Improvement of pre-harvest sprouting and grain quality in CMS line of hybrid rice using MAS

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◆The three-line system of hybrid rice breeding and production is an important milestone in the process of rice breeding followed by the application of dwarf varieties.

conventional semi-dwarf → Three-line *indica* hybrid rice

increase yield more than 20% yield

In 2006, the area growing three-line hybrid rice has reached 12.92 million hm², accounting for 84 percent of the total hybrid rice–growing area in China.

Ⅱyou 838, Gangyou 725, Gangyou 527 and Ⅱ you -7 have become the fourth update of the leading variety-group of medium *indica* hybrid rice in China.

- These varieties made an important contribution to the stability and development of rice production.
- However, most of them are criticized for poor appearance quality and bad eating quality, which was caused primarily by the poor quality of their cytoplasmic male sterile lines (CMS), G46 A and Ⅱ -32 A.

Rice grain quality of leading mediun hybrid rice

Variety	BR %	HR %	L/D	Chalky %	GC mm	AC %
Gyou 725	82	43.4	2.5	57	38	21.9
Ⅱyou 838	78.6	50.6	2.5	85	44	23.3

Breeding and application of an aromatic rice CMS line, Ch-29A

◆ **Aroma** is one of the most important characteristics for good quality rice. good quality, aromatic hybrid rice with a high yield would need to be developed by adding an aroma gene.

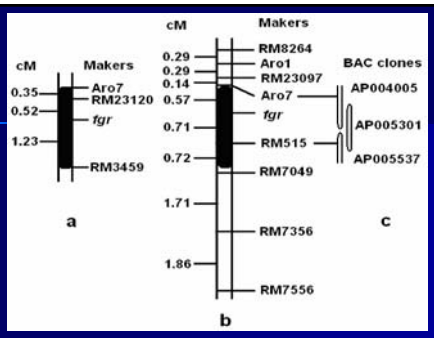
Year	Season	Location	Generation	Technical Measures
1992	Summer	Chengdu	II-32B/Xiangsimiao-2	Cross
1993	Winter	Hainan	F1	Mixed harvest
1993	Summer	Chengdu	F2	Select plants with good characters
1994	Summer	Chengdu	F3	Aroma evaluation Examination of agronomic traits and grain quality
1995	Winter	Hainan	Zhenshan97A/F4	test-cross
1995	Summer	Chengdu	F1/F5	Select plants with fine traits for backcross
1996	Winter	Hainan	BC1F1/F6	Examination for pollen sterility
1996-1998	Chengdu and Hainan			Pair backcross, Testing for yield GCAs
2000	Summer	Sichuan	BCF1/F15	seed production
			Ch-29A, Ch-29B	

Breeding procedure of a new CMS line Ch-29A

F1: non-aromatic

F2 non-aroma : aroma = 3:1.

F_2	Year	Total	Non-aroma	Aroma	χ^2 (3:1)*	P-value
Ch-29B/Le	2005	281	211	70	0.019	0.95 to 0. 99
	2006	1182	902	280	1.084	0.20 to 0.30
Ch-29B/R2	2006	2400	1823	577	1.176	0.20 to 0.30



Location of the fragrance gene in chromosome 8.

(a) Linkage map of the Ch-29B/R2 F2.

(b) Linkage map of the Ch-29B/Le F2.

(c) The bacterial artificial chromosome (BAC) clones covering Aro7 and RM515.

The aroma gene of Ch-29A might be the same gene reported by Bradbury et al (2005a).

Rice quality of Ch-29A

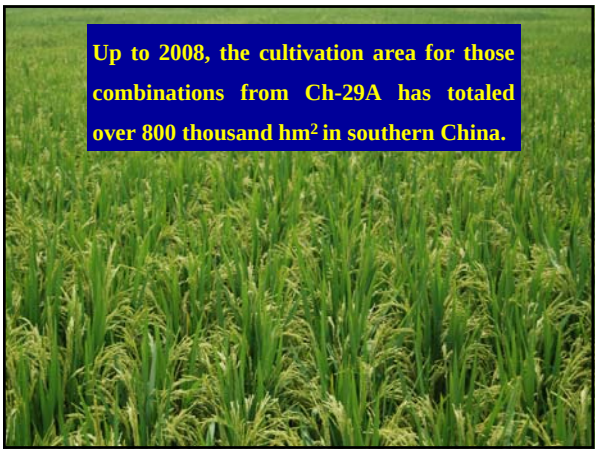
Brown rice rate	80.2 %
Milled rice rate	74.1 %
Head rice rate	59.4 %
Length / width ratio	2.4
Grain had a length	5.7
Chalky grain value	48
Chalkiness	7.6
Transparency	2
Gel consistency	54mm
Amylase content	21.6 %
Protein content	12.0 %

The yield and grain quality of fragrant hybrid rice from Ch-29A in the regional trial of China (1)

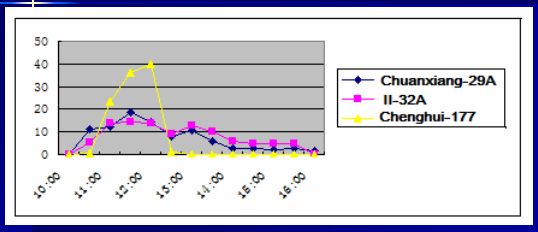
Variety	Trail Region	Yield t ha^{-1}	Over CK %	HR rate %	L/W Ratio	Chalky grains %	Transparency	AC (%)	GC (mm)
Chuanxiangyou-2	Sichuan	8.19	+2.46	67	2.9	24	1	20.0	70
	Upstream of Yangtze River	8.37	-0.63	67	2.7	24.5	1	21.8	48
	Middle and Downstream of Yangtze River	9.15	+5.57	63	2.8	31	1	22.1	53
Shanyou-63	Yangtze River	8.66	-	63	2.4	58	2	21.1	72
Gangyou 725	Sichuan	7.84	-	56	2.4	84	2	21.3	42

The yield and grain quality of fragrant hybrid rice from Ch-29A in the regional trial of China (2)

Variety	Trail Region	Yield t ha^{-1}	Over CK %	HR rate %	L/W Ratio	Chalky grains %	Transparency	AC (%)	GC (mm)
Chuanxiangyou-8	Sichuan	8.18	+4.25	64.7	2.8	12	1	23.3	55
Chuanxiangyou-993	Upstream of Yangtze River	8.87	0.40	66.5	2.8	18	1	21.9	68
Shanyou-63	Yangtze River	8.66	-	63.9	2.4	58	2	21.1	72
Gangyou 725	Sichuan	7.84	-	56.1	2.4	84	2	21.3	42



Ch-29 A has an earlier flowering time



Comparison of flowering time among Ch-29A , II-32A and the restorer line Chenghui177

High percentage of exposed stigma

The exposed stigma rate: 78 %

double-exposed stigma: 46%

seed production: 4.5 t ha^{-1}

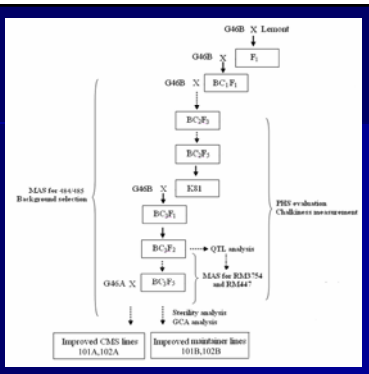
4.5 t ha^{-1} of seed production can be achieved with Ch-29A as a female parent. The high yield reached 6.6 t ha^{-1} for Chuanxiangdao no.5 seed production this year.

Pyramiding of good PHS resistance and nice grain quality into an elite *indica* CMS maintainer line, G46B

Gang 46A(G46 A) is a leading CMS line of medium hybrid rice production in Yangtze River and south-west China.

- the pre-harvest sprouting rate (PSR) of G46A in hybrid rice seeds was about 10%. in 2005, when the rainy weather lasted for a long time, the PHS of G46A has strong effects on hybrid rice seed quality due to a more than 50 % PSR.
- Therefore, hybrid rice seed production requires CMS maintainer and sterile lines with good resistance to PHS.

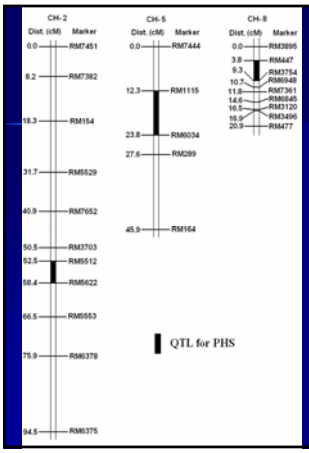
The PHS of G46A in seed production



Pedigree chart of the experimental materials used in this study

Pre-harvest sprouting resistance, amylose content and appearance quality of some near-isogenic introgression lines of G46B in BC2F5.

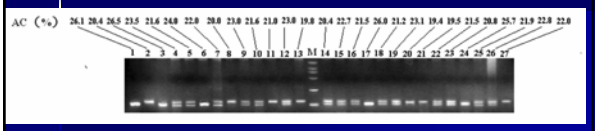
Lines	PSR %	Genotype of 484/485	AC %	Chalky grains %	Chalkiness
G46B	95.40	G	26.13	97.06	11.16
Lemont	2.98**	L	20.10**	2.64**	0.13**
K06	18.47**	L	20.03**	14.02**	1.11**
K23	10.85**	L	19.87**	12.22**	0.84 **
K45	16.35**	L	20.89**	22.99**	1.32 **
K65	11.82**	L	19.50*	26.96**	1.62**
K73	0.12**	L	20.40**	25.33 **	1.59**
K81	7.22**	L	20.03**	13.09 **	0.81 **
K89	6.95**	L	19.43**	14.99 **	1.06 **
K135	9.37**	L	20.15**	12.33 **	1.00 **
K152	13.24**	L	20.11**	20.55 **	1.51 **
K156	19.79**	L	20.12**	20.67 **	0.33**



SSR linkage map on the G46B / K81 F2 population and position of QTL for PHS.

Putative QTLs for PHS detected by CIM in the 'G46B' / 'K81'F2 population

Locus	Marker interval	Genetic distance[1])/cM	LOD value	Phenotypic variance %	Additive effects	Dominance effects
qPSR2	RM5512-RM5622	2.0	3.08	4.44	9.08	8.53
qPSR5	RM1115-RM6034	3.5	7.67	11.49	13.81	-5.4
qPSR8	RM447-RM3754	1.5	26.78	43.04	25.1	-5.34



PCR patterns of SSR marker 484/485. 1:G46B; 2: Lemont; 3-27: representative BC2F2 plants; M: DL-2000 DNA ladder.

Pre-harvest sprouting

Chuanmei-101B G46B Chuanmei-102B

Amylose Content

G46B : 26.1%

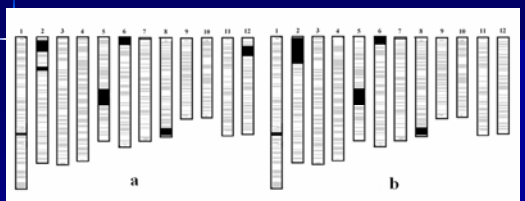
101B: 21.0%

102B: 19.4%

Grain chalkiness

Chuanmei-102B G46B Chuanmei-101B

10.3% 97.1% 7.0%



Graphical genotypes of two improved maintainer lines, (a) Chuanmei-101B and (b) Chuanmei-102B, from a G46B genetic background, with several introgressed segments (the black bars) from a U.S. japonica variety, Lemont.

Development of the introgression lines with intermediate amylose content by MAS

◆**Amylose content** (AC) is one of the most important determinants for the cooking and eating quality of rice grains.

However,most of CMS maintainer lines in China was observed to have high amylose content (more than 22%), their rice is not soft.

characteristics of the maintainer line selected as receptor parent

(1) F1 combinations should have strong heterosis. Some of them have been broadly used in rice production or performed excellently in regional trials.

(2) A higher head milled rice rate should be observed in hybrid rice.

(3) They should have good outcrossing rates and high yield of seed production and reproduction.

key maintainer receptors used in our improved breeding program

Region	Lines
medium *indica* hybrid rice	II-32B, Ch-29B, Chuan358B
double-cropping rice	Zhong-9B
late hybrid rice	Bo-IIB and Bo-IIIB
widely utilized in Indian hybrid rice production	IR58025B

The (CT)n of *Wx* gene and AC of donor varieties

Donor	Subspecies	AC (%)	CT repeats in exon 1 of *Wx* gene	Origin
KongYu131*	*Japanica*	17.2%	18	Japan
Lemont	*Japanica*	20.0%	20	America
KDML105	*Indica*	15.8%	17	Thailand
Basmati370	*Indica*	19.0%	17	India

The number of introgression lines with intermediate AC

Receptors	Number of introgression lines[1]	Total number
Ch-29B	K81[2](452),KDML105(83),Basmati370(116)，Kongyu131（early generation）	651
II-32B	K81(87),Basmati370(55)，（early generation）	142
Bo-IIB	K81(125),Basmati370(85)，（early generation）	210
Bo-IIIB	K81(266)	266
Chuan-358B	K81(513),KDML105(128),Basmati370(172)，Kongyu131（early generation）	813
Zhong-9B	K81(137),Basmati370(60)，Kongyu131（early generation），	197
IR58025B	K81(461)	461
F-32B	K81(167)，Kongyu131（early generation）	167

Rice quality of receptor maintainer lines and improved lines (2008, Hainan)

Lines	Combinations	length /width	The percentage of Chalky grains （%）	Chalkiness （%）	AC (%
Ch-29B	Ⅱ-32B/xiangsimiao2	2.5	51.0	9.0	23.5
29309	92037/K81////Ch-29B	2.7	24.2	4	20.0
29311	92037/K81////Ch-29B	3.0	10.1	1	19.3
29981	K81/92037////Ch-29B	2.7	14.4	2.3	19.6
F-32B	You 1B/FeigaiB//L301B	2.7	23.2	6.3	23.1
32716	K81/92097////F-32B	2.7	13.1	2.4	18.1
32720	K81/92097////F-32B	2.7	9.1	1.6	18.5
32789	K81/92037////F--32B	3.1	8.2	1.7	20.0
Bo-ⅢB	(Bo-B/1441)F_4/ Bo-ⅡB	2.4	12.0	2.1	14.4
Ⅲ-713	K81////BoⅢB	2.5	15.1	3.3	18.1

Discussion

Identification and application of aroma gene in rice

- The methods to evaluate aroma
  - Elution of leaves with dilute KOH
  - chewing grains

Identification and application of the major QTL for PHS

- QTLs for seed dormancy or resistance to PHS have been identified on all rice chromosomes.
- almost all QTLs reported for PHS are able to explain only a small portion of the phenotypic variation.
- MAS for the major QTL, qPSR8 is a highly efficient way to breed for PHS improvement.

Improvement of AC by using MAS for the SSR markers linked to the *Wx* gene

- The granule-bound starch synthase (GBSS), encoded by the *Wx* gene, plays an important role in determining AC in rice grains ;
- Two functional markers in the *Wx* gene, a (CT)n microsatellite (or SSR) and a G/T single nucleotide polymorphism (SNP), have been well characterized with different alleles differing in apparent amylose content (AAC);
- It is quite plausible to identify the genotype difference of rice varieties with different eating quality by MAS of the markers tightly linked to the *Wx* gene.

Recurrent parents		Donor parents
Ⅱ-32B, F-32B, Bo-B, Ch-29B, Zhong-9B, IR58025B	X	Basmati370, KDML10, Lemont and Kongyu131 Basmati370, KDML10, Lemont, Kongyu131

MAS with 484/485 was used to identify AC in the backcross and self-crossed generations. Subsequently, more than 2000 maintainer lines with intermediate AC, good appearance quality and high percentage of exposed stigmas have been developed.

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