The breeding effects and genetic mysteries of a milestone variety (Fengbazhan) of modern *indica* rice in China

Zhao lei

Rice Research Institute of Guangdong Academy of Agricultural Sciences.

Zhou Shaochuan (✉ xxs123@163.com)

Rice Research Institute of Guangdong Academy of Agricultural Sciences.

Wang Chongrong

Rice Research Institute of Guangdong Academy of Agricultural Sciences.

Li Hong

Rice Research Institute of Guangdong Academy of Agricultural Sciences.

Huang Daoqiang

Rice Research Institute of Guangdong Academy of Agricultural Sciences.

Wang Zhidong

Rice Research Institute of Guangdong Academy of Agricultural Sciences.

Zhou Degui

Rice Research Institute of Guangdong Academy of Agricultural Sciences.

Chen Yibo

Rice Research Institute of Guangdong Academy of Agricultural Sciences.

Gong Rong

Rice Research Institute of Guangdong Academy of Agricultural Sciences.

Pan Yangyang

Rice Research Institute of Guangdong Academy of Agricultural Sciences.

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Abstract

Huanghuazhan and Huazhan are the most widely used *indica* conventional rice and restorer line in China, respectively. In fact, both of them are all derivatives of Fengbazhan (FBZ), and many other FBZ-derived varieties have been widely planted in China, accounting 22% of China's rice paddy fields. Therefore, evaluating the breeding effects of FBZ and clarifying its genetic mysteries is an effective strategy to interpret the history of modern rice breeding. Here, we report FBZ could be regarded as a milestone for the renewal of China's modern *indica* rice. By comparing the variant information of genes that underlie quantitative traits (QTGs), we identified 48 QTGs showing allelic differences among different types of varieties. Further research found that favorable genes were gradually optimized during the breeding of the FBZ-series varieties, while the selection and utilization of *Hd1-Pi2-Pid3-Pid2* segment, directly contributed to the breeding of FBZ. Importantly, the application of favorable genes on chromosome 6 solves the problem of 'high-quality but susceptible to rice blast', enabling the FBZ-series varieties to achieve high and stable yield, good quality, and wide adaptability in production. We put forward thinking and insights from the perspectives of fertility restoration, defense and growth, germplasm resources, variety replacement and future breeding goals. Our study provides useful gene targets for molecular breeding, as well as a valuable reference for the development of breakthrough varieties.

Background

Rice is the staple food of an estimated 3.5 billion people worldwide. For low-income groups in many developing countries in Asia and Africa, rice not only provides the dietary caloric supply and a substantial part of the protein intake, but also provides a source of income and employment for more than 200 million households (Muthayya et al. 2014). Asian rice, *Oryza sativa*, is one of world's oldest and most important crop species, especially in east, southeast, and south Asia. Asian rice consists of two major subspecies, namely, *japonica* (*Geng*) and *indica* (*Xian*) (Wang et al. 2018b). The distribution of rice is mainly affected by water resources, the length of daylight, and the temperature. In general, *japonica* rice is mainly distributed in high latitude or high altitude areas, including Northeast China, Northwest China, Yunnan-Guizhou Plateau, Japan, South Korea, North Korea, etc. *Indica* rice is mainly distributed in low-latitude regions, including Southern China, South Asia and Southeast Asia. At present, many rice varieties with regional characteristics have emerged in various parts of Asia. IR64 was widely planted in South and Southeast Asian countries in the 1990s and 2000s. Indonesia was a major producer of IR64, which was grown on more than 40% of its total area for around a decade (Mackill and Khush 2018). Basmati has been cultivated for centuries at the foot of Himalayan mountain ranges. As a landmark Basmati rice variety, Pusa Basmati 1121 is grown in about 70% of the total area under Basmati rice cultivation in India (Singh et al. 2018). In Thailand, KDML105 and its mutagenized counterparts, RD15 and RD6, have been widely planted on 70% of its rice area (Vanavichit et al. 2018). In Japan, Koshihikari has been the most popular rice variety since 1979. The seven top-ranked cultivars after Koshihikari are also descendants of Koshihikari, accounting for 72.5% of the total rice production area in Japan (Kobayashi et al. 2018).

For several decades, tremendous efforts have been made by Chinese scientists in rice breeding in improving grain yield, nutrition quality, and environmental performance, achieving substantial progress for global food security. Several generations of rice breeding technologies have been developed, for example, semi-dwarf breeding, utilization of heterosis, launching ‘Super Rice Project’, developing green super rice and molecular design breeding (Qian et al. 2016; Bai et al. 2018; Tang and Cheng 2018; Yu et al. 2022). In Southern China, rice can be divided into early rice, middle rice and late rice, the rice cultivation in this region accounts for about 80% of China’s rice planting area. Currently, several distinctive rice varieties have been widely planted in Southern China. According to the 2019 statistics of National Agricultural Technology Extension Service Center (NATESC), *Indica* conventional rice is mainly planted in the early season, the representative varieties were Zhongjiazao17, Xiangzaoxian45 and Zhongzao39. In mid-season and late-season, both conventional *indica* rice and hybrid rice are widely planted. Among them, Huanghuazhan is the conventional *indica* rice with the largest planting area, while Huazhan and Wushansimiao are the restorer lines with the largest planting area. In addition, studies have also shown that Huanghuazhan and Huazhan have become the main force in the replacement of rice varieties in China (Zhou et al. 2016; Bai et al. 2018; Zeng 2018; E et al. 2019; Fang et al. 2020; Zhang et al. 2021; Yu et al. 2022). In particular, Huanghuazhan, Huazhan and Wushansimiao have been extensively used for genetics and genomics studies, and there are currently more than 50 papers using them as research materials according to the NCBI literature search. However, few people know that Huanghuazhan, Huazhan and Wushansimiao are all derived from FBZ, which can be named as the FBZ-series varieties. As far as we know, breeders have also bred many other elite rice varieties using FBZ as parents, such as Yuenongsimiao, Yuejingsimiao2, Huiliangyou898 and Yexiangyoulisi. Among them, Yuejingsimiao2 is the control variety for the regional trials of rice varieties in Guangdong Province, Yexiangyoulisi is a hybrid rice variety known for its high yield and excellent rice quality. These FBZ-series varieties have been planted in large areas in Southern China, but systematic studies have not yet been reported.

With the advent of high-throughput sequencing technologies, thousands of rice genomes have been sequenced (Chen et al. 2021). However, there are still gaps between genomic studies and breeding practice, because these studies generally use landraces as research materials. Recently, (Lv et al. 2020) resequenced 1,143 *indica* accessions selected from the parents of superior hybrid rice cultivars. A large-scale genomic for a collection of 1,275 rice accessions that mainly includes Chinese rice cultivars and parental lines were also reported (Li et al. 2020). In addition, 20 and 99 varieties in the Huanghuazhan pedigree were selected for high-throughput sequencing and SNP-chip analysis, respectively (Zhou et al. 2016; Chen et al. 2017). More importantly, high-quality genome of Huazhan were also assembled recently (Zhang et al. 2021). In general, these studies provide valuable genomic resource of modern varieties, including three-line sterile lines, two-line sterile lines, elite semi-dwarf varieties, iconic restorer lines and FBZ-series varieties.

However, these studies did not pay attention to the genetic basis in the improvement process of modern varieties breeding. In our previous studies, through the integrated use of bin strategy, selective sweep analysis and GWAS, we successfully identified the conservative selection segment in Huanghuazhan’s

breeding process (Zhou et al. 2016; Chen et al. 2017). Nevertheless, our understanding of the genetic basis involved in the breeding process of FBZ-series varieties is still insufficient, mainly due to the following three reasons. (i) In recent years, dozens of conventional rice varieties have been derived from Huanghuazhan; (ii) Using FBZ as core germplasm, we have also bred other series of varieties, including the Huazhan series and the Wushan series; (iii) The molecular mechanism of the conservative selected segments cannot be well understood due to the lack of integration of available functional gene data. Recently, 348 causative variants in 225 QTGs were identified from 299 papers published from 1995 to 2020 (Wei et al. 2021). This integrated information can be regarded as a rice gene encyclopedia, and making it possible to interpret the molecular basis in the breeding process of FBZ-series varieties.

In this study, we systematically evaluated the breeding effects of FBZ, mainly based on planting area provided by the NATESC. By comparing the allelic differences of QTGs between FBZ-series varieties and other type of varieties, and analyzing the genetic pattern, gene effects and genes flow of key selected QTGs in FBZ-derived varieties, the genetic basis of artificial selection during breeding process of FBZ-series varieties could be exploited. In summary, this study provides a comprehensive assessment of the breeding effect of FBZ, and analyzes the key molecular basis of this iconic breeding event, which will benefit modern breeding.

Material And Methods

Evaluation of FBZ's breeding effect

The planting area of rice varieties was extracted from the 2019 statistical table of the main varieties of crops in the country compiled by NATESC. This statistical table announced the planting area of 274 conventional rice varieties and 449 hybrid rice varieties in various provinces in China. We performed a one-by-one search of the pedigrees of these varieties using the National Rice Data Center website, and we finally found that 89 FBZ-series varieties were planted in 2019 (Additional file 2: Table S4). We used the map package in pyecharts to draw the planting distribution map of FBZ-series varieties.

Extraction of variant information

We collected sequencing data from three sets of rice germplasms (Additional file 2: Table S5). The first one are mainly landraces (Zhao et al. 2015). The second set of rice accessions consisting of widely planted modern cultivars and parental hybrid rice lines from China (Li et al. 2020). The third set of rice accessions are mainly rice varieties included in the Fengbazhan pedigree (Zhou et al. 2016). The paired-end reads of the samples were aligned against to Nipponbare reference genome (IRGSP 1.0) with BWA men (default parameters) (Li et al. 2009). GATK (v4.0) best practice pipeline (McKenna et al. 2010) was used for genotyping of 222-nuclear genes that underlie quantitative traits (QTGs) (Wei et al. 2021). We successfully identified 250 causative variants (QTNs) corresponding to 181 QTGs. However, there exist 51 SVs (large indels, presence/absence and copy number variation) in the QTN library (Wei et al. 2021), and it was unrealistic to calling genotypes of SVs from low-coverage sequencing data. Therefore, we selected 2-3 single nucleotide polymorphisms (SNPs) which exhibit polymorphism in each uncovered

QTGs based on DNA chips (Chen et al, 2017). This allows us to extract another 168 SNPs corresponding to 64 QTGs. In total, variant information of 418 sites were extracted (Additional file 2: Table S6), which uncovered 98.6% of the nuclear QTGs.

Analysis of the inheritance pattern and gene effects of loci on chromosome 6

Our diverse collection of 176 rice accessions is comprised of 5 foreign *indica* rice, 29 landraces, 83 FBZ-series varieties, 54 modern *indica* rice and 5 *japonica* rice (Additional file 2: Table S7). The seeds of these accessions were sown in the field, and the 10-day-old leaves were used for DNA extraction. We screen polymorphic SNP sites based on gene chips (Chen et al. 2017) or nucleotide sequence alignment. The primer design and genotyping of PARMS marker are based on previous report (Lu et al. 2020). The primer list used in this study is provided in Additional file 2: Table S8. To assess the impact of important functional genes on agronomic traits, we extracted the amylose content, whole growth period and rice blast resistance data from variety certification announcement. Since the amylose content trait is less susceptible to environmental influences factors, data from different provinces were used for comparison. For whole growth period and rice blast resistance, to ensure data comparability, we only extracted data from the Guangdong Variety Registration Committee. Statistical significance was evaluated with an independent Student's *t*-test using SPSS software (version 22, IBM, SPSS, Chicago, IL).

Sequence alignment of key genes

We downloaded high-quality genome sequence of Huazhan from Genome Warehouse in National Genomics Data Center (Zhang et al. 2021). Notepad++ software was used to extract the key gene sequence of Huazhan. We used the Rice Information GateWay (RIGW) website to extract the gene sequences of Minghui63 and Zhenshan97 (Zhang et al. 2016). We extracted sequences of different haplotypes of *Waxy* gene from MBKbase database (Peng et al. 2020). Multiple Sequence alignment was performed using Vector NTI version 10 (Invitrogen).

Results

Breeding effects of FBZ

Figure 1 shows the pedigree of FBZ and its relatives. FBZ was bred by crossing two key parents, Fengaizhan1 and 28zhan. Fengaizhan1 is a semi-dwarf rice variety with multiple tillers, high yield and slender grains, but it has poor blast resistance and high amylose content. 28zhan is the first rice variety with excellent blast resistance and low amylose content, but its appearance quality and grain yield are not ideal. FBZ inherited the advantages of both parents, it was widely accepted as the first rice variety with excellent milling, appearance and cooking qualities, resistances to blast and high yield in rice breeding in Guangdong.

Using FBZ as the core germplasm, the first certified rice variety bred by our team was Fenghuazhan (Fig. 1). The appearance quality and taste quality of Fenghuazhan were significantly improved compared to

FBZ. We bred another elite rice variety Huanghuazhan, which was derived from the cross of Huangxinzhan × Fenghuazhan and it was released in 2005. Huanghuazhan is the most common inbred rice cultivar planted in central and south China and has been widely grown across 9 provinces with semi-dwarf, super high yield, good eating quality and wide adaptability (Zhou et al. 2016; Deng et al. 2019). The cultivated area of Huanghuazhan ranged from 91,000 to 306,000 ha in China, and it was increased to 400,000 ha since 2015 (Big Data Platform of National Seed Industry Website). In the past two years, Huanghuazhan has become the *indica* conventional rice variety with the largest annual planting area in China (NATESC 2019, 2020). Huanghuazhan has been grown in China on a cumulative 3.4 million hectares from 1982 to 2019, and is currently the high-quality *indica* conventional rice variety with the largest cumulative planting area in China (Additional file 1: Table S1).

Nevertheless, the blast resistance of Huanghuazhan was significantly reduced compared to FBZ, which may limit its application in high-risk areas of rice blast. To meet such demand, we developed two mediate materials Wufengzhan2 and Fengsizhan, which directly derived from FBZ. On this basis, we bred Huazhan, Wushansimiao, Huanglizhan and Huangyuesimiao (Fig. 1). Those four varieties has been proved to be elite restorer lines with good quality, high resistance to rice blast and excellent combining ability. It should be noted that the breeding and popularization of hybrid rice were mainly completed by our partners, for example, China National Rice Research Institute, Longping High-Tech and Quanyin High-Tech. Data collected from NATESC showed that Jingliangyou534 (restorer line: Wushansimiao) and Jingliangyouhuazhan (restorer line: Huazhan) were the two rice varieties with the largest annual promotion area of hybrid rice in 2019. Moreover, 13 of the top 20 hybrid rice varieties are all direct descendants of FBZ-series restorers (Additional file 1: Table S2).

China covers a wide range of latitude, from 3°51'N to 53°33'N, the *indica* rice is mainly distributed in south of Huai River–Qinling Mountain line, and accounts for about 2/3 of the total rice planting area. Fig. 2a shows that FBZ-series varieties have been widely planted in all *indica* rice growing areas of China. In 2019, 79 hybrid rice derived from FBZ-series restorers are promoted in production, with 3,806,000 ha accounting for 35% of the total hybrid rice area in the country (Fig. 2b). Besides hybrid rice, 10 conventional rice derived from FBZ were also widely cultivated. In general, varieties derived from FBZ account for 22% of the total rice area in China (Fig. 2c). Taken together, these results suggest that the development of FBZ could be regarded as a milestone for the renewal of China's modern *indica* rice with wide adaptability, good quality and high resistance to rice blast.

Genetic features in Chinese modern *indica* rice breeding

To uncover the genetic basis of artificial selection during modern *indica* rice breeding, we collected genomic data of 138 varieties. According to the breeding time and variety type, the 138 varieties were divided into five groups, including 31 landraces (GroupI), 24 landmark semi-dwarf varieties (GroupII), 29 iconic restorer lines/conventional rice (GroupIII), 18 FBZ-series varieties (GroupIV), 23 male sterile/maintainer lines (GroupV) and 13 world-renowned varieties (GroupVI) (Additional file 2: Table S5). It should be pointed out that most of the varieties selected in this study have been widely used in China,

especially these varieties in GroupII (landmark semi-dwarf varieties) and GroupIII (iconic restorer lines/conventional rice) can be regarded as elite rice varieties. By comparing the variant information of QTGs, we identified 48 QTGs showing allelic differences among different types of varieties (Fig. 3, Additional file 2: Table S6). Among them, 10 QTGs are differentially used between GroupI (landraces) and GroupII (landmark semi-dwarf varieties), 33 QTGs are under artificial selection between GroupII (semi-dwarf varieties) and GroupIII (iconic restorer lines/conventional rice), allele types of 4 QTGs are exclusively used in GroupIV (FBZ-series varieties) (Fig. 3). This indicated that 91.6% QTGs identified in our study have been selected before FBZ was bred, whereas only a few QTGs directly contributed to the breeding of FBZ-series varieties.

After analyzing the gene function, we found that resistance (4/10) and plant type (3/10) related genes are mainly used in semi-dwarf breeding, while hybrid rice breeding mainly selected genes associated with resistance (15/33), quality (10/33) and fertility (4/33). Remarkably, four genes (*Hd1*, *Pi2*, *Pid3*, *Pid2*) involved in FBZ-series rice breeding are tandem distributed on chromosome 6. Among them, three genes confer resistance to rice blast disease, the remaining one gene *Hd1* mainly regulates rice heading date (Fig. 3). This suggests that resistance-related genes are usually the major selection targets during *indica* rice breeding in China. These resistance-related QTGs mainly control stresses resistance such as resistance to rice blast (*Pi21*, *Pb1*, *Pik*, *Pi2*, *Pid3*, *Pid2*), bacterial blight (*Xa13*, *Xa21*, *Xa23*, *Xa25*, *Xa1*, *Xa27*), and nutrients (*ARE1*, *Pup1*, *OsHKT2;1*, *TOND1*) (Fig. 3). Taken that most varieties used in this study are widely promoted in China, we speculate that these resistance genes may play an important role in improving the broad adaptability of rice.

We also found that the male sterile restorer gene *Rf3* has undergone selection before semi-dwarf breeding. Furthermore, *Rf17*, *Rf4* and *Rf1b* were also selected by Chinese breeders before FBZ was bred. For other traits, improvement of semi-dwarf (*sd1*) and tiller angle (*TAC3*, *D2*) in semi-dwarf breeding may contributed to the first leap in productivity. Subsequently, genes related to appearance quality (*GS3*, *GW5*, *GS5*, *OsSNB*, *Chalk5*) and taste quality (*OsACS6*, *Waxy*, *BADH2*, *OsGluA2*, *ALK*) were also under artificial selection during hybrid rice breeding (Fig. 3). Taken together, these results indicated that the wide adaptability, rice yield, grain quality and male sterility restoration ability have reached a high level after hybrid rice breeding. This provides the possibility to use a chromosome segment of *Hd1-Pi2-Pid3-Pid2* to breed FBZ-series varieties.

Inheritance pattern and gene effects of chromosome 6

Remarkably, in addition to *Hd1-Pi2-Pid3-Pid2* locus, 8 QTGs are also located on chromosome 6, which together account for 25% of all 48 QTGs (Fig. 3). Among them, several genes have been reported to play an important role in rice breeding, for example, *Waxy* and *ALK* are major genes controlling eating and cooking quality (Tian et al. 2009), while *APO1* affected both lodging resistance and productivity (Ookawa et al. 2010). We noticed that *APO1* has no functional variation in GroupIV (FBZ-series varieties) compared to other varieties, while causative variants corresponding to *ALK* also existed in other Groups. Two functional variants in *Waxy* was found differentially used in five groups (Additional file 2: Table S6).

Sequence alignment indicated that the FBZ haplotype is different from *Waxy* gene in other widely promoted varieties, such as Minghui63 and 9311. Compared to Nipponbare, Huazhan had 2 SNPs and 1 base deletion in the coding region (Additional file 1: Figure S1a), and FBZ haplotype are the closest to the *Wx^b* allele (Additional file 1: Figure S1b). By comparing with gene sequences of four tandem genes, we found that *Pi2* and *Pid3* sequence of Huazhan are identical to C101A51 (*Pi2*-carrying line), Gumei2 (*Pid3*-carrying line), respectively (Additional file 1: Figure S2a, b). We noted that there was only one non-functional SNP difference in the *Pid2* genes between Huazhan and Digu (*Pid2*-carrying line) (Additional file 1: Figure S2c), suggesting that Huazhan also carries the resistance allele of *Pid2*. We extracted the *Hd1* gene sequences of Huazhan and Huanghuazhan, and performed multiple sequence alignments with Minghui63 (nonfunction type) and Zhenshan97 (function type) (Zhang et al. 2016). Both Huanghuazhan and Minghui63 harbored a 4-bp frame-shift deletion at the 1904 site, however, Huazhan harbored a 2-bp frame-shift deletion at the 1686 site instead of 4-bp deletion at the 1904 site (Additional file 1: Figure S3).

Given that hundreds of varieties have been derived from FBZ, we wondered how these 12 QTGs inherited in latest FBZ-series varieties. Thus, we collected DNA samples from 176 rice varieties, including 5 foreign *indica* rice, 29 landraces (bred before 1970s), 83 FBZ-series varieties, 54 modern *indica* rice and 5 *japonica* rice (Additional file 2: Table S7). We designed 10 polymorphic PARMS markers based on DNA chips. Among them, 1.6 and 1.7 can be used together to distinguish FBZ-type *Waxy* allele (Additional file 1: Figure S1b), 10.4 is a functional molecular marker for resistance allele of *Pi2* (Yang et al. 2021), six PARMS makers are closely linked to *Hd3a*, *ALK*, *Hd1*, *Pid3*, *Pid2*, *Xa27*, respectively (Fig. 4a). According to the PARMS genotyping, 83 FBZ-series varieties can be divided into 13 groups, ranging from Group-a to Group-m. Surprisingly, 64 of 83 varieties derived from FBZ share the same genotype at locus Chr6:1.6–23.3 Mb, except for the variation in the *Waxy* locus (Fig. 4a). This strongly indicates that most of beneficial QTGs on chromosome 6 have been inherited during the breeding process of the FBZ-series varieties. We also found that 71 of 83 FBZ-series varieties have the same genotype at the *Hd1-Pi2-Pid3-Pid2* locus. In particular, 79 of 83 FBZ-series varieties carry the *Pi2* resistance allele, accounting for up to 95.2% (Fig. 4a). On the contrary, among the 93 non-FBZ pedigree varieties, we only found six varieties carrying the *Pi2* resistance allele. In addition, of these six varieties, 28zhan is the parent of FBZ, while the other five varieties have less clear pedigrees (Additional file 2: Table S7). Recent studies have shown that only 2 of the 400 varieties harbored *Pi2* resistance allele (Wei et al. 2021), suggesting *Pi2* was a rare variant. Hence, we speculate that breeding of FBZ may be the earliest breeding practice of inducing *Pi2* resistance allele to Chinese *indica* rice.

Considering the important influence of *Hd1*, *Waxy*, *Pi2*, *Pid3* and *Pid2* on agronomic traits, we extracted the amylose content, whole growth period and rice blast resistance data from variety certification announcement. Compared to *Hd1-G* (Huanghuazhan type), the whole growth period of *Hd1-T* (Huazhan type, exist in most FBZ-series varieties) has no significant difference in both early and late seasons (Fig. 4b), indicating variation of *Hd1* haplotype did not affect its gene effect in FBZ-series varieties. For *Waxy* gene, 75.9% FBZ-series varieties contained *Wx-FBZ* allele, while varieties carrying *Wx^b* allele accounted for 22.9%. There was no significant difference in amylose content between *Wx-FBZ* and *Wx^b*, but their

amylose content was significantly lower than that of *Wx^a* (Fig. 4c). This means that *Wx-FBZ* is also a favorable allele for rice quality improvement. We found 6 genotype combinations of *Pi2*, *Pid3* and *Pid2* in 176 varieties, among them, 3 combinations with a sample size greater than 5 (Fig. 4d). Varieties carrying *Pi2* resistance allele can significantly increase the frequency of resistance to ZB, ZC and total blast isolates, and the comprehensive resistance to rice blast was also significantly improved. However, *Pid3* and *Pid2* seems to have little effect on blast resistance in our collection (Additional file 1: Figure S4). Taken together, the application of favorable genes on chromosome 6 solves the problem of ‘high-quality but susceptible to rice blast’, enabling the FBZ-series varieties to achieve high and stable yield, good quality, and wide adaptability in production.

Genes flow of favorable genes involved in breeding of FBZ-series varieties

In order to identify the favorable genes involved in the breeding process of the FBZ-series varieties, we compared the allelic differences of 245 QTGs between FBZ-series varieties and their ancestors. A total of 16 QTGs which have beneficial functions in FBZ-series varieties were identified as key candidate genes according to functional variation information. These 16 QTGs are all included in the 48 QTGs that show haplotype differences among different types of varieties, except for *Hd3a*. This is due to the FBZ type *Hd3a* also exists in some varieties of GroupI (landraces) and GroupII (landmark semi-dwarf varieties). In addition, another heading date gene *Hd3b* at the *Hd3* locus was also specifically selected in the hybrid rice restorer line (Additional file 2: Table S6). The rice florigen genes *Hd3a* are homologs of *FT* in *Arabidopsis*, it function at the downstream of the photoperiodic and other flowering regulatory pathways (Chen et al. 2021). Therefore, *Hd3a/Hd3b* may have played an important role in modern *indica* rice breeding. Given that a single gene variations can only explain a limited part of photoperiod sensitivity change (Chen et al. 2021), the effect of *Hd3a/Hd3b* on heading date is not easy to judge.

We found that these 16 QTGs are generally well-known important functional genes, this allows us to understand their genetic effects. These QTGs have very obvious characteristics of gradual replacement, and they are mainly selected in four stages (Fig. 5). Firstly, 5 QTGs were selected during semi-dwarf breeding period, this makes Teqing exhibit semi-dwarf (*sd1*), delayed heading (*Hd3a*), partial fertility recovery (*Rf3*) and compact plant type (*d2*, *TAC3*); Secondly, 6 QTGs were integrated to breed Qingliuai1, including genes related to fertility restoration (*Rf4*, *Rf1b*), grain protein content (*OsGluA2*), blast resistance (*Pi21*) and bacterial blight resistance (*Xa13*, *Xa21*). Thirdly, Fengaizhan1 utilized 3 QTGs (*GW5/qSW5*, *GS3*, *Chalk5*), thereby exhibiting slender and chalk-free appearance quality. Lastly, 2 QTGs were used to breed FBZ, making it possible to breed cultivars with low amylose content (*Waxy*) and resistance to rice blast (*Pi2*).

In particular, Huanghuazhan did not carry the resistant *Pi2* allele. Huanghuazhan is widely used as conventional rice, while Huazhan is mainly used as a restorer line. Studies have shown that *Pi1*, *Pi2*, and *D12* showed a strong dosage effect on the resistance to blast in the hybrid background during the entire growth duration in the field condition (Jiang et al. 2012). Given that the resistance to rice blast of sterile lines is usually poorer than that of restorer lines, we estimate that the *Pi2* resistant allele may be the best

choice for breeding of restorer lines. However, for Huanghuazhan, excellent rice quality is more important than rice blast resistance, although no studies have shown that *Pi2* has an adverse effect on rice quality.

Discussion

Fertility restoration for cytoplasmic male sterility (CMS)

The annual planting area of the FBZ-series hybrids accounts for 35% of the hybrid rice area in China (Fig. 2b). Therefore, the ability to restore the fertility of CMS is very important for the application of FBZ-series varieties to hybrid rice system. Three-line hybrid rice system comprises CMS line, CMS maintainer line, and CMS restorer line. CMS-WA was derived from a male sterile plant of wild rice *O. rufipogon* discovered by team of Prof. Longping Yuan, and it is the most widely used type of CMS lines in China. Currently, three CMS systems are mainly used, including Boro II CMS (CMS-BT), Wild Abortive CMS (CMS-WA) and Hong-Lian CMS (CMS-HL). CMS is controlled by interactions between CMS genes and nucleus-encoded *restorer of fertility (Rf)* genes (Chen et al. 2021). In the CMS-HL system, the fertility restoration is controlled by *Rf5/Rf1a* and *Rf6*, while CMS-BT line carries *Rf5/Rf1a* and *Rf1b*. Fertility restoration of CMS-WA is controlled by two dominant nuclear loci, *Rf3* and *Rf4* (Tang et al. 2014). It should be noted that the fertility restoration QTL *Rf3* has not yet been cloned, and the causative variant (Chr1P5568692) provided by the previous study (Wei et al. 2021) should be a linkage SNP. In this study, we found that landraces and CMS-WA carried the 'T' allele at this locus, by contrast, the 'C' allele was present in the varieties after semi-dwarf breeding (Additional file 2: Table S6), suggesting that *Rf3* is likely has been selected in the process of semi-dwarf breeding. In particular, both CMS-WA and iconic restorer lines carried 'G' allele at the causative variation site (Chr10P19058375) of *Rf1b*, however, the 'A' allele was present in FBZ-series varieties. The possible explanation is that *Rf4*, *Rf1a* and *Rf1b* are closely linked genes, the favorable alleles of *Rf1a* and *Rf1b* were selected along with the application of *Rf4*. Since *Rf4*, *Rf1a* and *Rf1b* all encode PPR proteins (Chen et al. 2021), whether they have an additive effect in restoring CMS fertility remains unclear. In general, the favorable alleles of *Rf3* and *Rf4* were selected in the early period of the FBZ pedigree, making most of the FBZ-series varieties can restore the fertility of CMS-WA.

Fitness trade-off between defense and growth

Biotic and abiotic stresses are key factors threatening rice growth, leading to decreases in yield and quality. In this study, we found that the selection of *Hd1-Pi2-Pid3-Pid2* locus significantly improved the rice blast resistance, which directly contributed to the breeding of FBZ-series varieties (Additional file 1: Figure S4). Moreover, among 43 QTGs involved in semi-dwarf breeding and hybrid rice breeding, 19 QTGs were associated with resistance. However, by comparing the variant information of these QTGs, we found that only 3 QTGs (*Pi21*, *Xa13*, *Xa21*) are beneficial genes for FBZ-series varieties, while the gene effects of 8 QTG locus (*Bph9*, *Xa27*, *Xa23*, *OsPsbS1*, *Pb1*, *Pik-1/Pik-2*, *PSTOL1*, *SNORKEL1/SNORKEL2*) cannot be determined due to lack of functional variation information (Additional file 2: Table S6). On the contrary, 8 out of 19 QTGs showed unfavorable genetic effects in FBZ-series varieties, which may cause

susceptibility to sheath blight (*Xa1*, *Xa25*), UV-B (*OsUGT707A2*), herbicides (*CYP72A31*), drought (*DRO1*), low temperature (*qLTG3-1*), and nutrient deficiency (*OsHKT2;1*, *ARE1*) (Additional file 2: Table S6). We are curious why most of the resistance-related genes play an unfavorable function in semi-dwarf breeding and hybrid rice breeding. One possible explanation is that the adverse effects of these genes are supplemented by other favorable genes. Another possibility is that the adverse effects of these genes were obscured in growth environment without stress. For instance, *DRO1-kp* allele enables rice to produce more grains under drought-induced stress. However, under non-drought conditions, it could not show a yield advantage because *DRO1* alters only root growth angle and does not decrease either shoot or root biomass (Uga et al. 2013). On the other hand, it is well known that plant immune activation often leads to fitness cost, this makes plants trade-off between plant growth and immunity to ensure the best use of limited resources (Chen et al. 2021; Wang et al. 2021). For example, high-quality rice varieties usually have lower yields and poor resistance, such as KDML105, Pusa Basmati 1121 and Koshihikari (Kobayashi et al. 2018; Singh et al. 2018; Vanavichit et al. 2018). Recently, several genes (*ipa1-1D*, *Pigm*, *OsGRF6*, *WRKY45*) has been reported fine-tunes the balance between disease resistance and grain yield in rice (Wang et al. 2021). However, for most resistance genes, their adverse effects on yield or quality remains unclear. Given that semi-dwarf breeding and hybrid rice breeding are mainly aimed at improving yield and rice quality, we speculate that the selection of unfavorable alleles of these resistance genes may contribute to rice growth. To answer this question, it is essential to further elucidate molecular mechanism and coordination of complex agronomic traits, especially the relationship and balance among grain yield, grain quality, immunity, nutrient use efficiency, and stress tolerance.

Foreign germplasm resources contributed to breeding progress

Guangdong is the province with the largest consumption of high-quality rice in China, which makes many varieties with good quality have been developed before FBZ was bred, such as Mabayouzhuan and Zhengchengsimiao. However, before FBZ was bred, no varieties could have both excellent rice quality and blast resistance (Zhou et al. 2007). The lower amylose content is the basic requirement of high-quality rice. In this study, 28zhan carries the same genotype as most FBZ-series varieties at locus Chr6:1.6–23.3 Mb (Additional file 2: Table S7), indicating that the low amylose content and blast resistance of FBZ were inherited from its parent, 28zhan. 28zhan was derived from the cross of Qingxiangzao301×IR37704-131-2-1-3-2. Since two parents of 28zhan are all have low amylose content, it is difficult to determine the parent source of low amylose content traits through pedigree tracing. Previous studies have shown that compared with Nipponbare, there exist polymorphisms in the 5'UTR region of Huanghuazhan (Zhou 2015). Our research showed that FBZ carried T5 haplotype of *Waxy* gene, which was different from *Wx^b* in the coding region (Additional file 1: Figure S1a). We noticed that the T5 haplotype is a low-frequency variant according to MBKbase database (Peng et al. 2020) statistics. Given IR37704-131-2-1-3-2 showed good rice quality and resistant to rice blast in our field trial (Zhou et al. 2007), we speculate that the favorable allele *Pi2* and *Waxy* of 28zhan were inherited from IR37704-131-2-1-3-2. In the history of rice blast resistance breeding in Guangdong province, three resistance sources were mainly used, including Waixuan35, Qingliuai1 and Gengxian89, however, the linkage drag between rice quality and blast

resistance limited their breeding applications (Zhu et al. 2003). Therefore, International Rice Institute variety IR37704₋₁₃₁₋₂₋₁₋₃₋₂ provides a very rare germplasm resource for rice blast resistance breeding. Aizizhan was the core parent of semi-dwarf breeding in China, and it was introduced to China before 1949. Recent studies have shown that most modern *indica* rice varieties carry the same *sd1* allele as DGWG (Chen et al. 2020; Wang et al. 2020), supporting the view that Aizizhan was introduced from southeast Asian countries. In addition, foreign germplasm resources are also the main donors of restorer genes for wild abortive hybrid rice systems (Xie and Zhang 2018). We think that foreign germplasm resources carry very precious beneficial alleles, or allele combinations, which is an important reason why they can be applied to rice breeding in China.

Rice core germplasm breeding theory

The breeding of the FBZ-series varieties shows that the 'Rice Core Germplasm Breeding Theory' (Zhou and Ke 1998) has achieved remarkable success. Based on this theory, the ideal gene system will appear in the future, and the improvement of rice varieties is essentially a trajectory of gradual optimization from the original gene system to the ideal gene system.

Rice is a daily staple food crop for over 10 billion people, although breeding goals will be different in various periods and regions, the demand for high-yield, good-quality, multi-resistance, and mechanization will last a long time. In a certain period of economic and population development, regional development will be more balanced, and the demand for rice varieties will tend to be consistent. There will be a combination of genotypes that can adapt to this universal demand, and we define it as an ideal gene system. In this study, varieties derived from FBZ have been widely planted in all *indica* rice growing areas of China (Fig. 2a), indicating that broad adaptability is not the main limiting factor in the construction of ideal gene systems. In addition, 64 of 83 varieties derived from FBZ share the same genotype at locus Chr6:1.6–23.3 Mb (Fig. 4a), suggesting that the demand for rice breeding in recent years has begun to converge.

In this study, favorable genes were gradually optimized during the breeding of FBZ-series varieties (Fig. 5), which is consistent with the conjecture of the "Rice Core Germplasm Breeding Theory". Since Qingliuail was bred in 1990, it took our team fifteen years to bred the elite variety Huanghuazhan. We think it is incredible to breed a variety with an ideal genetic system through a single breeding event, mainly due to the following three reasons. (i) Favorable genes are usually distributed in different countries and subgroups, it is hardly to screen a germplasm resource that carries multiple favorable genes. For example, rice bacterial blight disease resistance gene *Xa21* was derived from *Oryza longistaminata* in West Africa (Song et al. 1995); (ii) Many adjacent genes tended to co-occur within the same interval of 2cM, which may lead to genetic drag, and the introgression of superior alleles at some genes may also introduce inferior alleles at linked loci during pedigree breeding (Wei et al. 2021). For example, blast resistance gene *Pi21* is tight linkage with genes that cause poor eating quality (Fukuoka et al. 2009), while chalkiness QTL *Chalk5* is linked to grain width effect QTL *GW5* (Li et al. 2014); (iii) The breeding goal is continuously optimized according to cultivation technology and market demand. For

instance, before chemical fertilizers were widely used in production, the semi-dwarf *sd1* allele was an unfavorable gene due to its low nitrogen efficiency (Li et al. 2018). In addition, China's demand for high-quality rice has only been increasing since the 1980s, which makes slender (*qSW5*, *GS3*), non-chalky (*Chalk5*), low amylose content (*Waxy*), and fragrance (*BADH2*) alleles become beneficial genes.

The renovation of Chinese *indica* rice varieties

In the past several decades, *indica* rice breeding has experienced three important technological advances in China, namely semi-dwarf breeding, three-line hybrid rice breeding and two-line hybrid rice breeding (Qian et al. 2016). In 1955, Prof. Yaoxiang Huang introduced the semi-dwarf variety Aizizhan from Guangxi Province, China. This allowed Guangchangai to be bred in 1959, which was 7 years earlier than the bred of the miracle rice IR8 (Qian et al. 2016). During the semi-dwarf breeding period, Chinese breeders bred many elite varieties, including Zhenzhuai, Shuanggui1, Guangluai4, Guichao2 and Teqing (Zeng 2018). The earliest male parents of the three-line hybrid rice were mostly imported from the International Rice Research Institute (IRRI), including Taiying 1, IR24, IR661, IR26 and IR36. Minghui63 was one of the most successfully developed male parents and played a key role for the promotion of hybrid rice production in China from the late 1980s (Xie and Zhang 2018). The widespread use of two-line hybrid rice in China began in 2000s, and 9311 was the most representative restorer lines in this period. Using 9311 and its derivative lines as restorer lines, many well-known hybrid rice varieties have been bred, including Liangyoupeijiu, Yangliangyou 6, Fengliangyou 1, and Shenliangyou 5814 (Zeng 2018). From 2010 to 2015, both conventional rice and restorer lines have undergone renovation. Huanghuazhan, Xiangzaoxian45 and Zhongjiazao17 were the three rice varieties with the largest annual promotion area of conventional rice, while the most widely used restorer lines became Huazhan, Bing4114 and Guanghui308 (Zeng 2018). In addition, recent studies have also shown that Huanghuazhan and Huazhan have now become important backbone parents or main recommended varieties of *indica* rice in China (E et al. 2019; Fang et al. 2020).

In this study, Huazhan and Wushansimiao were the two restorer lines with the largest annual promotion area of hybrid rice (Additional file 1: Table S2). The hybrids derived from FBZ were grown with area of 3.8 million hectares, accounting for 35% of the total hybrid rice area in the country (Fig. 2b). It should be pointed out that Shanyou63, a milestone for China's hybrid rice development, had a large planting area during the years from 1985 to 2001 with an average area of 3.6 million hectares and 28.3% of the national hybrid rice growing areas annually (Xie and Zhang 2018). This shows that the annual planting area of FBZ-derived hybrids has exceeded Shanyou63. Moreover, Huanghuazhan has been grown in China on a cumulative 3.4 million hectares from 1982 to 2019 (Additional file 1: Table S1), and is currently the high-quality *indica* conventional rice variety with the largest cumulative planting area in China. Hence, FBZ has become a milestone of Chinese modern *indica* rice, it's derived conventional rice and hybrids have greatly promoted the replacement of rice varieties in Southern China.

Perspective for breeding next generation rice

Under the guidance of ‘Rice Core Germplasm Breeding Theory’ (Zhou and Ke 1998), we have not only bred the FBZ-series varieties such as Huanghuazhan, Huazhan and Wushansimiao, but also the iconic aromatic rice variety Meixiangzhan2. Meixiangzhan2 was the only *indica* rice variety that has won the three gold medals in the national committee of evaluation on eating quality of high-quality rice varieties, and its taste quality has even surpassed that of Thai Hom Mali Rice KDML105. Meixiangzhan2 was released in 2006 and has been widely planted in China, with an annual promotion area of about 133,000 hectares (NATESC 2019). It has also been introduced to Myanmar, Vietnam, Laos, Thailand, Mozambique and other countries for cultivation (Li et al. 2021). As mentioned above, we believe that the ideal gene system will appear in the future. We are thinking about how to breed a variety that has the similar yield levels, disease resistance and combining ability of FBZ-series varieties, and the same eating quality as Meixiangzhan2. In order to achieve this goal, using molecular marker-assisted selection technology to manipulate some favorable genes, such as *Pi2*, *Waxy*, *Rf3*, *Rf4*, *BADH2*, will greatly improve breeding efficiency. In addition, it is necessary to introduce more germplasm resources, especially tropical or temperate *japonica* rice. This may help us discover more favorable alleles or combinations of favorable genes, thereby creating a new balance between yield, quality, and resistance.

Rice is the staple food for over half the world’s population and approximately 90% of the world’s rice is grown in Asia (Muthayya et al. 2014). China is the largest rice producer in the world, the annual production has stabilized at more than 200 million tons for 10 consecutive years (National Bureau of Statistics). On the contrary, China’s rice planting area is only about half of India’s, indicating that the high yield per unit largely contributes to China’s rice production. Except for China, there are six countries in Asia with rice planting areas more than 5 million ha, including India, Bangladesh, Indonesia, Thailand, Vietnam and Myanmar. The total rice planting area in these countries is about 90 million ha (FAO, Food and Agriculture Organization of the United Nations), which is about four times that of China. The current farm yield has reached about 7 metric tons per hectare (t ha^{-1}) in China (National Bureau of Statistics), however, the farm yields in these countries varied from 2.9 to 5.1 t ha^{-1} (FAO 2019), suggesting there is still a lot of room for improvement in potential yield. With the initiative and funding of ‘Green Super Rice’ (GSR) project, Huanghuazhan was released in Mozambique, Indonesia and India, another 15 varieties derived from Huanghuazhan were also released in some countries in Asia and Africa. Those FBZ-series varieties and other GSR varieties have been promoted in Africa, South Asian and Southeast Asian countries about 6.12 million hectares, which marked significant contributions to rice production and food security in these countries (Wang et al. 2018a; Yu et al. 2020; Yu et al. 2022)(Additional file 1: Table S3). It is estimated that the world food production needs to increase by 70% in 2050, driven primarily by population growth (FAO 2013). We are very willing to help these neighboring countries increase food production, because this is a win-win situation. China is advancing the ‘One Belt, One Road’ strategy. Scientists should try their best to help them increase the yield levels of rice, providing them with theoretical guidance, technical support, extension services, germplasm resources, and modern varieties.

Abbreviations

FBZ: Fengbazhan

SNP: Single nucleotide polymorphisms

GSR: Green Super Rice

FAO: Food and Agriculture Organization of the United Nations

IRRI: International Rice Research Institute

Rf: Nucleus-encoded restorer of fertility

CMS: Cytoplasmic male sterility

CMS-BT: Boro II CMS

CMS-WA: Wild Abortive CMS

CMS-HL: Hong-Lian CMS

QTNs: Causative variants corresponding to QTGs

QTGs: Genes that underlie quantitative traits

RIGW: Rice Information GateWay

NATESC: National Agricultural Technology Extension Service Center

Declarations

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Authors' contributions

L.Z. performed most of the research and wrote the paper; L.Z. and S.Z. designed the research; S.Z., Y.C., and C.W. provided support for this research; H. L., D.H., Z.W., D.Z., R.G., and Y.P. helped perform some experiments. All authors read and approved of the manuscript.

Availability of supporting data

All data generated or analyzed during this study are included in this published article and its supplementary information files.

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Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

Author details

Rice Research Institute, Guangdong Academy of Agricultural Sciences / Guangdong key Laboratory of New Technology in Rice Breeding / Guangdong Laboratory for Lingnan Modern Agriculture / Guangdong Rice Engineering Laboratory, Guangzhou 510640, China

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Figures

Figure 1

Breeding of FBZ-series varieties by our team.

The annual planting area of Fenghuazhan, Huanghuazhan, Huazhan, Wushansimiao, Huanglizhan and Huangyuesimiao are indicated below the variety name. We calculate the annual planting area by summing the planting area of a variety itself and the area of its directly derived hybrid rice. Big, short and wide. Small, long and narrow. High, >20%. Low, <18%. S, susceptible. MR, moderately resistant. R, resistant. Data source: Chinese national agricultural technology extension service center (2019), the unit is ×10⁴hm².

Figure 2

Breeding effects of FBZ in China.

a. Main areas where varieties derived from FBZ are cultivated in China. Planting area of Huanghuazhan, Wushansimiao and top seven planted hybrids were added up and used for drawing. b. The share of FBZ-series hybrids in total area of hybrid rice in China. c. The share of FBZ-series varieties (including hybrid rice and conventional rice) in total rice area in China. Data source: Chinese national agricultural technology extension service center (2019), the unit is $\times 10^4 \text{hm}^2$.

Figure 3

Allele types of QTNs involved in Chinese modern *indica* rice breeding.

For a particular gene, allele types are represented by different colors. One representative QTN site for each gene was used for genotyping. White colors represent unknown genotypes. Genes from chromosome 6 are labeled with red triangles. The picture only shows the genotype diagrams of 39 varieties, the detailed genotype data are listed in additional file 2: Table S6.

a

b

c

d

Figure 4

Inheritance pattern and gene effects of loci on chromosome 6 in breeding of the latest FBZ-series varieties.

a. Inheritance pattern of chromosome 6 in breeding of latest FBZ series varieties. Graphical genotypes were drawing based on the PARMS markers. The genes that contribute to modern indica rice breeding are

marked on top of chromosome. White (FBZ) and black (others) bars denote the parental origin of chromosome segments. Haplotypes of *Waxy*, *Hd1* and *Pi2-Pid3-Pid2* locus are marked with arrows. b. Allelic effects of *Hd1* locus on whole growth period. c. Effects of *Wx* allelic diversity on amylose content. d. Effects of *Pi2-Pid3-Pid2* locus on rice blast resistance. Whole growth period, amylose content and resistance frequency were obtained from the variety approval announcement. AC, amylose content. RF, resistance frequency to total rice blast fungus in guangdong province. The presence of different lowercase letter denotes a significant difference between them ($P < 0.05$).

Figure 5

Genes flow of key candidate genes involved in breeding of FBZ-series varieties.

QTNs which showed the same haplotype in progeny of FBZ, but distinct haplotypes in ancestors were selected. Only favorable QTN haplotypes in progeny of FBZ were retained. A total of 17 QTGs were identified as key candidate genes involved in breeding of FBZ series varieties.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalfile2.xlsx](#)
- [Additionalfile1.docx](#)