Journal of Integrative Agriculture 2016, 15(3): 493–501

Available online at www.sciencedirect.com

ScienceDirect

RESEARCH ARTICLE

A genetic diversity assessment of starch quality traits in rice landraces from the Taihu basin, China

AO Yan[1, 2], XU Yong[1], CUI Xiao-fen[1], WANG An[1], TENG Fei[1], SHEN Li-qun[1], LIU Qiao-quan[1]

[1] *Key Laboratory of Crop Genetics and Physiology of Jiangsu Province/Co-Innovation Center for Modern Production Technology of Grain Crops/College of Agriculture, Yangzhou University, Yangzhou 225009, P.R.China*

[2] *Suzhou Chien-Shiung Institute of Technology, Suzhou 215411, P.R.China*

Abstract

There are nearly 1 000 rice landrace varieties in the Taihu basin, China. To assess the genetic diversity of the rice, 24 intragenic molecular markers (representing 17 starch synthesis-related genes) were investigated in 115 Taihu basin rice landraces and 87 improved cultivars simultaneously. The results show that the average genetic diversity and polymorphism information content values of the landraces were higher than those of improved cultivars. In total, 41 and 39 allele combinations (of the 17 genes) were derived from the landraces and improved cultivars, respectively; only two identical allele combinations were found between the two rice variety sources. Cluster analysis, based on the molecular markers, revealed that the rice varieties could be subdivided into five groups and, within these, the *japonica* improved rice and *japonica* landrace rice varieties were in two separate groups. According to the quality reference criteria to classify the rice into grades, some of the landraces were found to perform well, in terms of starch quality. For example, according to NY/T595-2002 criteria from the Ministry of Agriculture of China, 25 and 33 landraces reached grade 1, in terms of their apparent amylose content and gel consistency. The varieties that had outstanding quality could be used as breeding materials for rice quality breeding programs in the future. Our study is useful for future applications, such as genetic diversity studies, the protection of rice variety and improvment of rice quality in breeding programs.

Keywords: intragenic molecular marker, starch synthesis, improved cultivars, cluster analysis, polymorphism information content

1. Introduction

Breeding success in crops is strongly related to the genetic variation of the materials available, that is, the germplasm resources; an understanding of the population structure and genetic variation in the germplasm is a prerequisite for crop genetic improvement (Xiao *et al*. 2012). Rice is one of the most important economic crops in China. However, a gradual reduction of the genetic diversity of breeding varieties could hinder future breeding programs. Thus, further investigation into the genetic diversity of existing landraces is critical for the sustainable development of this economic crop.

Since the 1950s, the genetic diversity of the breeding varieties of rice has been decreasing (Qi *et al*. 2006). Lin and

Received 6 February, 2015 Accepted 10 April, 2015

AO Yan, Mobile: +86-18051236202, E-mail: 350685056@qq.com; Correspondence LIU Qiao-quan, Tel: +86-514-87979242, E-mail: qqliu@yzu.edu.cn

doi: 10.1016/S2095-3119(15)61050-4

Min (1991) reported that several common rice varieties were descendants of the Aizizhan, Nantehao, Nongken 58, and Yinfangzhu varieties. Jin *et al*. (2006) found that the average similarity coefficient between rice varieties in the Taihu basin was 0.902. In fact, it is difficult to distinguish between the genotypes of several varieties of rice, such as Wuyunjing 7 and Wuyunjing 8. Continuous breeding programs have led to a trend of simplification in numerous agricultural crops, resulting in gene loss and a reduction in genetic diversity (Donini *et al*. 2000; Tian *et al*. 2005). Such narrow genetic bases pose potential risks to economic crop industries.

The Taihu basin is located at the juncture of Jiangsu, Zhejiang and Shanghai, between the lower reaches of the Yangtze River and Hangzhou Bay. Local residents have been cultivating rice in the basin for over 8 000 years (Wang *et al*. 2000). Both natural evolution and artificial selection have contributed to the high diversity of rice present in the Taihu basin (Guo *et al*. 1986; Jiang *et al*. 1986), including unique phenotypic, resistance and quality characteristics. However, while breeding programs to date have involved several local varieties, for example, Huangkezaonianri, Aininghuang and Laohu*,* numerous varieties have not been fully utilized as yet.

Improved living standards have raised expectations for high quality rice in many markets, facilitating an increase in research into rice quality traits in recent years. The quantity of starch in the endosperm of rice grains is a very important determinant of rice quality; it is essential to understand the genetic effects of starch synthesis-related genes in high-yielding rice varieties, to improve rice quality (Yan *et al*. 2007). Recent advancements in molecular biology have made it possible to identify genes relevant to starch formation, including the granule-bound starch synthase gene (*Wx*), starch branch enzyme genes (*Sbe1*, *Sbe3*), soluble starch synthase gene (*Sss1*), isoamylase gene (*Isa*), and the rice limit dextrinase or R synthase gene (*Pull*), amongst others (Wu 2006; Calingacion *et al*. 2014).

Recent research has primarily focused on the inheritance of resistance and tolerance, as well as agronomic and phenotypic characteristics of local rice varieties in the Taihu basin (Diao *et al*. 1999; Li *et al*. 2006; Dikshit *et al*. 2013; Ren *et al*. 2013). According to Shen *et al*. (2007, 2008), significant differences in traits exist among local *japonica* rice varieties; their research confirmed the high phenotypic diversity of local varieties in the Taihu basin (Shen *et al*. 2007, 2008). In a subsequent study at the molecular level, Jin *et al*. (2006) utilized microsatellite markers to conduct a polymorphic analysis of DNA in 129 local varieties from the Taihu basin. Their results showed that the germplasm of the local varieties of *japonica* rice contained a rich genetic diversity and large amounts of rare allelic variation (Jin *et al*. 2006). Similarly, Yu *et al*. (2009) utilized 45 pairs of simple sequence repeat (SSR) primers to analyze the genetic diversity of 224 local *japonica* rice varieties in the Taihu basin and revealed 162 allelic variations. Their research concluded that SSR diversity in local *japonica* rice varieties was relatively lower than that previously reported, though, conversely, more rare allelic variations existed (Yu *et al*. 2009).

In a number of studies into the diversity of rice resources, a variety of techniques, including amplified fragment length polymorphism (Sorkheh *et al*. 2016), restriction fragment length polymorphism (Sun *et al*. 2000), random amplified polymorphic DNA (Ali *et al*. 2014), SSR (Lin *et al*. 2012; Melaku *et al*. 2013; Roy *et al*. 2014; Umadevi *et al*. 2014; Liu *et al*. 2015), single nucleotide polymorphism (Sun *et al*. 2013), sequence tagged site (Lin *et al*. 2012), isozyme markers and DNA fingerprinting (Rekha *et al*. 2011), functional gene markers (Liu *et al*. 2015), have been used to examine polymorphic site ratios, allele numbers, genotype numbers, average heterozygosity and genetic diversity (Ren *et al*. 2003), polymorphism information content (PIC) values (Ren *et al*. 2003), and hereditary similarity coefficients. In addition, cluster analyses and unweighted pair-group method with arithmetic mean (UPGMA) dendrograms have been constructed using markers (Zhu *et al*. 2004; Ali *et al*. 2014). However, for example, these molecular markers were selected at random from rice chromosomes and they do not clearly reflect any particular trait or gene.

Some internal molecular markers within starch synthesis-related genes have previously been developed, derived from differences in sequence diversity, between the rice subspecies *indica* and *japonica*, by the Key Laboratory of Crop Genetics and Physiology of Jiangsu Province, Yangzhou University (Han *et al*. 2004; Wu 2006; Yan *et al*. 2007; Tian *et al*. 2010). In order to increase the pertinence of the present study, internal markers of starch synthesis-related genes were chosen, and the diversity of these genes and quality traits of rice landraces in the Taihu basin were studied. This research develops a new practice for diversity analysis, using internal markers that are related to a relevant gene. The results provide an optimized reference for the protection of rice varieties and good quality breeding program. The purpose of this study was to investigate the genetic diversity of starch quality traits of rice landraces in the Taihu basin and then compare with the improved varieties.

2. Results

2.1. Relationships between the different markers and quality traits

The influence of gene locus on the quality traits was studied using a multiple regression analysis; the numbers in Table 1

are defined on the basis of the path coefficient of each gene, whereby a smaller value indicates a greater influence of the gene on the trait. More genes related to quality traits were found when the rice was grown in Yangzhou than Hainan, which may be a result of environment and gene interactions. According to our results, *(CT)n*, *Wx(GBSSI)* and *(AATT)n* within *Wx* significantly influenced the starch quality traits. Notably, *(CT)n* was found to have the greatest influence on all tested traits, particularly in varieties grown in Yangzhou. The genes *SSSIV-1*, *SSSIV-2* and *Pull* had no significant influence on any quality traits. The genes *GBSSII*, *Sbe1*, *SSSIII-1*, *SSSIII-2*, and *AGPsma* influenced only one trait, while other genes tested had significant effects on more than one trait.

2.2. Cluster analysis of rice materials

A cluster analysis of the rice varieties was done based on the molecular markers and all the varieties tested were divided into five groups (named I–V; Fig. 1; Appendix A). Group I consisted of 19 improved *japonica* rice and 2 landrace *japonica* rice (Lujingqing and Danxuan 131) varieties. Group II consisted of 12 landrace *japonica* glutinous, 11 landrace *japonica* varieties, and 1 improved *japonica* glutinous (Guanglingxiangnuo) variety. Group III was the largest group (70.43% of the 115 landraces tested) and consisted nearly entirely of landrace *japonica* rice varieties, with the notable exception of two improved cultivars 73-208(62) and Jinwan 78101(63). It is interesting that landrace *japonica* rice (found in groups II and III) and improved *japonica* rice (in group I) were in different groups, this suggests that some genetic differences exist between the landrace *japonica* and improved *japonica* rice varieties. Group IV was the smallest and included only two *indica* glutinous rice varieties (Suyunuo and Youmangzaodao). Group V consisted of all remaining *indica* landrace varieties and check *indica* rice 9311.

2.3. Diversity of starch synthesis-related genes

Different alleles (all assigned unique identification numbers; Appendix B) were found in different rice varieties. The 115 landraces were classified using the 17 starch synthesis-related genes and 41 allele combinations were categorized (Appendix C; varieties corresponding to each of the allele combinations are shown in Appendix A). The differences between allele combinations varied widely; for example, genotypes 1 and 2 differed at only the two sites *(CT)n* and *Sbe1*. Conversely, a large difference was found between genotypes 18 and 19, with differences at 13 sites.

Table 1 Relationships between the genes and their relative importance on quality traits

Genes or markers	Quality traits[1)]																										No. of relevant quality traits	
	AAC		GC		RVA												TPS											
					PV		BDV		FV		PaT		PT		TV		SBV		T_o		T_p		T_e		W		*ΔH*			
	H	Y	H	Y	H	Y	H	Y	H	Y	H	Y	H	Y	H	Y	H	Y	H	Y	H	Y	H	Y	H	Y	H	Y	H	Y
Wx(GBSSI)	3	2		2	1	1	1	5	3	2	1		3	2	3	2		2					2	2		3	3	9	10
(CT)n	1	1	2	1	3	3	3	1	1	1		1	1	1	1	1	1	1		1		1		1	1	1	1	1	10	14
(AATT)n	4	5		3			5	3	4	4				4		3	3	4		2									4	8
GBSSII		3																											0	1
Sbe1																												5	0	1
Sbe3							4	4												3	1			4					2	3
SSSI					2	2			2	5					2	4													3	3
SSSII-1											2	3										3		3					1	3
SSSII-2	2		1							3		2	2	3															3	3
SSSII-3		6		6		4				6						5	5	5						5					1	7
SSSIII-1																			1										1	0
SSSIII-2																											2	4	1	1
SSSIV-1																													0	0
SSSIV-2																													0	0
Isa-1				4																								2	0	2
Pull																													0	0
AGPlar		4		5	4		2	2								6	2	3											3	5
AGPsma																						2							0	1
AGPiso			3														4						1						1	2

[1)] The sequence of the number is based on the path coefficient of the gene. Smaller values indicate greater gene influence on the respective trait. AAC, apparent amylose content; GC, gel consistency; RVA, RVA profile; TPS, thermodynamic characteristics of starch. H and Y, two locations, Hainan and Yangzhou, respectively. PV, peak viscosity; BDV, breakdown viscosity; FV, final viscosity; PaT, pasting temperature; PT, peak time; TV, trough viscosity; SBV, setback viscosity; T_o, initial temperature; T_p, peak temperature; T_e, final temperature; W, width at half maxima; *ΔH*, enthalpy change. The same as below.

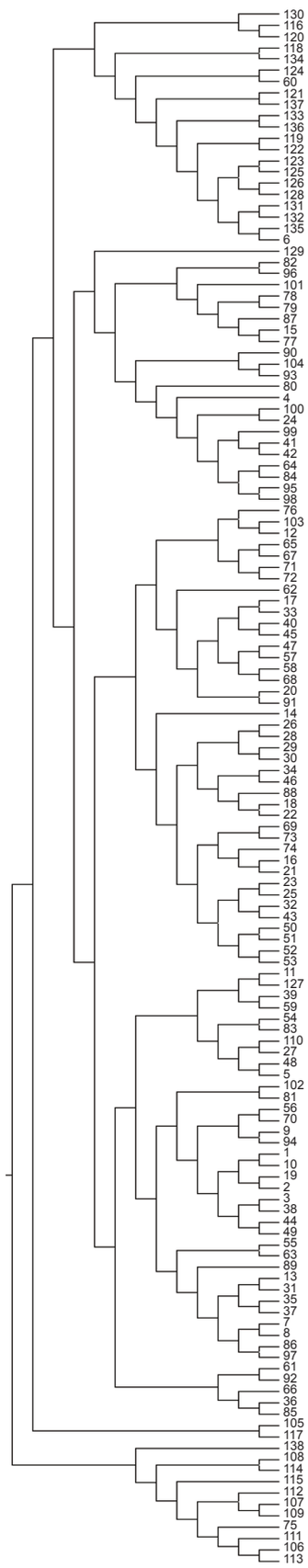

Fig. 1 Cluster analysis of rice landraces.

A total of 39 allele combinations were identified from the 87 improved cultivars, while only two identical allele combinations were found in the landraces (23 was not different from landrace allele combination 29). Similar to this, the allele combination 35 of improved cultivar Wujing 15 was identical to the allele combination 36 of the landrace varieties. The allele combinations of all other improved cultivars were distinct from the landrace varieties.

Among the 115 rice landraces tested, analysis of their genetic diversity revealed 49 alleles in the 17 starch sythesis-related genes (Table 2). On average, each marker locus had 2.58 alleles and the range was 1–7 alleles per locus. The average value of genetic diversity was 0.2402 and the range was 0–0.7078. The average PIC value was 0.2124 (with a range from 0 to 0.6547). PIC values at *(CT)n*, *Wx*(*GBSSI*), *Sbe1*, and *SSSII-1* were comparatively higher than values at other loci. *Pull* was found to be a monomorphic locus.

A total of 42 alleles were found in the improved cultivars. On average, 2.21 alleles were found at each locus. The average value of genetic diversity in the 87 improved cultivars was 0.1649, with a range from 0 to 0.5324. The average PIC value was 0.1450 and the range was 0–0.4228. *SSSIII-2* and *(CT)n* had the highest polymorphism, with PIC values of 0.4228 and 0.3474, respectively. Conversely, the locus *(AATT)n* exhibited the lowest polymorphism, with a PIC value of zero.

2.4. Phenotypic variations of quality traits in terms of allele combinations

Traits difference analysis of different allele combinations The rice quality of 41 different allele combinations was analyzed by performing analysis of variance (ANOVA) on the nested experiment data, to demonstrate differences in the rice quality between allele combinations and between varieties within allele combinations. Significant differences were found for final viscosity (FV) of the rapid viscosity analyzer (RVA) profiles among the allele combinations (F=382.37, P<0.01) and among varieties within the same genotype (F=103.13, P<0.01), although no other traits exhibited significant differences among varieties within the same allele combinations. With the notable exception of FV, the variance among varieties within the same allele combinations and the variance of the error were combined for the corrected ANOVA (Table 3). All the other traits exhibited very significant differences among the allele combinations.

Multiple comparisons were conducted for every two allele combinations (Table 3); among the 820 comparisons of the 41 allele combinations, the proportions of the significant differences of these traits were in the range of 10.12–40.49% (mean 28.40%).

Table 2 Diversity of internal genes- or markers-related starch synthesis

Genes or markers	Landrace			Improved cultivar		
	No. of alleles	Gene diversity	PIC[1)]	No. of alleles	Gene diversity	PIC
Wx(GBSSI)	3	0.6388	0.5635	2	0.0877	0.0839
(CT)n	7	0.7078	0.6547	4	0.4053	0.3474
(AATT)n	2	0.0989	0.0940	1	0.0000	0.0000
GBSSII	2	0.1588	0.1462	2	0.0666	0.0644
Sbe1	2	0.5000	0.3750	2	0.3541	0.2914
Sbe3	2	0.0989	0.0940	2	0.0877	0.0839
SSS I	4	0.1484	0.1449	2	0.0449	0.0439
SSSII-1	3	0.5072	0.3871	3	0.1094	0.1054
SSSII-2	2	0.1588	0.1462	2	0.0666	0.0644
SSSII-3	3	0.1161	0.1128	3	0.4056	0.3321
SSSIII-1	3	0.2610	0.2424	4	0.1924	0.1862
SSSIII-2	2	0.1600	0.1472	3	0.5324	0.4228
SSSIV-1	2	0.1588	0.1462	2	0.0666	0.0644
SSSIV-2	2	0.1588	0.1462	3	0.0452	0.0447
Isa-1	2	0.1588	0.1462	2	0.1083	0.1025
Pull	1	0.0000	0.0000	2	0.0666	0.0644
AGPlar	3	0.1470	0.1412	3	0.1099	0.1069
AGPsma	2	0.1588	0.1462	3	0.1297	0.1238
AGPiso	2	0.2268	0.2011	2	0.2542	0.2219
Mean	2.58	0.2402	0.2124	2.47	0.1649	0.1450

[1)] PIC, polymorphism information content.

Table 3 Analysis of variance (ANOVA) of quality traits and proportion of significant differences in multiple comparisons

Traits	*F*-value of among genotypes	Proportion of significance
AAC	4.21**	0.3372
GC	4.65**	0.3122
PV	3.41**	0.2640
BDV	3.07**	0.1348
PaT	2.90**	0.1860
TV	3.00**	0.2043
SBV	4.41**	0.2951
T_o	6.31**	0.3457
T_p	12.44**	0.4280
T_e	5.50**	0.4201
PT	4.00**	0.2579
ΔH	2.63**	0.1012

**, very significant difference (P<0.01).

Evaluation of starch quality traits of landraces According to the NY/T595-2002 grade classification criteria of the Ministry of Agriculture of China (Table 4; Jiao 2009), the cooking quality traits of some good quality landraces were graded for eating purposes (Table 5). Among those graded, 25 landraces achieved grade 1 in their apparent amylose content (AAC); these landraces belonged to 21, 22, 25, 28, 30, 31, 32, 33, 34, 35, 38, and 40 allele combinations. In addition, 33 landraces achieved grade 1 in their gel consistency (GC), which belonged to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 17, 22, 29, 30, 31, 32, 34, 35, 36, 38, and 41 allele combinations. The breakdown of the good quality rice was higher than 100 RVU (the viscosity unit for RVA profile), while the setback viscosity was below 25 RVU, and tended to be negative in most cases (Shu *et al*. 1998). Thus, among the landraces classified to be grade 1 in either AAC or GC, 33 have been recognized as good quality varieties, in view of their RVA profiles. The specific performance of the landraces, in terms of their starch quality traits are shown in Appendix D.

3. Discussion

3.1. Selection of markers in the analysis of genetic diversity

Understanding the genetic diversity of rice landraces will help to produce rice varieties that are better adapted to certain environments (Zhu *et al*. 2000). To date, few studies on genetic diversity in rice quality have been done to explore and target starch synthesis-related genes. So this study avoids the previous practice of randomly selecting markers to study genetic diversity and increases the pertinence of markers to the traits of interest; and thus improve the efficiency of rice genetic breeding programs. Of the selected genes, the genes *SSSIV-1*, *SSSIV-2* and *Pull* did not significantly influence the quality traits researched in this study; this result differs from the findings of Wu (2006). These differences in findings may have been a result of different experimental materials and interactions between the environment and genotypes. Other genes did significantly affect starch quality traits, in agreement with Wu (2006).

3.2. Genetic differentiation between landraces and improved cultivars

By sorting the rice varieties tested based on 17 starch synthesis-related genes, only two allele combinations of the improved cultivars were found in the landraces. Firstly, the (same) allele combinations present in the improved cultivars, Nanjing 44, Yinyu 2084, Jiaxing 04-33, Wu 2645, Yi 7001, Nanjing 46, Yang 4227, Yangjing 9538, Huaidao 11, Shengdao 14, Yanjing 48, Huaidao 5, Jingdao 7, Zhendao 609, 9520, 98-3, Wuxiang 9915, Wuxiangjing 9, Guanglingxiangjing, Wuxiangjing 14 (99-15), Wuyujing 3, and Yuncun 2544, were the same as the allele combination of the Luganqing landrace. Secondly, the allele combination of the improved cultivar Wujing 15 was identical to the Tianjiqing, Changzhong and Hongmangshajing landraces. Other allele combinations in the improved cultivars were not found in the landraces. This phenomenon suggests a gradual widening of the genetic gap between the improved cultivars and the landrace varieties tested, in terms of their starch synthesis genes. In addition, our cluster analysis of rice varieties,

Table 4 Reference criteria for grade classification NY/T595-2002 (NY/T595-2002 2003)

Type	Grade	AAC (%)	GC (mm)	GT (ASV)[1)]	RVA[2)]		
					Grade	BDV	SBV
japonica	1	15.0–18.0	≥80	≥7.0	#	>100	<25
	2	13.0–14.9 or 18.1–20.0	70–79	6.0–6.9			
	3	11.0–12.9 or 20.1–22.0	60–69	5.0–5.9			
	4	9.0–10.9 or 22.1–24.0	50–59	4.0–4.9			
	5	<9.0 or >24.0	<50	<4.0			
indica	1	17.0–22.0	≥70	≥6.0	#	>100	<25
	2	15.0–16.9 or 22.1–24.0	60–69	5.0–5.9			
	3	13.0–14.9 or 24.1–26.0	50–59	4.0–4.9			
	4	11.0–12.9 or 26.1–28.0	40–49	3.0–3.9			
	5	<11.0 or >28.0	<40	<3.0			
Glutinous	1	≤1.0	≥100	≥7.0	#	>100	<25
	2	1.1–2.0	95–99	6.0–6.9			
	3	2.1–3.0	90–94	5.0–5.9			
	4	3.1–4.0	85–89	4.0–4.9			
	5	>4.0	<85	<4.0			

[1)] Alkali spreading value (ASV) is a key indicator to measure rice gelatinization temperature (GT).
[2)] #, the breakdown viscosity was higher than 100 RVU (the viscosity unit for the RVA profile), while the setback viscosity was below 25 RVU. The same as below.

Table 5 Grades of the cooking quality traits of some good quality landraces

Varieties	Grade	AAC	ASV	GC	RVA	Varieties	Grade	AAC	ASV	GC	RVA
Taihuqing	38	1	3	2	#	Pudongqing	5	4	3	1	#
Aijiaotaihuqing	38	1	3	3		Yazihuang	36	2	5	1	
Luoshuangqing	1	5	2	1	#	Zhoujiazhong	38	1	5	3	
Tiejingqing	31	1	3	3		Huangkezaonianri	21	1	3	5	
Lujingqing 43	28	1	3	2		Hongmangxiangjingnuo	3	5	3	1	#
Lujingqing 44	32	1	3	2		Xiangjingnuo	3	5	3	1	#
Daichangqing	38	1	3	2	#	Xiangzhunuo	3	3	4	1	#
Gankeqing	38	1	3	3		Xiangzhunuoxuan	6	3	5	1	#
Tianjiqing	35	1	3	3	#	Jinggunuo	7	3	4	1	#
Honggudao	32	1	3	3		Hongkenuo 741	31	2	3	1	#
Jijiaohong	3	5	3	1	#	Baikenuo 748	32	3	3	1	#
Niumanghuang	38	1	3	3		Jintannuo	9	3	5	1	#
Bodao	22	5	3	1	#	Shuangjiangqingnuodao	41	3	4	1	#
Xiaohuangdao	32	1	3	3		Aiqinuo	29	3	3	1	#
Changqiyedao	25	1	3	5		Shuijingnuo	2	3	5	1	#
Wanyangdao	32	1	3	2		Wushinuo	38	3	4	1	#
Wujing 15	40	1	3	2	#	Putaonuo	5	3	4	1	#
Huangdao	38	1	3	2	#	Guozinuo	7	3	2	1	#
Changzhong	35	1	3	2		Bainuodao	5	3	4	1	#
Zaoguangtou	5	5	3	1	#	Maonuo	5	2	2	1	#
Zaoxiqiu	22	5	3	1	#	Zhimanuo	10	2	2	1	#
Changqiguang	22	1	3	2		Niaoxiunuo	4	4	3	1	#
Luganbai	31	1	3	2		Zaonuodao	2	3	5	1	#
Juziguang	31	1	3	3		Wuxidao	11	5	5	1	
Xiaohuangzao	33	1	3	3		Datougui	31	4	1	2	
Buliuming	38	1	3	2		Huangganxian	17	3	5	1	
Hongmangshajing	35	2	3	1		Majinnuo	11	4	5	1	#
Dongting 2	30	1	3	1		Buxuenuo	8	4	5	1	#
Jinwan 78101	34	1	3	1	#						

based on molecular markers, demonstrated *japonica* rice landraces and improved *japonica* rice varieties were in two different groups; this finding suggests genetic differentiation has occurred between these two groups.

3.3. Genetic diversity of landraces and improved cultivars

The average genetic diversity and PIC values of the tested

landraces were higher than those of the improved cultivars. Notably, modern rice breeding has reduced the diversity of the genes that are important for rice quality, in particular (for this study) producing a narrower range of genes important for starch synthesis quality in the improved, cultivated rice varieties. To achieve further quality improvements in commercial rice crops, the higher diversity in the genes important for quality found in the Taihu basin could be used to expand the hereditary basis of the improved rice varieties used in rice breeding programs. Compared with the improved varieties, each of the landraces tested has a much longer history, a higher plant height, and an increased susceptibility to lodging during harvesting. Despite these limitations, some of these landraces have performed very well, in terms of their starch quality. For example, of the studied landraces, 25 and 33 reached grade 1 standard in their AAC and GC, respectively; among the landraces classified as grade 1 in either AAC or GC, 33 were recognized as good quality varieties in view of their RVA profiles. Therefore, these varieties, with their outstanding quality, could be used as breeding material to improve rice quality breeding for the future.

This study provides a new methodology for studying genetic diversity of a population. Our results also demonstrate that in the future, there is a great potential to improve the conservation and utilization of the diverse rice quality genes found in landraces. Thus, there is an urgent need for the large scale collection and utilization of landrace rice species with high quality characteristics, which should then be used to broaden the rice hereditary basis in rice quality breeding programs.

4. Conclusion

In this study, cluster analysis confirmed that some genetic differences exist between landrace *japonica* and improved *japonica* rice varieties. The average genetic diversity and PIC values of the landraces were higher than those of improved cultivars studied. Of the varieties selected in this study, many had excellent quality characteristics, for example, four landraces (Hongmangshajing, Dongting 2, Jinwan 78101 and Yazihuang) achieved grade 1 in both their AAC and GC traits, according to the reference criteria of the Ministry of Agriculture of China (NY/T595-2002 2003). The varieties identified to have outstanding quality traits in this study could be used as breeding material for rice quality breeding programs in the future.

5. Materials and methods

5.1. Rice varieties and marker selection

A total of 115 rice landraces (evenly selected from 897 samples in Taihu basin, based on the difference in their AAC), 23 reference materials (Appendix A), and 87 improved cultivars (from diverse sources outside of the Taihu basin; Appendix E) were planted in Hainan and Yangzhou (China). Many landrace varieties that are commonly recognized by a single name still exhibit genetic variation among different areas within this region (Yu *et al*. 2010). Therefore, varieties with a single name were studied simultaneously in the current study, for example, Luganqing and Wanzhongqiu. The improved cultivars were selected from a wide variety of sources, including American rice, *japanese* rice, Taipeidao, Taihudao, Zhendao, Yujing, and Yangdao, as well as some check rice varieties like Nipponbare, 9311, Guanglingxiangnuo and Nongken 57, amongst others. The pedigree of some improved varieties and the relationships between landrace and the improved varieties are shown in Appendix F; a more complex genetic relationship exists between the improved varieties. On the other hand, the Aiqibaikenuo landrace is an ancestor of the Taihunuo, Guanglingxiangjing and Wujing 15 varieties, and the Huangkezaonianri landrace is an ancestor of Taihunuo, Guanglingxiangjing, Wujing 15, Zhendao 9424 and Yangjing 9538 varieties. Other varieties not included in the list of improved varieties in Appendix F have no ancestry in all of our rice materials.

Markers within starch synthesis-related genes were selected to assess the rice specimens (Appendix G); these molecular markers had previously been developed in the Key Laboratory of Crop Genetics and Physiology of Jiangsu Province, Yangzhou University, China (Han *et al*. 2004; Wu 2006; Tian *et al*. 2010; Yan *et al*. 2011). The markers are related to 17 starch synthesis genes: *WX* (*GBSSI*), *GBSSII*, *Sbe1*, *Sbe3*, *SSSI*, *SSSII-1*, *SSSII-2*, *SSSII-3*, *SSSIII-1*, *SSSIII-2*, *SSSIV-1*, *SSSIV-2*, *Isa-1*, *Pull*, *AGPlar*, *AGPsma*, and *AGPiso*. The DNA repeats *(CT)n* and *(AATT)n*, located in *WX* genes, are very important markers that affect quality traits and were also studied in this research.

5.2. Detection of marker genotypes

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was used to extract DNA and relevant primers were used for gene amplification using polymerase chain reaction (PCR). In the case of cleaved amplified polymorphic sequence (CAPS) molecular markers, PCR products were subjected to enzymatic digestion. PCR products or the products of the enzymatic digestion were divided by size through electrophoresis using a 1% agarose or 6% polyacrylamide gel. The conditions of electrophoresis were dependent on the polymorphism band size, with agarose gel for bands >50 bp and polyacrylamide gel, followed by ethidium bromide (EB) staining for bands <10 bp. After electrophoresis, the DNA bands were revealed by Ag staining,

and the results analyzed using a ScanMaker 3830 (Microtek, Shanghai, China).

5.3. Evaluation of quality traits

All rice specimens were harvested in autumn for measurement of AAC, GC, the thermodynamic characteristics of the starch, and RVA. The GC was selected in accordance with the GB/T17891-1999 high quality rice standard. Measurement of the AAC was similarly conducted according to the Standard NY147-88 (Ministry of Agriculture of China). A three-dimensional rapid viscosity analyzer (RVA; Newport Scientific Instrument Corp., Australia) was used to measure the starch viscosity, and Thermal Cycle for Windows (TCW) software was used for further analysis of the recorded data. Operating procedure 61-02 (1995), provided by the American Association of Cereal Chemistry (AACC), was applied for all analyses of the RVA profiles (AACC 1995). Briefly, the RVA profile included the peak viscosity, trough viscosity, breakdown viscosity, FV, setback viscosity, peak time, and pasting temperature. All viscosity measurements were recorded in units of centipoise (cP). A DSC200F3 differential scanning calorimeter (DSC) (Netzsch, United Kingdom) was used to measure the thermodynamic characteristics of starch. The DSC heat effect curve of each sample was used to calculate the enthalpy change, initial temperature, peak temperature, width at half maxima and final temperature during gelatinization.

5.4. Statistical analysis

Statistical tests, including regression analysis and ANOVA, were conducted using SAS ver. 9.1.3 (IBM, USA) software. The influence of gene locus on the quality traits was analyzed using regression analysis. A nested ANOVA was conducted to demonstrate differences in rice quality between allele combinations and between varieties within each genotype. Measures of variation include the PIC, genetic diversity and the number of alleles; these analyses were performed using the software program PowerMaker (North Carolina State University, USA).

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (30800603), the Science and Technology Plan Projects of Taicang City, China (TC214YY3) and the Building Program of the Science and Technology Innovation Team of Chien-Shiung Institute of Technology, China (2013CX02).

Appendix associated with this paper can be available on http://www.ChinaAgriSci.com/V2/En/appendix.htm

References

AACC (American Association of Cereal Chemist). 1995. *Approved Method of the AACC*. 9th ed. Methods 61-02 for RVA. The Association, St Paul, MN.

Ali M A, Islam M S, Mandal S K, Nasrin Z, Rahman M M, Kuddus R H, Prodhan S H. 2014. Genetic diversity among salt-tolerant rice (*Oryza sativa* L.) landraces cultivated in the coastal districts of Bangladesh. *Journal of Bioscience and Biotechnology*, **3**, 15-22.

Calingacion M, Laborte A, Nelson A, Resurreccion A, Concepcion J C, Daygon V D, Mumm R, Reinke R, Dipti S, Bassinello P Z, Manful J, Sophany S, Lara K C, Bao J S, Xie L H, Loaiza K, El-hissewy A, Gayin J, Sharma N, Rajeswari S. 2014. Diversity of global rice markets and the science required for consumer-targeted rice breeding. *PLOS ONE*, **9**, e85106.

Diao L P, Wang J F, Li Y S, Zhang H N, Zhang H S, Zhai H Q, Yang T N. 1999. Analysis of main agronomic characters for *japonica* rice from Tai Lake Region. *Journal of Nanjing Agricultural University*, **22**, 1–4. (in Chinese)

Dikshit N, Das A B, Sivaraj N, Kar M K. 2013. Phenotypic diversity for agro-morphological traits in 105 landraces of rice (*Oryza sativa* L.) from Santhal Parganas, Jharkhand, India. *Proceedings of the National Academy of Sciences, India* (Section B: Biological Sciences), **83**, 291–304.

Donini P, Law J R, Koebner R M D, Reeves J C, Cooke R J. 2000. Temporal trends in the diversity of UK wheat. *Theoretical and Applied Genetics*, **100**, 912–917.

GB/T 17891-1999. 1999. High quality rice. China Bureau of Quality and Technical Supervision. pp. 6–12. (in Chinese)

Guo E N, Hua G H, Gao J C, Yu J P, Wan J W, Wang C L, Zhong D X. 1986. Evolution and utilization of rice cultivars in Taihu region. *Jiangsu Journal of Agricultural Sciences*, **2**, 42–44. (in Chinese)

Han Y P, Xu M L, Liu X Y, Yan C J, Korban S S, Chen X L, Gu M H. 2004. Genes coding for starch branching enzymes are major contributors to starch viscosity characteristics in waxy rice (*Oryza sativa*). *Plant Science*, **166**, 357–364.

Jiang H, Wang G L, Wu J L. 1986. Japonica rice germplasm in Tai Lake region. *Crop Germplasm*, **2**, 5–8.

Jiao G A. 2009. Cooking quality assessment and diversity analysis of RVA profiles of aromatic rice. MSc thesis, Zhejiang University, China. p. 21. (in Chinese)

Jin W D. 2006. Heterosis and genetic diversity of Japonica rice (*Oryza Sativa* L.) in Taihu Lake valley. Ph D thesis, Nanjing Agricultural University, China. (in Chinese)

Li Y S, Wang Z F, Tang H J, Guan R Z, Zhang H S. 2006. Genetic analysis of some traits related to yield in Japonica rice landrace from Taihu lake region. *Chinese Journal of Rice Science*, **20**, 272–276. (in Chinese)

Lin H Y, WU Y P, Hour A L. 2012. Genetic diversity of rice germplasm used in Taiwan breeding programs. *Botanical Studies*, **53**, 363–376.

Lin S C, Min S K. 1991. *Chinese Rice Varieties and Its Pedigree*. Shanghai Science and Technology Press, China. pp. 282–292. (in Chinese)

Liu D, Wang J Y, Wang X X, Yang X L, Sun J, Chen W F. 2015. Genetic diversity and elite gene introgression reveal the *japonica* rice breeding in northern China. *Journal of Integrative Agriculture*, **14**, 811–822.

Melaku G, Haileselassie T, Feyissa T, Kiboi S. 2013. Genetic diversity of the African wild rice (*Oryza longistaminata* Chev. et Roehr) from Ethiopia as revealed by SSR markers. *Genetic Resources and Crop Evolution*, **60**, 1047–1056.

NY/T595-2002. 2003. *Indica* type cooking rice. Ministry of Agriculture. *China Rice*, **9**, 43-44. (in Chinese)

Qi Y W, Zhang D L, Zhang H L, Wang M X, Sun J L, Wei X H, Qiu Z G, Tang S X, Cao Y S, Wang X K, Li Z C. 2006. Genetic diversity of rice improved cultivars bred in China and theirs changing trend on fifty years. *Chinese Science Bulletin*, **51**, 681–688.

Rekha T, Martin K P, Sreekumar V B, Madassery J. 2011. Genetic diversity assessment of rarely cultivated traditional indica rice (*Oryza sativa* L.) varieties. *Biotechnology Research International*, **2011**, 7.

Ren F G, Lu B R, Li S Q, Huang J Y, Zhu Y G. 2003. A comparative study of genetic relationships among the AA-genome *Oryza* species using RAPD and SSR markers. *Theoretical and Applied Genetics*, **108**, 113–120.

Ren J S, Yu Y C, Gao F Y, Zeng L H, Lu X J, Wu X T, Yan W G, Ren G J. 2013. Application of resistance gene analog markers to analyses of genetic structure and diversity in rice. *Genome*, **56**, 377–387.

Roy S, Banerjee A, Pattanayak A, Roy S S, Rathi R S, Misra A K, Ngachan S V, Bansal K C. 2014. Chakhao (delicious) rice landraces (*Oryza sativa* L.) of North-east India: collection, conservation and characterization of genetic diversity. *Plant Genetic Resources: Characterization and Utilization*, **12**, 264–272.

Shen X P, Shen M X, Gu L, Gong L P, Ji H J, Yao Y M, Wang J P, Gao F, Gu Q Q. 2007. Assessment on superficial heredity of late *japonia* rice local germplasm resources in Taihu Lake area. *Acta Ecologica Sinica*, **27**, 189–196.

Shen X P, Shen M X, Gu L, Ji H J, Gong L P, Wang J P, Yao Y M. 2008. Research on diversity analysis of the of RVA profile of in the Taihu Lake area. *Scientia Agricultura Sinica*, **41**, 2513–2519. (in Chinese)

Shu Q Y, Wu D X, XiaY W, Gao M W. 1998. Relationship between RVA profile character and eating quality in *Oryza sativa* L. *Scientia Agricultura Sinica*, **31**, 1–4. (in Chinese)

Sorkheh K, Masaeli M, Chaleshtori M H, Adugna A, Ercisli S. 2016. AFLP-based analysis of genetic diversity, population structure, and relationships with agronomic traits in rice germplasm from North region of Iran and world core germplasm set. *Biochemical Genetics*, doi: 10.1007/s10528-016-9711-7

Sun C Q, Wang X K, Yoshimura A, Iwata N. 2000. A study of the genetic diversity of common wild rice (*O. rufipogon* Griff.) and cultivated rice (*O. sativa* L.) by RFLP analysis. *Acta Genetica Sinica*, **27**, 227–234. (in Chinese)

Sun X Y, Kang S, Zhang Y J, Tan X Q, Yu Y F, He H Y, Zhang X Y, Liu Y F, Wang S, Sun W X, Cai L, Li S J. 2013. Genetic diversity and population structure of rice pathogen ustilaginoidea virens in China. *PLOS ONE*, **8**, e76879.

Tian Q Z, Zhou R H, Jia J Z. 2005. Genetic diversity trend of common wheat (*Triticum aestivum* L.) in China revealed with AFLP markers. *Genetic Resources and Crop Evolution*, **52**, 325–331.

Tian Z X, Yan C J, Qian Q. 2010. Development of gene-tagged molecular markers for starch synthesis-related gene in rice. *Chinese Science Bulletin*, **55**, 3768–3777.

Umadevi M, Veerabadhiran P, Manonmani S. 2014. Assessment of genetic diversity of rice (*Oryza sativa*) cultivars using simple sequence repeat (SSR) markers. *African Journal of Biotechnology*, **13**, 3547–3552.

Wang C L, Zou J S, Tang L H, Ding J L, Zhang M, Zhou Y X, Udatsu T, Fujiwara H. 2000. Rice cultivation at the Neolithic age in Taihu region. *Jiangsu Journal of Agricultural Sciences*, **16**, 129–138. (in Chinese)

Wu H K. 2006. The effects of the starch-synthesizing genes on cooking and eating quality in rice (*Oryza sativa* L.). Ph D thesis, Yangzhou University, China. (in Chinese)

Xiao Y L, Lei J G, Yu C Y, Cruz Q D D, Cstillo J M, Tabanao D A. 2012. Genetic similarity and population structure in a rice drought stress adaptation panel. *Acta Agriculturae Universitatis Jiangxiensis*, **34**, 886–892. (in Chinese)

Yan C J, Tian S, Zhang Z Q, Han Y P, Chen F, Li X, Gu M H. 2007. The source of genes related to rice grain starch synthesis among cultivated varieties and its contribution to quality. *Agricultural Sciences in China*, **6**, 129–136.

Yan C J, Tian Z X, Fang Y W, Yang Y C, Li J, Zeng S Y, Gu S L, Xu C W, Tang S Z, Gu M H. 2011. Genetic analysis of starch paste viscosity parameters in glutinous rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, **122**, 63–76.

Yu P, Li L, Hou L Y, Xu Q, Wang Y P, Yu H Y, Wei X H, Yuan X P. 2010. Genetic variation within landraces with the same name of japonica rice in the Taihu lake region. *Journal of Plant Genetic Resources*, **11**, 535–539. (in Chinese)

Yu P, Li L, Lu J Z, Yuan X P, Xu Q, Wang Y P, Yu H Y, Wei X H. 2009. SSR analysis on *japonica* rice landraces from the Taihu Lake Region, China. *Chinese Journal of Rice Science*, **23**, 148–152. (in Chinese)

Zhu M Y, Wang Y Y, Zhu Y Y, Lu B R. 2004. Estimating genetic diversity of rice landraces from Yunnan by SSR assay and its implication for conservation. *Acta Botanica Sinica*, **46**, 1458–1467.

Zhu Y, Chen H, Fan J, Wang Y Y, Li Y, Chen J B, Fan J X, Yang S S, Hu L P, Leung H, Mew T W, Teng P S, Wang Z H, Mundt C C. 2000. Genetic diversity and disease control in rice. *Nature*, **406**, 718–722.

(Managing editor WANG Ning)