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THE PROGRESSION OF PERENNIAL RICE BREEDING AND GENETICS RESEARCH IN CHINA

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ABSTRACT

Soil erosion is a worldwide problem of increasing concern, and perennial grain crops could be an important part of the solution. In Southeast Asia, upland rice (*O. sativa*) contributes to regional soil erosion problems because it is an annual crop grown on hilly lands. The perennial cultivars of upland rice could reduce soil erosion and meet the needs of subsistence farmers. From the viewpoint of breeding, *O. longistaminata*, with same genome, AA, similar to *O. sativa*, would be the most logical donor of genes for rhizome expression for perennial rice cultivar development,

several donor traits, such as rhizome and stolon have been employed for perenniality. Up to now, there are some results as following: 1) based on the fine mapping of the rhizome genes (*Rhz*), via genomic library (BAC, Fosmid, rhizome cDNA library) construction and analysis, confirming the genetic regularity that the rhizome was controlled by two pairs of dominant complementary genes, *Rhz2*、*Rhz3*, and obtaining 15 rhizome locus and candidate functional genes; 2) the perennial rice breeding is on the way and some breeding lines that hold the rhizome genes were made. There are five perennial rice (PR) lines, namely PR23, PR57, PR129, PR137 and PR139, that have been bred; 3) the potential perennial materials were screened at different sites, such as Lao, Africa and China for perennial ability investigation. Thus development of perennial rice cultivars from *O. longistaminata* faces two large challenges: 1) the need to pyramid in an *O. sativa* background multiple rhizome QTL in order to get strong rhizome expression, and 2) the need to get rid of QTLs for low pollen fertility without losing linked QTLs for rhizomes.

Keywords: perennial rice, breeding, genetics research, *Oryza longistaminata*

INTRODUCTION

Soil erosion is a serious problem in the uplands of Southeast Asia (Figure 1). Once forest is cleared on sloping uplands and replaced with annual crops such as upland rice, nutrients are rapidly leached and soils are eroded, so lands need to return to forest for some years before being suitable again for crop production. Population pressure is reducing the duration of fallow, so performance of upland rice, associated crops and livestock are declining, as soil quality and fertility progressively deteriorate. A way is needed to stabilise these fragile soils (IRRI, 1998).

FIGURE 1. SOIL EROSION IN UPLAND RICE FIELD IN YUNNAN, CHINA

Soil erosion in uplands of southeast Asia has been a serious problem that led to the project of developing perennial upland rice at IRRI (IRRI 1989)

TABLE 1. *ORYZA* SPECIES, THE SPECIES COMPLEX, CHROM., GENOME GROUP AND DISTRIBUTION

SECTION	COMPLEX	SPECIES	CHROMOSOME NUMBER	GENOME GROUP	DISTRIBUTION
ORYZA	*O. sativa* complex	*O. sativa* L.	24	AA	worldwide
		O. nivara Sharma et Shastry	24	AA	Tropical and Sub. Asia
		O. rufipogon Griff	24	AA	Tropical and Sub. Asia
		O. meridionalis Ng	24	A^mA^m	Tropical and Australia
		O. glumaepatula Steud.	24	$A^{gl}A^{gl}$	South America
		O. glaberrima Steud	24	A^gA^g	Africa (mainly West)
		O. barthii A. Chev.	24	A^gA^g	Africa
		O. longistaminata Chev. et Roher	24	A^lA^l	Africa
	O. officinalis complex	*O. officinalis* Wall ex Watt	24	CC	Tropical and Sub. Asia
		O. minuta Presl. et Presl.	48	BBCC	Philippines
		O. eichingeri Peter	24	CC	Sri Lanka, Africa
		O. rhizomatis Vaughan	24	CC	Sri Lanka
		O. punctata Kotschy ex Steud.	24, 48	BB, BBCC	Africa
		O. latifolia Desv.	48	CCDD	Latin America
		O. alta Swallen	48	CCDD	Latin America
		O. grandiglumis (Doell) Prod.	48	CCDD	South America
		O. australiensis Domin	24	EE	Australia
RIDLEYANAE TATEOKA		*O. brachyantha* Chev. et Roehr.	24	FF	Africa
		O. schlechteri Pilger	48	HHKK	Papua New Guinea
	O. ridleyi complex	*O. ridleyi* Hook. f.	48	HHJJ	SE Asia
		O. longiglumis Jansen	48	HHJJ	Irian Jaya, Indonesia
GRANULATA ROSCHEV.	O. meyeriana complex	*O. meyeriana* Baill	24	GG	SE Asia
		O. granulata Nees et Arn. ex Watt	24	GG	S and SE Asia

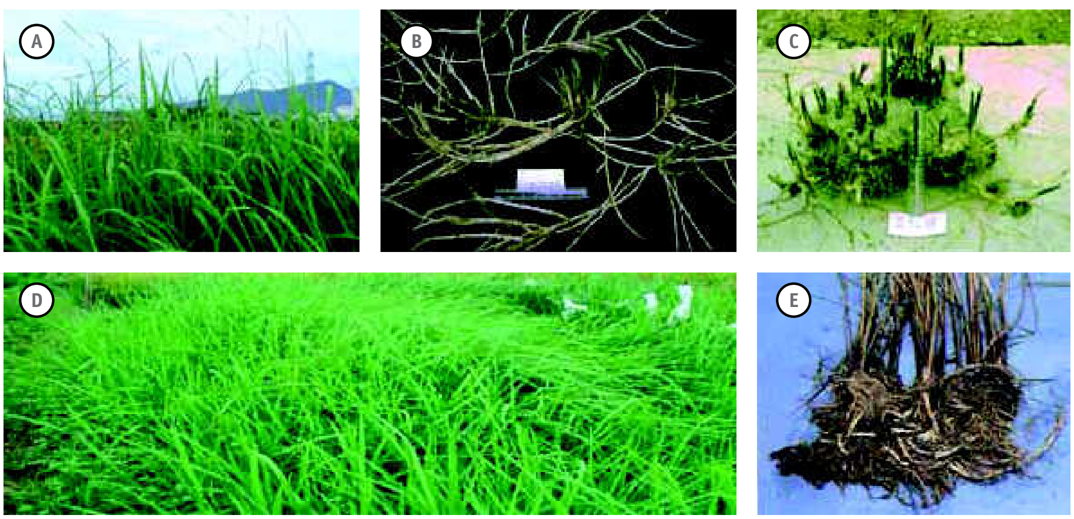
Following reports of a successful cross between *Oryza sativa* and *O. longistaminata* at the Yunnan Academy of Agricultural Sciences (Tao, 2000), development of perennial rice was proposed as one way to maintain surface cover after clearing, with potential benefits to nutrient and soil retention, and rice and livestock performance (IRRI, 1998). With donor support from Europe, research commenced at IRRI, with several reports explaining the concept, outlining the approach and reporting some initial results (Xiu, 1995; Schmit, 1996; Tao, 2000; Sacks, 2003).

METHODS

The *Oryza* species are quite diverse, as indicated in Table 1, which lists the species with their chromosome number, genome group and distribution by *Oryza* complex. The species arrowed express perenniality, including *O. longistaminata* in the *O. sativa* complex, and *O. rhizomatis* and *O. australiensis* in the *O. officianalis* complex.

Initially, *O. sativa* was crossed with *O. longistaminata* and *O. rufipogon*, but the crosses were more successful with *O. longistaminata*, as it is in the same complex as *O. sativa*. Consequently, it was decided to use *O. longistaminata* predominantly as the source of perenniality. *O. longistaminata* is AA genome like *O. sativa*, and develops extensive rhizomes in its native wetland habitat (Figure 2).

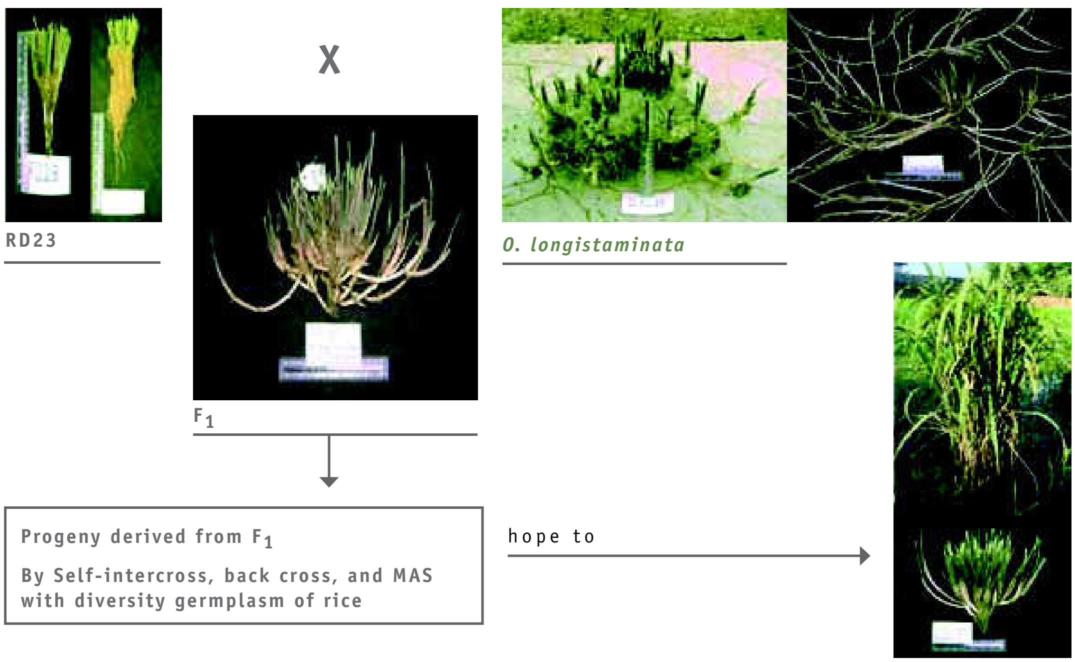
FIGURE 2. THE *O. LONGISTAMINATA* (AA GENOME)



The *Oryza longistaminata*
A. The panicle of the *O. longistaminata*
B,C,E. The strong rhizomes of *O. longistaminata*
D. The performance of *O. longistaminata* in field

The breeding strategy adopted to develop perennial rice at Yunnan Academy of Agricultural Sciences was as follows. RD23 was chosen as the *O. sativa* parent, as this cultivar was widely grown in lowland or upland, high yielding, good grain quality glutenous, and with disease resistance to rust etc. It was crossed with *O. longistaminata*, and the F_1 was intermediate in characteristics between the parents (Figure 3).

FIGURE 3. THE STRATEGY FOR PERENNIAL RICE BREEDING



From a combination of intercrossing among the F_1 progeny, backcrossing to RD23, and selection for desired traits, progress was made in developing perennial rice (Figure 3). This process was repeated a number of times using a wide range of successful cultivars as the *O. sativa* parent, but to date, the most successful has been with RD23. Selection, including marker-aided selection (MAS), was used to assist in transferring the perenniality traits from *O. longistaminata* into perennial rice. This followed successful research to identify QTL associated with rhizome development in *O. longistaminata*.

RESULTS

1. Genetic analysis of rhizome production

Genetics of rhizome expression was explored with Simple-Sequence Repeats (SSR) markers on the F_2 population from RD23/ *O. longistaminata*, using field and marker data. The PCR-based molecular genetic map (Figure 4) revealed three regions on chromosomes 3 and chromosome 4 of rice that indicated the two dominant complementary genes for rhizome expression, which were designated as *Rhz2* and *Rhz3*, respectively (Hu, 2001; Hu, 2003).

FIGURE 4. MOLECULAR GENETIC MAP OF RD23_LONGI BY SSR MARKERS

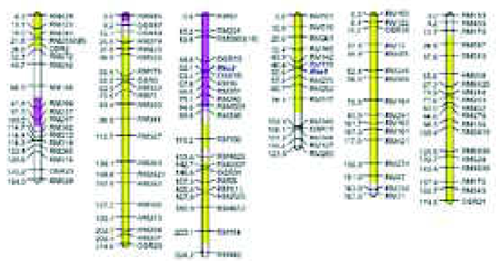
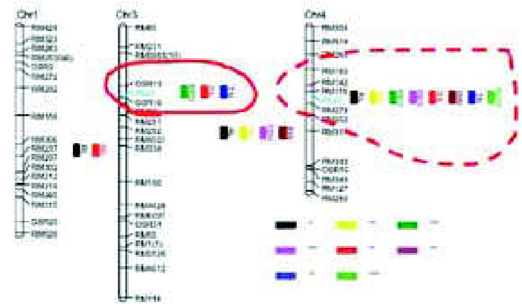


FIGURE 5. QTLs ANALYSIS OF RHIZOME RELATED TRAITS



Rhz3 on chromosome 4 co-located with many related root traits, including root length, root number, root branching density, root branching number, root internode length, root internode number, tiller number and root dry weight (Figure 5). Likewise, *Rhz2* on Chromosome 3 was co-located with root branching density, root internode length and tiller number, with the other traits closely located on the same arm of chromosome 3. The strong association between rhizome QTLs and related root QTLs lends confidence, as rhizome formation should lead to changes in the other parameters (Hu, 2003).

Subsequent fine mapping for *Rhz3* identified flanking markers RM14603 and OSR16 about 35 kb apart for *Rhz2* in 2008, and in 2012, a functional 5 scaffold was determined (Figure 6). Likewise, for Rhz3, the flanking markers were RM119 and RM17000 at 9.528 kb apart, with a 6 scaffold determined (Figure 7).

FIGURE 6. THE *RHZ2* FINE MAPPING

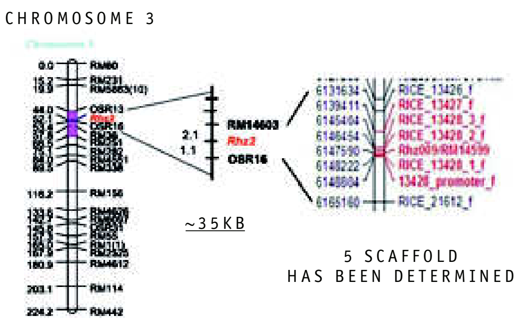
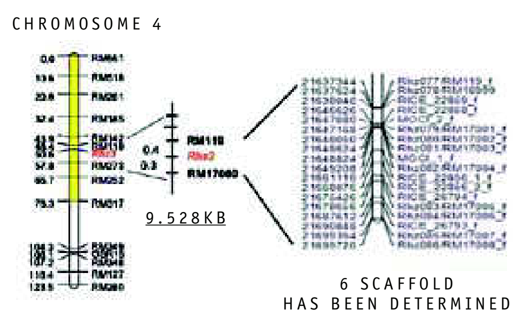


FIGURE 7. THE *RHZ3* FINE MAPPING



2. Breeding of perennial rice

Materials were initially evaluated and selected in the greenhouse, before being evaluated and selected in several field environments. Sanya on Hainan Island in the south of China was used from 2007 to 2010 to allow rapid generation advance under tropical lowland paddy conditions, as two crops per year were possible there. From 2011, however, the breeding station was moved from Sanya to Jing Hong in southern Yunnan Province, with additional field sites established on different soils at Puer and Menglien, which were perceived to be more appropriate environments for perennial rice.

Field evaluation demonstrated that plants were able to regrow successfully in the field for at least three seasons under these conditions, demonstrating not only regrowth and survival, but also reproductive success and grain set in the field.

The outstanding line was PR23 derived from RD23/ *O. longistaminata*, although PR57, PR129 and PR137 were also promising. Field performance of PR23 is after grain harvest in Simao, during regrowth in Simao and Jing Hong, and as a mature crop close to harvest in its third year in Jing Hong (Figure 8). A close-up of PR23 regrowth relative to senesced stems cut in the previous year is shown (Figure 9), along with a close-up of dry season survival at Puer (Figure 10).

FIGURE 8. THE PR LINES: PR23

The phenotype of PR23 in Jinghong, 2012/5 during Len Wade visiting there

FIGURE 9. THE REPRODUCTION ABILITY OF PR23

RE-GROWTH FROM UNDERGROUND THE OLD STEMS

The growth situation of PR23 after cutting the stub back to 10-15 cm 2 months in Jinghong (The third growth season)

FIGURE 10. THE REPRODUCTION ABILITY OF PR23

PR23 leave in field for next season growth 2011/12, Simao, Yunnan

TABLE 2. YIELD VARIATION OF PR23 BETWEEN DIFFERENT GROWTH SEASONS AND SITES

CROP SEASON	PHENOLOGICAL STAGE	YIELD OF PER UNIT AREA (KG/HA)	DEATH RATE (OVER YEAR)	SITE
First growth season	2011/1/10-2011/6/10 150d	5619.83		Jinghong, Yunnan, China
Second growth season	2011/6/13-2011/10/22 131d	3905.05		
Third growth season	2012/2/10-2012/6/5 116d	4027.57[a]	3.5%	
First growth season	2011/3/7-2011/8/10 157d	7350.00		Simao, Yunnan, China
Second growth season	2011/8/30-	The temperature was too low that the grain setting rate was not very good		
Third growth season	2012/3/28-2012/8/15 137d	6720.00	5.4%	

The yield performance of PR23 perennial rice over three seasons in the field at Jing Hong and Simao is shown in Table 2. Yields from the first wet season were impressive at 5.6 and 7.4 tonnes/ha. Not surprisingly, yields were lower in the drier second season when temperatures were lower in winter. Survival in the second wet season was impressive, with about 5 percent loss in plant stand over the first year. Nevertheless, wet season yields in the second year declined to about 70 and 90 percent of yields in the first wet season, respectively, which may reflect different seasonal conditions, some decline in plant vigour, or both. What factors may contribute to such changes needs further investigation, even though yields were still impressive in season three at 4.0 tonnes/ha (with some rat damage) and 6.7 tonnes/ha.

As a result of the strong performance of PR23 in the field, and farmer and district interest at the field sites, PR23 has entered pre-release testing for release as a perennial rice cultivar in Yunnan Province. At this stage, an individual farmer can try a small area, but a further three years of field evaluation data at several sites in Yunnan is needed before formal release of the cultivar, and any approval to increase and release seed to farmers. Nevertheless, the entry of PR23 to pre-release testing in Yunnan is a first for the perennial crops community. While some perennial species have been improved, and some fortuitous discoveries of unknown origin have been identified and grown by a few farmers, this is the first report of the intentional selection of a perennial cultivar from a cross with a related wild perennial species. This represents a milestone in combining a capacity to regrow with a capacity to set grain in subsequent generations. Hopefully, this heralds the advent of further breeding success in the perennial grains community.

3. Collaborations in perennial rice

In 2011, collaboration was established in neighbouring Lao, PDR via the ACIAR project on developing improved farming and marketing systems for rainfed regions of southern Lao PDR (Wade and Sengxua, 2014). A set of 13 recombinant inbred lines from the cross RD23/ *O. longistaminata* was planted at Xepon in Savannakhet Province and Phone Ngam in Champassak province in southern Lao PDR (Figure 11). The lines performed well in the first wet season (Table 3), but there was some stand loss in the harsher conditions encountered on light-textured soils in southern Lao, PDR, despite life-saving applications of water during the dry season. Some plants of most entries did regrow at both sites in the second wet season, but at one site, the farmer allowed livestock to graze at break of wet season, and heavy rain and flooding shortly thereafter resulted in total crop loss at Xepon. At the other site, the crop survived the typhoon, but plant vigour was affected and greater weed competition resulted, so yields at Phone Ngam were much lower in the second wet season (Table 3). A second set of 22 recombinant lines has now been sent to Lao, PDR for evaluation, and with the experience provided by this initial attempt, should be conducted with better management next time. Nevertheless, the capacity to regrow and produce forage and grain in the second year was recognised by the collaborators, who wish to continue this research in perennial rice. The plant breeders in particular were keen to collaborate in this research.

FIGURE 11. PERENNIAL RICE LINES GROWN IN XEPON, SAVANNAKHET AND PHONENGAM, PAKXE, CHAMPASAK, FOR ONE (2011) AND TWO (2011-2012) YEARS, RESPECTIVELY

TABLE 3. ANALYSIS OF VARIANCE OF GRAIN YIELD (g/m^2) OF 13 PERENNIAL RICE LINES GROWN IN XEPON, SAVANNAKHET AND PHONENGAM, PAKXE, CHAMPASAK, FOR ONE (2011) AND TWO (2011-2012) YEARS, RESPECTIVELY

SOURCE	df	TYPE II SS	MS	F	P
MAIN EFFECTS					
Year	1	1172206.515	1172206.5	1138.8705	.0000***
Site	1	413.1565707	413.15657	0.4014069	.5283 ns
Entry	12	140679.6121	11723.301	11.389905	.0000***
INTERACTION					
Year x Entry	11	39082.49769	3552.9543	3.4519128	.0007***
Site x Entry	11	30768.72232	2797.1566	2.717609	.0053**
Error	74	76166.06598	1029.2712<-		
Total	110	1893016.645			
Model	26	1816850.579	50468.072	49.032824	.0000***

R^2 = SSmodel/SStotal = 0.95976471407

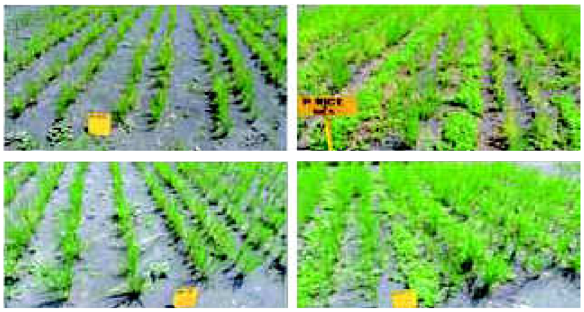
Root MSerror = sqrt (MSerror) = 32.0822561844

Mean Y = 188.996693703

Coefficient of Variation = (Root MSerror)/ abs (Mean Y) * 100% = 16.975036%

A related evaluation conducted in Nairobi, Kenya has confirmed the initial set of materials could not cope well with dry periods and their associated water deficits (Figure 12). Further research is needed to develop perennial rices able to cope with the severity of abiotic stresses under rainfed conditions, including drought in rainfed lowland and especially rainfed uplands. An optimistic note was provided by plant survival after three seasons on rainfall alone at Puer. The soil there is heavier with greater water-holding capacity, and temperatures in the dry season are cooler at higher elevation, so water loss is reduced, and plants survived. This observation lends confidence to the prospect for further improvements in dry season survival.

FIGURE 12. THE PERENNIAL ABILITY TEST OF PR23 IN AFRICA



DES: DESMODIUM

did not respond well to extreme drought conditions

CONCLUSIONS

A successful perennial rice breeding program has been established at Yunnan Academy of Agricultural Sciences, with the line PR23 now in pre-release testing in Yunnan Province. This success is based on a strategy of intercrossing F_1 lines, backcrossing to the cultivated rice parent, and rigorous selection for survival and seed set in the field. Development of perennial rice is consequently at the forefront of perennial grain development, and will hopefully act as an incentive to success in other species. The time is ripe to build on this success by establishing a consortium of perennial crop researchers, supported by a suite of donors to ensure the continuity of efforts needed for success in this challenging but important endeavour. Despite some success in developing a perennial rice phenotype which may be suitable for more favourable lowland conditions in which abiotic stresses are minimal, significant challenges remain in developing a robust perennial rice for the harsher rainfed lowland and especially upland ecosystem, where perennial rice is really needed.

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