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Molecular Marker Assisted Breeding of a New *Japonica* Hybrid Rice 'Shenyou28' with Good Quality and Disease Resistance

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Abstract The development of *Japonica* hybrid rice is of great significance to ensure supply of good quality *Japonica* rice and food security in China. Aiming at the problems of late growth period, unstable yield, and the need to improve rice quality and seed production yield of *Japonica* hybrid rice, a new *Japonica* hybrid rice combination 'Shenyou28' with early maturity, good quality, blast resistance, stable and high yield and easy seed production was developed by using molecular breeding techniques including high-density rice gene chip. 'Shenyou28', bred by Shanghai Academy of Agricultural Sciences, is a three-line *Japonica* hybrid rice with 'Shen21A' as sterile line and 'Shenhui26-28' as restorer line. For two-year regional trials from 2018 to 2019, the average output were 10 588.5 kg/hm^2, which was 11.6% higher than the control variety 'Huayou14'. For the one-year production trial, the output reached 10 473.0 kg/hm^2, which was 4.8% higher than the control variety 'Huayou14'. 'Shenyou28' has good quality, reaching the second grade of national standard, and has fragrance because it carries fragrance gene *badh2-p-5'UTR*. It also has good resistance to rice blast and bacterial blight because it aggregates the rice blast resistance genes *Pi2*, *Pita*, *Pib*, *Pi9*, *Pi54*, *Pikm*, *Pit*, and bacterial blight resistance gene *Xa21*. It was approved by the Crop Variety Certification Committee of Shanghai in 2020. The breeding process, variety characteristics and key points of high yield mechanized seed production technology of 'Shenyou28' are introduced in this study.
Keywords *Japonica* hybrid rice; 'Shenyou 28'; Molecular marker; Good quality; Disease resistance

Rice (*Oryza sativa* L.) is the largest grain crop in China, and its output accounts for about 40% of the total grain production in the country, which makes an important contribution to ensuring the food security in China. With the improvement of people's living standard, the consumption structure of rice has undergone great changes, and began to change from full to eating well. *Japonica* rice is increasingly favored by consumers because of its soft and delicate taste, but only a few countries such as China, Japan and South Korea grow and export *Japonica* rice. Domestic *Japonica* rice yield already could not satisfy people's consumption demand, ensuring the supply of *Japonica* rice will be the focus of grain security in the future. Developing *Japonica* hybrid rice is an important way to increase rice yield and satisfy rice supply. Although *Japonica* hybrid rice has made remarkable achievements, the development of *Japonica* hybrid rice is slow. At present, the planting area of *Japonica* hybrid rice only occupies about 5% of that of *Japonica* rice, which has great potential for development (Niu et al., 2019a).

Traditional breeding mainly selects progeny based on breeding experience and phenotype, the selection accuracy is low and easy to be affected by environment. Molecular markers can be used to directly identify sample genotypes at the DNA level, thus significantly improving the selection efficiency and accuracy. At present, biological breeding techniques based on molecular marker-assisted selection are playing an increasingly important role in the cultivation of rice varieties (Lu et al., 2017; Guo et al., 2019). SSR and other traditional molecular markers have defects such as small number of markers, low genome coverage and laborious detection. With the rapid development of DNA sequencing technology, the cost of sequencing is greatly reduced, which strongly promotes the development of rice re-sequencing and simplified genome sequencing. SNP markers have been widely used in rice molecular breeding. Gene chip is an important method to detect SNP. In recent years, the development of rice high density gene chip for breeding has realized the rapid and accurate detection of functional genes of important traits in rice. By detecting multiple functional SNP variation sites of specific genes in the samples, this technology can help breeders quickly find the good genes of the varieties, and further understand the good characteristics of the varieties, thus significantly improving the breeding efficiency (Yu et al., 2016; Qiu et al., 2018). Aiming at the problems of late growth period, unstable yield, poor meeting of parents in seed production and other problems of the main japonica hybrid rice combination in Shanghai, we developed a new *Japonica* hybrid rice combination 'Shenyou 28' based on traditional hybrid technology and with the help of rice high density gene chip technology, which is early maturing, high quality, resistant to rice blast, stable and high yield and easy to seed production. In 2020, it has passed Crop Variety Certification Committee of Shanghai (Approval number: Hushendao 2020002), which has great potential for popularization and application in the middle and lower reaches of the Yangtze River. This paper introduces the breeding process, characteristics and high yield mechanized seed production technology of ‘Shenyou 28’.

1 Results and Analysis

1.1 The breeding process of ‘Shenyou 28’

According to the identification, the sterile line 'Shen 21A' showed stable sterility, large panicle, medium grain density, compact plant type, strong tillering ability, green leaf color, straight blade leaf, and beautiful late ripening phase, and the total number of grains per panicle was about 130, the weight of 1 000 grains was 24 g lodging resistance, and the rice quality was excellent. It has a fragrance (Detected by molecular markers, it carries the badh2-p-5 'UTR fragrance gene), good flowering habits, and early and concentrated flowering. The flowering time is mainly concentrated between 10:30 and 12:30, with large glume opening angle, good recovery and high out-crossing seed setting rate. A new restorer line of *Japonica* hybrid rice 'Shenhui 26-28' showed moderate plant height, strong tillering ability, compact plant type, large panicle and many grains, dense grains, green straw when harvesting, 1000-grain weight of 26 g, high combining ability, and strong yield advantage (Niu et al., 2019b). In addition, it has excellent rice quality, transparent appearance, good outcrossing traits, abundant spikelet and large pollen amount (Table 1). It has a long flowering period and a concentrated flowering period. On sunny days, it usually blooms between 10:30 and 13:00, and can maintain a large population pollen amount from beginning to end, which is easy to mechanized seed production.

Table 1 Comparison of pollen number of single anther, spikelets per panicle and effective panicle number between restorer lines ‘Shenhui26-28’ and ‘Fan 14’

Name	Shenhui 26-28	Fan14/CK	Percentage increase (%)
Pollen number of single anther	2306.0	1820.0	26.7
Spikelets per panicle	176.2	165.3	6.6
Effective panicle number ($10^4/hm^2$)	267.0	253.5	5.3

Rice blast is a frequent rice disease in Yangtze River Delta. The haplotype analysis of nine blast resistance genes by high density rice microarray showed that the sterile line 'Shen 21A' carried *Pita* and *Pi2*, and the restorer line 'Shen Hui-28' carried *Pi9*. Further molecular markers were used to detect *Pib*, *Pi54*, *Pit* and *Pikm* of four rice blast resistance genes, and it was found that 'Shen 21A' also contained *Pit* and *Pib*, and 'Shen Hui 26-28' also contained *Pikm* and *Pi54*. Therefore, seven major blast resistance genes (*Pita*, *Pib*, *Pi2*, *Pi9*, *Pi54*, *Pikm* and *Pit*) could be integrated into the hybrid 'Shen 21A' and 'Shen Hu 26-28', and it was easy to select a new *Japonica* hybrid rice resistant to rice blast. The haplotype analysis of five bacterial blight resistance genes using rice high density microarray showed that 'Shen 21A' and 'Shen Hui 26-28' both contained *Xa21*.

The hybrid group of 'Shenhui 26-28' and 'Shen 21A' was tested. The hybrid combination showed good ripe green culm, good comprehensive resistance and strong yield advantage, and passed Crop Variety Certification Committee of Shanghai in July 2020.

1.2 Output performance of 'Shenyou 28'

From 2018 to 2019, ‘Shenyou 28’ was planted in Zhuanghang, Fengxian (Zhuanghang); Chongming Guangming Seed Industry (Guangming); Pandian, Fengxian (Pandian); Zhuqiao, Pudong (Zhuqiao); Chongming Shanghai Industry Investment (Holdings) company (Shangshi); Quzhou, Zhejiang (Quzhou); Hefei, Anhui (Hefei) and other places for multi-point identification demonstration. The average yield in 2018 was 10 880.4 kg/hm^2, and there is an increase of 12.6% compared with the control 'Huayou 14', and the average yield in 2019 was 10 705.7 kg/hm^2, an increase of 8.1% compared with the control 'Huayou 14' (Table 2). In 2019, 'Shenyou 28' was demonstratively planted in Shanghai, Zhejiang, Anhui, Jiangxi, Jiangsu and other places with good performance, among which the output of Jiashan Tianning Base in Jiaxing, Zhejiang reached 10 731.0kg /hm^2.

‘Shenyou 28’ was tested in *Japonica* hybrid rice regional experiment in Shanghai from 2018 to 2019. Its average yield was 10 588.5 kg/hm^2, which was 11.6% higher than that of the control. In 2019, it participated in the Shanghai *Japonica* hybrid rice production experiment, and the yield reached 10 473.0 kg /hm^2, an increase of 4.8% compared with the control (Table 3). ‘Shenyou 28’ showed high and stable yield and showed wide adaptability in the middle and lower reaches of the Yangtze River.

Table 2 Yield performance of multi-point identification of the new *Japonica* hybrid rice combination 'Shenyou28' in 2018 and 2019, respectively

Year	Test place	Yield (kg/hm^2)		Yield increase (%)
		Shenyou 28	Huayou 14	
2018	Zhuanghang	11 524.5	9 126.0	26.3
	Guangming	10 920.2	9 567.3	14.1
	Pandian	10 479.0	9 793.5	7.0
	Zhuqiao	11 355.0	10 417.5	9.0
	Shangshi	11 130.0	9 228.0	20.6
	Quzhou	9 337.5	8 962.5	4.2
	Hefei	11 416.5	10 645.5	7.2
	Average	10 880.4	9 677.2	12.6
2019	Zhuanghang	10 276.5	9 975.0	3.0
	Guangming	10 899.0	9 706.5	12.3
	Pandian	9 352.5	9 295.5	0.6
	Zhuqiao	11 607.0	10 867.5	6.8
	Shangshi	10 375.5	9 082.5	14.2
	Quzhou	10 192.5	9 186.0	11.0
	Hefei	12 237.0	11 260.5	8.7
	Average	10 705.7	9 910.5	8.1

Table 3 Regional trials and production trial yield of 'Shenyou28' in 2018 and 2019, respectively

Group	Year	Yield (kg/hm^2)		Yield increase (%)
		Shenyou28	Huayou14	
Municipal regional trial	2018	10 126.5	9 018.0	12.3
	2019	11 050.5	9 955.5	11.0
	Mean value	10 588.5	9 486.8	11.6
Municipal production trial	2019	10 473.0	9 993.0	4.8

1.3 Agronomic characters of 'Shenyou 28'

'Shenyou 28' was planted as single-season late rice in Shanghai, the whole growth period was about 155 days, 6 days earlier than the control 'Huayou 14'. It shows early maturity, which is beneficial to ease the contradiction with the later crop. This variety has the characteristics of moderate plant type, strong tillering ability, green leaf color, green stalk live ripe. Its panicle length is 17.1 cm, and has the characteristics of large panicle and many grains, medium grain type, dense grain, fast grain filling speed. The seed setting rate was as high as 92.0%, the plant height was 115.0 cm and strong lodging resistance. The number of effective panicles per 667 m^2 was 17.4×104, the total number of grains per panicle was 193.6, and the 1 000-grain weight was 26.8 g.

1.4 Rice quality identification of 'Shenyou 28'

According to the inspection by the Rice and Product Quality Supervision and Testing Center of the Ministry of Agriculture and Rural Affairs entrusted by Shanghai Seed Management Station, all the test indexes of 'Shenyou 28' have reached the national standard of first-class quality rice (According to the standard of NY/T593-2013 *Cooking Rice Variety Quality*) except the brown rice rate and amylopectin content. Among them, the chalkiness rate is 3%, the chalkiness is 0.4%, and the transparency is Grade 1 (Table 4). The appearance quality is especially outstanding. The rice quality of 'Shenyou 28' is excellent, and on the whole, it meets the quality requirements of high-quality second-class edible *japonica* rice varieties.

Table 4 Quality test results of ‘Shenyou28’ from rice and product quality inspection center of Ministry of Agriculture

Project	First class standard of high quality rice	Test result
Brown rice rate (%)	≥83	81.9
Milled rice rate (%)	-	74.0
Head rice rate (%)	≥69	72.3
Chalkiness rate (%)	-	3
Chalkiness degree (%)	≤1.0	0.4
Transparency	≤1	1
Amylose content (%)	13-18	18.4
Alkali dissipation value	≥7.0	7.0
Gel consistency (mm)	≥70	79

1.5 Identification of disease resistance of 'Shenyou 28'

The new *Japonica* hybrid rice combination 'Shenyou 28' had good comprehensive resistance and lodging resistance. It has good resistance to rice blast and leaf blight. In 2019, Shanghai Agricultural Technology Extension Service Center commissioned Jiangsu Academy of Agricultural Sciences to carry out inoculation evaluation. ‘Shenyou 28’ showed high resistance to seedling blast (HR), panicle blast (R), moderate resistance to leaf blast (MR). The comprehensive resistance of rice blast was evaluated as resistance (R). Inoculation and identification of bacterial blight showed that 'Shenyou 28' had good resistance to the representative pathogenic strains PX079 and JS49-6 of bacterial blight.

1.6 Key points of high yield mechanized seed production technology of 'Shenyou 28'

(1) Reasonable arrangement of parents’ delay time: When the parents of 'Shenyou 28' were seeded and transplanted at the same time, the heading date of male parent was 4 d later than that of female parent. In order to bring the parents together during the blooming period of seed production, the male parent 'Shenhui 26-28' was sown in two phases on May 15 and May 22, while the female parent 'Shen 21A' was sown in a single phase on June 7. The female parent was sown on rice plates with 90 g of dry grain per plate. The male parent raised seedlings in the rice field, and the planting amount of the rice field is 225 kg/hm^2. The rice plate cover film or non-woven fabric in order to promote early growth. (2) Reasonably setting male and female parents’ row ratio and transplanting period: In tractor-assisted pollination, the row ratio of parents should be between 2:7 and 2:8, the row spacing should be 25×13 cm, and the basic seedlings of the female parent should be about 75 × 10^4 plants /hm^2. The male parent 'Shenhui 26-28' was planted about 10 days earlier than the mother plant (Around June 20), and when it was green again, the female parent 'Shen 21A' was implanted by machine and the seedlings were

inserted shallowly. (3) Timely management of water and fertilizer to build a good seed production community: The female parent of 'Shenyou 28' was transplanted late, and the fertilization required sufficient base fertilizer and early topdressing, so as to promote early tillering and ensure that the maximum seedling of the female parent reached 300 to 330×10^4 seedlings /hm^2 and the male parent reach to 90 to 105×10^4 seedlings /hm^2 25 to 30 days after transplanting. Water slurry management should be conducive to the growth of tillers, and the strategy of light shelving and multiple shelving should be adopted when shelving fields. (4) Timely forecast and adjustment of flowering period: At the end of July, the female parent 'Shen 21A' was put into the field with fertilizer, and urea was applied to the female parent at 225 kg/hm^2. After the beginning of young panicle differentiation, the planting field was continuously filled with deep water. At the same time, the related personnel were organized every 3 days to carry out young panicle stripping and development progress analysis since the end of July. If the young panicle differentiation of the female parent is faster or similar than that of the male parent, the female parent will continue to apply nitrogen fertilizer. Before fertilization, drain the field water slurry, fertilize the middle 5 rows of the female parent, and try not to let the male parent absorb fertilizer. The final regulation effect was the best when the first male parent entered the first heading stage, which was 1 to 2 days earlier than the female parent. (5) Spraying gibberellin and mechanized leaf cutting and powder driving: The blade of the female parent "Shen 21A" is short, so it can be cut mechanically with special machine for garden cutting at 5% spike, and 1/3 to 2/3 of the blade length can be cut. In order to facilitate powder dispersion, 2/3 of the blade can be cut off by the male parent (Cheng et al., 2017). After leaf cutting at spike stage, the male parent was treated with gibberellin 90 g/hm^2 alone, and the parents were treated with gibberellin 90 g/hm^2 the next day. The powder was driven mechanically on the second day after leaf cutting. On sunny days, powder time is generally from 10:50 to 12:30, with the mother flowering time. Mechanical powder drive only a single trip, not back and forth, every 20 to 30 minutes to drive powder one time, rainy days are normal to drive powder. (6) Mechanized harvesting, drying and selection: Carefully and strictly remove miscellaneous plants in every stage of seed production (Mainly during tillering stage, flowering stage, before mature harvest and after male parent harvest) to ensure seed purity. After the harvest of the male parent and the acceptance of the field, the female parent will be harvested mechanically. The seeds of the planting field should be harvested in time and early, and dried and selected in time after harvesting to ensure germination rate.

2 Discussion

SNP markers developed based on sequencing technology have been widely used in rice molecular breeding. The rice high density microarray developed in recent years realizes the rapid and accurate identification of functional genes for important traits in rice through the combined analysis of a set of polymorphic markers (haplotype analysis) of gene regions and adjacent gene segments (Qiu et al., 2018). In this study, high density rice microarray was used to identify some important blast and blight resistance genes in *Japonica* hybrid rice backbone parents. The results laid a solid foundation for the breeding of a new hybrid rice combination resistant to disease.

Rice blast is one of the most serious rice diseases in the world, which can cause 35% to 50% loss in production every year (Singh et al., 2015), and even no grain harvest in severe cases. Using high-quality genetic resources to breed new varieties of disease-resistant rice has been proved to be the most economical, environmentally friendly and effective method for rice blast control (Liang et al., 2019). Because the interaction between blast fungus and rice is consistent with the gene-to-gene hypothesis, the directional mutation of blast strain will cause the gradual loss of resistance of varieties carrying a single or few blast resistance genes (Li et al., 2017; Yang et al., 2019). At least 35 blast resistance genes have been cloned (Zhu et al., 2020), most of which are dominant. Studies have shown that the aggregation of multiple resistance genes in the same variety can not only broaden the resistance spectrum, but also improve the resistance level of the variety to specific physiological subspecies to a certain extent (Sakowicz et al., 2004; Sun et al., 2020), therefore, it is an effective way to improve blast resistance in *Japonica* hybrid rice by combining more major blast resistance genes from both parents. The molecular detection of blast resistance genes of both parents can be performed before the rice hybridization group, and much blast resistance genes can be gathered through the reasonable hybridization group, so as to predict the rice blast resistance of the hybrid generation. In this study, 'Shenyou 28' not only aggregated blast resistance genes *Pita Pib*, *Pi54*, *Pikm*, *Pit*, but also aggregated complex alleles *Pi2* and *Pi9*, which showed good blast resistance in production. The application of complementary breeding strategy of dominant blast resistance genes in disease resistance breeding of hybrid japonica rice is beneficial to improve the long-term resistance level of new varieties of rice blast.

The popularization and application of hybrid rice made a contribution to ensuring the steady increase of grain output for more than ten years (Tang et al., 2020). However, in recent years, the planting area of indica hybrid rice showed a declining trend. The main reasons were low seed production yield and high cost, which seriously affected the promotion speed of the new hybrid combination. The whole process mechanization of hybrid rice seed production is the only way to improve hybrid rice seed production efficiency and promote the further development of hybrid rice (Xu and Huang, 2010). The female parent 'Shen 21A' of the new *Japonica* hybrid rice combination 'Shen You 28' has the characteristics of good flowering habit, large opening Angle and high outcrossing seed setting rate, while the male parent 'Shen Hui 26-28' has the characteristics of large pollen amount, concentrated flowering time and long flowering duration, so 'Shen You 28' is easy to mechanized seed production and has the basis of high yield seed production. Through systematic studies on the sowing difference period of 'Shenyou 28' 's parents, the ratio of parents' row and the management of water and fertilizer in planting field during mechanized seed production, mechanical leaf cutting, gab spraying and mechanized powder driving, we established the high yield mechanized seed production technology of hybrid rice' Shenyou 28 ', which is conducive to realizing the whole process mechanization of hybrid rice 'Shenyou 28' seed production and improving seed production yield. The cost of seed production is reduced, which lays a solid foundation for the large-scale popularization and application of ‘Shenyou 28’.

The genetic mechanism of rice quality traits is complex. Although the phenotype of hybrid generation is relatively stable, its rice is already F2 generation. Due to the separation of rice quality characters, rice quality improvement of *Japonica* hybrid rice was difficult to conventional *Japonica* rice, especially the appearance quality. General *Japonica* hybrid rice lagged behind conventional *japonica* rice (Min et al., 2007). In theory, both the sterile line and restorer line have good rice quality, and the difference of rice quality indexes between them is small, so it is easier to prepare a new hybrid combination of high quality. According to this principle, we selected high quality sterile line 'Shen 21A' and high quality restorer line 'Shen Hui 26-28' as the mating group, and selected a high quality japonica hybrid rice new variety 'Shen You 28', which showed good quality, especially the appearance quality has made an obvious breakthrough. The chalky kernel percentage is 3%, the chalkiness degree is 0.4%, and the transparency is first level. All the three indexes have reached the national standard of first-class high-quality rice. 'Shenyou 28' shows early ripening period, excellent rice quality, high and stable yield, strong disease resistance, easy mechanization seed production, and has a broad application prospect in the middle and lower reaches of the Yangtze River.

3 Materials and Methods

3.1 Materials for test

The test materials included 'Shen 21A' and 'Shen Hui 26-28'. 'Shen 21A' is a japonica male sterile line formed by hybridization of line selected line 'Shen 21' and japonica male sterile line 'Shen 9A' in the spring of 2015 and continuous backcross replacement. In 2017, the fertility of the sterile line was stable, the plant height was 90.8 cm, and the photosensitivity was strong. Japonica restorer line 'Shenhui 26-28' is an excellent line selected from the breeding nursery of 'Shenhui 26' in the normal season of 2016. The sowing time is about 104 days, 1~2 days earlier than 'Shenhui 26'.

3.2 Molecular marker detection of resistance and fragrance genes of 'Shen 21A' and 'Shen Hui 26-28'

The rice blast and blight resistance genes of 'Shen 21A' and 'Shen Hui 26-28' were analyzed by using high density rice microarray. Resistance gene haplotype analysis is to determine whether the sample contains a certain resistance gene through a set of polymorphism markers specific to the gene region and 100 kb region upstream and downstream of the gene. The resistance genes analyzed included rice blast resistance genes *Pi1*, *Pi2*, *Pi5*, *Pi9*, *Pia*, *Pid2*, *Pid3*, *Pigm*, *Pita* and resistance gene to bacterial blight *xa13*, *Xa21*, *Xa23*, *xa5* and *Xa7*. The analysis and detection methods were referred to the literature (Chen et al., 2014).

In order to detect the distribution of more blast resistance genes in two parents, functional molecular markers reported in the literature were used for rice blast resistance gene *Pib* (Fjellstrom et al., 2004; Liu et al., 2008), *Pi54* (*Pikh*) (Sharma et al., 2010; Ramkumar et al., 2011), *Pit* (Hayashi et al., 2010) and *Pikm* (Ashikawa et al., 2008) performed molecular marker detection. Primer information, PCR amplification and detection methods of rice blast resistance genes *Pib*, *Pi54* and *Pit* were referred to the literature (Chu et al., 2018). *Pikm* detection methods refer to literature (Song et al., 2017). The detection methods of the two parents' fragrance genes were also referred to the literature (Shi et al., 2014).

Authors' contributions

NFA was the executor of this study and responsible for the data analysis of experimental results and the writing of the paper. CHW participated in molecular marker detection and data analysis; SB participated in data collection, analysis and revision of the first draft of the paper. ZJH, ZAP, HWW, LY and YY participated in data collection and analysis. CLM and CC were the leaders of the project, supervising the experimental design, paper writing and revision. All authors read and approved the final manuscript.

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