F_2 population from crosses between ZHE226 and SC4 were used to validate the three KASP markers and showed consistent results as the previously reported SSR marker CAP-134 and well-distinguished in the genotyping diagram by the KASP platform (Fig. 2). Based on the results from BN900051 and BN900053, 132 plants were identified as clubroot-resistant (*PbBa8.1*/-), of which 44 were homologous at the locus (*PbBa8.1*/*PbBa8.1*). Two recombinants of the locus between *PbBa8.1* and *FAE1* were identified (*PbBa8.1*/*FAE1*) using BN900052. The erucic acid content of selfing seeds from the two recombinants varied between 0.34 and 0.56% (undetectable), while the erucic acid value varied between 16.25 and 18.95% for the non-recombinant lines (Table S3).

Breeding strategies for obtaining CR and minimizing erucic acid content

In the BC_1F_1 population, 731 plants were subjected to foreground selection, 179 containing heterozygous *PbBa8.1* (*PbBa8.1*/*pbBa8.1*) and homozygous *Rfp* (*Rfp*/*Rfp*) loci, and were used for genetic background analysis. The recovery ratio of the recurrent parent genome ranged from 64.7 to 88.5%, with an average of 75.0% (Fig. 3a). The two plants with the highest recovery ratio of the recurrent parent genome (> 87%) were used for further backcrossing with SC4 to produce BC_2F_1 (Fig. 1; Fig. 3b). For the BC_2F_1 generation, foreground selection and recombinant

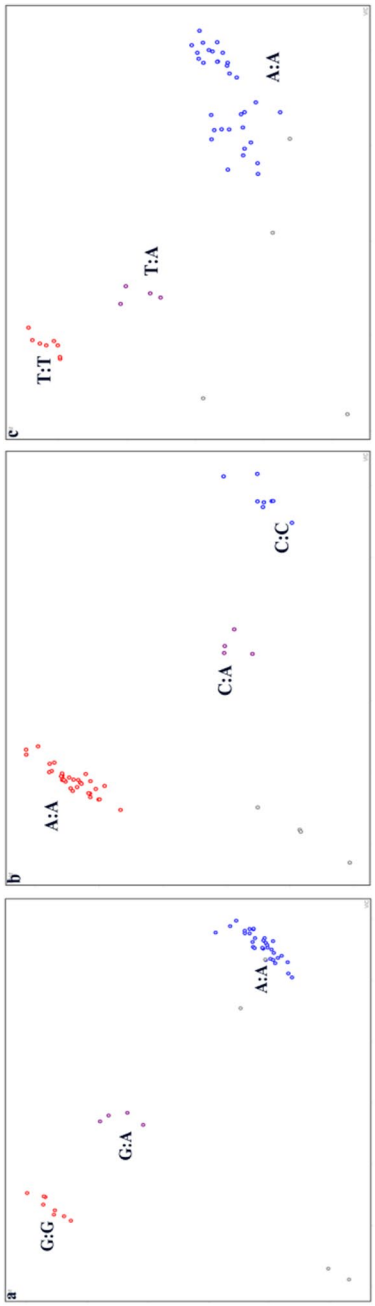

Fig. 2 Genotyping diagram of BN900051 (**a**), BN900053 (**b**), and BN900052 (**c**). In BN900051, G:G, A:A, and G:A represent clubroot resistant, sensitive, and hybrid genotypes, respectively, while C:C, A:A, and C:A represent the corresponding genotypes in BN900053, respectively. In BN900052, T:T, A:A, and T:A represent high erucic acid, low erucic acid, and hybrid genotypes, respectively

selection were applied to 198 plants, identifying 2 recombinants with a heterozygous *PbBa8.1* locus (*PbBa8.1*/*pbBa8.1*) and homozygous recessive *BnA.FAE1* locus (*fae1*/*fae1*), which were used for selfing to produce BC_2F_2. In the two populations of BC_2F_2, 50 of 224 plants contained the homozygous *PbBa8.1* locus (*PbBa8.1*/*PbBa8.1*); these were subjected to further selfing to BC_2F_3, before testing for low erucic acid and CR.

CR test of improved lines at BC_2F_3 generation, and SC4 at seedling stage

At 50 dpi, seedlings from two improved BC_2F_3 lines (BC_2F_3-L49; BC_2F_3-L59), SC4, and ZHE226 were examined for disease infection. Infections occurred in all four lines, with a 100% infection rate for SC4 and 5.88%, 7.70%, and 2.17% for BC_2F_3-L49, BC_2F_3-L59, and ZHE226, respectively. The disease indices were calculated as 61.90 for susceptible SC4, 1.96 for BC_2F_3-L49, 2.56 for BC_2F_3-L59, and 1.40 for ZHE226. There were no significant differences between the two BC_2F_3 lines and the ZHE226 control (Table 1).

Microscopic and phenotypic examination of all the tested lines (Fig. 4) found disease introgression into the roots of SC4 (Fig. 4a) and clearer root cells in the BC_2F_3 improved lines (Fig. 4b,c), as well as the corresponding enlarged and healthy roots (Fig. 4d). As a result, the improved lines of BC_2F_3-L49 and BC_2F_3-L59 had significantly improved CR compared to the original SC4, with comparable resistance levels to the donor parent ZHE226.

Evaluation of agronomic performance between FY306, FY737, FY792 and their corresponding updated version (FY306R, FY737R, FY792R)

The updated versions of the three hybrids were produced by crossing SC4R with their corresponding maternal lines (H20A, X5A, and 167A). Field trials were conducted in five locations in Hunan Province to determine whether the updated version retained the original characters. Plant height, branch number, main inflorescence length, total pod number, seed number per pod, 1000-seed weight, seed yield, oil content, glucosinolate, and erucic acid content were measured and evaluated across five environments. The average value of each trait is presented in Table 2.

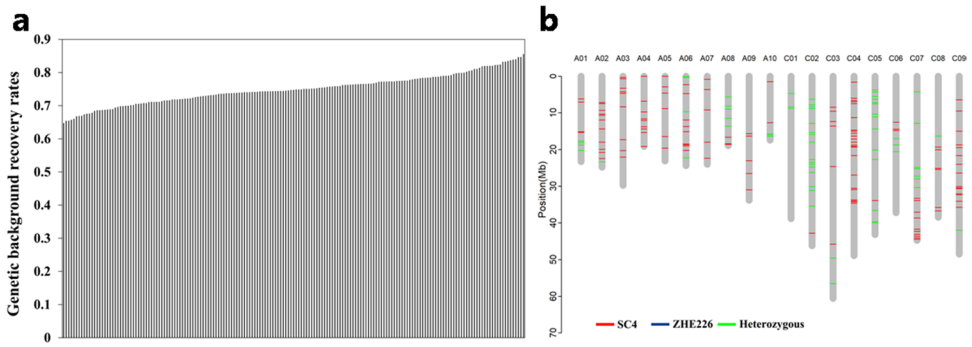

Fig. 3 **a** Distribution of the recovery ratio of recurrent parent genome at BC_1F_1 generation and **b** the SNP sources of the 330 polymorphic loci from different parents at the individual with the highest recovery ratio. The red, blue, and green bars represent the genotypic resources from SC4, ZHE226, and heterozygous in the BC_1F_1, respectively

Table 1 Survey of *Plasmodiophora brassicae* inoculation in BC_2F_3 lines and ZHE226 at seedling stage

Tested lines	Disease infection rate (%)	Disease index
BC_2F_3-L49	5.88	1.96
BC_2F_3-L59	7.70	2.56
SC4	100	61.90
ZHE226	2.17	1.40

Student's *t*-test between the three varieties and their updated version showed no differences for any of the agronomic traits nor the quality traits, except for the most variable plant pod number, indicating that the updated version was similar to the original version.

Discussion

An increasing number of clubroot disease outbreaks in rapeseed growing areas have caused severe yield loss, resulting in urgent demand from growers for updated CR varieties with local adaptations to control the threat (Pang et al. 2018). Pol-CMS derived hybrids are the dominant varieties and transfer CR genes into the parental lines, and the corresponding hybrids are promising approaches to limit the threat of disease. Compared to sterile maternal lines, paternal lines with good combining ability are shared among hybrids; therefore, the common recovery line of three local varieties (FY306, FY737, and FY792) was chosen as the recurrent parent in the breeding program.

The donor parent ZHE226 is an earlier version of Huashuang5R (HS5R) and shares the same CR locus as HS5R, which has been widely applied as a CR variety in China. Unlike HS5R, ZHE226 has a high erucic acid content due to the *FAE1* gene (Zhan et al. 2020). A combined KASP-assisted foreground selection and an SNP panel-assisted background selection strategy were applied to the breeding program, achieving the breeding goal within five generations. Ultimately, the homologous CR gene *PbBa8.1*, which was successfully transferred from ZHE226 to SC4, retained the original agronomic and quality traits of SC4 while improving CR to a level similar to that of ZHE226 (Fig. 1).

Efficient indoor selection by KASP and SNP panel based-genotyping

Backcrossing breeding is a traditional method of disease resistance breeding, transferring targeted R-genes from a donor parent into a recurrent parent by crossing and backcrossing (Swathi et al. 2019). It typically requires 7–8 generations, and field phenotypic selection during the process is often constrained by individual development, leading to low selection efficiency. In this study, in addition to the CR locus (*PbBa8.1*), the fertility gene of Pol-CMS (*Rfp*) and the high erucic acid determining gene *FAE1* were also target genes during selection, making it even harder to complete the breeding goal in the same period. MAS was required to shorten breeding time

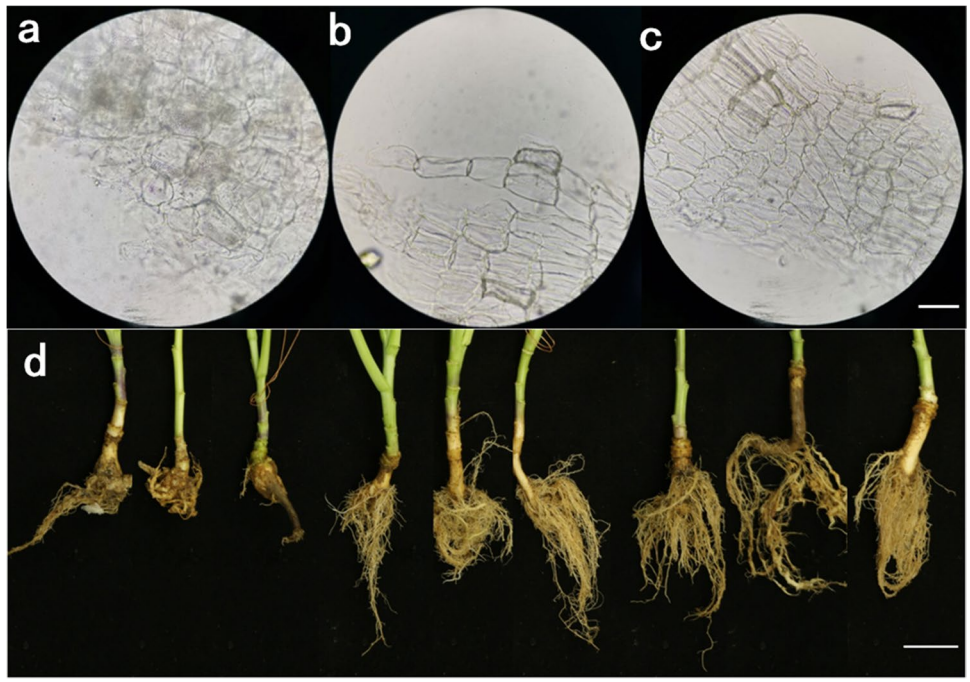

Fig. 4 Microscopic examination (scale bar: 10 μm) and their corresponding phenotypes (scale bar: 1 cm) of *Plasmodiophora brassicae* inoculated roots at 50 dpi for three tested lines: **a** SC4; **b** BC_2F_3-L49; **c** BC_2F_3-L59; and **d** the corresponding phenotypes of the above 3 lines with 3 individual plants per line

Table 2 Agronomic, yield, and quality performances of Fengyou 306, Fengyou 737, and Fengyou 792 and their improved versions Fengyou 306R, Fengyou 737R, and Fengyou 792R respectively in five environments in Hunan Province during 2020–2021

	FY306R	FY306	FY737R	FY737	FY792R	FY792
PH (cm)	182.4 ± 23.2	186.1 ± 19.6	168.6 ± 13.7	166 ± 15.6	180.7 ± 15.7	186.6 ± 20.7
BN	7.4 ± 2.0	7.0 ± 1.3	7.6 ± 1.5	7.9 ± 1.7	7.5 ± 1.5	7.5 ± 1.5
MIL (cm)	58.3 ± 9.4	58.8 ± 7	58 ± 10	61.7 ± 11.5	56.8 ± 8	59.4 ± 7.5*
PN	309.7 ± 129.7	309.4 ± 116.2	304.1 ± 107.5	378.3 ± 144.1*	257.4 ± 84.9	299.3 ± 99.8**
SN	19.4 ± 5.1	19.9 ± 4.9	20.4 ± 5.3	21.0 ± 4.1	21.7 ± 3.6	21.0 ± 4.3
SW (g)	3.6 ± 0.5	3.5 ± 0.5	3.6 ± 0.4	3.5 ± 0.6	3.5 ± 0.4	3.5 ± 0.4
SY (g)	17.7 ± 7.5	17.6 ± 7.1	19.2 ± 6.9	21.4 ± 6.7	16.2 ± 6.4	18.1 ± 7.7
OC (%)	45.3 ± 2.4	44.1 ± 2.1	43.8 ± 3.8	44.4 ± 2.3	42.7 ± 1.7	42.5 ± 1.2
GC (μmol/g)	30.4 ± 2.6	28.3 ± 4.1	22.3 ± 1.4	23.4 ± 2.0	25.2 ± 4.2	26.9 ± 4.1
EA (%)	0.06 ± 0.01	0.14 ± 0.03	0.00 ± 0	0.09 ± 0.01	0.08 ± 0.02	0.15 ± 0.03

Agronomic trait data presented as the means ± standard deviations (SD) were obtained from 10 plants with three replicates under natural conditions in Changsha, Hengyang, Changde, Jishou, and Huaihua, while quality trait data represent the means ± SD from five environments

PH, plant height; BN, branch number; MIL, main inflorescence length; PN, plant pod number; SN, seed number per pod; SW, 1,000-seed weight; SY, seed yield; OC, oil content, GC, glucosinolate content; EA, erucic acid

* and ** represent significant differences at $p < 0.05$ and $p < 0.01$ respectively based on Student's *t*-test

and co-dominant SSR markers for those loci that had been previously developed (Liu et al. 2016; Wang et al. 2020a, b, c; Zhan et al. 2020). Nevertheless, standard procedures using SSR and running agarose gel for gene validation are labor-intensive and time consuming, not accessible for the current breeding scale, and require a large population to screen three genes and the recombinant individual between *PbBa8.1* and *FAE1* loci because they were closely linked (Zhan et al. 2020). Compared to SSR and other molecular markers, KASP can complete genotyping in 96-well or 384-well plates simultaneously and distinguish the allele sources accurately; moreover, it is very flexible and can genotype sequences containing multiple SNPs if mixed bases are added to the primer sequences (Patterson et al. 2017). Therefore, in this study, the SNP-based KASP platform enabled utilization of polymorphic SNPs near the targeted genes and development of five specific KASP markers for high-throughput screening of the BC_1F_1 and BC_2F_1 generations (Tables S1 and S2).

High recovery ratio of recurrent parent genome to shorten the backcrossing cycle

Traditional MAS may result in the introduction of undesirable genes or loss of desirable traits with unclear genetic backgrounds. In addition to foreground selection, background selection is essential as it is more accurate and efficient (Feng et al. 2019). Recently, a Bnapus50K array that provides precise genomic information on Chinese local accessions of *B. napus* was developed (Xiao et al. 2021). The recovery ratio of the recurrent parent genome was the key value required prior to further backcrossing or selfing. In this study, 330 polymorphic SNPs widely distributed across the 19 chromosomes of *B. napus* were used to identify the recovery ratio compared to the SC4 genome at the BC_1F_1 generation; the values ranged from 64.7% to 88.5% for 179 individual plants, with the average at 75% (Fig. 3a). Two plants that had the highest recovery ratio of the recurrent parent genome (> 87%) were used for further backcrossing with SC4 to produce BC_2F_1 (Fig. 3b), making us confident in obtaining a > 95% recovery ratio for the BC_2 generation. Moreover, recombinant selection between *PbBa8.1* and *FAE1* was possible at BC_2, which is earlier than at BC_4 as reported by Zhan et al. (2020), and is consistent with their discussion.

Similar phenotypic and yield performance and necessity of pyramiding more CR genes

FY306, FY737, and FY792 are the representative local varieties, which are Pol-CMS hybrids and share the same parental line as SC4. Within five generations, an updated version of SC4 was produced (SC4R), as well as the corresponding hybrid varieties (FY306R, FY737R, and FY792R). The field trials of the updated hybrid varieties in the five environments showed that phenotypic and yield-quality performance was consistent with previous versions, and the artificial inoculation during CR testing led to confidence in the next stage of resistance testing in the field.

Large-scale cultivation of CR-improved local varieties tends to be the main approach to control loss caused by soil-borne root diseases in the rapeseed industry. In the long term, and with the development of mechanized production in China and other countries, the spread of *P. brassicae* spores will continue, resulting in different pathotypes with a wider distribution. Therefore, pyramiding different sources of CR genes into one variety is necessary to provide alternative durable resistance to a broader spectrum of pathotypes. Following a similar strategy, introducing other CR genes into maternal lines and obtaining combined CR genes in hybrid varieties would be accessible and efficient strategies for future CR breeding.

In conclusion, the breeding strategy introduced here combined specifically designed KASP markers and a 1000 + SNP panel for foreground selection, recombinant selection, and background selection to precisely introduced a CR gene (*PbBa8.1*) into a Pol-CMS recovery line within five generations while retaining the original phenotypic traits of the recurrent parent. The demonstrated process not only provides accessible CR-improved varieties for disease-infected rapeseed growing regions, but also sets a model reference for efficient breeding for more traits in the future.

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Author contribution Yiming Guo and Bao Li conducted the experiments and drafted the manuscript. Mei Li, Liang Qu, and Lianyi Fan were involved in the agronomic evaluation of the previous and updated varieties. Qian Yang took part in data analysis and plotting. Hongjian Zhu and Xinhong Liu participated in the artificial inoculation of seedlings. Tonghua Wang designed the experiment and had oversight of the entire process. All authors read and approved the final manuscript.

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Data availability Additional datasets generated by this study are included in the Supplementary Materials.

Declarations

Ethics approval and consent to participate Not applicable.

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Competing interests The authors declare no competing interests.

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