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# Practice of Research and Application of Two-Line Hybrid Rice

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**Abstract**

The main problems about research and application of two-line hybrid rice were reviewed, including the confusing nomenclature and male sterile lines classification, the unclear characteristics of photoperiod and temperature responses and the unsuitable site selection for male sterile line and hybrid rice seed production. In order to efficiently and accurately use dual-purpose genic male sterile lines, four types, including PTGMS (photo-thermo-sensitive genic male sterile rice), TGMS (thermo-sensitive genic male sterile rice), reverse PTGMS and reverse TGMS, were proposed. A new idea for explaining the mechanism of sterility in dual-purpose hybrid rice was proposed.

**Keywords:** Traditional rice**;** Bioactive compounds**;** Nutraceuticals**;** Therapeutic properties**;** Biological diseases

## Introduction

Rice is one of the most important cereal crops in the world. With nearly 10% protein and more than 80% starch in the endosperm, it serves as a primary food for more than half of the world's population. Modern human population numbers and living standards have urgently increased demand for high-quality food. Rice quality mainly covers eating and cooking quality (ECQ), milling quality, nutritional quality, appearance quality (AQ), and hygiene quality. ECQ mainly reflects the physicochemical characteristics and edible quality of rice grains in the process of cooking and eating. Consumer preference for cooked rice largely depends on its ECQ; it is the key quality factor most considered by consumers, producers, farmers and breeders [1].

ECQ, also known as sensory and cooking quality, refers to the comprehensive evaluation of the smell, colour, shape, taste (hardness, stickiness, cohesiveness, and elasticity), and other sensory indicators of a food after cooking. The taste profiles of cooked rice are often evaluated manually or by machine. Because both methods are often cumbersome and time-consuming, the grain physicochemical properties: amylose content (AC), gel consistency (GC), gelatinization temperature (GT) and protein content (PC) is widely used for rice ECQ evaluation. Among them, AC is regarded as the most important determinant. Generally, AC has a negative correlation with the taste score, stickiness, and cohesiveness of cooked rice, but it has a positive correlation with hardness and elasticity [2]. As for GC and GT, both have a minor effect on ECQ parameters compared with A.

## Literature

In terms of PC, it is the second principal component in rice grain, and it was found to have a negative correlation with taste score., In general, rice AC is measured by the iodine blue colorimetric method, and thus expressed as apparent amylose content (AAC). The AAC of rice varieties varies greatly (0–35%), and the differences in the requirements for rice palatability in different countries and regions mainly stem from differences in AAC. It is generally accepted that the AAC of rice grains can be divided into five grades. High amylose rice has more than 25% AAC, and when cooked, these grains have good expansibility, are dry and loose, harden after cooling and are hard to digest. Medium amylose rice has 20–25% AAC, and when cooked, these grains have a certain viscosity and are fluffy. Low amylose has approximately 10–20% AAC, and when cooked, these grains have low swelling and high water content, and they are relatively soft and sticky and easy to digest. Cooked rice with very low amylose (2–9% AAC) has poor expansibility and is wet, sticky and lustrous [3]. Cooked waxy or glutinous rice (<2% of AAC) is extremely soft and lustrous, but it is so sticky that it is mainly used in various kinds of processed or traditional foods, so it is less often eaten directly compared to nonluminous rice.

Amylose synthesis is mainly controlled by the *Waxy* (*Wx*) gene, which is the "one main gene". This gene consists of 14 exons and 13 introns, encodes granule-bound starch synthase I (GBSSI), and is mainly responsible for the synthesis of amylose in endosperm and pollen sacs. The *Wx* gene is in the center of AC control.

The multiple minor genes include three kinds of genes: (1) starch synthesis-related genes (*SSRGs*), such as *SSs*, *SBEs*, and *DBEs*, for amylopectin synthesis in the starch synthesis pathway, which indirectly regulate the synthesis of amylose with micro-effects; (2) genes found to genetically interact with the *Wx* gene, including Type I genes encoding transcription factors binding to cis-elements of the *Wx* gene (*Wx-TFs*) such as *OsBP-5*, *OsEBP-89*, *OsbZip58*, *OsREB*, *OsRPBF*, *NF–Y*, *OsMADS7*, *OsSERF1*, *OsRSR1*, and *ETR2*, and Type II genes having functions related to pre-mRNA splicing (*RSFs*) such as *Du1*, *Du3*, *qAC2*, and *LowAC1*; in addition, *qSAC3* can influence AC by interacting with the *Wx* gene; and finally, (3) genes encoding other regulators, such as starch localize factors (*SLFs*), including *OsGBP* and *FLO6*, which can help the GBSS protein localize to starch and thus influence amylose synthesis[4]. Thus, multiple minor genes are helpers or aids in AC regulation.

$Wx^{in}$ can be selected as the potential allele in *indica* rice breeding because of its function in decreasing AC compared with that of $Wx^{a}$ and its stable effect on ECQ under high temperature compared with that of $Wx^{b}$. Thus, this allele will likely become widely used in the very near future. In addition, further efforts may be needed to decrease AC, since the AC of these rice varieties is still relatively high.

$Wx^{mp}$ and $Wx^{op/hp}$ are widely applied alleles in soft rice because of their association with low AC and good edible characteristics. It will

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be necessary to improve AC moderately to approximately 10%–13% to overcome or relieve the translucent or dull appearance of these lines, as accompanied by $Wx^{mq}$. Therefore, to best address ECQ and AQ together, $Wx^{mw/la}$ is more optimal. Other *Wx* alleles originating from gene editing, $Wx^{a-dc2,3}$ and $Wx^{a-ds1,2,3}$ from $Wx^{a}$ $Wx^{b-d8}$, $Wx^{m6,7}$ and $Wx^{a-be2}$ from $Wx^{b}$ and $Wx^{b(P415S)}$ from $Wx^{b}$ from other transgenic methods decrease ACs to proper levels and may be used as resources for future utilization.

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Two single nucleotide substitutions, G/A in exon 4 (different from the SNP in $Wx^{op}$) and T/C in exon 5, that cause missense base changes resulting in amino acid substitutions from arginine to histamine and from tyrosine to histidine, respectively, occurred in $Wx^{mq}$ compared to its wild-type allele $Wx^{b}$, which determines the change in enzymatic activity of GBSSI and leads to a decrease in the AC of this rice; the AC is approximately 10% or less in a Koshihikari mutant line, Ko271 (Milky queen, a commercialized variety with good taste in Japan). The mature grains show a translucent-endosperm appearance, which can be readily distinguished from waxy and nonluminous endosperm. Another allele, $Wx^{mp}$, derived from Ko272 (the male parent of Milky princess), a sibling of Milky queen, has a missense base change of G/A in exon 4 compared to $Wx^{b}$, resulting in an amino acid substitution from arginine to histamines, which is located in the starch synthase catalytic domain and near the substrate binding region, and may cause a change in the enzymatic activity of GBSSI and lead to a decrease in AC. The AC of this rice is approximately 10% or less, and the grain endosperm has a translucent appearance. Using $Wx^{mp}$, breeders in China and Japan focused on breeding *japonica* varieties with a low AC of approximately 10% due to their good eating quality. Newly bred soft rice varieties carrying $Wx^{mp}$, such as the *japonica* rice Nangeng46, Nangeng9108, Nangeng5718, Nangeng5055, Ninggeng8, and Huruan1212, have been planted in very large areas of more than 6 million hectares in the last ten years, mainly in the middle-lower reaches of the Yangtze River region, one of the major *japonica*-producing regions in China[7].

## Genetic Manipulation of the Minor Genes

In addition to the direct function of *Wx* in amylose synthesis, dozens of minor genes/QTLs are involved in the regulation of the *Wx* gene at transcription and post-transcription levels [8]. Therefore, the identification and utilization of minor genes provide another way to improve the AC trait and ECQ of rice.

## Other Genes/Qtls Not Allelic with *Wx*

A number of minor-effect genes/QTLs that are not allelic to *Wx* have been identified in recent years, such as *qAC*, *qHAC*, *QAc*, *amy*, *ac*, and *dull*, which are distributed throughout the whole rice genome. Many of these genes were found to genetically interact with the *Wx* gene. Specifically, the *Wx* gene can be finely regulated by different genes mainly at three levels, including transcriptional, posttranscriptional, and posttranslational pathways, as found in recent decades [9,10].

## Utilization of the Favorable *Wx* Alleles

The gap in ACs between *indica* and *japonica* is becoming smaller. The gap is approximately 1% between the two subspecies of rice, and perhaps in a few years, the gap may no longer exist. People from countries whose staple food is rice pay more attention to the ECQ, and even in Japan and China, for example, rice breeders have focused on the breeding of varieties with a low AC of approximately 1%–15% in the last decade.

## Conclusion

In rice production, the AC is the main determinate of the ECQ of directly consumed rice grains. The breeding improvement of the AC in rice is helped by the progress of its genetic research. The strategy for AC and ECQ improvement is based on genetics, current status and prospects for breeding and utilization, including allele replacement of the main gene *Wx*-determined AC, control of *SSRGs* other than the *Wx* gene for amylopectin synthesis, cooperation with other minor-effect genes/QTLs involving *Wx* mRNA splicing factors, transcription factors binding to the *Wx* gene (*Wx-TFs*), starch localization factors (*SLFs*) and other factors found to genetically interact with the *Wx* gene. We hope this review will aid the improvement of rice ECQ, especially the genetic improvement of traits related to its palatability from the perspective of AC.

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