Genetic improvement of rice varieties for Africa under new research collaboration between JIRCAS and Africa Rice Center

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Abstract
Japan International Research Center for Agricultural Sciences (JIRCAS) has been collaborating with the Africa Rice Center (AfricaRice) since 1998. In order to contribute to achieving goals set by the Coalition for African Rice Development (CARD), JIRCAS and AfricaRice have set new themes to improve rice varieties for Africa, namely phosphorus-deficiency tolerance and rice blast resistance.

Phosphorus deficiency is a widespread problem in west Africa where rice is cultivated on acidic soils. Through ongoing work at JIRCAS and partners related to the major quantitative trait locus (QTL) for tolerance, *Pup-1*, suitable markers for use in marker-aided introgression are being developed. In addition, joint efforts are directed toward the identification of new sources of tolerance using screening sites for P-deficiency in Japan and Africa.

Rice blast can severely damage rice production worldwide. JIRCAS and AfricaRice will evaluate the diversity in blast races and the variation in resistance of rice germplasm in Africa aiming to develop a differential system consisting of a set of differential varieties that each carries a single resistance gene, and a set of standard differential blast isolates. This would involve identifying new sources of resistance, but in the short term, allow us to focus on the development of resistant varieties through marker-assisted breeding, because DNA markers linked with several resistance genes are already available.

In future, by reviewing available research assets, other new traits will be incorporated to further enhance the value of rice varieties, thus contributing to increasing rice production in Africa.

Introduction

Japan International Research Center for Agricultural Sciences (JIRCAS) started collaboration with Africa Rice Center (AfricaRice; then known as the West Africa Rice Development Association, WARDA) in 1998. The target areas were genetic and eco-physiological characterization of tolerance to drought and soil acidity in indigenous rice varieties and interspecific progenies between *Oryza glaberrima* and *O. sativa*. Socioeconomics in relation to sustainability of lowland rice cultivation in west Africa was another target of collaboration. Researchers conducted fundamental research surveys in sub-Saharan African countries under a collaborative research plan.

At the Fourth Tokyo International Conference on African Development (TICAD IV) in 2008, the Yokohama action plan was announced. One of the goals of that plan was to double rice production in 10 years "through developing capacities to adopt systematic crop management, and new methodologies including wider use of New Rice for Africa (NERICA)" (TICAD IV, 2008). To contribute to achieve this goal, JIRCAS and AfricaRice have reviewed the research needs in Africa and developed a set of new research themes related to genetic improvement of rice varieties for Africa. By taking into account the research assets of both organizations, tolerance to phosphorus deficiency and resistance to rice blast have been identified as new target traits.

Improving tolerance to phosphorus deficiency

Phosphorus (P) is the second most important inorganic plant nutrient after nitrogen and one of the least available nutrients because of its tendency for tight binding in the soil, especially in acidic soils. Phosphorus deficiency can substantially impact rice growth and development. Plants grown on P-deficient soils appear stunted with dark green leaves, suppressed root development and reduced number of tillers (Doberman and Fairhurst, 2000). Delayed maturity, high sterility and poor grain quality are also common under P deficiency. The deficiency can be corrected through P fertilizer application, but resource-poor farmers often do not have access to P fertilizers (Kirk *et al.*, 1998). Furthermore, suitable high-grade rock phosphates represent a finite resource that could be depleted towards the end of the 21st century (Runge-Metzger, 1995). It is therefore essential to develop rice cultivars that are P efficient, i.e. that produce acceptable yields with limited P supply.

Existing sources of tolerance — Pup1

Screening trials under P deficiency have been conducted independently by several research groups and it appears that ample genotypic variation for tolerance to P deficiency is present in the *Oryza* gene pool. However, the only tolerance source to have been mapped with high enough precision to be directly used in marker-assisted selection (MAS) is the major quantitative trait locus (QTL) for P-deficiency tolerance *Pup1* (for *P-up*take *1*).

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Pup1 was identified in the Nipponbare × Kasalath mapping population and fine-mapped to the region 15.1–15.6 Mb on chromosome 12 (Wissuwa *et al.*, 2002). Near isogenic lines (NILs) carrying the *Pup1* allele from tolerant donor parent Kasalath consistently show higher P uptake, dry weight and grain yield (Fig. 1) compared to the intolerant recurrent parent Nipponbare (Wissuwa and Ae, 2001; Wissuwa, 2005).

Figure 1. Phenotypic effect of *Pup1* in a Nipponbare background when grown on a highly P-fixing Andosol without addition of P fertilizer

Precision mapping (Heuer *et al.*, 2009) placed *Pup1* in a 150 kb interval at around 15.3–15.5 Mb on Nipponbare chromosome 12. Potentially useful markers have been developed based on the Nipponbare–Kasalath sequence comparison. Screening of 159 rice accessions with *Pup1*-specific markers suggests that the tolerance allele of *Pup1* may already be present in many upland cultivars developed for drought-prone environments (Chin *et al.*, 2010). Breeders have therefore selected for *Pup1* in the field unknowingly, which indirectly confirms its effectiveness, but also means that most likely candidates for recipient parents would have to come from lines with high yield potential or desired grain quality that have not yet been adapted to low-input environments, e.g. have been selected under more favorable conditions. Several *Pup1*-specific markers have been identified (Chin *et al.*, 2010) and are being used in MAS for upland varieties in Indonesia. A similar project could be initiated with NERICA and new generation rice varieties for Africa after an initial screening to confirm the presence or absence of *Pup1* in target genotypes.

Potential new sources of P-deficiency tolerance

Screening experiments conducted in west Africa have shown that CG14 (*Oryza glaberrima*) possesses some tolerance to P deficiency (Tobita *et al.*, 2003). Initial phenotypic scoring on the highly P-fixing upland soil type (Andosol) at Tsukuba (Japan) revealed variations in terms of the tolerance to P-deficiency among the 18 upland NERICA varieties — some upland NERICA lines may have inherited tolerance to P-deficiency from CG14. Through genotyping of upland NERICA, it should be possible to pinpoint which CG14 introgression confers this tolerance. Subsequently, it would be necessary to verify that this is not simply another allele of *Pup1*, but a truly novel source. Further characterization of upland NERICA varieties carrying the introgression corresponding to P-deficiency tolerance and others lacking it in screening environments may allow us to deduce (at least in part) what tolerance mechanism had been affected or enhanced by the introgression. Subsequent screening experiments should include parental genotypes used in the development of second-generation NERICA materials and possibly wild rice accessions.

Research on and breeding for rice blast resistance

Rice blast is a serious disease caused by a fungal pathogen, *Pyricularia grisea* (Cooke) Sacc., the anamorph of *Magnaporthe grisea* Hebert (Rossman *et al.*, 1990), which occurs in every rice-growing region in the world (Bonman and Mackill.,1988; Latterell and Rossi, 1986; Ou, 1985), including Africa. One outbreak was reported in Kenya in 2008. Many resistance genes for rice blast have been reported in scientific journals in the past (Fig. 2); however, their characterization and effects were not clearly presented in the papers (Koide *et al.*, 2009). Moreover, almost all of the blast resistance found has been broken down because new races of blast have appeared after the release of new resistant varieties. Thus, inconsistencies always exist between farmers' fields and scientific findings.

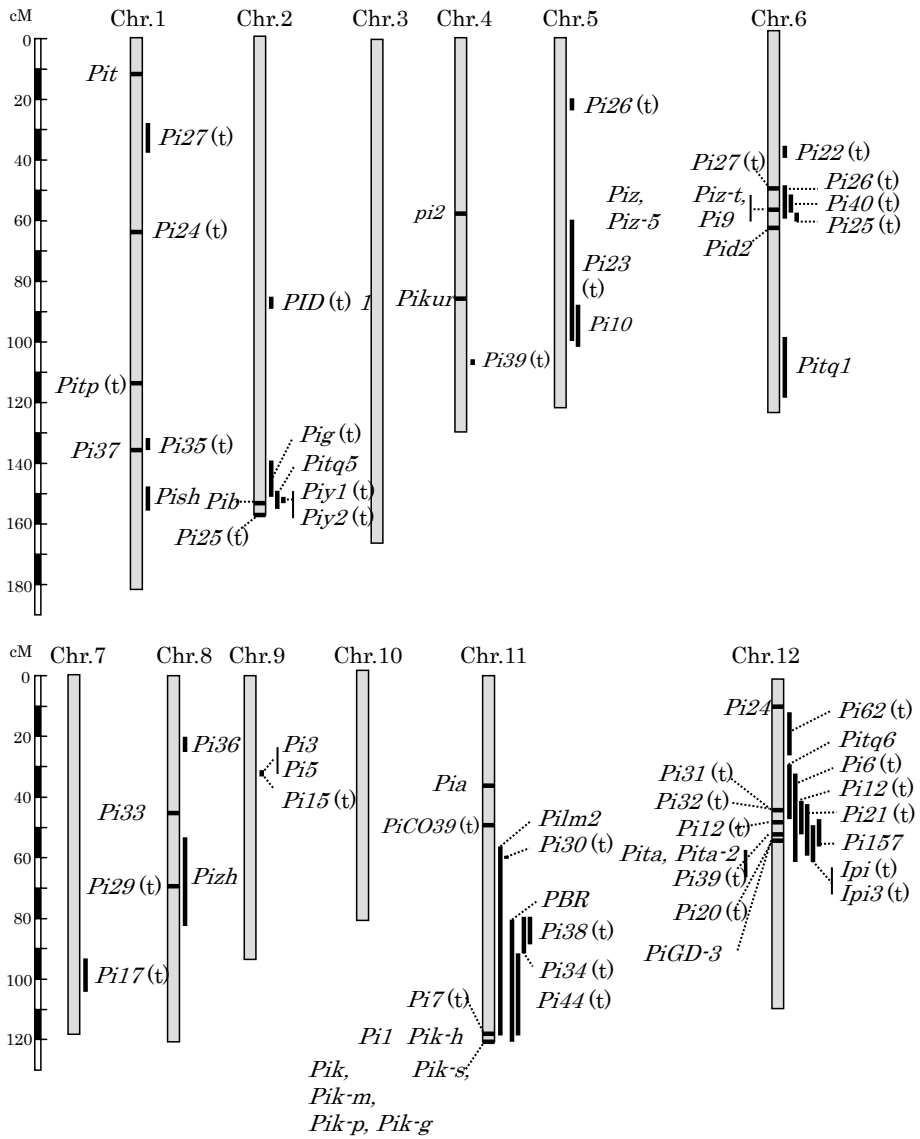

Figure 2. Putative location of the blast resistance genes reported by 2008 (Koide *et al.*, 2009). The genetic locations of the each gene are based on the public databases (Oryzabase and Gramene) and published papers.

Differential system for rice blast

Even though new blast races break resistance, rice has an advantage since no new race has broken all resistant genes at one time. Therefore, it is necessary to develop a system to monitor or identify the dominant races of rice blast in rice cultivation areas and thereby identify the resistance genes that will be effective against them to prevent devastation of crops. One of the solutions is the differential system, which is composed of rice varieties or lines each carrying (ideally) a single resistance gene and blast isolates differing in (corresponding) avirulence/virulence to each of the resistance genes. Several groups of varieties have been developed as differential varieties on the basis of the gene-for-gene theory (Flor, 1971; Silue *et al.*, 1992). The differential variety groups have been useful for understanding host–pathogen interaction and will contribute to the development of durable protection against blast disease as a tool for genetic, pathological and breeding studies.

International Rice Research Institute (IRRI) and JIRCAS have developed four universal differential variety sets under the IRRI–Japan Collaborative Research Project. Each set comprises monogenic lines (MLs) with the *japonica*-type variety Lijianxintuanheigu (LTH) genetic background, and near isogenic lines (NILs) of LTH, *indica*-type variety CO39 and universal susceptible line US-2 genetic backgrounds. The sets target 24 resistance genes (Fukuta *et al.*, 2009). Using this differential system as a key tool, JIRCAS has established the Blast Research Network for Stable Rice Production with national agricultural research systems (NARS) in Asian countries. Ten research institutes and one university from eight countries (Bangladesh, China, Indonesia, Japan, Korea, Lao PDR, Philippines, Vietnam), and two international organizations (AfricaRice and IRRI) are

members of this research network. Information on blast disease, methods of genetic and pathological analysis, resistance genes and their selection markers, blast isolates collected and rice varieties in each country, will be exchanged within this network. Each participating country is conducting diversity studies for blast races (Table 1) and rice resistance, developing a differential system, and identifying novel resistance genes, to use in rice breeding for durable resistance or protection system (e.g. a multiline system).

Toward blast-resistant varieties for Africa

In the case of Africa, only limited information is available for differentiation of blast fungus, and therefore, limited information is available regarding predominant blast races in specific regions. A set of differential varieties would facilitate the gathering of such important information. In parallel, differential blast isolates should be collected to complete a differential system for Africa, which will contribute to the construction of a breeding strategy for each region in Africa. The complete establishment of a differential system will take a long time. However, using the information on predominant blast races in Africa, our collaboration will focus in the short term on the development of resistant varieties. Since there are several DNA markers linked with blast resistance, marker-assisted breeding will be used.

Prospects

For collaborations on genetic improvement of rice varieties to increase rice production in Africa, JIRCAS and AfricaRice have chosen improving tolerance to P deficiency and blast resistance as new target areas. In a long-term perspective, since varieties and tools applicable to rice breeding will be developed and accumulated, we will be able to incorporate other new traits through review of available research assets and needs for rice varieties. We at JIRCAS hope that this collaborative research with AfricaRice will contribute greatly to increase rice production in Africa.

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Table 1. Reaction patterns of monogenic lines to blast isolates from the Philippines

Target gene	Designation†	Blast isolates and pathotypes																		
		Ca89	Ca41	IK81-3	M64-1-3-9-1	M39-1-3-8-1	V850256	V86010	BN111	PO6-6	M39-1-2-21-2	JMB8401	43	M36-1-3-10-1	BN209	M101-1-2-9-1	IK81-25	JMB840610	V850196	B90002	C923-49
		Ib	Id	II	IIIa	IIIa	IIIc	IVe	Va	Vb	VIb	XIIa	XVIII	XIXb	XXIIa	XXIIa	XXIII	XXIVb	XXVII	XXVIIIb	XXVIIIb
Pia	IRBLa-C	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R
Pita	IRBLta-K1	S	R	R	S	R–M	R	S	S	MS	S	M	S	MS	S	S	R	S	R	R	S
Pik-s	IRBLks-F5	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	R	S	S
Pit	IRBLt-K59	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Pi3	IRBL3-CP4	S	S	S	S	M	S	S	R–M	R	R	R	R	S	S	S	R	S	R	S	S
Pii	IRBLi-F5	S	S	S	S	M	S	S	R	R	R–M	R	R	S	S	S	R	S	R	S	S
Piz-t	IRBLzt-T	S	S	S	R	R	S	R	S	S	S	S	S	S	S	S	S	R–M	S	R	R
Pib	IRBLb-B	S	S	S	S	S	S	M	S	S	S	S	S	S	R–M	R	S	R	S	R	R
Piz-5	IRBLz5-CA	M	R	R–M	M	M	M	M	R	R	R–M	R	M	R	M	S	R	R	R–M	M	M
Pita-2	IRBLta2-Re	S	R	R	R–M	R	R	R	R	S	R	R	S	R	R	R	R	R	R	R	S
Piz	IRBLz-Fu	M	M	R	R–M	R–M	M	R–M	S	R–M	R–M	R–M	R	R	R–M	R	R	M	R	M	R
Pik	IRBLk-ka	R	R	R	S	S	R	R	R	R	S	S	R	S	R	R	S	R	R	R	R
Pi1	IRBL1-CL	R	R	R	S	S	R	R	R	R	S	S	R	S	R	R	S	R	R	R	R
Pik-h	IRBLkh-K3	R	R	R	S	S	R	R	R	R	S	S	R	MS	R	R	M	R	R	R	R
Pik-m	IRBLkm-Ts	R	R	R	S	S	R	R	R	R	S	S	R	M	R	R	MS	R	R	R	R
Pik-p	IRBLkp-K60	R	R	R	S	S	R	R	R	R	S	S	R	S	R	R	S	R	R	R	R
Pi20(t)	IRBL20-IR24	S	S	S	S	S	S	R	R	S	R	S	S	R	S	S	MS	S	S	R	R
Pi5(t)	IRBL5-M	S	M	R–M	M	R	MS	S	R–M	R	R	R	R	R–M	MS	R	R	M	R	M	MS
Pi7(t)	IRBL7-M	R	R	R	S	S	R	S	R	R	S	S	R	S	R	R	S	R	R	R	R
Pi9(t)	IRBL9-W	R	R	R	R–M	M	R	S	R	R	R–M	R	R	R	R	R	R	R	R–M	R	R
Pi11(t)	IRBL11-Zh	S	S	M	S	S	S	R	S	S	S	MS	S	MS	R–M	R	S	R	S	R	R
Pi12(t)	IRBL12-M	S	S	S	S	S	S	M	S	S	S	S	S	S	R	R	S	R	M	R	R
Pi19(t)	IRBL19-A	S	S	S	S	S	S	S	S	S	S	MS	S	MS	S	S	S	S	S	S	S
Pish	IRBLsh-S	M	R–M	R–M	M	M	R–M	M	R–M	R–M	R	S	M	R	M	R–M	R	M	R–M	R–M	M

The reaction data of monogenic lines are partially modified from Kobayshi *et al*. (2007).

† Monogenic lines are designated as IRBL followed by the resistance gene then the abbreviation of the resistant donor variety.

R, resistant; M, moderately resistant; MS, moderately susceptible; S, susceptible; R–M, varied from moderately resistant to resistant.

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