Transferring Translucent Endosperm Mutant Gene $Wx$-$mq$ and Rice Stripe Disease Resistance Gene $Stv$-$b$ by Marker-Assisted Selection in Rice ($Oryza$ sativa)

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Abstract: A high-yielding japonica rice variety, Wuyunjing 7, bred in Jiangsu Province, China as a female parent was crossed with a Japanese rice variety Kantou 194, which carries a rice stripe disease resistance gene $Stv$-$b$ and a translucent endosperm mutant gene $Wx$-$mq$. From $F_2$ generations, a sequence characterized amplified region (SCAR) marker tightly linked with $Stv$-$b$ and a cleaved amplified polymorphic sequence (CAPS) marker for $Wx$-$mq$ were used for marker-assisted selection (MAS). Finally, a new japonica rice line, Ning 9108, with excellent agronomic traits was obtained by multi-generational selection on stripe disease resistance and endosperm appearance. The utilization of the markers from genes related to rice quality and disease resistance was helpful not only for establishing a MAS system of high-quality and disease resistance for rice but also for providing important intermediate materials and rapid selection method for good quality, disease resistance and high yield in rice breeding.

Key words: rice; translucent endosperm mutant gene; rice stripe disease resistance gene; marker-assisted selection

Breeding of good grain quality, high yield and multi-resistant rice varieties is of great significance for improving the rice production efficiency and promoting its sustainable development. In recent years, with the improvement of living standards, people have become increasingly demanding for rice quality, especially eating quality. Studies have demonstrated that rice endosperm amylose content was one of the key factors determining the grain texture and eating quality of rice (Matsuo et al, 1990; Hushibuchi, 1992). Low amylose content rice, with cloudy and milky white endosperm appearance, slightly worse transparent, was the middle type between the ordinary sticky rice and glutinous rice in amylose content. Therefore, it is also known as semi-glutinous rice (Zhu et al, 2004). The rice grain surface is gloss translucent, soft and flexible. By combining the softness of glutinous rice and the flexibility of japonica rice, low amylose content rice is of better eating quality with no retrogradation and good puffing.

So far, a total of 14 rice mutant genes in relation to low amylose content have been reported (Heu 1986; Yano et al, 1988; Heu and Kim, 1989; Kaushik and Khush 1991; Suto et al, 1996; Koh et al, 1997; Sato et al, 2001; Sato, 2002). According to the allelic relationships to the $Wx$ gene, these genes could be divided into allelic and non-allelic types. The translucent endosperm mutant gene $Wx$-$mq$ belongs to a low amylose content gene allelic to $Wx$. The $Wx$-$mq$ gene has been successfully used in Japanese rice varieties and some low amylose content rice varieties with excellent eating quality have been bred, such as ‘Milky Queen’ (Suto et al, 1996; Sato et al, 2001), ‘Kantou 194’ and ‘New-hikari’ (Tomit et al, 2007). According to the difference between $Wx$-$mq$ and other $Wx$ alleles in exon 4, a cleaved amplified polymorphic sequence (CAPS) marker was developed to distinguish the homozygous and heterozygous genotypes of $Wx$-$mq$ gene, and it was successfully used by MAS in the development of a japonica variety, Nanjing 46, with good eating quality (Wang et al, 2009).

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In recent years, rice stripe disease was a major rice disease successively epidemic in the middle and lower Yangtze River region in China, which made a serious threat to rice production (Wang et al, 2007). Numerous studies confirmed that the development of rice varieties resistant to stripe disease was the most economic and effective way to control the disease (Sun and Jiang, 2006). Analysis of the source of rice stripe disease resistance showed that the resistance of most of rice varieties bred and released to the farm’s paddy in home and abroad was controlled by the same pair of incomplete dominant gene \(Stv-b^i\) (Washio et al, 1967; Washio et al, 1968a; Washio et al, 1968b). The gene is very important in rice stripe disease resistance breeding. Its importance lay not only in direct resistance to the disease rather than to the insects transmitting the stripe virus, but also in remaining the good stripe disease resistance in the application in production for over 40 years. Hayano-Saito et al (1998) mapped the \(Stv-b^i\) gene in the interval about 286 kb of two overlapping BAC in rice chromosome 11, and it was closely linked with one RFLP (restriction fragment length polymorphism) marker ST10. Tsuji (2000) further transferred the ST10 into a SCAR (sequence characterized amplified region) marker and applied the SCAR marker in rice stripe resistance breeding by MAS.

Development of rice varieties resistant to the stripe disease and with low amylose content is one of the goals of modern rice breeding. Such varieties could not only get rid of the serious threat to rice production by stripe disease, but also meet the growing consumer demand of urban and rural residents for good rice quality. In this study, the SCAR marker tightly linked with the rice stripe resistance gene \(Stv-b^i\) and the CAPS marker co-segregated with low amylose mutant gene \(Wx-mq\) were used in the detection of \(Stv-b^i\) and \(Wx-mq\) for the segregation progenies of rice breeding materials. A japonica rice line Ning 9108 with good rice stripe disease resistance and taste quality was quickly and accurately bred by field selection for generations, identification of resistance to stripe disease and observation of endosperm appearance. The selection process provided a simple and quick selection method, and new rice genetic resources for breeding of rice varieties with good quality and disease resistance as well.

**MATERIALS AND METHODS**

**Rice materials**

Two rice varieties and their progenies of segregated generations of the combination Wuyunjing 7/Kantou 194 were used. Wuyunjing 7 with high-yielding but highly susceptible to rice stripe disease was developed by Wujin District Institute of Agricultural Sciences, Changzhou City, Jiangsu Province, China. Kantou 194 with low amylose content and resistant to stripe disease was bred by Ibaraki Prefecture Crops Research Institute, Kantou, Japan. According to the pedigree of Kantou 194, the stripe disease resistance gene \(Stv-b^i\) was from a Pakistan rice variety Modan, and the low amylose content gene \(Wx-mq\) was from the endosperm mutant Ko 272 (Wang et al, 2008).

**DNA extraction**

At the tillering stage, genomic DNA from fresh young rice leaves was isolated following the procedure described by Dellaporta et al (1983).

**Molecular detection of the translucent endosperm mutant gene \(Wx-mq\)**

The translucent endosperm mutant gene \(Wx-mq\) was detected by a CAPS marker using a pair of specific primers (forward: 5′-TGTTGCTGAGGTAGGAGCA-3′; reverse: 5′-AAGGATCTGGTTGTCTTTG-3′ (Wang et al, 2009). PCR reaction was performed as described by Chen et al (1997) with minor modifications. PCR reaction mixture consisted of DNA template (10 ng/μL) 1 μL, Primer (4 pmol/ μL) 0.2 μL, 10×Buffer (free MgCl₂) 2 μL, dNTPs (2.5 mmol/L) 0.4 μL, MgCl₂ (25 mmol/L) 0.6 μL, Taq enzyme (5 U/μL) 0.2 μL and ddH₂O 12.6 μL. PCR reaction was performed on the MJ Research PTC-200 thermal cycler with the reaction process as follows: pre-denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 60 s with a final elongation step of 72°C for 10 min. A 20 μL...
reaction system digested by a restriction endonuclease Nla III consisted of PCR product 2 μL, Nla III 2 μL (5 U/μL), 10×NEB Buffer 2 μL, 100×BSA 0.2 μL, ddH2O 13.8 μL. Reaction was performed at 37°C for 4 h, and digestion products were visualized in 2.5% agarose gels and stained with ethidium bromide.

Molecular detection of the stripe disease resistance gene Stv-b1

A SCAR marker from the stripe disease resistance gene Stv-b1 was used for molecular detection (Tsuji, 2000) using the specific primers (reverse: 5′-CGAAAGATGTTTCTCCACC-3′; reverse: 5′-GACCAAGCAACTAATGACGC-3′). The PCR reaction system was the same as the detection of the Wx-mq gene. The PCR reaction was performed as follows: pre-denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52.2°C for 30 s, and elongation at 72°C for 60 s with a final elongation step of 72°C for 10 min. Amplification products were analyzed on 1% agarose gels stained with ethidium bromide and photographed using UVP Bioimaging Systems.

Phenotype identification of starch characteristics

The phenotype of starch characteristics of endosperm for each plant was visually identified after maturity. The plant whose grains showed transparent was recognized as normal endosperm and its genotype was \( W_{x}^{b}W_{x}^{b} \). The plant whose endosperm appearance of all grains was cloudy, milky white, poor transparent was recognized as low amylose content plant and its genotype was \( W_{x-mq}W_{x-mq} \). The plant that contained transparent and non-transparent grains for the endosperm appearance could be identified as heterozygous plant and its genotype was \( W_{x}^{b}W_{x-mq} \).

Identification of field resistance to stripe disease

The field resistance to stripe disease was identified by natural inoculation in serious stripe disease region. In order to ensure the accuracy of the results, rice seedling bed was selected in the area close to wheat field rich in brown planthopper. In the whole growth period of rice, any insecticides were not sprayed. Thirty-five days after transplanting, the incidence of stripe disease was investigated. Disease grade criterion was referenced the method developed by Washio et al (1967) and slightly improved. 0, Asymptomatic; 1, Slightly yellowish green mottle symptoms, infected leaves do not curl, and growth normal; 2, Diseased leaves on the chlorosis extended connected into a white or yellowish green with irregular yellow stripe, diseased leaves do not curl or slightly curly, normal growth; 3, Severe chlorosis diseased leaves, diseased leaves were twirling curl like a small number of diseased leaves appear yellow wilt symptoms; 4, Most of the diseased leaves was twisting like curling, yellow leaves of dead, dry heart-shaped plant was false or whole plant dead. Among them, from levels 2 to 4, it was recorded directly for the disease and the levels 0 and 1 were recorded for the non-disease. For level 1, the plant survey was confirmed once again in seven days. Resistance evaluation was performed according to the method proposed by Zhou et al (2007). Based on the average incidence rate calculated: immune (I), the incidence rate was 0.0%; high resistance (HR), the incidence rate was less than 5.0%; resistance (R), the incidence ranged was from 5.1% to 15.0%; moderate-susceptible (MS), the incidence rate was between 15.1%–30.0%; susceptible (S), the incidence rate was between 30.1%–50.0%; high-susceptible (HS), the incidence rate was higher than 50.0%.

RESULTS

Screening for the plants with both Wx-mq and Stv-b1 genes from segregated generation of F2

In the winter of 2004, Wuyunjing 7 as a female parent was crossed to Kantou 194 as a male parent in Hainan Province, China. F1 generation was grown in the planting season of 2005 in Nanjing, Jiangsu Province, China and seeds of F2 generation were mixed after harvested. In the winter of 2005, 184 plants of F2 segregating population were grown in Hainan Province. At the tillering stage, the CAPS marker co-segregated with translucent endosperm mutant gene Wx-mq and the SCAR marker closely linked to the stripe disease resistance gene Stv-b1 were used to detect the two genes respectively in segregated plants of F2 population. Restriction endonuclease
digestion results with the CAPS marker showed that

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Then, the SCAR marker was used to detect the gene $Stv-b$ in 45 plants with two bands of 215 bp and 170 bp. As a result, 29 plants were found with a 727 bp band (Fig. 2), which should have both the $Stv-b$ gene (homozygous or heterozygous) and the $Wx$-$mq$ gene (homozygotes).

Phenotypic identification of the homozygous plants with both $Stv-b$ and $Wx$-$mq$ genes

In 2006, the 29 plants containing both $Stv-b$ and $Wx$-$mq$ genes were grown as 29 lines for phenotype identification. The parents of Kantou 194 and Wuyunjing 7 were used as resistant and susceptible controls for the identification of field resistance to stripe disease. The natural incidence rates of stripe disease of Kantou 194 and Wuyunjing 7 were 0% and 62.5%, respectively, indicating that the incidence of stripe disease was fully and can effectively distinguish the resistance levels of F3 family lines in the field. Identification results showed that among 29 plant-derived F3 families, 8 families were not segregated in the resistance with the incidence rate from 0% to 5%, belonging to immunity or high resistance level and the resistance level was the same as the resistance parent Kantou 194 (Table 1). The result implied that MAS is feasible in rice stripe disease. After maturity, 40 plants were selected from each family for the observation of starch properties of rice endosperm. The results showed that all the plants of 29 F3 families had cloudy, milky white and non-transparent endosperm. Thus, the genotype could be confirmed as $Wx$-$mq$$Wx$-$mq$. The phenotypic results were completely consistent to the molecular identification using the $Wx$-$mq$ gene marker; which further verified that the CAPS marker from the
translucent endosperm mutant gene $Wx-mq$ is feasible and accurate.

**Breeding of yield and agronomic traits for double homozygous offspring with both $Stv-b'$ and $Wx-mq$ genes**

The eight $F_3$ families which were obtained in 2006 and did not segregate in stripe disease resistance were homozygous progenies with both $Stv-b'$ and $Wx-mq$ genes. According to the test results of yield and agronomic traits of 10–15 plants selected from each $F_3$ family, a total of 105 plants were selected from eight $F_3$ families. The selected plants were grown as $F_4$ plots in the winter of 2006 in Hainan Province and each plot had 40 plants. At the maturity stage, selection was carried out in 48 plots with excellent yield and agronomic traits. From each plot, 5 to 10 plants were selected and a total of 350 plants were collected. The selected plants were grown as $F_5$ families in 2007 in Nanjing, Jiangsu Province and each plot had 40 plants. Among 350 $F_5$ families, 12 families showed stable and consistent agronomic traits and better yield performance. In 2008, the 12 plots were identified and four plots were selected for further yield testing in 2009. As a result, JD9108 showed outstanding performance and was tentatively named as Ning 9108. The whole breeding process is shown in

![Fig. 3. Breeding process of Ning 9108.](image)

**Fig. 3.** To prevent the loss of homozygous genotype of $Stv-b'$ and $Wx-mq$ genes in the breeding and harvesting, 20 plants were randomly selected from JD9108 at the tillering stage in 2009 for molecular detection. The detection results of the SCAR marker showed that all the plants had a 727 bp band, indicating that Ning 9108 was $Stv-b' Stv-b'$ homozygous (Fig. 4). The CAPS marker test results confirmed that all the plants had homozygous translucent endosperm mutant genotype (Fig. 5). These results were completely

![Fig. 4. Molecular detection of $Stv-b'$ genotype in the stable line Ning 9108.](image)

M, DL2000; Lane 1, Kantou 194; Lane 2, Wuyunjing 7; Lane 3, ddH2O (negative control); Lanes 4 to 23, Twenty randomly selected plants of the line Ning 9108.

<table>
<thead>
<tr>
<th>Line</th>
<th>No. of plants</th>
<th>Incidence rate (%)</th>
<th>Resistance level</th>
</tr>
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<tbody>
<tr>
<td>L3</td>
<td>40</td>
<td>0.0</td>
<td>I</td>
</tr>
<tr>
<td>L5</td>
<td>40</td>
<td>0.0</td>
<td>I</td>
</tr>
<tr>
<td>L9</td>
<td>40</td>
<td>0.0</td>
<td>I</td>
</tr>
<tr>
<td>L13</td>
<td>40</td>
<td>2.5</td>
<td>HR</td>
</tr>
<tr>
<td>L14</td>
<td>40</td>
<td>2.5</td>
<td>HR</td>
</tr>
<tr>
<td>L18</td>
<td>40</td>
<td>0.0</td>
<td>I</td>
</tr>
<tr>
<td>L20</td>
<td>40</td>
<td>5.0</td>
<td>HR</td>
</tr>
<tr>
<td>L26</td>
<td>40</td>
<td>0.0</td>
<td>I</td>
</tr>
<tr>
<td>Kantou 194</td>
<td>40</td>
<td>0.0</td>
<td>I</td>
</tr>
<tr>
<td>Wuyunjing 7</td>
<td>40</td>
<td>62.5</td>
<td>HS</td>
</tr>
</tbody>
</table>

I, Immunity; HR, High resistance; HS.: High susceptible.
consistent with the field investigation results on the stripe disease resistance and the endosperm characteristic observation.

**Agronomic performance of Ning 9108 with resistance to stripe disease, good eating quality and high yield**

The new rice line Ning 9108 with the comprehensive characters of stripe disease resistance, good eating quality and high yield, had the whole growth duration of 146 d, which was 1–2 d later than the control Zhendao 88 and belonged to the middle season japonica rice with medium maturity. Its plant height was 95–98 cm, with erect plant type and strong lodging resistance. It had good tillering ability with 8 to 10 panicles per plant. It also had large panicle with 140 to 160 spikelets per panicle. Its seed setting rate was 95%–97% and the 1000-grain weight was 27–29 g. The grain length and width ratio was about 1.5 with less chalky. Inoculation results showed that Ning 9108 was resistant to stripe disease, moderately resistant to bacterial blight, susceptible to panicle blast and sheath blight. Its endosperm was semi-glutinous, cloudy and milky in appearance. The cooked rice of the new line was crystal-clear, soft, texture-smooth, elastic and flexible by combining the softness of glutinous rice and flexibility of normal sticky rice. Therefore, its eating quality was excellent. Generally, its grain yield was more than 9 000 kg/hm² and up to 10 500 kg/hm² in high yielding cultivation conditions.

**DISCUSSION**

Kantou 194 is a new rice variety with good quality and resistant to rice stripe disease bred by Crops Research Institute of Ibaraki Prefecture, Kantou, Japan. As it has the stripe resistance gene $Stv-b'$ and the low amylase mutant gene $Wx-mq$, it has strong resistance to stripe disease and good eating quality (Wang et al, 2008). However, limited by geography, climate and other environmental factors, it was difficult to show better overall character when it was grown in Jiangsu Province, China. Therefore, it was a suitable parent to cross with local high-yielding rice varieties to develop new varieties with good quality, high yield, disease resistance and suitable for local cultivation.

However, traditional breeding is mainly based on the phenotype to indirectly select the genotype. For the rice stripe disease, Wang et al (2008) considered that it was possible to obtain new rice strains with stable stripe disease resistance by artificial selection for 2–3 generations on the progenies of the hybrids containing the source of resistance to stripe disease under the full incidence of the natural conditions. Indeed, it is a time-consuming and laborious work to process on a large number of breeding materials in the field. It is also difficult to combine several target characters in a variety such as stripe disease resistance, good quality and high yield. Therefore, if you want to pyramid several favorable genes in an individual and to ensure these genes can genetically transfer from generation to generation and do not lose and obtain stable rice lines or varieties, there must be a rapid, accurate and practical method for identification of breeding materials.

Today, MAS is considered as an important means for plant breeding. It is a method to select genotypes by using molecular markers closely linked to the target gene or directly from the gene. So it is not subject to environmental conditions and can shorten the breeding period to improve selection efficiency. One of the applications of MAS is gene pyramiding which is to combine several useful genes scattered in different germplasms into the same genome. These can be different genes or QTLs related to yield, quality or resistance. Therefore, gene pyramiding
could improve a number of characters simultaneously, resulting in more valuable breeding materials by overcoming the shortcomings or limitations of the backcross which can only modify individual character (Wu et al, 1999). There were many reports on aggregation of multiple disease resistance genes in rice pyramiding breeding. Huang et al (1997) and Hittalmani et al (2000) aggregated rice bacterial blight and blast resistance gene by using of MAS technology, respectively. Naoki et al (2004) bred the new stripe disease resistance variety Aichi 106 by pyramiding stripe disease resistance gene Stv-bi from Modan and blast resistance gene Pib1 by MAS. Narayanan et al (2002) got together three major genes of Pi-1, Piz-5 and Xa21 into Co39, two main effect genes of Xa21 and Piz-5 into IR50. Jiang et al (2004) cooperated Xa21 and Bt genes to the restorer line Minghui 63. Ni et al (2005) aggregated Xa21 and Pib9(t) gene by MAS. Chen (2005) developed a material with broad-spectrum, durable resistance to rice blast by pyramiding three blast resistance genes using the MAS technology. The above research has focused on pyramiding resistance genes, however, combining other traits such as good quality, high yield with disease resistance genes were rarely reported.

In this study, a new rice line Ning 9108 with resistance to stripe disease, good quality and high yield was developed by using the SCAR marker tightly linked to the rice stripe disease gene Stv-bi and the CAPS marker co-segregated with the translucent endosperm mutant gene Wx-mq through MAS combined with conventional breeding techniques. To ensure the accuracy of two genes in the whole process of gene transfer and polymerization, phenotypic identification and molecular detection for the two genes have been done in the two key generations. Practice has proved that plants with target genes could be selected quickly and effectively by detection of the target genes using molecular markers directly from the target gene or tightly linked to it, combined with traditional breeding techniques, and by resistance identification, the appearance identification of mature endosperm and the selection of agronomic traits. These plants can be directly applied to rice breeding in good quality, high yield and disease resistance. It has important practical significance on rice eating quality and resistance improvement.

With the progress of molecular biology and development of bioinformatics, gene functions will be further understood. A multidisciplinary cooperation of functional genomics, proteomics, cell physiology and bio-informatics and other disciplines will further strengthened by the use of genetic resources obtained. By combining of transgenic biotechnology, MAS and conventional breeding methods, it is possible to realize co-expression of multiple genes in the same plant. In the future, to develop new rice varieties with multi-genes by molecular design breeding, excavation and innovation of germplasm will be the focus of rice breeding.

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