Improvement of Grain Quality in Hybrid Rice

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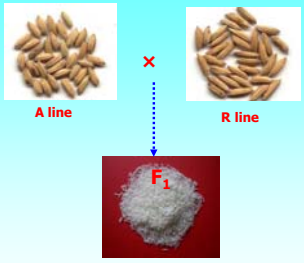
1. Introduction

- Rice is one of the most important crops in the world that provides staple food for half of the world's population.
- Hybrid rice has yield advantage of more than 20% over conventional pure line varieties (Yuan,1994). Its potential in increasing rice production has motivated many countries to attempt exploiting this technology.

It is known that the seeds borne on F_1 hybrid plants are F_2 seeds. Most of the quality traits, such as

- Endosperm translucency
- Amylose content (AC)
- Gelatinization temperature (GT)
- Gel consistency (GC)
- Chalkiness

are bound to show genetic segregation.



Thus the grain quality of the hybrid rice is genetically determined by both the male and female parental lines.

So, it is of importance for developing high-quality hybrid rice to study the genetics of the quality traits and understand how the female and male parental lines influence their F_1 hybrids.

2. Genetics of quality traits and strategies of improvement

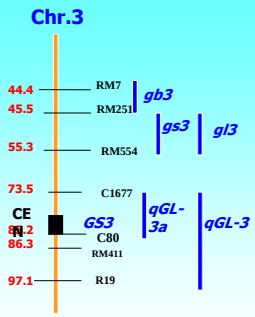
Grain size, shape and weight

- Since rice is consumed and processed mainly in whole kernel form, the grain length, breadth, shape and weight are always of foremost importance for its market price.
- Grain length, breadth and shape are determined by maternal genotype, and the long grain length is dominant over the short grain length.

- We also have found that the grain shape of hybrid rice is mainly determined by the MS line, and the grain length, breadth and weight are co-determined by both the male and female parents.
- Many QTLs associated with grain Size, shape and weight have been mapped:

Table 1. Some QTLs for grain size, shape and weight identified

Trait	QTLs	Chr.	Marker interval or linked markers	NLM (cM)	LOD score	PVE (%)	Reference
GL	*qGL-1*	1	R210-C955		5.3		Wan et al. 2005
	qGL-3	3	R19-C1677		5.9		
	qGL-3a	3	C80-C1677		27.79	34.6	Harushimaet al. 1998 Wan et al. 2006
	grl1-1	1	RM431–RM10	0.01	5.75		Amarawathi et al.2007
	gl3	3	RM251-RM554	6	6.32	19.3	Rabiei et al. 2004
GS	*gs3*	3	RM251-RM554	2	11.29	27.1	
	lbr7-1	7	RM11–RM505	0.01	6.20		Amarawathi et al.2007
	lbr7-2	7	RM505–RM336	9.88	10.8		
GL/GW	*GS3*	3	GS09-MRG5881	0.3			Fan et al. 2006
GB	*gb3*	3	RM7-RM251	5	15.02	34.1	Rabiei et al. 2004
	grb7-1	7	RM11–RM505	0.01	6.15		Amarawathi et al.2007
	grb7-2	7	RM505–RM336	9.88	9.20		
GW	*Gw8.1*	8	RM23201.CNR151-RM30000.CNR99				Xie et al.2006



Major-effect QTL's for grain size, shape and weight identified on chromosome 3

If incorporating the long-grain allele *GS3* or *qGL-3a* into the medium grain parental lines by using MAS, to make both male and female parents carry the long-grain allele, both the yield and grain quality of rice hybrids could be ,to some extent, improved.

Endosperm appearance

- Endosperm appearance is primarily decided by the amylose content. It varies from waxy or dull endosperm to translucent as amylose content increases.
- The appearance of the endosperm of seeds borne on hybrid rice plants varied in crosses involving waxy or dull endosperm, on the one hand, and translucent endosperm, on the other.

- White centers and white bellies are reported to be governed by a single recessive gene or white bellies are also reported to be controlled by a dominant gene (Nagai, 1958), and by polygenes (Chang, 1979), and the low chalkiness is partial dominant over the high chalkiness (Xu et al. 1989).

- Three QTLs *qPGWC*-8, *qPGWC* -12 and *qSWC* -3 for ratio of chalky grain and square of the white core have been detected on chromosome 3, 8 and 12, respectively (He et al 1998) .

- Zhu et al (2002) also reported that the male sterile line had greater effects on the chalkiness than the restorer line in Hybrid rice.

So, it is crucial to develop and use a male sterile line with less or no chalkiness in hybrid rice breeding program.

Amylose content, Gel consistency and Gelatinization temperature

- Amylose content

Amylose content is one of the major factors that influence the cooking and eating quality.

Grains harvested from hybrid rice plants are a bulk F_2 seeds and show segregation for amylose content. Thus the amylose content of hybrid rice is actually the mean value of the bulk F_2 seeds and is reported to be generally between that of the parents.

Amylose content is genetically controlled by a major gene *Wx* on chromosome 6 and some minor QTLs *qAC-2 and qAC-5* on chromosome 2 and 5, respectively .

Blight et al.(1995) found there was a simple repeat sequence (CT)n in this gene, and then developed a SSR marker 484/485 (RM190) based on it. The number of (CT) repeat is highly correlated with the amylose content of rice.

So, SSR marker RM190 is useful in molecular marker-assisted selection for improving the AC of parental lines in hybrid rice breeding.

- **Gel consistency (GC)**

GC in rice determines softness or hardness of cooked rice after cooling.

Hard GC is to be dominant over intermediate or soft. Similarly, intermediate GC was found to be dominant over soft GC in analysis of F_1 seeds.

GC is genetically controlled by a major-effect QTL *qGC-6* ,and some minor-effect QTLs *qGC-2* and *qGC-7* .

And the major-effect QTL *qGC-6* is locate at the vicinity of waxy locus on chromosome 6.

This provides a perfect explanation why a variety of low AC generally has soft GC. The coincidence of QTLs in the vicinity of the *Wx* gene for AC and GC may be due to pleiotropy or linkage.

- **Gelatinization temperature (GT)**

GT is known to affect the cooking quality of rice.

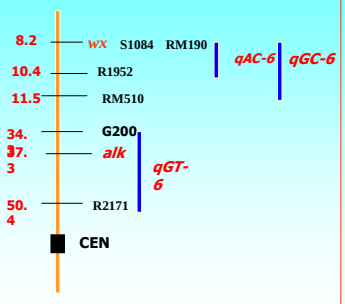
It is also reported to be genetically controlled by major- and minor-effect QTLs. The major gene (QTL) *alk*, *qGT-6* or/and *qASS-6* have been mapped to the same interval on Chromosome 6.

Just like AC and GC, GT is governed by one major gene and some modifier genes in rice.

Table 2 some major- and minor-effect QTLs for eating and cooking quality traits

Trait	Gene/QTLs	Chromosome	Marker interval or linked markers	LOD score	PVE (%)	Reference
AC	*Wx*	6	Wx	28.39	91.1	He et al. 1999
	qAC-6	6	RM190-RM510 S1084-R1952	35.5 20.34	61.8 74.67	Tian et al. 2005 Sun et al. 2006
	qAC-2 *qAC-5.*	2 5	R1843-G132 RG573-C624	4.20 2.67	5.83 11.8	Sun et al. 2006 He et al. 1999
GC	*qGC-6*	6	RM190-RM510	33.7	59.7	Tian et al. 2005
	qGC-2	2	R712-R1843 RG171-G243A	6.38 4.14	18.99 20.2	Sun et al. 2006 He et al. 1999
	qGC-3	3	R2856-R3226	4.73	12.7	Sun et al. 2006
	qGC-7	7	TCT122-RG769	3.26	14.2	He et al. 1999
GT	*qGT-6*	6 6	RM276-RM121 G200-R2171	34.0 29.83	80.3 64.42	Tian et al. 2005 Sun et al. 2006
	qASS-6	6	CT201-RZ450	6.19	24.6	He et al. 1999
	alk	6	CT506-C235	27.04	82.4	
	asv6-1	6	RM3–RM21	2.6	6.9	Amarawathi et l.2007
	qGT-3-1 *qGT-3-1*	3	R663-S14055 R2856-R3226	3.85 5.18	3.14 8.31	Sun et al. 2006
		6	C1478-RZ667	/	60.30	Lanceras et al. 2000

Chr.6



The segment harboring *Wx* and *alk* gene on Chromosome 6 plays a very important role in determining the cooking and eating quality of rice.

Thus the improvement of AC, GC and GT for the parental lines of hybrid rice could be made by substitution of the targeted segment (allele) on Chromosome 6 with a desirable one from a high-quality parent through backcross breeding and molecular marker-assisted selection.

Using this method, Zhou et al (2003) have successfully improved a high AC maintainer Zhenshan 97B through substituting the segment harboring the high-AC *Wx* allele with a segment carry the low-AC *Wx* allele from the restorer Minghui 63, that has medium AC, soft GC and high GT. And the improved line has not only low AC, but also soft GC and high GT as the donor.

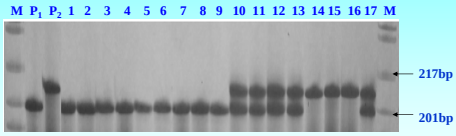
Aroma

Aroma is an important quality characteristics of high-quality rice.

- It is reported to be controlled by a single recessive gene *fgr* on chromosome 8 (Sood and Siddiq, 1978; Ahn et al, 1992; Lorieux et al.1996 ; Li et al.2006) , and 2 minor-effect QTL located on chromosome 3 and 4 (Amarawathiet al. 2008).

- Various markers tightly linked with *fgr* have been developed, such as
  - RG28 (Ahn et al.1992),
  - RSP04 (Jin et al.2003),
  - SCU015RM (Cordeiro et al. 1996),
  - GR01 (Li et al., 2006), and
  - GRFM04 (Wang et al.,2008)

In order to improve the precision of marker-assisted selection for fragrance in rice , we have developed a functional marker GRFM04 based on the deletion of 8bp in DNA sequence of BAD2 gene in the fragrant rice variety.

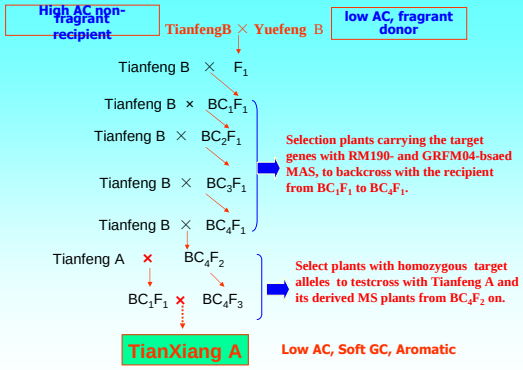


The fragrance gene can be easily incorporated into the parental lines of hybrid rice by using backcross and MAS with this functional marker.

3. Advances in development of hybrid rice with fine grain quality

(1) Improvement of Amylose content and aroma by using MAS

Tianfeng A is a newly developed MS line. It has long grains, resistance to blast and good combining ability, by using it, more than 10 heterotic hybrids have been developed and released for commercial production in China, However, and it has high amylose content and no aroma.



Breeding procedure of pyramiding two genes to improving the quality of Tianfeng B by MAS

Five improved lines with the low-AC allele and *fgr* gene have been successfully developed:

Improved lines	GL (mm)	GB (mm)	L/B	Brown Rice (%)	Milled Rice (%)	AC (%)	GC (mm)	Alkali value (grade)	Aroma
D211	9.6	2.5	3.8	71.4	67.4	15.7	72	2.0	moderate
D221	9.5	2.5	3.8	81.3	73.5	15.5	73	2.0	strong
D223	9.6	2.5	3.8	80.8	73.0	15.6	72	2.1	strong
D225	10.3	2.5	4.0	80.7	72.8	15.2	76	2.0	strong
D238	10.0	2.4	4.0	79.8	71.5	13.0	81	2.0	strong
Yuefeng B (donor)	9.9	2.4	4.1	82.0	73.7	17.4	75	5.8	strong
Tianfeng B (recipient)	9.2	2.4	3.8	81.2	73	27.0	32	3.8	no

The other agronomic traits of improved lines showed no significant difference from that of the original Tianfeng B. By backcrossing one of the improved lines D221 with the MS lines, a new MS line **Tianxiang A**, which possesses low AC, soft GC and strong aroma, has been developed.

it is suggested that the improvement of the AC, GC and aroma for the parental lines in hybrid rice by using MAS with marker RM190 and GRFM04 is successful.

(2) MS lines with fine grain quality

Most of the MS lines previously developed in China have short or bold grains, chalky endosperm, high amylose content and hard gel consistency. And the hybrids derived from these lines usually having poor grain quality. Therefore, breeding fine-quality MS lines is of prime importance.

Yuefeng A and **Taifeng A** are two newly developed MS lines in Guangdong, China. Both of the MS lines have slender grain, less or no chalkiness, translucent endosperm, low amylose content and soft gel consistency.

Table 4 Quality traits of the elite MS lines and their hybrids

Quality traits	Tai feng A	Yue Feng A	Xie qing Zao A	Tai feng you 3922	Tai feng you 368	Feng you 998	Feng you xiang zhan	Shan you 63 (CK)
Brown rice (%)	81.6	80.3	80.7	83.8	83.8	82.6	79.2	82.6
Milled rice (%)	77.6	71.2	71.4	75.4	74.8	76.4	72.6	76.2
Head rice (%)	74.1	28.2	24.6	52.4	70.4	66.5	63.5	67.9
kernel length (mm)	8.1	6.7	6.5	8.7	7.3	7.0	7.1	6.3
kernel breadth (mm)	1.8	1.8	2.3	1.8	1.9	1.94	2.0	2.5
kernel shape (L/B)	4.3	3.7	2.8	4.8	3.8	3.6	3.5	2.5
Chalky kernel (%)	1	0	100	10	17	30	7	74
Chalkiness area (%)	0.1	0	25.2	3.9	6.0	5.4	0.7	16.1
Translucency (grade)	1	1	3	1	2	2	1	2
Gel consistency (mm)	82	92	58	53	80	83	84	44
Amylose content (%)	13.9	14.3	23.4	21.3	16.5	17.0	13.5	20.1
Aromatic	-	+	-	-	-	+	+	-

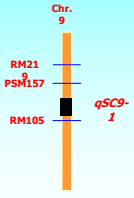
Although Taifeng A has long kernel length of 8.1 mm, and its head rice recovery is up to 74.1%. Yuefeng A has aroma in both leaf and kernel. Hybrids derived from these two MS lines, such as Taifengyou 3922, Taifengyou 368, Fengyou 998 and Fengyouxiangzhan, also have better appearance and eating and cooking quality.

(3) Restorer lines with fine grain quality

Restorer line is another parental line which determines the grain quality of hybrid rice. Breeding new restorers possessing fine grain quality is of importance, too. Much progress has been made in Guangdong province, China. Some restorer lines with better grain quality, such as Guanghui 122, Guanghui 998, Guanghui 290, and Guanghui 368, have developed and widely used in recent years .

Restorer lines Guanghui 122

- Guanghui122 has slender grain , soft GC , lower AC and storable character of seed (seed longevity)
- It also has strong resistance to blast and medium resistance to bacterial blight.



The grain quality of the restorer lines Guanghui 122 and its hybrids

Quality traits	Guang hui 122	Tianyou 122	Hua you 122	You you 122	Yue za 122	Bo you 122
Brown rice (%)	81.9	81.3	81.2	82.8	83.8	80.0
Milled rice(%)	73.7	74.3	73.7	75.3	76.1	72.0
Head rice (%)	58.8	45.4	65.4	55.6	61.3	65
kernel length (mm)	6.8	/	6.2	6.4	6.9	6.01
kernel breadth (mm)	2.2	/	2.07	2.7	2.22	2.2
kernel shape (L/B)	3.2	3.1	2.6	2.5	3.2	2.7
Chalky kernel (%)	16	5	32	37	10.0	44.0
Chalkiness area (%)	3.3	0.5	5.4	10.4	2.5	27
Translucency (grade)	2	1	2	3	1	1
Gel consistency (mm)	76	85	50	48	94	57
Amylose content (%)	15.3	18.7	21.0	19.4	22.1	22.7

Released in 2000

Released in 2001

Released in 2005

Released in 2001

Restorer lines Guanghui 998

- fine grain quality
- resistant to blast
- good combining ability

Table5 The grain quality of Guanghui998 and their hybrid rice

Quality traits	Guang hui 998	Qiuyou 998	Huayou 998	Tianyou 998
Brown rice (%)	80.6	80.9	80.0	78.9
Milled rice(%)	71.8	73.7	76.9	76.1
Head rice (%)	52.1	56.5	64.2	61.5
kernel length (mm)	6.9	6.2	6.5	6.9
kernel breadth (mm)	2.2	2.07	2.24	2.22
kernel shape (L/B)	3.4	3.0	2.9	3.1
Chalky kernel (%)	22	16	6.0	10.0
Chalkiness area (%)	3.6	2.5	0.6	2.5
Translucency (grade)	2	2	1	1
Gel consistency (mm)	76	63	80	65
Amylose content (%)	15.5	22.9	17.6	22.1

Using this R line, more than 10 hybrids, such as

Tianyou998 Huayou 998 You you 998

Boyou 998 Wufengyou998 Shanyou 998

Qiuyou998 II you998 Fengyou 998

Zhenyou998

have been developed and released for commercial production, All these hybrids have better grain quality, 4 of them are up to the China National standard of rice grain quality.

Released in 2003 (national)

Released in 2004 (national)

Released in 2006 (national)

Released in 2006 (national)
With high yield potential

Released in 2003 (national)

Released in 2005 (national)
with fine grain qaulity

Hybrids developed from Guanghui998 have been widely planted more than 2 million hectares in South China. It has become the most widely used restorer line in Guangdong Province in recent years.

4.Conclusions

- The grain quality of hybrid rice is co-determined by both parents, thus the improvement of the parental lines in quality traits is of importance in hybrid rice breeding.
- The inheritance of the main quality traits has been elucidated and most of them have been precisely mapped on the linkage groups with molecular markers.

It makes us possible to deploy the "Breeding by design" strategy and pyramid the favorable genes or QTLs conferring the desirable grain quality traits into the elite parental lines, especially into the MS lines by MAS, and to establish a high-efficient platform for hybrid rice breeding and accelerate the rapid development of fine-quality hybrid rice.

Thanks for attention!