Establishment of an implementation methodology for an afforestation/ reforestation clean development mechanism project targeting small-scale farmers

The Clean Development Mechanism (CDM) is a system that emission reductions or removals of greenhouse gases (GHGs) to be achieved by GHG emission reduction projects carried out in developing countries are converted to carbon credits which can be added to the emission reduction targets of the Annex I countries (developed countries) of the Kyoto Protocol. The purpose of the study is to establish a practical methodology to obtain carbon credits (CERs) by implementing an afforestation/ reforestation CDM (A/R CDM) project targeting small-scale farmers (SSFs) in Paraguay as a part of rural development.

The CDM Executive Board of UNFCCC issued 6,819 tCO_2 of CER to the A/R CDM project of JIRCAS titled as "Reforestation of croplands and grasslands in low income communities of Paraguarí Department, Paraguay" in August 2013 (Table 1, Figure 1). The CERs were the first in Latin America issued to the A/R CDM project targeting SSFs. This CDM project realized the effective use of SSFs' lands through introducing agro-forestry and silvo-pasture based on the needs of SFFs (Figure 2).

In spite of expected large social significance of A/R CDM project targeting SSFs, the growth of trees was poor or irregular due to low capability of participants and degraded lands susceptible to drought. Carbon stocks were quantified by establishing sample plots not with a unit area but with a certain number of trees to correspond to the irregularity of SSFs' forests. The study clarified that the A/R CDM project targeting SSFs generated quite less carbon stocks or CERs than the planned amount of them (Table 2).

The implementation methodology of an A/R CDM project targeting SSFs from formulation to implementation including the results of monitoring activity conducted in 2012 was recommended and developed as manuals which were uploaded in the JIRCAS homepage. In addition, the necessary documents for the issuance of CERs such as project design document and monitoring report as well as validation and verification reports prepared by the designated operational entity were published on the web site of the UNFCCC.

Expecting high sustainability, the methodology of JIRCAS is intended to promote reforestation by self-responsibility and beneficiary pays principle of participant farmers. The methodology can be used for carbon sequestration projects applied to A/R CDM, REDD+ and voluntary carbon offset system which involve unorganized SSFs in Latin America.

If an A/R CDM project is proposed, a break-even CER unit value of the proposed A/R CDM project should be calculated by financial analysis; then, should be compared to an assumed market value or the minimum unit cost obtained by JIRCAS in order to ensure the viability of CER acquisition. The keys to realize sustainable reforestation project with the beneficiary-pays-principle are to select project area with farmers' high needs for tree plantation and to promote self-help efforts by means of awareness raising activities.

(E. Matsubara, M. Watanabe, S. Shiraki)

Table 1. Increase of GHG removals by sinks in the A/R CDM project in Paraguay

Item	Tree species	Carbon stock (tC)	Baseline (tC)	Leakage (tC)	Net carbon stock increase (tC or tCO2)[1]
Carbon stocks					
	Eucalyptus grandis	882.2	263.2	132.3	486.7
	Eucalyptus camaldulensis	2,471.2	557.6	370.7	1,542.9
	Grevillea robusta	74.5	57.7	11.2	5.6
	Sub total	3,427.8	878.5	514.2	2,035.2
Deduction[2]		∆ 205.9	-	∆ 30.6	∆ 175.3
Total					1,859.8
Convert to tCO_2 [3]					6,819.4

Note (1) Net carbon stock increase= Carbon stock - Baseline - Leakage.
Note (2) Deduction rate is determined according to margine of error. The margine of error of the project is 11.4 %, which is corresponding with 6% of deduction rate.
Note (3) The conversion rate of tC to tCO2 is 44/12 (or 3.667).

Table 2. Comparison of planned reforestation area with monitored reforested area

Stratum	Tree species (Planted year)	Plan (2009)		Monitoring (2012)		Baseline (2009)	
		Planted Area (ha)	No. of Parcel	Crediting Area (ha)	No. of Parcel	Cropland (ha)	Grassland (ha)
S1	*Eucalyptus grandis* (2007)	30.05	56	13.59	23	11.61	1.99
S2	*Eucalyptus grandis* (2008)	31.17	41	9.59	15	3.80	5.79
	Sub total	61.22	97	23.18	38	15.41	7.78
S3	*Eucalyptus camaldulensis* (2007)	16.36	17	7.71	9	2.75	4.96
S4	*Eucalyptus camaldulensis* (2008)	64.48	21	45.63	17	1.91	43.72
	Sub total	80.84	38	53.34	26	4.66	48.68
S5	*Grevillea robusta* (2007)	5.59	9	0.67	2	0.67	0.00
S6	*Grevillea robusta* (2008)	15.16	14	2.13	1	2.13	0.00
S7	*Grevillea robusta* (AF) (2007)[1]	14.05	28	1.13	1	1.13	0.00
S8	*Grevillea robusta* (AF) (2008)	38.30	54	1.06	2	0.08	0.97
	Sub total	73.10	105	4.99	6	4.01	0.97
Total		215.16	240	81.51	70	24.08	57.43

Note (1) AF: Agroforestry

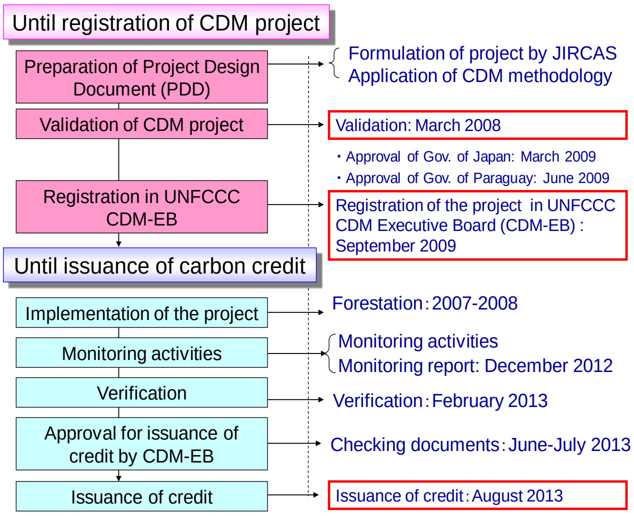


Figure 1. Flow of processes from the start to the acquisition of CER of the A/R CDM project in Paraguay

Figure 2. Photographs of established forest in the A/R CDM project in Paraguay

QTL for total spikelet number per panicle is detected on chromosome 7 in the genetic background of *Indica*-type rice cultivar

An *Indica*-type rice cultivar, IR64, has been widely accepted as high quality rice with good eating quality and high yield in many countries. Total spikelet number per panicle (TSN) is one of the most important traits to increase grain productivity in rice (*Oryza sativa* L.). To improve yield potential of IR64, a Japanese high-yielding cultivar, Hoshiaoba, was backcrossed to IR64 for three times. We attempted to detect quantitative trait loci (QTL) for TSN by using the developed introgression line. Furthermore, we developed a near isogenic line (NIL) to characterize the effect of the QTL for increasing TSN.

A putative QTL, *qTSN7.1*, was detected between two markers, RM1132 and RM505, on the long arm of chromosome 7 (Fig. 1A and 1B). For developing NILs, plants which have an introgression in the target chromosomal region of *qTSN7.1*, were selected from 144 F_2 plants derived from a cross between IR64 and its introgression line used for the QTL mapping. Whole-genome survey was conducted using 480 SSR markers distributed throughout the 12 rice chromosomes to generate the graphical genotype of NIL with *qTSN7.1* (Fig. 1C). To characterize agronomic traits of NIL, 11 traits were evaluated and compared with those of IR64 (Table 1). NIL showed significantly higher TSN than IR64. In contrast, NIL had significantly shorter SL than IR64. There was no significant difference between IR64 and NIL in other agronomic traits (except GW in 2012DS) across the seasons.

In this study, we succeeded to detect a QTL for TSN, *qTSN7.1*, using a Japanese high-yielding cultivar, Hoshiaoba, as a donor parent. The developed NIL for TSN with tagged DNA markers would be useful to improve the yield potential of *Indica*-type rice cultivars through increase in TSN.

(N. Kobayashi [NARO-NICS], Y. Koide [JSPS], D. Fujita [JSPS], A.G. Tagle [IRRI], , K. Sasaki, Y. Fukuta, T. Ishimaru)

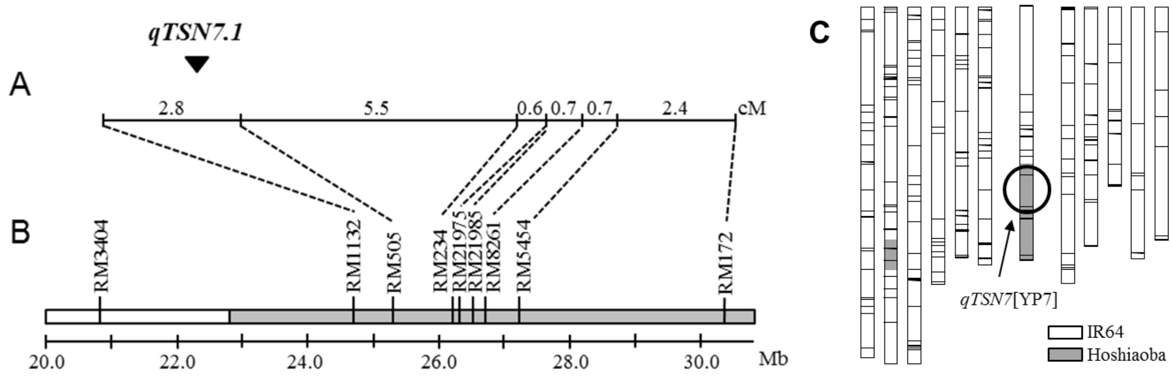


Fig. 1. Chromosomal location of *qTSN7.1* and graphical genotype of NIL with *qTSN7.1*.

(A) Genetic map of the marker on chromosome 7. ▼ ; LOD peak

(B) Physical map of the DNA markers. The physical chromosomal position is based on Nipponbare genome sequence.

(C) Graphical genotype of the NIL with *qTSN7.1*. White boxes show the chromosomal segments of IR64, while grey boxes show the chromosomal segments of Hoshiaoba.

Table 1. Characterization of agronomic traits of IR64 and NIL with *qTSN7.1* in the wet season of 2010 and the dry season of 2012.

Line	Season	TSN	DTH	CL (cm)	PL	LW (cm)	LL (cm)	PN
IR64	2010WS	141.8 ± 30.1	88.8 ± 1.8	78.6 ± 3.0	25.2 ± 1.5	1.3 ± 0.1	38.2 ± 5.3	18.0 ± 5.3
NIL		176.4 ± 21.4**	86.0 ± 1.0	77.8 ± 2.5	25.1 ± 1.2	1.3 ± 0.0	39.7 ± 5.4	22.6 ± 6.8
IR64	2012DS	106.9 ± 18.9	79.8 ± 3.4	65.5 ± 1.9	23.5 ± 1.1	1.3 ± 0.2	26.1 ± 4.0	19.2 ± 4.4
NIL		150.0 ± 34.2**	78.5 ± 2.1	69.7 ± 5.2	23.0 ± 1.7	1.5 ± 0.1	28.6 ± 3.3	14.6 ± 5.6

Line	Season	GW (g)	SL (mm)	SW (mm)	ST (mm)
IR64	2010WS	2.7 ± 0.1	10.0 ± 0.4	2.5 ± 0.1	2.0 ± 0.0
NIL		2.6 ± 0.1	9.7 ± 0.4**	2.6 ± 0.1	2.0 ± 0.0
IR64	2012DS	2.8 ± 0.1	9.9 ± 0.5	2.4 ± 0.1	2.0 ± 0.1
NIL		2.5 ± 0.1**	9.3 ± 0.5**	2.4 ± 0.1	1.9 ± 0.1

TSN, Total spikelet number; DTH, days to heading; CL, Culm length; PL, Panicle length; LW, Leaf width; LL, Leaf length; PN, Panicle number; GW, 100-grain weight; SL, Seed length; SW, Seed width; ST, Seed thickness. **, significant difference at 1% by *t*-test.

Gene discovery of *SPIKE*

-A unique gene from a rice landrace increases grain yield of *Indica*-type cultivars-

Increasing crop production is essential for securing the future food supply in developing countries. Total spikelet number per panicle (TSN) is one of the most important traits to increase grain productivity in rice (*Oryza sativa* L.). We previously reported the detection of *qTSN4*, a QTL for increasing TSN on the long arm of chromosome 4 derived from new plant type (NPT) cultivars with the genetic background of IR64 (Refer the Research Highlight in 2012). In this study, we attempted to clone the gene for *qTSN4*. We further conducted characterization of agronomic traits of a near isogenic line (NIL) with the gene. To understand the effect of the gene in different genetic backgrounds, we introduced it into six *indica* cultivars popular in South and Southeast Asian countries by marker assisted selection.

We successfully identified a causative gene for *qTSN4*, designated here as *SPIKE* (*SPIKELET NUMBER*) by map based cloning using 7996 BC_4F_3 plants of an NPT cultivar as a donor and IR64 as a recurrent parent (Fig. 1A). NIL for *SPIKE* had higher TSN (Fig. 1B), wider flag leaf (Fig. 1C) and heavier root dry weight (Fig. 1D) than those of IR64. Rate of filled grain were also higher, but panicle number per plant and 1000-grain weight were lower in the NIL (data not shown). Notably, the grain appearance of the NIL was significantly improved (Fig. 2A), presumably owing to a strengthening of assimilate supply to the larger number of spikelets by an increase in vascular bundle number (Fig. 2B). Consequently, the grain yield of the NIL was consistently higher by 13-36% than that of IR64 over four cropping seasons, significantly so in three of the four seasons (Fig. 2C). *SPIKE* also increased TSN in six cultivars popular in South and Southeast Asia (Fig. 3), confirming its effectiveness in various genetic backgrounds.

SPIKE would be a unique gene to increase grain yields of *indica* cultivars in South and Southeast Asia through molecular marker-assisted breeding, thus contributing to food security in these regions.

(N. Kobayashi [NARO-NICS], D. Fujita [NARO-NICS, JSPS], A.G. Tagle [IRRI], Y. Koide [JSPS], K. Sasaki, R. B. Gannaban [IRRI], Y. Fukuta, T. Ishimaru)

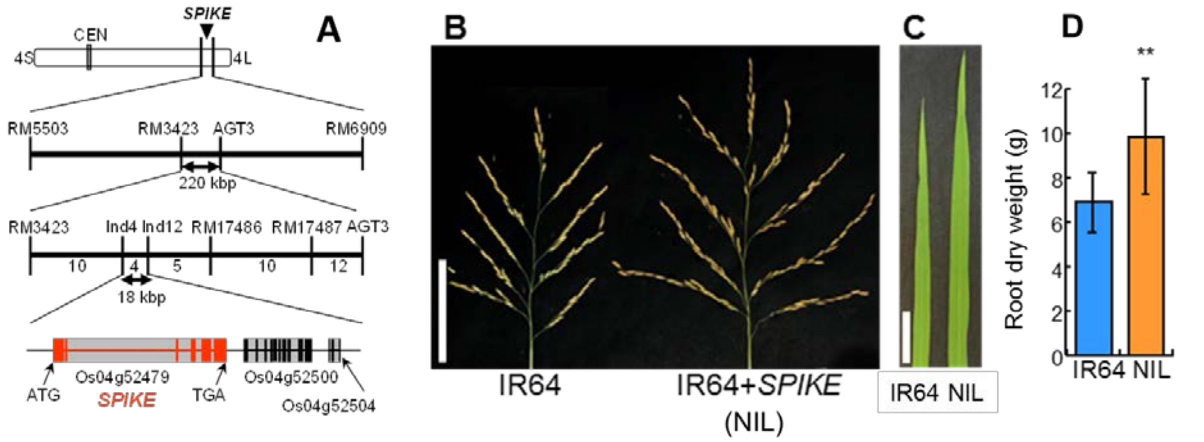


Fig. 1 Chromosomal location of *SPIKE* (A), panicle architecture (B), flag leaf width (C) and root dry weight (D). Values are mean ± SD. **Significant at 1% level by *t*-test.

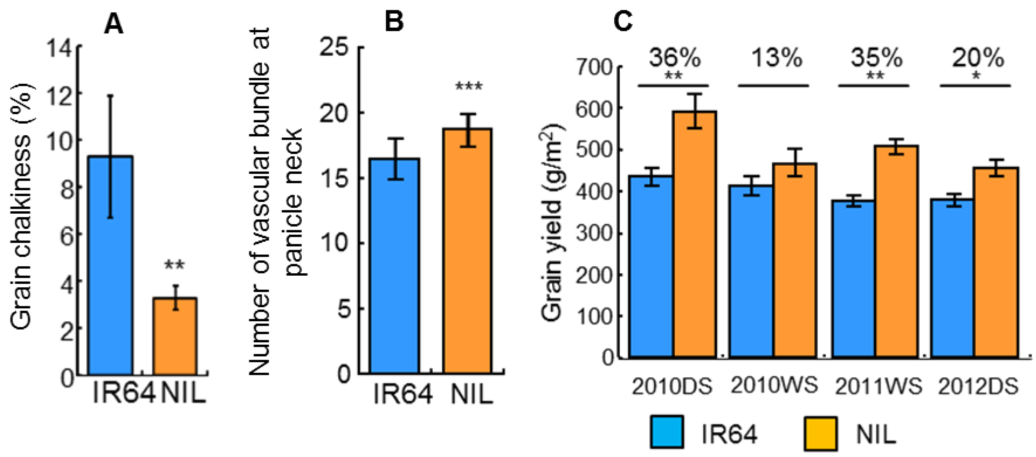


Fig. 2 Percentages of grain chalkiness (A), number of vascular bundle at panicle neck (B) and grain yield (C). Values are mean ± SD (A, B) or SE (C). Significant at ***0.1%, **1% and *5% level by *t*-test.

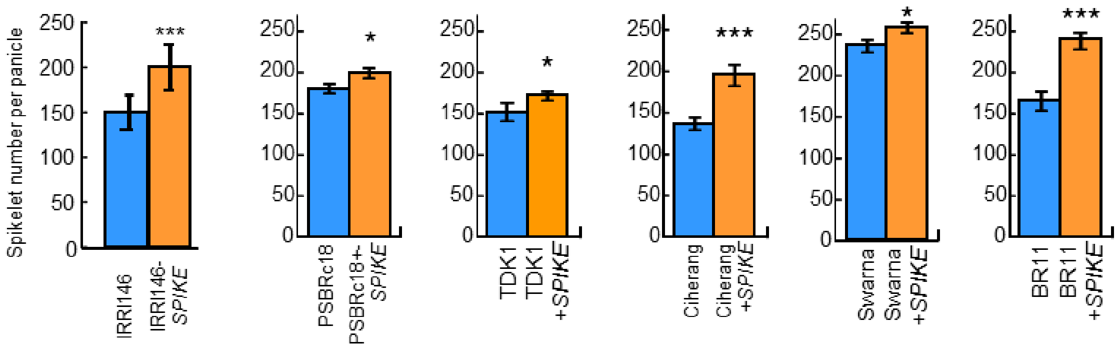


Fig. 3 Total spikelet number per panicle between *Indica*-type cultivars with and without *SPIKE*. Values are mean ± SE. Significant at ***0.1%, **1% and *5% level by *t*-test.

Hydrological impacts of full-dyke system in flood-prone rice granary areas in the Mekong Delta

Mekong Delta is the rice granary area which produces 90% of exporting rice of Vietnam which is the world's second largest rice exporter. It is feared as one of the mega deltas strongly affected by the most risk from climate change. The objective of the study is to clarify the effect of full-dyke systems constructed for triple rice cropping in flood-prone rice area in the Mekong Delta on hydrological environment in the region through interviews with government agencies and residents, the analysis of river water level and satellite image and to provide a basic knowledge for sustainable rice cultivation to cope with the increasing flood risk under climate change.

Two types of dyke systems are constructed to reduce vulnerability in high-flood areas adjacent to the Cambodian border in the Mekong delta: a high embankment called "full-dyke”, which completely prevents farmland from flooding (August – November) ; and a low embankment called "semi-dyke", which prevents flooding up to harvest period (August) of spring-summer rice but allows flood inflow after harvest (Fig.1). Triple rice cropping is feasible in the farmlands enclosed by full-dykes, as rice can grow even in the peak flood season from September to October. In response to farmers’ request, the Vietnamese government has promoted construction of full-dykes, hence the areas with full-dyke systems expanded rapidly in the past 10 years especially in An Giang Province (Fig.2).

The comparison of MODIS Terra images between flood in 2011 with 10 years of return period when full-dyke has widely spread in the study area and flood in 2000 with 60 years of return period when full-dyke has constructed only a little. Both inundated area and flooding period are shown larger in 2011 flood than those in 2000 in Kien Giang province located in the west side downstream of An Giang Province (point A) and in Cambodia border located in the upstream of full-dyke area (point B), (Fig. 3). Three points in Fig. 3 were selected for verification, (a) the point with significantly prolonged inundation, (b) with slightly prolonged and (c) the point with little change of inundation and carried out interview survey to the farmers. The result shows farmers view for the changes on flooding period have a good accordance with the results of the satellite images. The water level at CanTho station in Hau River one of main-streams in the Mekong River in recent years shows rising trend from the water level analysis. Comparing the relationship of annual maximum water level between CanTho and ChauDoc from 1979 to 2011 separating into two groups before 2004 and after 2005, the rising trend of water level after 2005 was observed in CanTho compared with the water level before 2004 (Fig. 4).

The research output will be utilized for the study on adaptation measures in the mega deltas with progressive flood risk and also useful as the validation data for hydro-hydraulic model in flood inundation area caused by the expansion of full-dyke system in the Mekong Delta. We should take note land subsidence in urban areas and sea level rise due to global warming also the major causes of water level rise in CanTho, more detailed investigation is required to separate the impact of full-dike systems.

Fig.1 Semi-dyke and Full-dyke (Up: Both sides are semi-dyke area, Middle : Left; Semi-dyke area, Right: Full-dyke area Down : Both sides are full-dyke area)

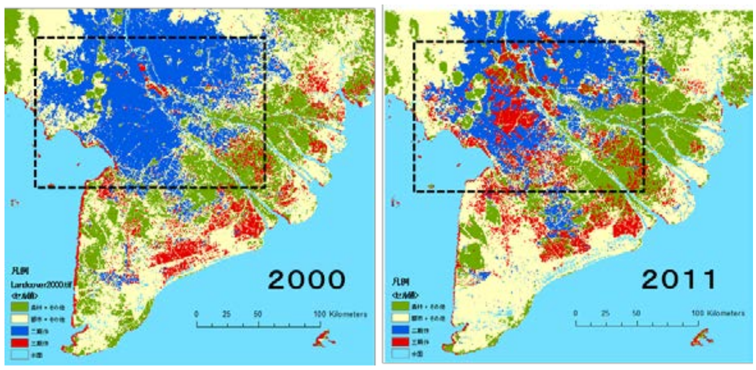


Fig.2 Increase in triple rice cropping in the flood-prone area (dashed line shows the area of Fig.3) Blue : double cropping, Red : Triple cropping, Green : Forest and others

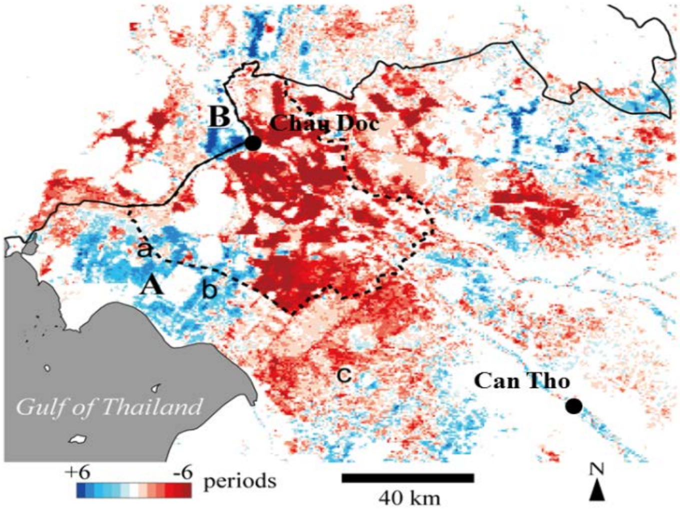


Fig.3 Comparison of inundation duration between 2000 and 2011 flood evaluated by NDWI of MODIS. (Dashed line shows the border of An Giang Province and solid line shows Vietnam and Cambodia border. Blue: inundation period in 2011 is longer than that in 2000; Red: 2011 is shorter than 2000: White: little difference between two years. Prolonged inundation is observed, such as Cambodia (B point) located upstream of An Giang Province and Kien Giang Province (A point) located in the west side of An Giang Province. a, b, c shows verification points. a: prolonged much, b: slightly prolonged, c: not so much change.

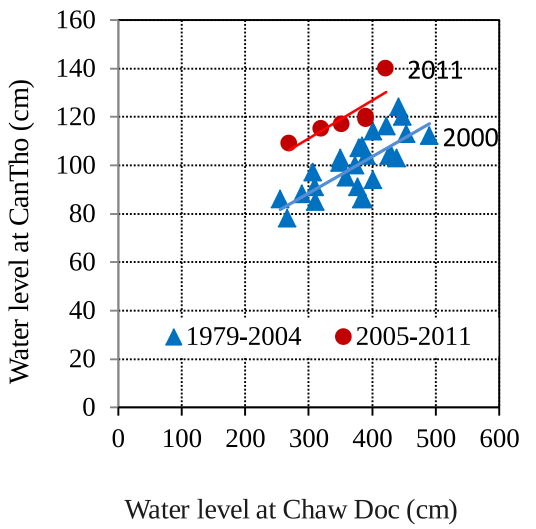


Fig.4 Relationship of yearly maximum water level at ChauDoc and CanTho (2007: missing data)

H. FUJII, Y. FUJIHARA [Ishikawa Prefectural University], K. Hoshikawa [Center for Integrated Area Studies, Kyoto University] and S. Yokoyama

Effects of no-till maize cultivation after leguminous hairy vetch cropping on fertilizer saving and nitrogen leaching

No-till cultivation associated with residue mulch after leguminous cropping, has several advantages such as decrease in water runoff and soil erosion, fertilizer saving (JIRCAS research highlight 14). Decrease in water runoff as an advantage, on the contrary, may increase water percolation into the soil; therefore, there is a higher risk to increase nitrogen leaching if nitrate generated due to decomposition of organic matter is not effectively taken by plant. In the present study, we elucidate nitrogen leaching and balance taking into account the relationship between water runoff and water percolation in sloping field when no-till maize cultivation with residue mulch of hairy vetch（*Vicia villosa* Roth.）, which is cropped during the fallow period.

As shown in Figure 1, maize yield with 50 kg dose of nitrogen after hairy vetch cropping, either tilled or no-tilled, is equal to or more than that after fallow (conventional treatment; nitrogen dose: 100 kg ha^{-1}). This results confirm possible nitrogen fertilizer saving. In addition, when zero dose of nitrogen, maize yield after the natural fallow records almost zero yield, while that after hairy vetch cropping obtains 70 % of yield for the conventional cultivation (nitrogen dose: 100 kg ha^{-1}). No-till cultivation after hairy vetch cropping turnovers the residue as mulch. Table 1 shows an example of water movement and nitrogen leaching at main rainfall events. The concentration of nitrate-nitrogen in the percolated water at the hairy vetch treatment increases 23.5 times more than that at the natural fallow treatment, and the amount of percolated water increases only 1.5 times, resulting 37 times of the amount of leached nitrogen. This means that increase in nitrogen leaching is mainly due to the increase in the concentration of nitrate-nitrogen, while the effect of increase in percolation, which is the result of no-till cultivation associated with the residue mulch, is limited. Available nitrogen provided (total amount of nitrogen derived from fertilizer, hairy vetch, and soil) is almost equal to the sum of the amount of nitrogen taken up by maize and that leached. It can be explained that excess nitrogen generated results leaching. Regarding nitrogen balance at the harvest, it is much positively higher for no-till cultivation after hairy vetch cropping than that for conventional cultivation, suggesting that hairy vetch cropping increases soil fertility in sustainable way. It is noted that nitrate-nitrogen output associated with water runoff is not considered in nitrogen balance since it was not detected in the runoff water.

This cropping system shows advantages such as decrease in water runoff, fertilizer saving as described. Therefore, it is expected to be adopted in regions where rainfall is limited, but with high rainfall intensity, and where dose of fertilizer application is limited. Excess turnover of biomass into the soil, however, have a risk of ground water pollution due to nitrogen leaching as shown in the present study. This requires considering quantitative relationship of the amount between fertilized nitrogen and hairy vetch derived one in order to adjust the dose of nitrogen fertilizer.

The present study is conducted at sloping fields (2º, 3.5º, 5.0º in slope gradient, 14 m long), one of the open research facilities, at Japan International Research Center for Agricultural Sciences (JIRCAS), Tropical Agriculture Research Front (TARF), Ishigaki Island, Okinawa prefecture, Japan.

(F. Nagumo, K. Nakamura [National Institute for Agro-Environmental Sciences])

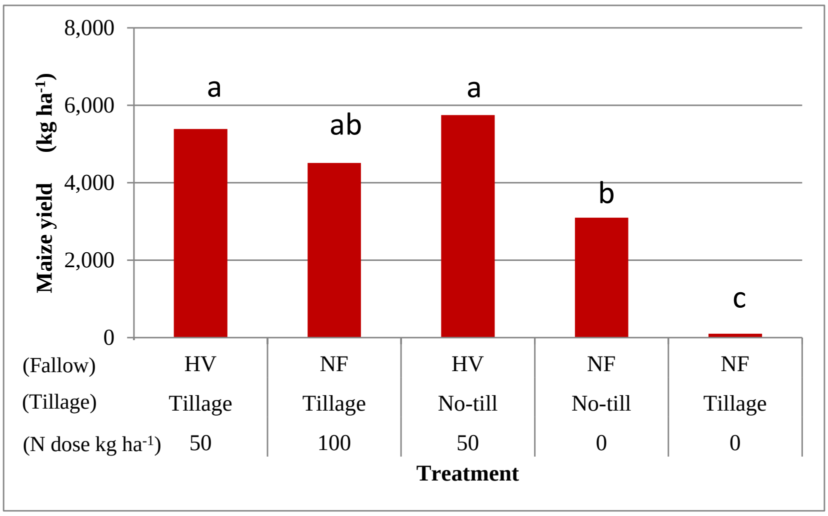


Figure 1 Effects of the treatment combination on maize yield.
HV: hairy vetch cropping during fallow period, NF: natural fallow
Different alphabets indicate significantly different at 5%

Table 1 Example of water runoff and percolation into the soil during a main rain event (Slope gradient: 5^{o}, 27-28 days after planting)

Treatment	(Fallow)	NF	HV
	(Tillage)	Tillage	No-till
	(N dose: kg ha^{-1})	100	50
Rainfall mm		145.0	145.0
Water runoff mm		30.6	7.1
Deep percolation mm		86.0	130.3
NO_3-N Concentration g m^{-3}		1.1	25.8
NO_3-N leached kg ha^{-1}		0.9	33.6

Note: Percolated water was collected by a lysimeter installed at 60 cm deep.
HV: hairy vetch cropping during fallow period, NF: natural fallow

Table 2 Nitrogen balance at the maize cropping (kg ha^{-1})

Treatment	(Fallow)	NF		HV	
	(Tillage)	Tillage		No-till	
	(N dose: kg ha^{-1})	100		50	
Biomass-N		13.2	(weeds)	150.1	(HV)
Fertilizer-N		100.0		50.0	
Input-N		**113.2**		**200.1**	
Available-N		**108.7**		**133.7**	
Uptake N		95.6		96.2	
Leached N		11.1		36.5	
Output-N		**106.7**		**132.7**	
Available N-Output N		**2.0**		**1.0**	
N balance		**6.5**		**67.4**	

Identification of the fatty acid and the fatty acid methyl ester as the new nitrification inhibitors

The tropical pasture grass, *Brachiaria humidicola* (Rendle) Schweick, produces nitrification inhibitory compounds (termed biological nitrification inhibitors or BNIs) in its shoot and root tissues, and releases BNIs from its roots. During this study, two BNI compounds were isolated and identified from the shoot tissue of *B. humidicola* using activity-guided fractionation.

The BNI compounds in the shoot tissue were identified as linoleic acid (LA) and linolenic acid (LN) using authentic chemicals (ED_{80} 16.0 μg ml^{-1} for both LA and LN) for verification (Fig. 1). None of the other tested free fatty acids namely stearic acid, oleic acid, arachidonic acid, and vaccenic acid showed any inhibitory effects on nitrification. Among the fatty acid methyl esters (FAME) evaluated [methyl oleate, methyl linoleate (LA-ME) and methyl linolenate (LN-ME)], only LA-ME showed any inhibitory effect (ED_{80} 8.0 μg ml^{-1}) (Figs. 1, 2). The inhibitory effect of LA, LN and LA-ME on soil nitrification was stable for 120 days at 20ºC (Fig. 3). Soil treated with LA, LN and LA-ME showed a very low accumulation of NO_3^- and the maintenance of soil inorganic N in the NH_4^+ form (Fig. 3). The inhibitory effect of LA-ME on soil nitrification was greater than that of LA, LN or nitrapyrin (commercial nitrification inhibitor) (Fig. 3). Both LA and LN suppressed soil nitrifier activity by blocking AMO (ammonia monooxygenase) and HAO (hydroxylamine oxidoreductase) enzymatic pathways in *Nitrosomonas europaea*. Commercial nitrification inhibitors (such as nitrapyrin or dicyandiamide (DCD)) are not effective (nitrapyrin is volatile at temperatures >5C, thus is not effective in tropical environments; DCD is highly soluble in water, thus leaches out of fertilizer zone, thus is not effective in field environments) in tropical environments, thus are not adopted for production agriculture in tropics.

Since LA, LN and LA-ME can be produced from vegetable oils such as soybean, flax or sunflower and are more effective and stable (these compounds are bound to the soil thus do not leach out from the point of application to the fertilizer zone, which is in addition to their stability at tropical temperatures) in tropical soils, they have the potential for use as nitrification inhibitors in production agriculture.

(*G. V. Subbarao, K. Nakahara, T. Ishikawa, T. Yoshihashi, Y. Ono [National Food Research Institute], M. Kameyama [National Food Research Institute], M. Yoshida [National Food Research Institute]*)

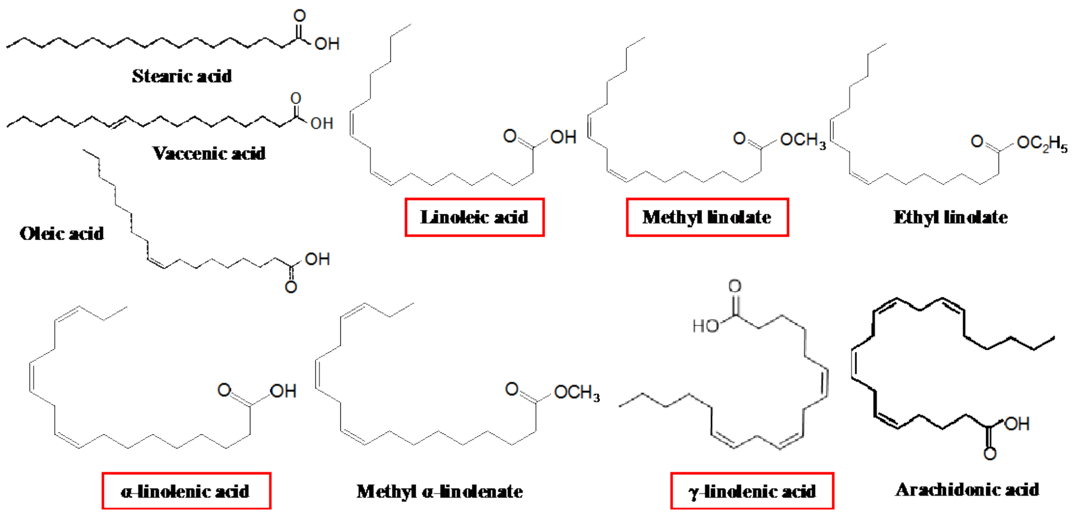


Fig. 1. Structure formulae of various fatty acid and fatty acid ester. Substance with an enclosure has nitrification inhibitory activity.

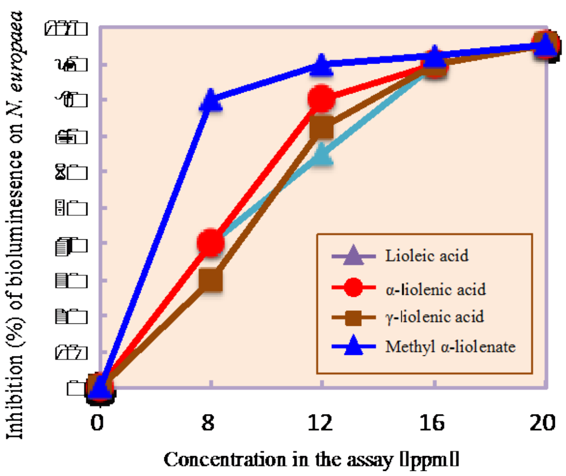


Fig. 2. Relative effectiveness of substances in inhibiting *Nitrosomonas europaea* activity in an *in vitro* assay.

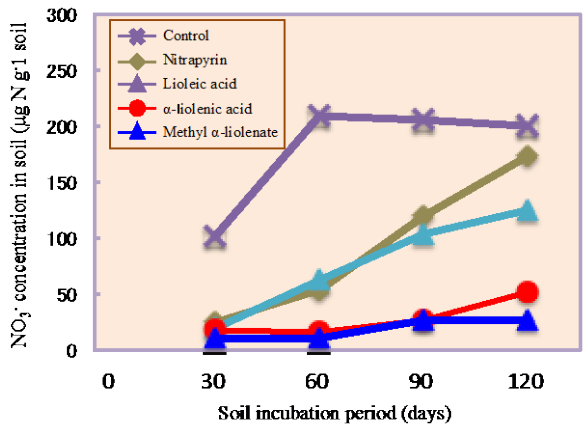


Fig. 3. Relative stability of the inhibitory effects on soil nitrification from linoleic acid (1,000 μg g^{-1} soil), linolenic acid (1,000 μg g^{-1} soil) , methyl linoleate (1,000 μg g^{-1} soil) and nitrapyrin (4.5 μg g^{-1} soil) during 120-day incubation period at 20°C.

Analysis of the secretion mechanism of the biological nitrification inhibitors from sorghum root

The ability to suppress soil nitrification by releasing nitrification inhibitors from plant root systems is termed 'biological nitrification inhibition' (BNI). We have reported earlier that sorghum roots release higher BNI-activity when grown with NH_4^+, but not with NO_3^- as N source. Also, for BNI release, rhizosphere pH of <5.0 is needed; beyond this, a negative effect on BNI release was observed with nearly 80% loss of BNI activity at pH $\geq$7.0. This study is aimed at understanding the inter-functional relationships associated with NH_4^+ uptake, rhizosphere-pH and plasma membrane H^+-ATPase (PM H^+-ATPase) activity in regulating the release of biological nitrification inhibitors (BNIs).

Sorghum was grown hydroponically and root exudate was collected from intact plants using a pH-stat system to separate the secondary acidification effects from NH_4^+ uptake on BNIs release. A recombinant luminescent *Nitrosomonas europaea* bioassay was used to determine BNI-activity. Root plasma membrane was isolated using a two-phase partitioning system. Hydrolytic H^+-ATPase activity was determined. Split-root system is used to understand the localized responses to NH_4^+, H^+-ATPase-stimulator (fusicoccin) or H^+-ATPase-inhibitor (vanadate) on BNI release by sorghum. The results presented suggest that the presence of NH_4^+ in the rhizosphere stimulated the expression of H^+-ATPase activity and enhanced the release of BNIs from sorghum roots compared to NO_3^- (Fig. 1a, b). NH_4^+ levels (in rhizosphere) positively influenced BNIs release and root H^+-ATPase activity in the concentration range of 0 - 1.0 mM, indicating a close relationship between BNIs release and root H^+-ATPase activity with a possible involvement of carrier-mediated transport for the release of BNIs in sorghum (Fig. 2). Split-root system studies show that part of the root system exposed to NH_4^+ released substantially higher levels of BNI activity than the root system that was exposed to NO_3^-; similarly, part of the root system which was exposed to fusicoccin, stimulated BNI release, whereas, the part of the root system exposed to vanadate suppressed BNI release (Fig. 3a, b). These results indicate that NH_4^+ uptake, PM H^+-ATPase activity, and rhizosphere acidification are functionally inter-connected with BNI release in sorghum and a hypothesis is proposed (Fig. 4).

Such knowledge is critical to gain insights into why BNI function is likely to be more effective in light-textured and mildly acidic soils [such as Alfisols (of SAT regions), Ultisols (predominant in South America) or Sandy-loams (of West-African SAT region)] compared to heavy-textured soil types such as Vertisols.

(Y. Zhu, H. Zeng [Nanjing Agricultural University], Q. Shen [Nanjing Agricultural University], T. Ishikawa, G. V. Subbarao)

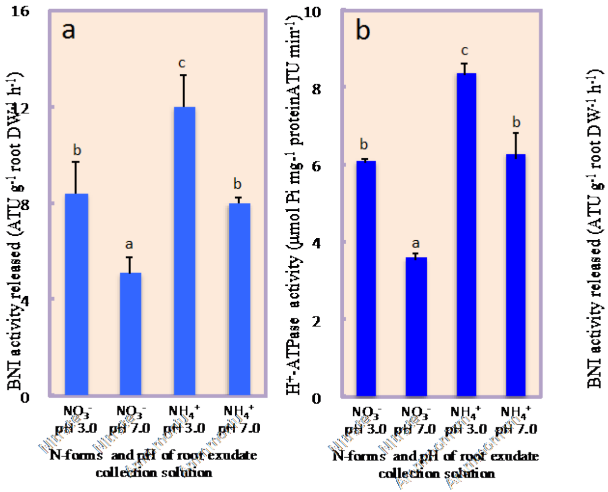


Fig. 1. Influence of N-forms (i.e. 1 mM N as NH_4^+ *vs.* NO_3^-) and root exudate collection solution pH (solution pH 3.0 vs. 7.0) on biological nitrification inhibition (BNI) release and root plasma membrane (PM) H^+-ATPase in sorghum grown hydroponically for 14 days with NH_4^+ or NO_3^- as N source.

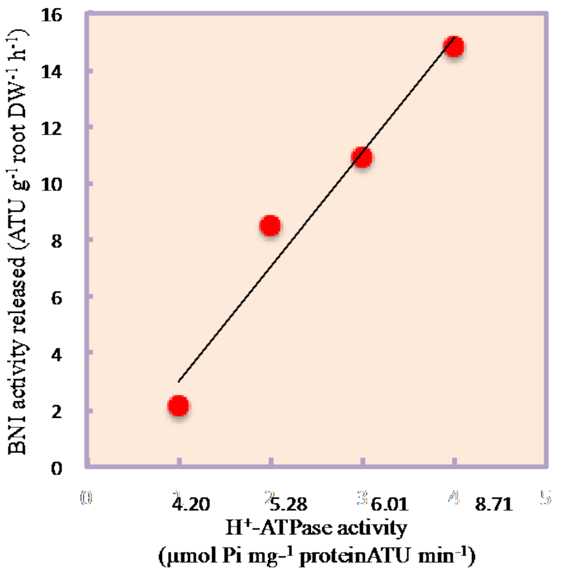


Fig. 2. The relationship between BNI release from sorghum roots and root PM H^+-ATPase activity at various concentrations of NH_4^+ (0 to 1.0 mM) in the root exudate collection solutions.

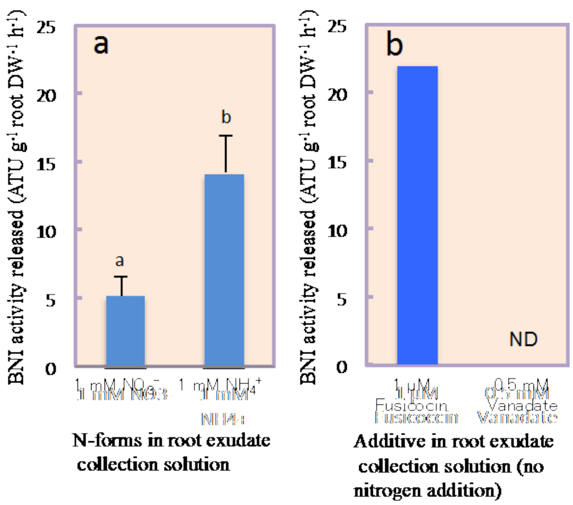


Fig. 3. Influence of N-forms (1 mM N as NH_4^+ *vs.* NO_3^-) and H^+-ATPase stimulator, fusicoccin (1 μM) or H^+-ATPase inhibitor, vanadate (0.5 mM) on BNI release in sorghum in a split-root system setup.

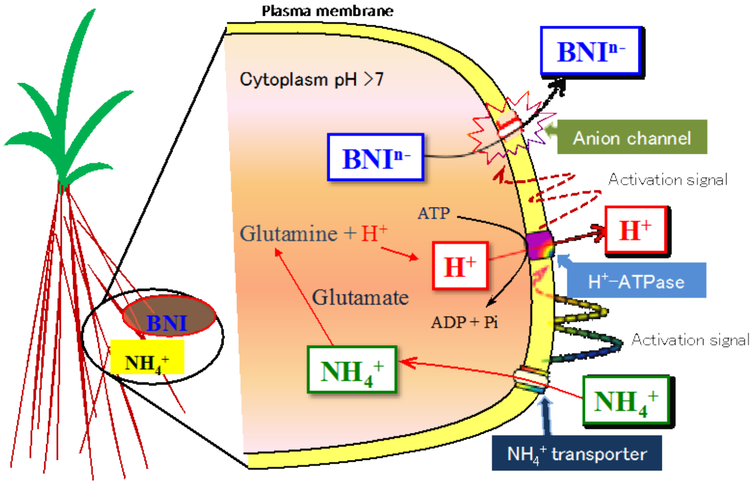


Fig. 4. Hypothesis on the transport of biological nitrification inhibitors (BNIs), driven by plasma membrane H^+-ATPase, associated with NH_4^+ uptake and assimilation in sorghum.

Laboratory manual for studies on soybean rust resistance

Soybean [*Glycine max* (L.) Merrill] is an economically important legume crop, with more than 80 million tons exported to the world market mainly from North and South American countries. Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi* Sydow & P. Sydow, is one of the biggest threats to stable soybean production in South America and in other tropical and sub-tropical regions.

Various studies related to the pathogenic variations of ASR and the development of ASR-resistant cultivars in each country have been done. Since ASR pathogens are widespread across borders, each country has had to cope with this disease based on information using a common resistance evaluation method. However, it was difficult to compare the pathogenicity of ASR pathogens and the degree of ASR resistance in soybean genotypes among countries because the evaluation method related to ASR resistance was not standardized. Therefore, a uniform procedure for conducting ASR studies is necessary.

First, we standardized the experimental protocols – i.e., 1) multiplication of ASR urediniospores, 2) single-lesion isolation, 3) inoculation of soybean with spore suspension, 4) evaluation of ASR pathogenicity, 5) evaluation of ASR resistance in soybean genotypes, and 6) evaluation of ASR tolerance of soybean genotypes (Figure 1, Table 1) -- to obtain experimental results that are reproducible. South American ASR pathogens and the differential varieties were utilized for this work (Table 2). Then, we optimized the experimental protocols related to SSR marker analysis for marker-assisted selection (MAS) so that domestic institutions in South America can carry out their soybean breeding programs for ASR resistance. Finally, we compiled these standardized experimental protocols into a single manual, titled "Laboratory manual for studies on soybean rust resistance," which can be accessed from the JIRCAS website: http://www.jircas.affrc.go.jp/english/manual/soybean_rust/JIRCAS_manual_soybean_rust.pdf.

The data for the pathogenicity of ASR pathogens and for the degree of ASR resistance in soybean genotypes obtained by following this manual can be compared with previously obtained data (Akamatsu et al., 2013). Therefore, this manual is expected promote research related to pathogenic variations of ASR pathogens and marker-assisted soybean breeding for ASR-resistant cultivars.

(N. Yamanaka, H. Akamatsu, Y. Yamaoka [University of Tsukuba])

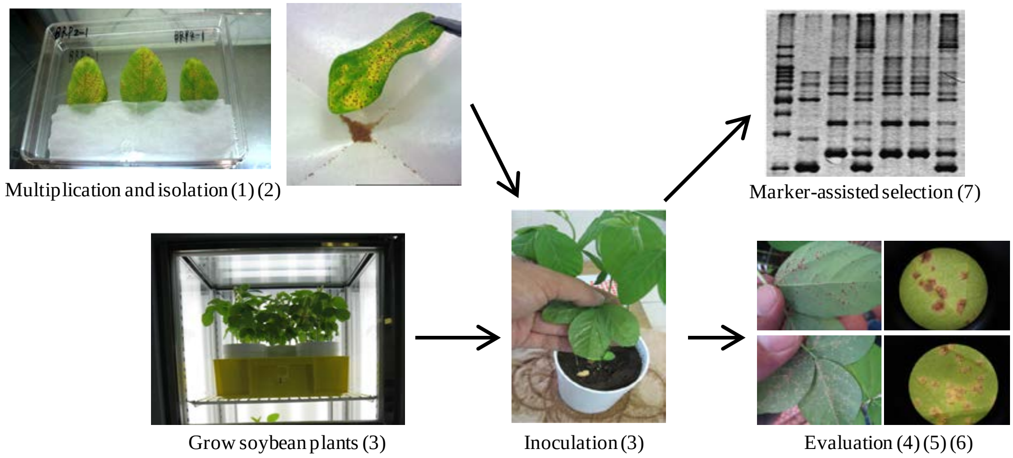


Fig. 1. Evaluation process for Asian soybean rust (ASR) resistance. The numbers in the figure correspond to that of Table 1.

Table 1. Contents of the manual for Asian soybean rust (ASR) resistance

No.	Item	Details
1	Multiplication of ASR urediniospores	Method to multiply ASR urediniospores for the following experiments
2	Single-lesion isolation	Method to isolate ASR pathogens from the ASR population that may contain various races
3	Inoculation of soybean with spore suspension	Methods to grow soybean plants, to prepare urediniospore suspension, and to inoculate urediniospore suspension to soybean plants
4	Evaluation of ASR pathogenicity	Method to evaluate virulence of ASR pathogens based on the lesion type
5	Evaluation of ASR resistance in soybean genotypes	Method to evaluate resistance of soybean genotypes based on the lesion type
6	Evaluation of ASR tolerance of soybean genotypes	Method to evaluate tolerance of soybean genotypes based on infection index and degree of leaf-yellowing
7	Marker-assisted selection of ASR resistance	Method of SSR marker analysis for the marker-assisted soybean breeding of ASR resistance

Table 2. Example of pathogenic data for Asian soybean rust (ASR) pathogens based on the resistance reactions of the differential varieties

			Differential varieties*	PI 200492	PI 368039	PI 230970	PI 417125	PI 462312	PI 459025	PI 200562	PI 416764	PI 587855	PI 587880A	PI 587886	PI 587905	PI 594767A	BRS 154	TK5	PI 548628
Country	Location	Season	No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Argentina	Pergamino,	2007/200		S	S	S	S	nd	S	R	R	nd	R	S	R	R	nd	S	S
	Buenos Aires	2009/201		S	S	S	S	S	R	IM	S	I	R	S	S	IM	S	S	S
Brazil	Passo Fundo,	2007/200		S	S	R	IM	S	S	R	IM	IM	S	S	IM	R	S	S	S
	Rio Grande do	2008/200		S	S	IM	S	I	IM	R	S	I	I	S	I	R	S	S	S
		2009/201		S	S	S	IM	I	R	R	S	I	I	S	R	R	S	S	S
Paraguay	Capitán Miranda,	2007/200		S	S	S	S	S	S	R	S	I	R	S	I	R	S	S	S
	Itapúa	2008/200		S	R	S	S	S	R	R	R	I	I	S	I	R	S	R	S
		2009/201		IM	S	IM	IM	I	IM	R	IM	I	I	S	I	R	S	R	S
Japan	Tsukuba	2007		R	R	S	S	R	R	R	R	nd	I	S	R	R	nd	S	S
		2008		I	I	R	R	R	R	I	I	nd	R	R	R	nd	nd	S	R

I: Immune; R: Resistant; IM: Intermediate; S: Susceptible; nd: no data
* Latest set includes 17. PI517602B and 18. No6-12-1.

Enhancement of porosity and aerenchyma formation by nitrogen deficiency in rice roots (*Oryza sativa* L.)

Lysigenous aerenchyma is formed by cell collapse accompanied with cell death. The aerenchyma in roots provides oxygen from the ground portion to the roots, and is concerned with waterlogging tolerance in plants. Field crops such as wheat show generally poor tolerance to water logging, whereas semiaquatic crops such as rice show high tolerance to water logging. One of the major reasons for the difference in tolerance levels is the initiation of aerenchyma formation between these crops. In wheat, aerenchyma is inducibly formed by multiple environmental factors such as oxygen and nutrient (N, P, K) deficiencies. On the other hand, rice forms two kinds of aerenchyma: constitutive and inducible aerenchyma. Thus, the mechanism of aerenchyma formation in rice is more complicated compared with field crops. Moreover, the mechanism of aerenchyma formation induced by nitrogen deficiency has remained unknown, although the resulting aerenchyma is likely to reduce energy loss. In this study, we attempted to (1) establish reliable growth conditions to estimate aerenchyma formation, and (2) reveal the pattern of aerenchyma formation induced by nitrogen deficiency in rice roots.

Before evaluating aerenchyma formation, we modified the growth conditions, e.g., hydroponic solution and growth period, to estimate precisely the response by nitrogen deficiency alone. We could then establish precise growth conditions demonstrating the recovery of growth vigor caused by pH reduction in hydroponic solutions (Fig. 1). Compared with nitrogen sufficiency, nitrogen deficiency facilitated the formation of air space in whole roots, i.e., an increase in porosity (Fig. 2). In order to determine the spatial and temporal patterns of aerenchyma formation induced by nitrogen deficiency, cross-sections from seminal roots of seedlings grown only on nitrogen-deficient and oxygen-deficient conditions were prepared at several positions, from the root tip to the root base. Microscope observations revealed that aerenchyma formation was enhanced in both nitrogen- and oxygen- deficient conditions compared with reference condition (Fig. 3). In nitrogen-deficient conditions, aerenchyma formation initiated close to root base. Conversely, in oxygen-deficient conditions, the initiation was observed close to root tip (Fig. 3).

As far as we know, this is the first evidence that nitrogen deficiency in rice roots enhances porosity and aerenchyma formation. It strongly distinguishes the physiological roles of nitrogen deficiency and oxygen deficiency on induced aerenchyma formation, demonstrating the different initiation patterns of aerenchyma between nitrogen and oxygen deficiency. Aerenchyma induced by nitrogen deficiency may function in reducing respiration and remobilization of nitrogen, or both. Furthermore, our established growth condition is expected to isolate causal genes associated with aerenchyma (formed either constitutively or induced) toward developing molecular breeding techniques for conferring waterlogging tolerance in field crops in the near future.

(M. Obara, T. Abiko)

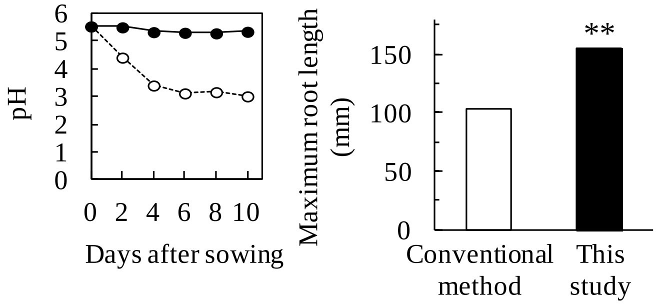


Fig.1. pH maintenance of nutrient solutions (left) and recovery of root elongation (right) in this study (improved method). ○ : conventional method, ●: this study

Asterisks (**) represent a significant difference in maximum root length between conventional method and improved method at *P*-value of 1% level (one-way ANOVA).

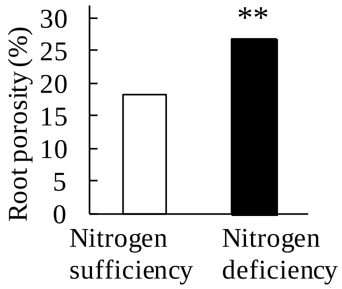


Fig. 2. Increased root porosity by nitrogen deficiency.

Root porosity refers to the size of air space including aerenchyma. Ten-day-old seedlings were used. Asterisks (**) represent a significant difference in root porosity between nitrogen-sufficient conditions and nitrogen-deficient conditions at P-value of 1% level (one-way ANOVA).

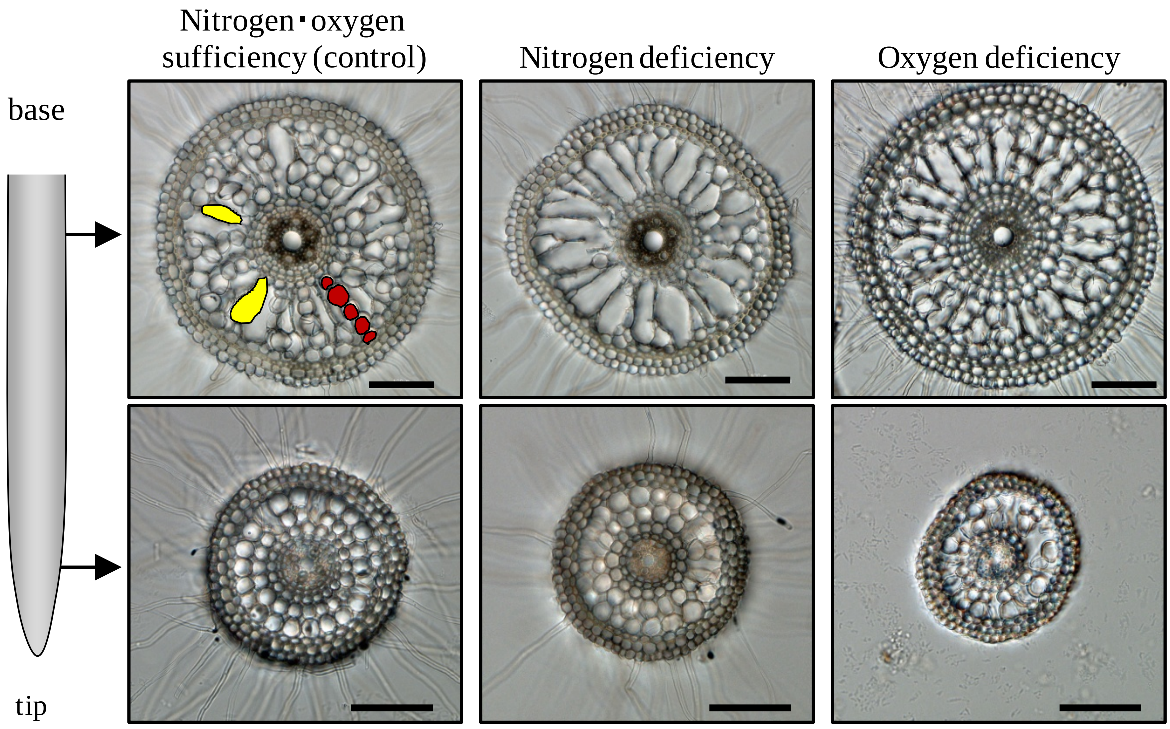


Fig. 3. Root aerenchyma in rice.
Increased aerenchyma was formed by nitrogen deficiency or oxygen deficiency. Examples of cortical cell, which are living cells, were illustrated in red. Examples of aerenchyma, which are dead cells, were illustrated in yellow. Scale bar in individual pictures indicates 100 µm.

Direct application of Burkina Faso phosphate rock is highly effective on lowland rice cultivation

Phosphorus (P) is a critical nutrient for crop production all over the world. In Sub-Saharan Africa (SSA), deficit of soil P is one of the most serious constraints on crop production. This shortfall has resulted from the high P fixation capacities of highly weathered acidic soils. The lack of soil P impacts on a range of agricultural lands, including paddy soils for rice cultivation. However, few farmers in SSA can use commercial water-soluble P fertilizers to cope with this P deficiency. Resource-poor farmers can especially find it difficult to apply these chemical fertilizers because of very limited accessibility and affordability.

Phosphate rock (PR) is considered as the resource that can be an cheaper alternative to water-soluble P fertilizers such as triple super phosphate (TSP). And it was verified that PR deposits are existing in SSA. So it is imperative to propose a proper PR application method with use of local PRs. But generally, PR produced in SSA was considered to have lower effect because of its low solubility, especially on upland crop production.

However, in the direct application on paddy field, it seemed that PR solubility will be affected by its unique soil conditions. So it cannot be denied that PR direct application will show the positive effect on lowland rice production. Elucidation of the effect of PR direct application will be fundamental information for PR utilization in SSA.

This study examined the impact of direct application of fine ground sedimentary PRs produced in Kodjari, Burkina Faso (BPR) on rice production under two agro-ecological conditions of the Guinea Savanna and Equatorial Forest zones of Ghana, with several application levels as shown in Table 1. Effects of BPR direct application on rice grain yield were observed to be comparable to TSP in both agro-ecological zones (Figure 1). In BPR direct application plots, rice grain yield has increased with increase of BPR application rate. These results suggest that BPR direct application is effective on lowland rice production in Ghana. And positive correlation between plant P uptake and rice grain yield has been indicated (Figure 2). It means that applied P has increased rice grain yield effectively. Moreover BPR direct application shows high residual effect on lowland rice production (unpublished data).

Results shown in this study will have great impact on lowland rice cultivation in SSA, because BPR reserve is estimated that 100 million ton (as P_2O_5) are existing in Burkina Faso, and 600 million tons are estimated in the deposit around Burkina Faso. Moreover, BPR are sold at the 1/4 price against TSP in the marcket in Burkina Faso.

(S. Nakamura, M. Fukuda, S. Tobita)

Table 1. Summary of fertilizer application rate in each treatment of PR direct application experiment

Trearment	P source	Savanna zone			Equatorial forest zone		
		P_2O_5	N	K_2O	P_2O_5	N	K_2O
Zero†	None	0	0	0	-	-	-
Control	None	0	60	30	0	90	60
PR-L	BPR*	67	60	30	67	90	60
PR-M	BPR*	135	60	30	135	90	60
PR-H	BPR*	270	60	30	270	90	60
TSP	TSP**	270	60	30	270	90	60
TSP-rec†	TSP**	-	-	-	60	90	60

†In Savanna zone, absolute zero application (Zero) plot was settled, and in Equatorial Forest zone, TSP in recommended rate (60 kg P_2O_5 ha^{-1}) was set-upped (TSP-rec), respectively.
*BPR: Burkina Faso phosphate rock (P_2O_5 26%, Ca 32%, Si 6%)
**TSP: Triple Super Phosphate

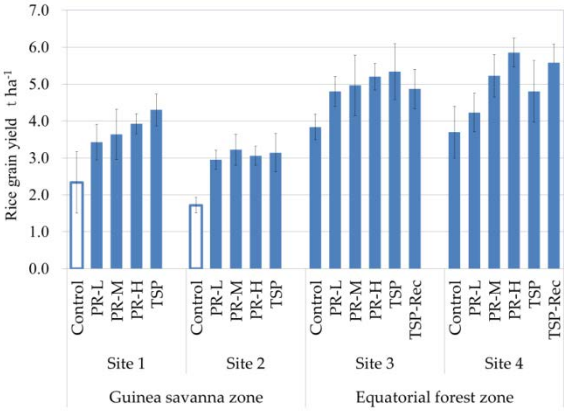


Figure 1. Effect of BPR direct application on lowland rice yield in Ghana
Error bars indicate standard error (n=3). Soil pH in each site were as follows, Site1; 5.60, Site 2; 5.83, Site 3; 4.53, Site 4; 5.70.

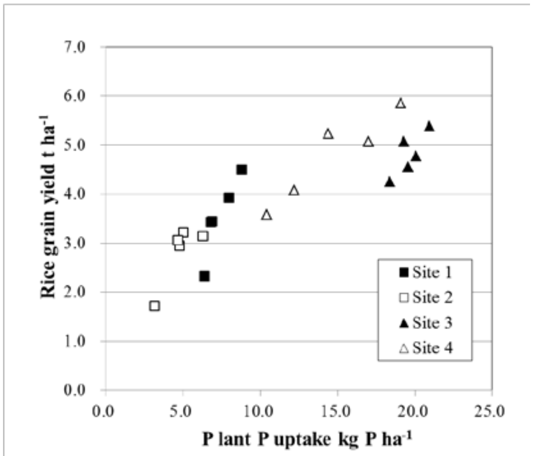


Figure 2. Relationship between plant P uptake and rice grain yield under BPR direct application.
Plant P uptake was calculated as multiplication of P concentration in flag leaf determined by dry combution method and above ground biomass.

Rice seed priming improves germination speed, germination uniformity, and seedling emergence

Improving seedling establishment and emergence rates is extremely important in the development of a direct seeding rice cultivation technology and for maintaining sustainable production for the rainfed lowlands of Africa. However, individual differences in initial growth rates due to variations in seedling emergence affect the dry matter production and light-receiving posture of rice. These defective conditions decrease rice yield. Strategies involving techniques for good seedling establishment in a wide range of soil moisture condition are thus required because controlling soil moisture at the time of seeding is difficult, particularly in rainfed lowlands. Seed priming, a treatment method that allows artificial germination to proceed by soaking the seeds in water over a certain period and drying them back to their initial weight, has demonstrated that germination and emergence rates are accelerated and that seedling vigor is enhanced. If this technology becomes applicable to rainfed lowlands, it can contribute greatly to the establishment and expansion of a stable rice production system.

Shortened germination time was more pronounced when seeds were soaked at 24h and 48h at 20 ℃ water temperature, and at 12h at 30 ℃. Soaking the seeds for 12h at 30 ℃ water temperature (similar to African conditions) reduced germination time by about 18 hours compared with non-priming (Table 1). With priming, shoot elongation rates after germination improved 1.2 times in sandy soils with moisture content from 3 to 20% (field capacity = 22.2%) inside growth chambers (Figure 1). Emergence time, meanwhile, was shortest at 8% soil moisture content, equivalent to dry conditions (Figure 2). Emergence uniformity was also improved by seed priming except at soil moisture contents equal to 6% and 20% (Figure 3). These results indicate that emergence speed is improved and stabilized by increasing the growth rate of the seedlings and thus accelerating germination.

Seed priming is practical because it can be accomplished by simply soaking and re-drying the rice seeds, eliminating the need for dedicated facilities. In conclusion, this study has shown that stabilization of emergence with fast germination rates can be obtained in the rainfed lowlands of Africa, and that primed seeds can be stored for several months as they have already been pre-soaked.

(K. Matsushima, J.-I. Sakagami)

Table 1. Germination time (h) after seed priming at varying temperatures and soaking times

Soaking time (h)	Soaking temperature (℃) 15	20	25	30
6			39.7 ± 4.6 ns	35.5 ± 1.7 **
12	46.0 ± 0.5 ns	37.7 ± 2.3 *	35.6 ± 0.8 **	34.4 ± 0.7 **
24	39.0 ± 0.7 **	34.4 ± 1.3 **	35.6 ± 1.0 **	35.6 ± 1.8 **
36	41.6 ± 2.0 *	36.7 ± 1.2 **	39.7 ± 0.7 **	
48	36.0 ± 0.5 **	34.3 ± 0.3 **	-	
60	37.9 ± 1.7 **	36.5 ± 1.2 **		
96	36.2 ± 1.4 **	44.1 ± 3.8 ns		
120	40.0 ± 2.0 *	-		
Control	51.9 ± 2.4			

The time to achieve 50% germination is germination time. "-" in the tables indicates less than 50% germination. Mean±SE. **, *: Significantly different at P<0.01, 0.05 (*T*-test). After soaking at several temperatures and at multiple time points, the seeds were dried back to attain initial seed weight before submerging in water at 25 ℃. Germination was investigated below 30 ℃ under dark conditions.

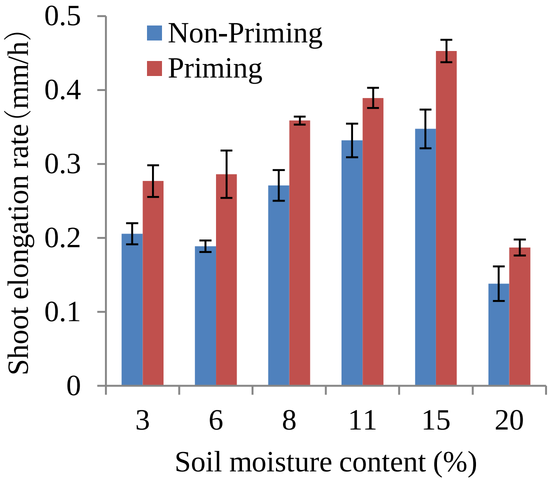


Figure 1. Effect of seed priming on shoot elongation rate
**, *: Significantly different at P<0.01, 0.05 (*T*-test)

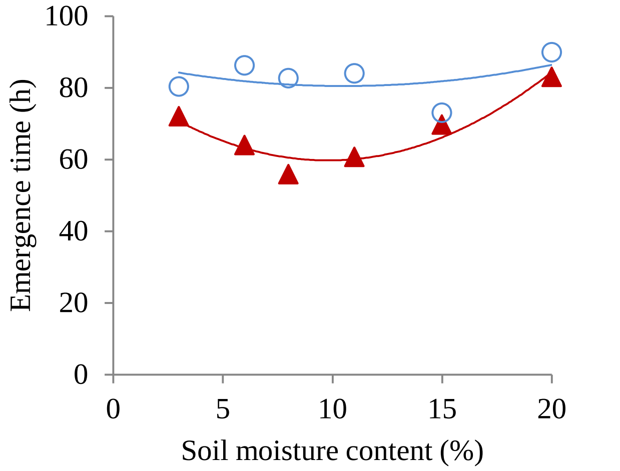


Figure 2. Shortened emergence time caused by seed priming
Non-priming: ○, $y = 0.066x^2 - 1.385x + 87.814$, r=0.395
Priming: ▲ $y = 0.237x^2 - 4.630x + 82.442$, r=0.957**

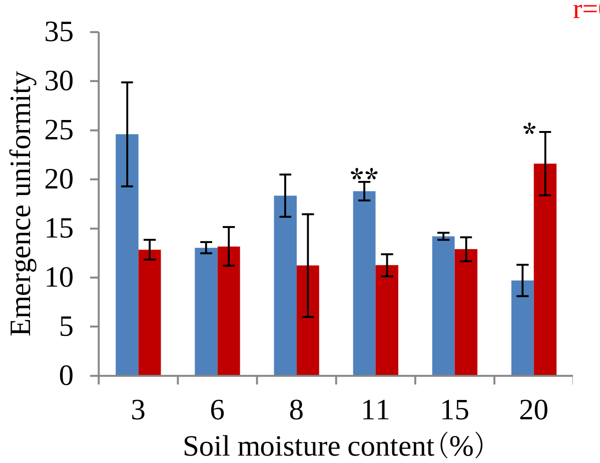


Figure 3. Effect of seed priming on germination uniformity (formula shown at right)
※Emergence is uniform when 'priming' value is lower

Germination Uniformity (GU) is the standard deviation of the mean of germination time. The formula used to calculate is:

$$GU = \sqrt{\frac{\sum\{(\bar{D}-D)^2 \times n\}}{\sum n - 1}}$$

where
$\bar{D}$: mean of germination time, D: time after sowing, and n: number of germinate at the time of D

Pathogenicity of Rice Blast (*Pyricularia oryzae* Cavara) Isolates from Cambodia and its geographical distribution

Evaluation of 122 blast (*Pyricularia oryzae* Cavara) isolates collected from the Tonle Sap and Mekong River regions of Cambodia revealed a wide variation.

Using a new designation system, the blast isolates were categorized into 92 races based on the reaction patterns of rice differential varieties (DVs) harboring 23 resistance genes and of 1 susceptible cultivar, Lijiangxintuanheigu (LTH).

Cluster analysis was used to classify the blast isolates into 3 groups — I, IIa, and IIb — using data from the reaction patterns of the DVs and LTH (Fig. 1). We used the classification method established under the new designation system, alongside cluster analysis and the geographical distribution of blast isolates, to investigate the diversity and differentiation of blast races in the Tonle Sap and Mekong River regions.

The distribution of the blast races differed between the 2 regions. Blast isolates of group IIa were distributed in both regions, and groups I and IIb occurred at higher frequencies in the Tonle Sap region rather than in the Mekong region. The blast isolates in groups I and IIb were also less diverse than those in group IIa. Accordingly, Group II blast isolates overall were distributed in both regions with high diversity, but some modified blast isolates were additionally distributed in the Tonle Sap region (Fig.2).

(Y. Fukuta [TARC, JIRCAS])

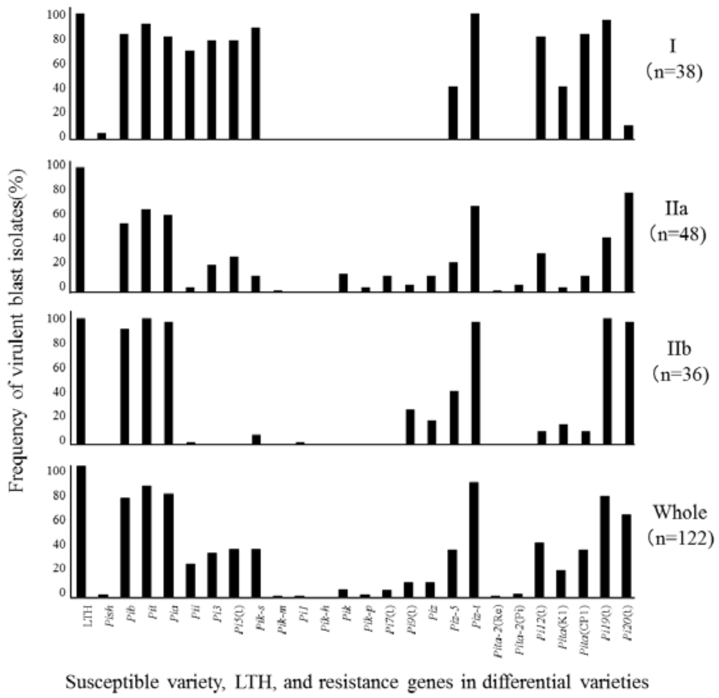


Fig. 1. Frequency of virulent blast isolates from Cambodia against differential varieties.

Blast isolates from Cambodia were classified into three groups--I, IIa, and IIb--==by cluster analysis based on the reaction pattern data of differential varieties harboring 23 resistance genes and of 1 susceptible cultivar, Lijiangxintuanheigu (LTH).

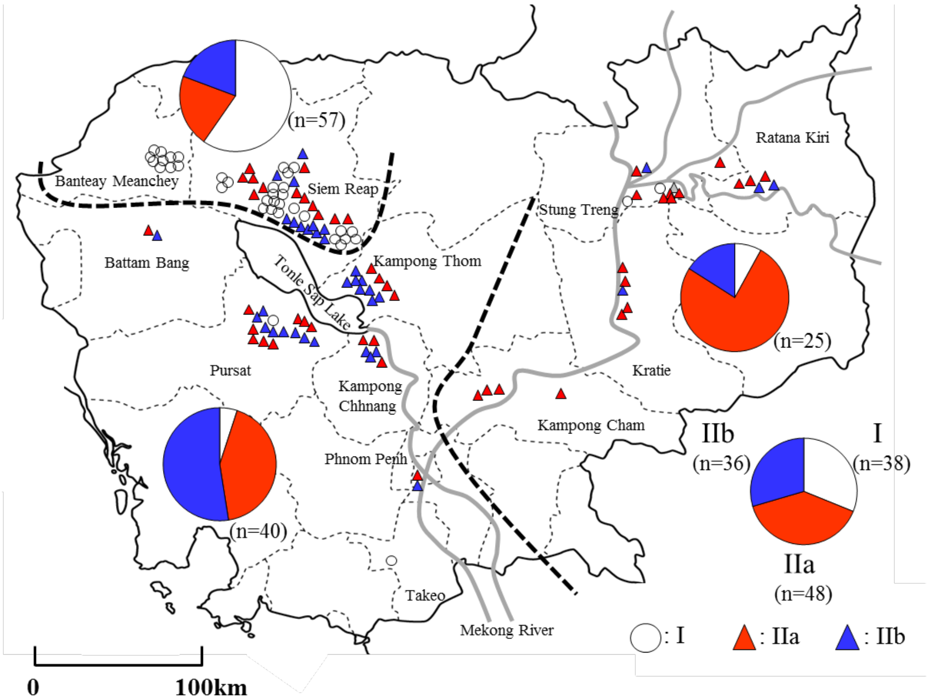


Fig. 2. Distribution of blast isolates, classified into three groups--I , IIa, and IIb--in Cambodia.

Blast isolates of group I was distributed at a high frequency in Siem Reap. In the Mekong River region, group IIa was found at a high frequency, whereas groups I and IIb were at low frequencies. In the Tonle Sap region, the frequency of groups IIa and IIb were similarly high. High diversity group IIa probably comprises the basic population of blast races in Cambodia and that groups I and IIb were modified from IIa. These unique distributions of blast races among the 3 regions might occur and be attributable to differences in genotypes of blast resistance genes in the cultivated rice varieties.

Novel blast resistance genes from a landrace rice variety in Myanmar

The use of broad-spectrum resistance genes is an effective way to achieve durable resistance against rice blast (*Pyricularia oryzae* Cavara) in rice (*Oryza sativa* L.).

We previously surveyed the diversity of blast resistance in 948 rice varieties and found Haoru (International Rice Research Institute genebank acc. no. IRGC33090), a Myanmar rice landrace with broad-spectrum resistance against blast.

We examined the genetic basis of Haoru's broad-spectrum resistance using the standard blast differential system consisting of the standard isolates and differential varieties.

For genetic analysis, we used the BC_1F_1 population and BC_1F_2 lines derived from crosses of Haoru with a susceptible variety, US-2. Co-segregation analysis of the reaction pattern in the BC_1F_1 population against the 20 standard isolates suggested that Haoru harbors three resistance genes.

Using bulk-segregant and linkage analysis, we mapped two of the three resistance genes on chromosomes12 and 6, and designated them as *Pi58*(t) and *Pi59*(t), respectively.

Pi58(t) and *Pi59*(t) were differentiated from other reported resistance genes using the standard differential system. The estimated resistance spectrum of *Pi58*(t) corresponded with that of Haoru, suggesting that *Pi58*(t) is primarily responsible for Haoru's broad-spectrum resistance.

In addition, *Pi59*(t) and the third gene were also proven to be new and useful genetic resources for studying and improving blast resistance in rice.

(Y. Fukuta [TARF, JIRCAS])

Table 1. Reaction patterns of Haoru and segregation lines harboring new resistance genes to standard differential blast isolates

Line	Resistance gene	Chr.	Reaction patterns																			
			Standard differential blast isolates from the Philippines																			
			PO6-6	CA89	43	CA41	M64-1-3-9-1	M39-1-3-8-1	M39-1-2-21-2	M36-1-3-10-1	JMB8401	IK81-25	IK81-3	BN111	V850256	V850196	V86010	JMB840610	BN209	M101-1-2-9-1	B90002	C923-49
Haoru	-	-	R	R	S	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R
US-2	-	-	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
BC_1F_2 line (US-2/Haoru//US-2)	***Pi58* (t)**	**12**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
IRBL12-M	*Pi12* (t)	12	S	S	S	S	S	S	S	S	S	S	S	S	S	M	M	R	R	R	R	R
IRBL19-A	*Pi19* (t)	12	S	S	S	M	S	S	S	M	M	S	S	S	S	S	S	S	S	S	S	S
IRBLta-CP1	*Pita*	12	S	S	S	R	M	M	S	S	M	R	R	S	R	R	S	S	S	S	M	S
IRBLta2-Pi	*Pita-2*	12	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S
IRBL20-IR24	*Pi20* (t)	12	S	S	S	S	S	S	R	R	S	M	M	R	S	S	R	S	S	S	R	R
BC_1F_2 line (US2/Haoru//US2)	***Pi59* (t)**	**6**	**S**	**S**	**S**	**S**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**S**	**S**	**R**	**R**
IRBLz-Fu	*Piz*	6	R	M	R	M	R	R	R	R	R	R	R	S	M	R	R	M	R	R	M	R
IRBLz5-CA-1	*Piz-5*	6	R	M	M	R	M	R	R	R	R	R	R	R	R	R	M	R	M	S	M	M
IRBLzt-T	*Piz-t*	6	S	S	S	S	R	R	S	S	S	S	S	S	S	S	R	R	S	S	R	R
IRBL9-W	*Pi9*	6	R	R	R	R	R	M	R	R	R	R	R	R	R	R	S	R	R	R	R	R

R: Resistant, M: Moderately resistant, S: Susceptible

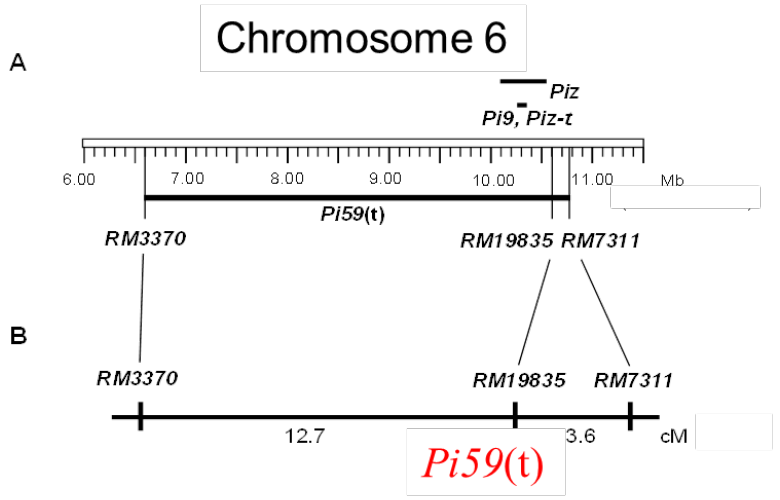


Fig. 1a. Position of resistance gene, *Pi59*(t), on chromosome 6.
A: Physical map. Position based on the Nipponabare's genome sequence.
B: Genetic map. Genetic distances between the gene and markers were estimated using the BC1F2 lines of US-2/Haoru//US-2 (n=55).

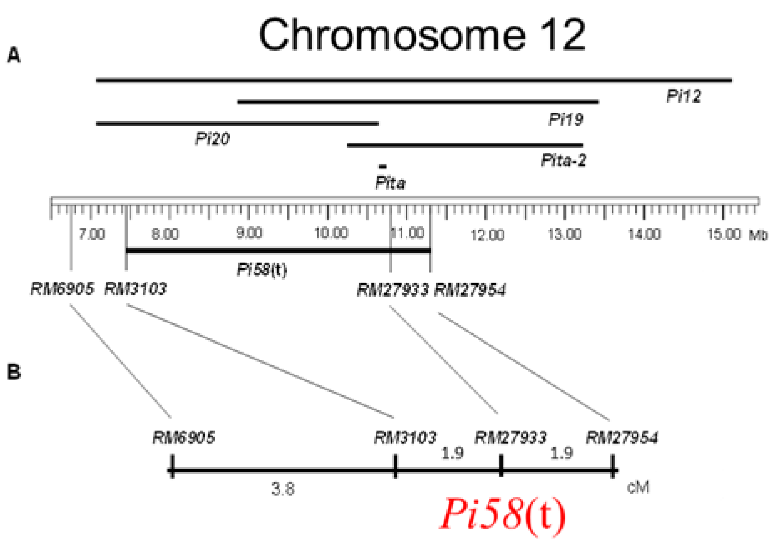


Fig. 1b. Position of resistance gene, *Pi58*(t), on chromosome 12.
A: Physical map. Position based on the Nipponabare's genome sequence.
B: Genetic map. Genetic distances between the gene and markers were estimated using the BC1F2 lines of US-2/Haoru//US-2 (n=106).

Identification of RNA-binding protein that regulates growth, senescence, and stress tolerance in rice

Because plants cannot move freely, they must grow and live in place even under environmental stress conditions such as drought. Thus, plants have developed a mechanism to survive under such conditions by controlling the function of various genes. Under drought conditions, expression of the gene coding hydrophilic proteins, transcription factors, and various proteins involved in stress tolerance is induced. The role of stress-induced CCCH-type zinc finger proteins was not well understood. In this study, we revealed that a stress-inducible CCCH-type zinc finger protein OsTZF1 binds to RNA, and is involved in the regulation of growth, senescence, and stress tolerance.

Expression of *OsTZF1* was induced by drought, high-salt stress, hydrogen peroxide, abscisic acid, methyl jasmonate, and salicylic acid (Fig. 1A, B). The expression was observed in callus, coleoptile, young leaf, and panicle tissues under normal growth condition. OsTZF1-green fluorescent protein localization was observed in the cytoplasm and cytoplasmic foci under stress condition (Fig. 1C). OsTZF1 binds to RNA, suggesting that OsTZF1 might be associated with RNA metabolism (Fig. 1D). Transgenic rice plants overexpressing OsTZF1 (OsTZF1-OX) exhibited delayed seed germination, growth retardation at the seedling stage, and delayed leaf senescence (Fig. 2A, B). OsTZF1-OX plants also showed improved tolerance to high-salt and drought stresses (Fig. 2C). Microarray analysis revealed that genes related to stress were regulated in the OsTZF1-OX plants.

OsTZF1 may serve as a useful biotechnological tool for the improvement of stress tolerance in various plants through the control of RNA metabolism of stress-responsive genes. Since the delay-like growth was observed when we overexpressed OsTZF1 constitutively, it might be necessary to utilize the suitable promoters such as stress-responsive promoters to improve environmental stress tolerance without causing growth retardation.

(A. Jan, K. Maruyama, D. Todaka, S. Kidokoro [The University of Tokyo], M. Abo [Meiji University], E. Yoshimura [The University of Tokyo], K. Shinozaki, [RIKEN], K. Yamaguchi-Shinozaki and K. Nakashima)

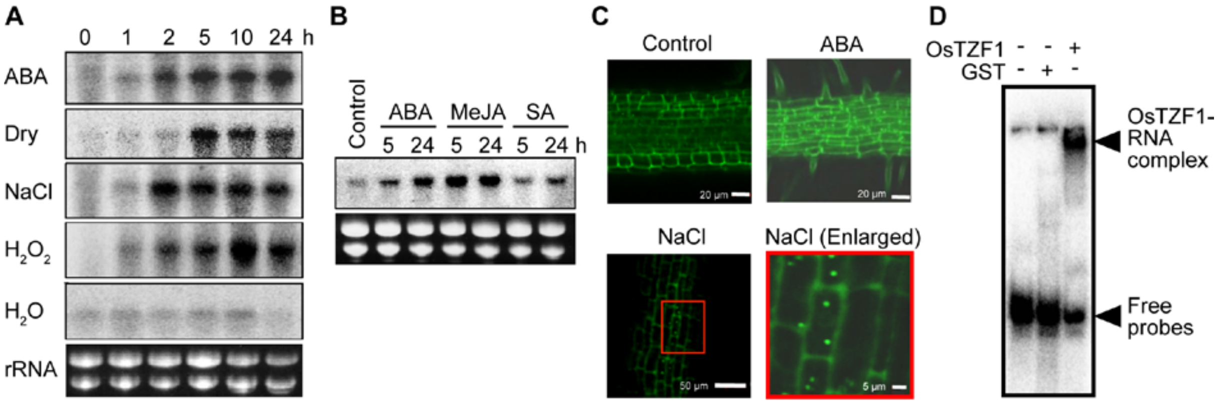


Fig. 1. Expression of *OsTZF1*, subcellular localization, and RNA-binding activity of OsTZF1.
(A) RNA gel-blot analysis of *OsTZF1* expression under different stress conditions. Expression was induced by abscisic acid (ABA), dry, high-salt (NaCl), and hydrogen peroxide (H_2O_2). Water (H_2O) was used as the control.
(B) Hormone-dependent expression analysis of OsTZF1. Expression was induced by methyl jasmonate (MeJA) and salicylic acid (SA) as well as ABA.
(C) Subcellular localization of OsTZF1-green fluorescent protein (GFP).
(D) Binding analysis of the RNA fragment of stress-related gene and OsTZF1-GST fusion protein. OsTZF1-GST can bind to RNA but GST protein does not bind to RNA.
These pictures were adopted from Jan et al. (2013) [1] (Copyright American Society of Plant Biologists; www.plantphysiol.org).

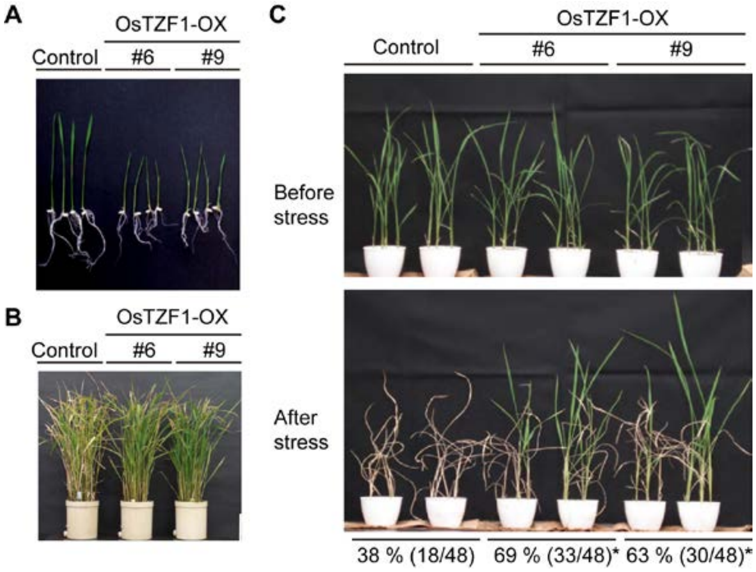


Fig. 2. Phenotypes of transgenic rice plants overexpressing OsTZF1 (OsTZF1-OX).
(A) Growth retardation observed in seedling stage.
(B) Delayed senescence observed in matured plants.
(C) Improved drought tolerance. Survival rates were shown below the pictures. Asterisks showed significantly higher survival rates than the control (P<0.01).
These pictures were adopted from Jan et al. (2013) [1] (Copyright American Society of Plant Biologists; www.plantphysiol.org).

1) A. Jan, K. Maruyama, D. Todaka, S. Kidokoro, M. Abo, E. Yoshimura, K. Shinozaki, K. Nakashima, K. Yamaguchi-Shinozaki (2013) OsTZF1, a CCCH-Tandem Zinc Finger Protein, Confers Delayed Senescence and Stress Tolerance in Rice by Regulating Stress-Related Genes. Plant Physiology 161:1202-16.

Development of stress-tolerant rice plants without growth defect using Oshox24 promoter

Rice production is largely inhibited by environmental stresses such as drought and high salinity. Developing transgenic rice plants with enhanced stress tolerance is therefore necessary. Stress-responsive promoters with low expression under normal growth conditions are needed to minimize the adverse effects of stress-tolerance genes on rice growth. We aim to find stress-inducible promoters with low expression levels under normal growth conditions, and develop the technology to produce rice plants showing enhanced stress tolerance without growth inhibition.

We conducted expression analyses of drought-responsive genes in rice plants using a microarray, and selected *Oshox24* for promoter analysis. Transient assays using the rice promoters indicated that AREB/ABF (abscisic acid (ABA)-responsive element-binding protein/ABA-binding factor) transcription factors enhanced expressions of the gene. We generated transgenic rice plants containing the *Oshox24* promoter and the β-glucuronidase (GUS) reporter gene. GUS assays revealed that the *LIP9* and *OsNAC6* promoters that have been used were induced by drought, high salinity, and ABA treatment, and both promoters showed strong activity under normal growth conditions in the root (Fig. 1A). The *Oshox24* promoter was strongly induced by stresses and ABA, but showed low activity under normal growth conditions (Fig. 1A). In seeds, GUS staining showed that *Oshox24* expression was low and expressions of the other genes were high (Fig. 1B). Transgenic rice plants overexpressing a stress-tolerant gene under the control of the *Oshox24* promoter showed increased tolerance to drought and high salinity, and no growth defects (Fig. 2).

These data suggest that the *Oshox24* promoter is useful for overexpressing stress-tolerance genes without adversely affecting growth. Verification of the transgenic plants expressing stress-tolerant genes and the *Oshox24* promoter in fields is required.

(K. Nakashima, A. Jan, D. Todaka, K. Maruyama, S. Goto, K. Shinozaki [RIKEN], K. Yamaguchi-Shinozaki)

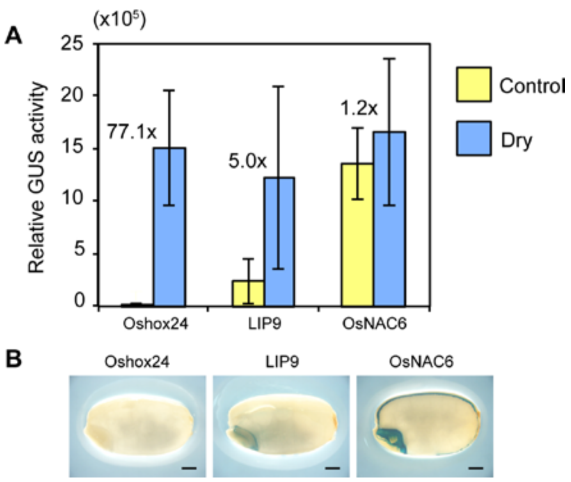


Fig. 1. Expression analysis of the newly isolated rice *Oshox24* promoter and rice *LIP9* and *OsNAC6* promoters that have been used.

We generated transgenic rice plants containing each promoter and the β-glucuronidase (GUS) reporter gene.

(A) GUS activity at 0h (Control) and 5h drought (dry) condition in the shoot. Error bars: SD.

(B) GUS staining in seeds of transgenic plants. Bars: 1mm.

These were adopted from Nakashima et al. (2014) (Copyright Springer; http://link.springer.com/journal/425).

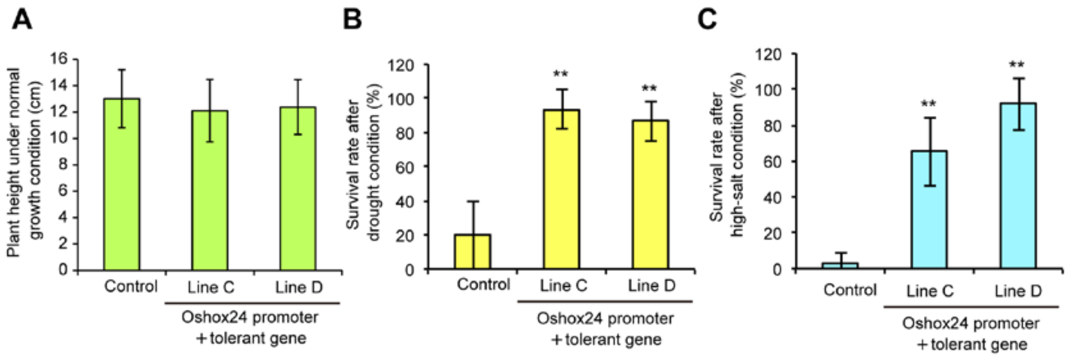


Fig. 2. Phenotype of transgenic plants expressing a stress-tolerant gene using the *Oshox24* promoter.

(A) Plant heights of 14-day-old plants under normal growth condition.

(B) Drought tolerance of 14-day-old plants.

(C) High-salinity tolerance of 14-day-old plants.

Error bars: SD. Asterisks indicate significant increase compared with the control (P<0.01).

These were adopted from Nakashima et al. (2014) (Copyright Springer; http://link.springer.com/journal/425).

1) K. Nakashima, A. Jan, D, Todaka, K. Maruyama, S. Goto, K. Shinozaki, K. Yamaguchi-Shinozaki. (2014) Comparative functional analysis of six drought-responsive promoters in transgenic rice. Planta. 239:47-60.

Evaluations of genetic diversities and population structures of Laotian two small-sized fishes using microsatellite DNA markers

In Indochinese countries, including Laos, greatly diversified indigenous fish fauna is present, and many of them are the important food materials, particularly in the remote rural areas. However, settlements and habitat expansion of invasive alien fishes in recent years and/or agricultural exploitations / urbanization in such areas are now the concerns for decline in species diversity / stock level of indigenous fishes and risk of inbreeding of each species. This situation leads the necessity to investigate the genetic diversities and soundness of each species in the region.

Among the indigenous fish species, *Esomus metallicus* (Cyprinidae) and *Parambassis siamensis* (Ambassidae) are the most common and widely distributed species over the Indochina Peninsula (Fig. 1). Both of them are small-sized (60 – 70 mm of maximum standard length) and often utilized in fermented and dried forms. In the present study, we developed the microsatellite DNA markers of these two species collected from 2 sites in Vientiane City and 4 sites in the west coast of Nam Gum River for evaluating the genetic diversities and soundness of local population of fish species.

24 and 40 microsatellite DNA markers were developed each for *E. metallicus* and *P. siamensis* (Fig. 1). Using these markers, levels of genetic diversities of two species (evaluated based on the number of alleles and heterozygosity) were confirmed to be high enough as of this moment in the investigated area (Fig. 3), and the efficiency of the markers was verified. In addition, based on the genetic cluster analysis of the genotype data for each specimen, the presence of three and two distinct genetic clusters for *E. metallicus* and *P. siamensis* was estimated in the investigated area.

By means of further applications of microsatellite DNA markers over the widespread areas, more detailed genetic characteristics of local populations of the species are confirmable, and the network situation between genetic clusters by migration and breeding can be estimated. Furthermore, this method is applicable not only for the two species above but for the various other species, including the endangered species and the ones for aquaculture for the proper understandings of their strains and management.

(S. Morioka, N. Koizumi [National Institute of Rural Engineering], B. Vongvichith [Living Aquatic Resources Research Center]

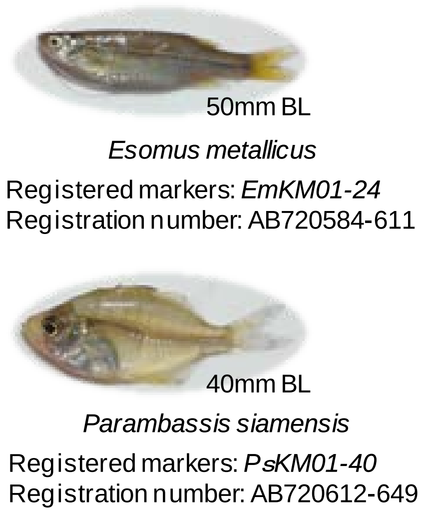


Fig. 1. Two fish species used in this study and registered DNA markers.

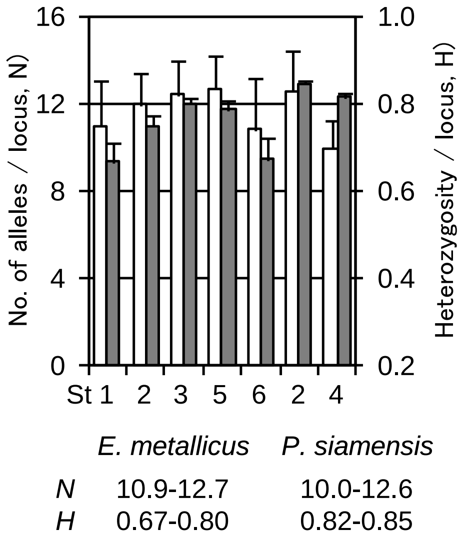


Fig. 2. Genetic diversities of two species based on no. of alleles (white bars) and heterozigosity (grey bars).

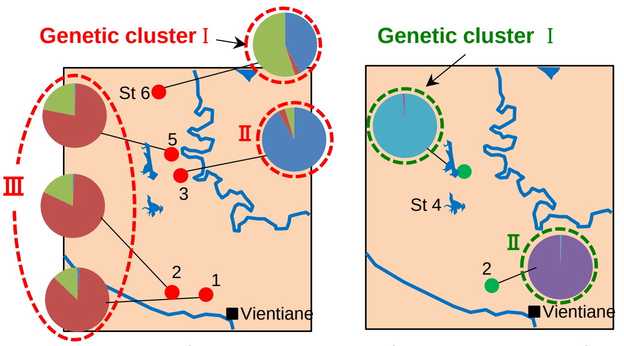


Fig. 3. Structures of genetic clusters of *E. metallicus* (left) and *P. siamenssis* (right).

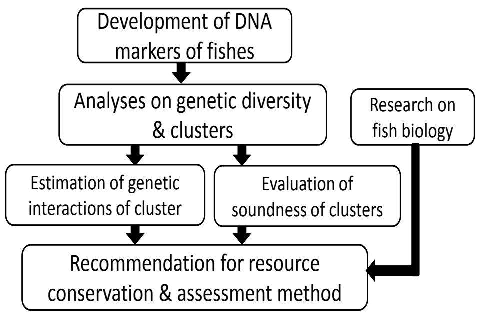


Fig. 4. Significance in usage of DNA markers for resource conservation & sustainable exploitation.

Local farmers employ bat guano to overcome soil acidity in a semi-mountainous area of Lao PDR

Rainfed paddy soils in a semi-mountainous area in Lao PDR show strong acidity, i.e., pH(KCl) <4, despite belonging to a calcareous zone. Under such conditions, aluminum injury often becomes a major constraint in cultivating non-rice crops in rice-based cropping systems (Fig. 1). To remedy this situation, local farmers collect bat guano from limestone caves and mix them with soil of planting hall at seed sowing depth of upland crops. This study was conducted to clarify the effect of such practice.

Application of bat guano to the soil decreases the soil pH(H_2O) but increases pH(KCl). Also, it markedly reduces exchangeable aluminum in acidic soils (Fig. 2), which corresponds to the results of soil survey~~s~~ in farmers' fields that received bat guano treatment. In spite of low aluminum-bound phosphorus (Al-P) content in bat guano itself, there is a marked increase of Al-P in soils treated with bat guano regardless of small variations in Ca-P or Fe-P (Fig. 3), which suggests the fixing of exchangeable aluminum with the phosphorus present in bat guano. Through such effects, plant growth in the early stages of tested upland crops are commonly enhanced even when the soils are enriched in exchangeable aluminum with aluminum chloride treatment (Fig. 4). These observations confirm that the bat guano improves soil fertility and mitigates aluminum toxicity.

Bat guano is collected from limestone caves in the neighboring village; however, depletion of this natural resource within a few decades is expected. Therefore, methods for efficient utilization based on the knowledge of its effectiveness must be established.

(K. Matsuo, N. Ae [Rakuno-gakuen Univ.], S. Vorachit [Agriculture Research Center, NAFRI, Lao PDR])

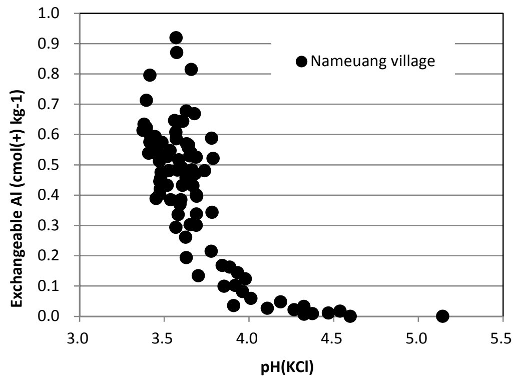


Fig. 1. Relationship between soil pH(KCl) and exchangeable Al in the paddy soil.

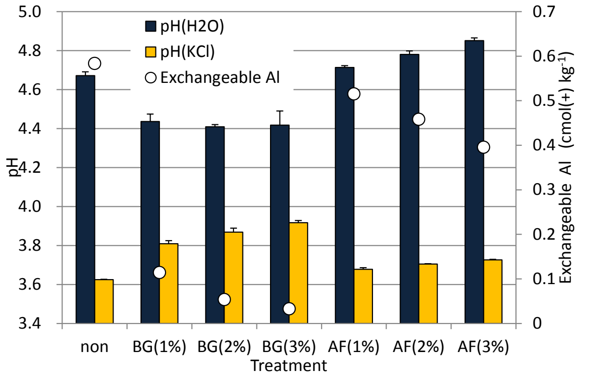


Fig. 2. Effect of bat guano (BG) and animal feces (AF) on soil pH--pH(H_2O) and pH(KCl)--and exchangeable Al in the soil.

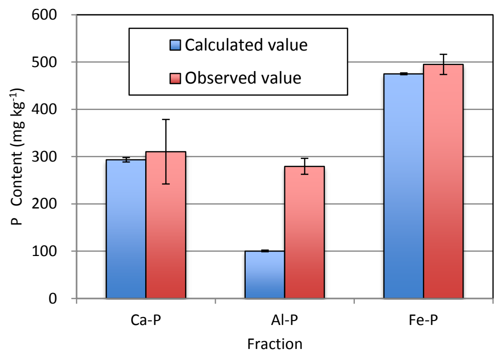


Fig. 3. Composition of inorganic phosphorus in soils treated with bat guano.

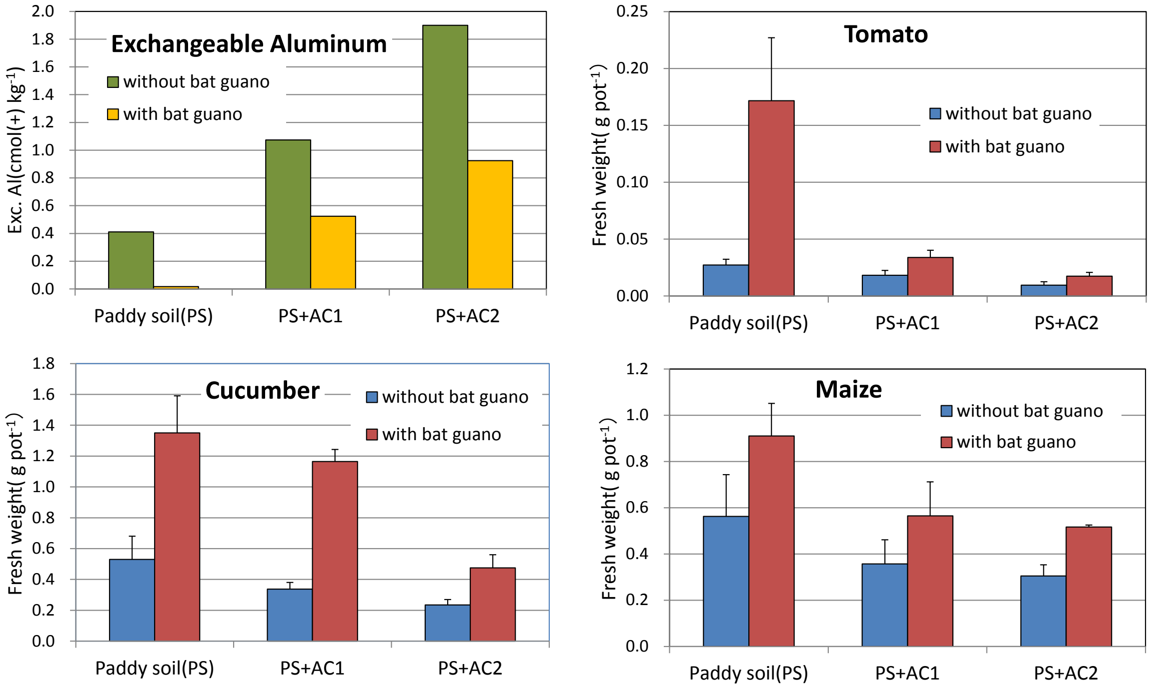


Fig. 4. Effect of bat guano application on exchangeable Al on the soil and on the growth of upland crops in early stage under different aluminum chloride treatment conditions. AC1: aluminum chloride@0.74 cmol kg^{-1}; AC2: aluminum chloride@1.48cmol kg^{-1} soil.

The effectiveness of introducing organic vegetable cultivation to the small-sized dairy farms in the semi-arid regions, China

The cost of feed grains had risen precipitously in recent years; it resulted in a critical situation for the viability of the small-sized dairy farms in the semi-arid regions, China. In order to deal with this problem, some of the dairy farmers have started planning to independently grow corn by using dairy manure as fertilizer. It must occur however to waste water resources by extensive farming and to increase wind erosion phenomena in soil condition improved by manure.

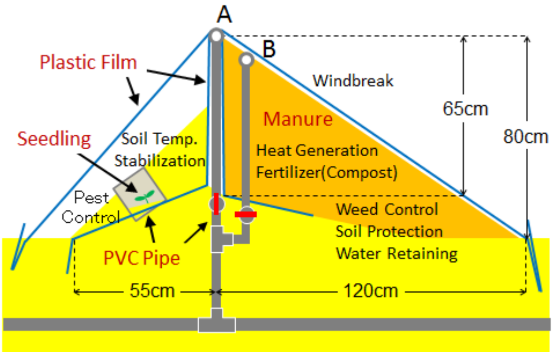
Cultivating organic vegetables as high value added products is an effective method against feed grain price rise and for sustainable dairy farming, but it is necessary for dairy farmers living in the regions far away from market to introduce the cost-saving and labor-saving cultivation system. Because only 11.9% of the consumers were willing to pay over twice as much for organic vegetables as for vegetables produced in conventional farming practice, when we conducted a survey in which 1,200 consumers from four large cities (Beijing, Shanghai, Guangzhou and Ha'erbin) were asked how much they would be willing to pay for organic vegetables.

We have introduced a low cost multifunctional system utilizing regional resources for cultivating organic vegetables in Suniteyouqi, Inner Mongolia (Fig.1); for example, heat released from fermenting manure promotes germination of vegetable seeds; barrier of piled manure protects seedlings, which have just sprouted, from strong wind; and PVC pipe prevents cutworms from damaging the plants. This cultivation system provides cost-saving without building a greenhouse for raising seedling and planting a forest for windbreak; and also results in labor-saving not to constitute an additional heavy burden on dairy farmers in only switching irrigation pump on-off as daily operation.

We have cultivated 170 plants of mini pumpkin (Beibei) in $500m^2$ experimental field, and have harvested 993.0kg (9pieces per plant, 649g per piece in average) by using composted dairy manure and liquid fertilizer in 2012 (Table 1). The mini pumpkins harvested in 2012 and 2013 have been provided to the consumers through the producing and selling organic vegetable company in Beijing, and 65.8% of the consumers are willing to pay more than 16.0 RMB/kg. The income derived from cultivating mini pumpkins can be evaluated by this willingness to pay and the production and marketing costs for organic mini pumpkins (Table 2); that is, $1021.4RMB/100m^2$ as the effectiveness of introducing organic vegetable cultivation.

The significant points to consider when introducing this system are to control the irrigation water delivered on dairy manure under the toxic level of sodium for plants and to adopt a monitoring system at low cost for providing information about the organic farming practice to customers.

(K. Nakamoto, N. Li [Institute of Agricultural Economics and Development, Chinese Academy of Agricultural Sciences], Tana [Institute of Grassland Research, Chinese Academy of Agricultural Sciences], L. Li [Institute of Agricultural Economics and Development, Chinese Academy of Agricultural Sciences])



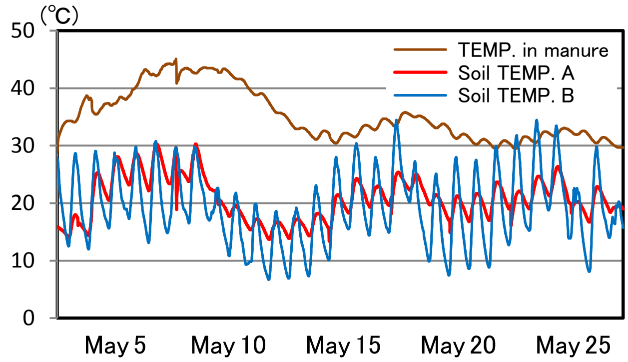


Fig. 1. Multifunctional vegetable cultivation system utilizing dairy manure

* To minimize the effects of strong winds in spring the system should be built against west-northwest, which is the direction of the heavy winds in Suniteyouqi, Inner Mongolia.

* Vegetable plants will be supplied fresh water when the valve of pipe A is opened, and will be applied liquid fertilizer through fermented manure when the valve of pipe B is opened.

* In graph, Soil TEMP. A is the temperature at 5cm below the surface of the nursery in the system and Soil TEMP. B is the temperature at 5cm below the surface of the ground outside the system.

Table 1. Components of dairy manure as a fertilizer resource (%)

	N	P_2O_5	K_2O
Composted dairy manure			
Upper parts of compost	1.65	0.98	0.40
Lower parts of compost	0.87	0.56	0.34
cf. Fresh dairy manure	1.50	1.02	1.12
Liquid fertilizer from manure			
Applied on July 24	0.01	0.05	0.11
Applied on July 29	0.01	0.03	0.10
Applied on July 31	0.01	0.03	0.12
Applied on August 4	0.01	0.01	0.05
Applied on August 8	0.01	0.02	0.07

* Only 5 date, which were measurable in amount, were presented in this table, though liquid fertilizer was applied 18 times during cultivating mini pumpkins.

Table 2. Production and selling cost of mini pumpkins

	RMB/100m^2	Ratio
Materials cost	734.1	34.0
Seed	166.2	7.7
Fertilizer	0.0	0.0
Pesticide	0.0	0.0
Energy	47.3	2.2
Supply	482.9	22.4
Machine rental	37.6	1.7
Land rent	61.9	2.9
Labor cost	209.9	9.7
Transportation cost	514.5	23.9
Selling cost (estimated)	635.8	29.5
Total	2156.2	100.0

* Expected earnings (RMB/100m^2) can be calculated as (Expected selling price x Quantity – Total cost); that is, 16.0RMB/kg x 198.6kg/100m^2 – 2156.2RMB/100m^2 = 1021.4RMB/100m^2 .

Potassium deficiency in fertilizer budget for crop production in China

There are several concerns about the negative environmental impact of excessive fertilizer use, especially nitrogen use, on Chinese agricultural production systems. Therefore, we calculated the nutrient intake by crops and the nutrient budget in China to obtain information on fertilizer demand and its effective use, food production, and the management of fertilizer resources from the view point of apparent nutrient (nitrogen (N), phosphorus (P) and potassium (K)) balance.

Data sets on chemical fertilizer types, crop production, livestock population, and human population came from “China Agriculture Yearbook,” with 2000, 2005, and 2010 chosen as the target years. In this study, we assumed the rural population as half of total population. Basic unit values of N, P, and K excretion by human and livestock were obtained from “China Organic Fertilizer Ingredients”; N, P, and K concentration in crops were from “China Fertilizer”; and chemical fertilizer application rates for cereals were from “Fertilizer Guideline for Crops.”

The budgets of chemical N and P fertilizers are larger than that of chemical K but smaller than that of organic fertilizers. Also, based on the output or crop yield, K output of cereal by-products (stems and leaves) occupies a significant portion of total K output.

The apparent N, P, and K balance shows a large surplus of N, an even amount of P, and a deficiency of K. For K, the crop K output was larger than the K budget; therefore, it is believed that the K deficiency will reduce soil K fertility.

The cereal K output includes by-products, leaving the farmland soil largely deficient in K fertilizer. However, if only the product part is removed and the by-products returned to the soil, then the amount of N, P, and K would be enough. Therefore, K deficiency for cereal will disappear when the by-product is sown back into the soil or used for mulching.

In this study, basic units of excretion, crop N-P-K contents, and statistical data were entered into a Microsoft Excel spreadsheet so the apparent nutrient balance in each province can be calculated. The results can be used for the effective management of fertilizer resources and for policy-making. On the other hand, we did not calculate the loss of N, P, and K during storage and composting and during volatilization of N when N was applied to alkali soils. We may incorporate these factors in future studies to obtain a more accurate balance measurement and a quantitative assessment of its environmental impact.

(S. Mishima [National Institute for Agro-Environmental Sciences], Chien H.)

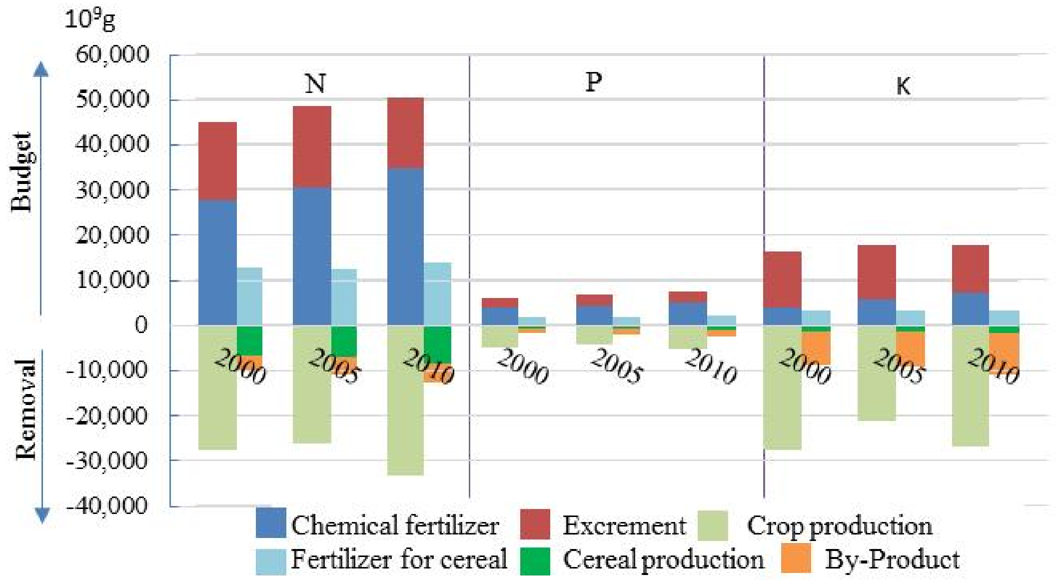


Figure1. Nutrient budget and removal

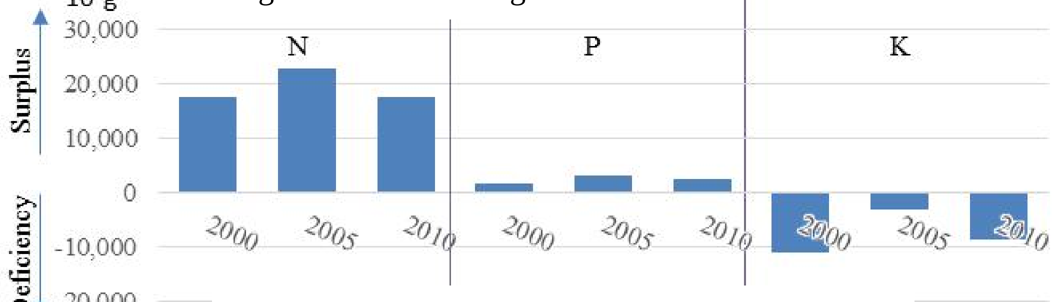


Figure 2. Apparent N, P, and K balance for all crops

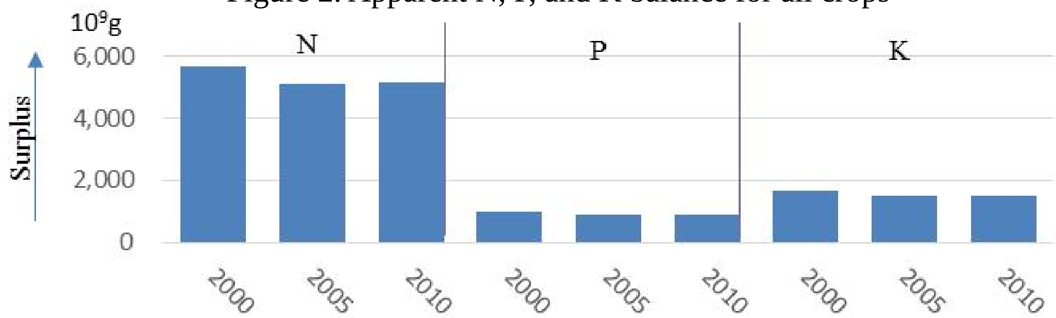


Figure 3. Nutrient balance for cereals without by-product removal

Renin and chymase inhibitory activities of edible lichen, *Sulcaria sulcata* and *Lobaria kurokawae*

In East and Southeast Asia, there are various food resources such as native agricultural and marine products and traditional fermented foods. These food resources are able to utilize as materials for functional foods and new processed foods. Unknown physiological active substances may be found from the foods produced by regionally specific materials, production methods and microorganisms. If we utilize these active substances as materials of functional foods, we can promote high value-addition to traditional agricultural products or conventional processed foods. On the other hand, the large-scale flow of population from farming villages to the cities and the increase in middle-income groups in the cities are occurring in these regions. In connection with it, diversification and quality improvement of agricultural products are demanded increasingly. In this study, renin and chymase inhibitory activities in foods were investigated. The activity promises inhibitory effect on elevated blood pressure.

We performed screening of the inhibitors from foods in East Asia. As results, it became clear that edible lichen, *Sulcaria sulcata* and *Lobaria kurokawae* (Fig. 1), contained the active substances. *S. sulcata* and *L. kurokawae* are used as foods in the partial area in Japan or China. Strong renin and chymase inhibitory activities exist in methanol, ethanol and water extracts of *S. sulcata* and *L. kurokawae* (Table 1). On the other hand, there extracts did not inhibit angiotensin converting enzyme (ACE). The inhibitory activities of the water extracts were retained after boiling or autoclave treatment (Table 1). Therefore, the inhibitory substances of the water extracts are very thermostable. Moreover, the water extracts decreased blood presser of spontaneous hypertensive rats (Table 2).

These results showed that newly functional food materials with renin and chymase inhibitory activities could be produced using edible lichen, *S. sulcata* and *L. kurokawae*. These food materials may be utilizable for the manufacture of nutrition foods with inhibitory effect on elevated blood pressure levels.

(S. Nirasawa, Y. Q. Cheng [China Agricultural University] and S. Takahashi [Akita Research Institute for Food and Brewing])

Sulcaria sulcata *Lobaria kurokawae*

Fig. 1. Pictures of *Sulcaria sulcata* and *Lobaria kurokawae*

Table 1. Renin, chymase and ACE inhibitory activities of extracts of *Sulcaria sulcata* and *Lobaria kurokawae*

Material (Origin)	Solvent	Inhibitory activities		
		Renin	Chymase	ACE
Sulcaria sulcata (Japan)	Methanol	+++	+++	±
Sulcaria sulcata (Japan)	Ethanol	++	+++	±
Sulcaria sulcata (Japan)	Water (boiling)	++	+++	±
Sulcaria sulcata (Japan)	Water (autoclave)	+++	+++	±
Sulcaria sulcata (China)	Methanol	++	+++	±
Sulcaria sulcata (China)	Water (autoclave)	+++	+++	±
Lobaria kurokawa (China)	Methanol	++	+	±
Lobaria kurokawa (China)	Water (autoclave)	+++	+++	-

Table 2. Effects of blood presser levels of spontaneous hypertensive rats (SHR) for extracts of *Sulcaria sulcata* and *Lobaria kurokawae*

Material (Origin)	Relative blood-pressure value	
	Administrated group	Control group
Sulcaria sulcata (Japan)	94% ± 2	101% ± 2
Sulcaria sulcata (China)	94% ± 2	102% ± 2
Lobaria kurokawa (China)	94% ± 1	102% ± 2

Data are means ± standard error. Blood pressure of SHR was measured 4 or 6 hours after administration.

Bio-Ethanol Production from Oil Palm Trunk Fiber.

Oil palm (*Elaeis guineensis*) used in palm oil production must be replanted at 20 to 25-years intervals in order to maintain oil productivity (Yamada et al. 2010). Consequently, the felled palm trunks represent one of the most important biomass resources in Malaysia and Indonesia (Shuit et al. 2009; Sumathi et al. 2008). To utilize the felled palm trunks specifically for bioethanol production, we characterized the sugars in the sap of the felled trunks and found large quantities of sap with a high glucose content (Kosugi et al. 2010). This study reports on ethanol production using separated PA and VB from oil palm trunk (Fig.1.). For efficient utilization of cellulosic materials as well as starchy materials, oil palm trunk was separated into PA and VB. Separated PA, alkali-pretreated starch-free PA (sfPA) and VB resulted in high ethanol conversion yields (Table 1). Separated PA and VB from oil palm trunk is a promising fermentation strategy for producing ethanol, without loss of starchy and cellulosic materials (Prawitwong et al. 2012).

(A. Kosugi)

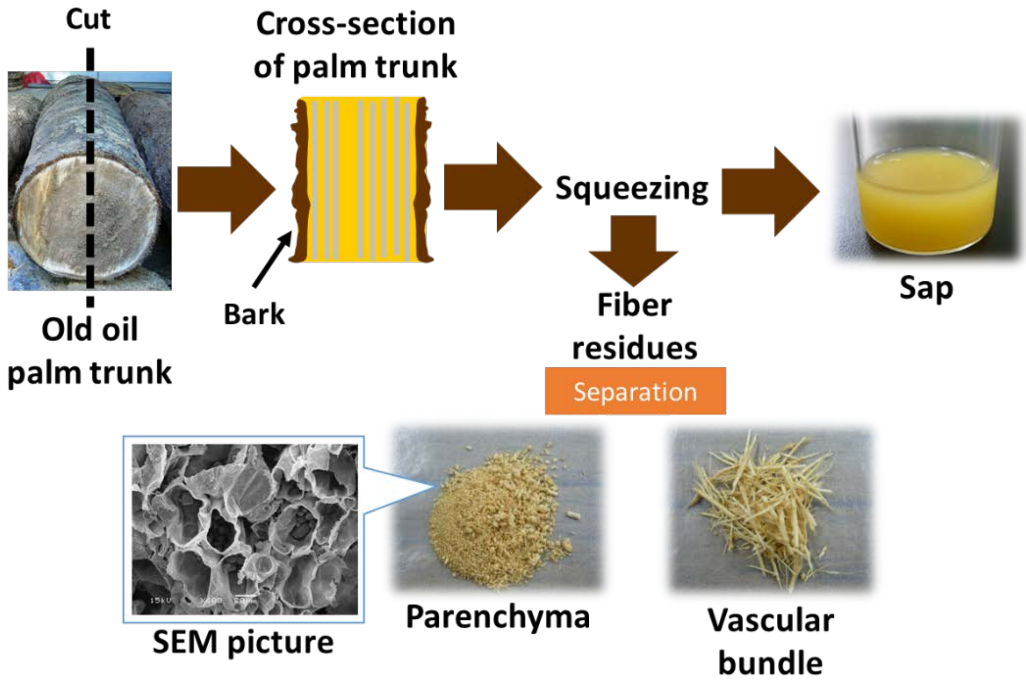


Fig. 1. Sap and fiber residues from oil palm trunk. Oil palm trunk was separated into parenchyma and vascular bundle components. The fractions were easily and distinctly separated. The ratio of PA and VB in the trunk was estimated as approximately 55:45 (dry weight %).

Table 2. Potential ethanol production from oil palm trunk fiber using a separation process (Prawitwong et al. 2012).

Source material	Input[a] (g)	SR[b] (g)	Pretreatments	Available sugars[d] (g)		Ethanol[e] (g)
				Starch	Glucan	
Trunk fiber	100.0	-	-	25.8	34.0	-
Separated PA	55.0	29.3	Autoclave	25.7	-	11.2
Pretreated sfPA	-	16.9	5% NaOH	-	13.1	5.1
Separated VB	45.0	44.9	-	0.08	-	0.03
Pretreated VB	-	27.1	5% NaOH	-	22.0	8.6
Total						25.0

[a] Calculated assuming 100 g of squeezed oil palm trunk is used in the separation process.
[b] SR (solids remaining) after each pretreatment, calculated from the data in Table 1.
[d] Available sugars calculated from the data in each component.
[e] Ethanol, calculated from theoretical maximum yield for each saccharification and fermentation.

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Net energy balance of ethanol production from sap squeezed from old oil palm trunks

In this study, a method was developed to estimate the NER or net energy ratio (i. e., ratio of energy output to energy input) of ethanol production from sap squeezed from oil palm trunks. A bench-scale shredder and compressed mill apparatus was constructed to squeeze the sap containing fermentable sugars from oil palm trunks. Energy input and energy output for squeezing sap were estimated through squeezing trials, and the NER was calculated.

Old oil palm trunks (30-45cm in diameter, 12m in length) were processed into trunk cores (20cm in diameter, 1.2m in length) by peeling the bark and the outer parts. The total amount of energy spent for processing was estimated to be the energy input (Table 1). Energy output, meanwhile, was estimated from the sum of calories derived from ethanol produced from sap and squeezed residues (50% moisture) (Fig. 1). From the study, it was determined that the ratio of energy input to energy output was 4.8 (Fig. 2A).

The energy ratio in ethanol from sugar cane was 8.3 because of self-sufficient energy from bagasse. The energy ratio in ethanol from oil palm sap was also high enough for practical use.

We can estimate the energy from large trunks (40cm in diameter, 10m) by using a multiplying factor of 33.74 on the trunk core.

Energy for transportation was estimated to be 47.2MJ, based on the assumption that the distance from plantation to ethanol plant was 8km (Fig. 2B). On the other hand, energy for cultivation of oil palm was not counted because the materials used were waste products of palm oil production. Likewise, energy for transportation of trunk cores was not counted because the materials used were wastes from the timber factory.

(*Yoshinori Murata, Takamitsu Arai, Akihiko Kosugi*)

Table1. Energy input involved in squeezing sap (core: 20cm in diameter, 1.2m in length)

	core
Peeling, kWh	0.24
Shredder, kWh	0.17
Mill, kWh	0.23

	core
Sum of energy, kWh	0.64
Sum of energy, MJ*1	2.3
Total sum of energy, MJ*2	5.8

*1Conversion kWh to MJ: Wh x 3,600s h^{-1}

*2 MJ = (Sum of energy : 2.3MJ) x (100/40)

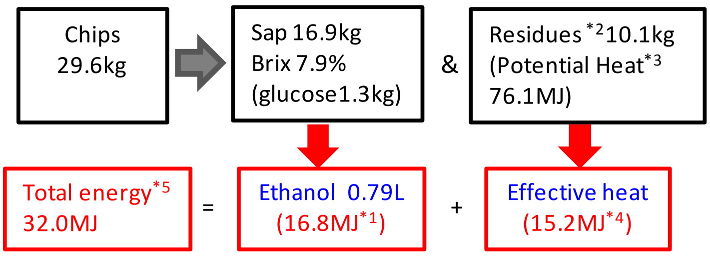

Fig. 1. Energy flow in ethanol production and in sap-squeezed residues.

Blue: production Red: energy from production *1 Ethanol: 21.2 MJ L-1, *2 Recovery of the residues is 80%, *3 Low heat value (LHV) of residues (50% moisture) is 7.5 MJ kg-1, *4 Effective heat is estimated as 20% of potential heat. *5 Total energy = (EtOH, MJ)+(the residues, MJ)

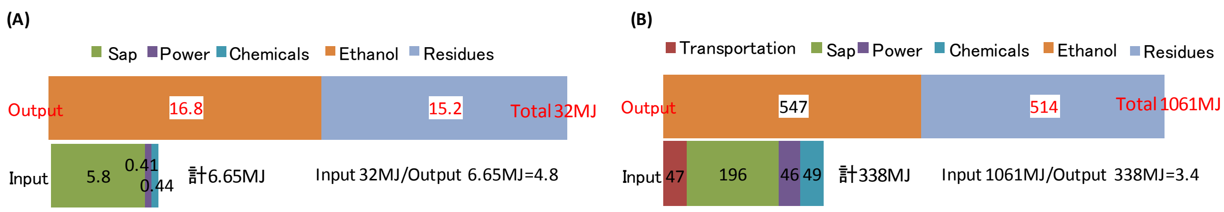

Fig. 2. The energy involved in sap squeezing. (A) Trunk core (20cm in diameter, 1.2m in length); (B) Total trunk (40cm in diameter, 10m in length). The energy for transportation from plantation to mill is needed in the case of total trunk.

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Production of binderless particleboard and compressed lumber using oil palm trunk as a feedstock

Palm oil, which is produced from fruit of oil palm (*Elaeis guineensis*), is one of the most important agricultural products in Southeast Asia, particularly in Malaysia and Indonesia. Oil palm is replanted every 25 to 30 years for to maintain the fruit production, and a large amount of biomass, namely oil palm trunk (OPT), is generated when they are felled. At present, this trunk is not efficiently used. On the other hand, for wood-based industries, the shortage of wood from natural forests is becoming a major concern. So research has been actively undertaken on unutilized biomass for to find substitute for some existing usages and decrease the demand for limited natural resources. Considering such situations, we have developed two types of products using OPT as a feedstock, namely binderless particleboard and compressed lumber.

Binderless particleboard is a self-binding particleboard without the addition of adhesives. It is produced simply by hot pressing formed particles at appropriate temperature and pressure. Since it does not used synthetic adhesives, cost necessary for raw materials and impact toward environment is smaller, compared with typical particleboard with synthetic adhesives. Also, by reducing feedstock to particles, it is assumed to overcome heterogeneity of OPT depending on the section of that used (interior or exterior; bottom or top). The binderless board produced by laboratory scale experiment is shown in Fig. 1. Under the conditions we have tried, the physical properties of board become maximum at the press temperature 200 °C.

Compressed lumber is the densified board produced by hot pressing block of feedstock. Normally in the case of wood, the strength of the product enhances by this densification. This was also valid for compressed lumber made from OPT. The compressed lumbers produced are shown in Fig. 2. A steam process in a closed chamber at a temperature of 130 °C before compression made physical properties and dimensional stability better than those without the process.

Unlike wood, the main components of which are cellulose, hemicellulose and lignin, OPT also contains sugars and starch at certain levels as sap or in parenchyma tissues. When binderless particleboards were produced with monomeric and dimeric sugars extracted OPT, properties of the board obtained was inferior to the board made from non-extracted sample (Fig. 3). On the other hand, addition of glucose and sucrose increased properties of the produced board. Since such effects were not observed with starch added sample, it is suggested that such sugars in sap contribute to self-binding mechanism of binderless particleboard and probably to features of compressed OPT lumber.

Regarding OPT binderless particleboard, the weak point is water durability. So at moment, the board should be used for indoor use, such as interior finishing or furniture.

(Tomoko Sugimoto, Rokiah Hashim [Universiti Sains Malaysia (USM)], Othman Sulaiman [USM], Masatoshi Sato [the University of Tokyo])

Fig. 1. Image of the binderless particleboard made from oil palm trunk
(The magnified image of the surface is shown inside the circle.)

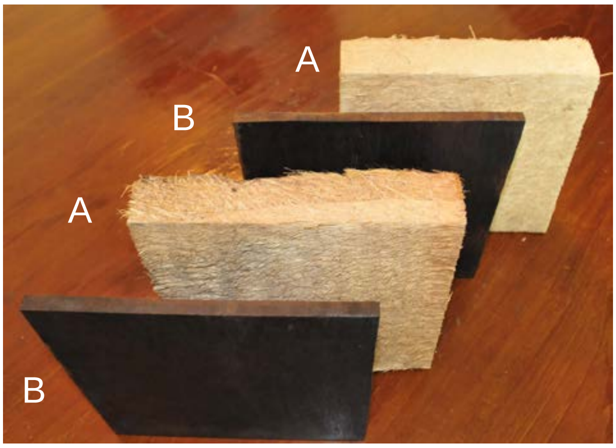


Fig. 2. Image of the compressed lumber made from oil palm trunk (B)
(A: The raw material before compression.)

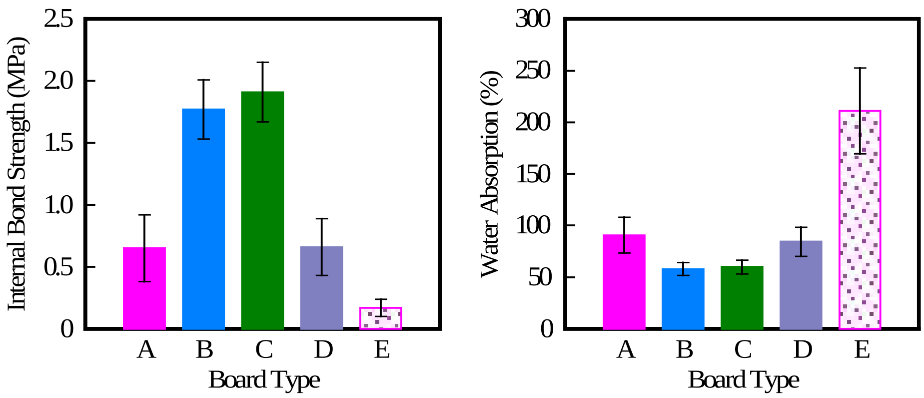


Fig. 3. Internal bond strength (left) and water absorption (right) of various types of board
(A: unextracted OPT board (control), B: unextracted + 20% glucose board, C: unextracted + 20% sucrose board, D: unextracted + 20% starch board, E: extracted OPT board)

Selective logging criteria to ensure healthy seed production for dipterocarp species that depend their pollination on strong flyer insects

Timber in Malaysia has been produced from tropical rainforests that were recognized as one of the highest biodiversity hotspots in the world. Trees of timber species larger than 50 cm in trunk diameter at breast height (dbh) have been harvested in ongoing selective logging operation in Malaysia. The harvesting lowered remaining adult tree density, which might inhibit pollen travel from a tree to others by pollinator insects. This is a critical issue to produce healthy seeds that shall be source of forest regeneration, because it has been reported that mother trees at lowered population density show frequent self-fertilization that produces less vigorous seeds. A group in genus *Shorea* known as 'red meranti' in forestry trading name is belonging to some sections, *Mutica*, *Brachypterae* etc., which is most abundant in lowland and hill dipterocarp forests. These species produce small-sized flower, which is for symbiosis with thrips, main pollen vector of these species, characterized by weak flying ability. The reproductive system ensured by pollination of thrips is vulnerable to reduction of tree density, such as the selective logging activity.

On the other hand, recent studies on pollen dispersal pattern of dipterocarps revealed that pollen dispersal pattern was mainly regulated by pollinator and conspecific tree density. Another group in genus *Shorea* known as 'balau' in forestry trading name is belonging to section *Shorea*, which produces the second highest valuable timber in Peninsular Malaysia. The species belonging to this section produces relatively larger flower than 'red meranti' species, which putatively attract small beetles belonging to Chrysomelidae. The small beetles are generally characterized as energetic strong flyer, which should represent different pollen dispersal pattern from 'red meranti' with thrips pollination. We estimated pollen dispersal pattern by using paternity analysis of seeds and a reproductive model (JIRCAS Research Highlight No. 15, 2011), and evaluated selective logging criteria by simulating pollen dispersal before and after selective logging for *S. maxwelliana*, one of the 'balau' species.

When the pollen dispersal pattern was compared between mass flowering season (in 2005) and sporadic flowering season (in 2002), the strong flying ability of small beetles achieved active pollen dispersal even though flowering tree density was reduced in the sporadic flowering season (Fig. 1). However, seeds with higher genetic diversity were produced in the mass flowering season, because more flowering trees in the mass flowering seasons contributed as pollen donors, which was shown by number of effective pollen donors (N_{ep}) in Table 1. We calculated rate of outcrossing pollen over the mother trees in after the logging to in before logging. The criterion of selective logging was increased with 1 cm from 40 cm to 100 cm at dbh in the simulation. Only more than about trees with 80 cm dbh can be harvested to conserve 50% of outcrossing pollen over the mother trees in *S. curtisii* (one of the 'red meranti' species). However, our result showed that trees with more than about 60 cm dbh can be harvested for 'balau' because of the small beetles' stronger flying ability (Fig. 2).

Our results showed that the selective logging criterion should be determined for every timber trading group of dipterocarps because reproductive characteristics within the timber trading group were similar. Our reproductive model and the simulation assumed similarity of pollinator and conspecific tree density. When the results would be applied to different type of forests, the simulation results couldn't be directly applicable to the practices.

(Naoki Tani, Yoshihiko Tsumura [FFPRI], Soon Leong Lee [FRIM] Norwati Muhammad [FRIM])

Table 1. The comparison of number of effective pollen donors between 'balau' and 'red meranti'

Species	Section	Timber trading name	Flowering year	Flowering magnitude	Effective numver of pollen donors (N_{ep})	Effective number of pollen donors per a tree (N_{ep}/N)
Shorea maxwelliana	*Shorea*	Balau	2002	Sporadic	28.808	0.200
	Shorea	Balau	2005	Mass	44.154	0.307
Shorea leprosula	*Mutica*	Red meranti	2002	Sporadic	8.817	0.134
Shorea parvifolia	*Mutica*	Red meranti	2002	Sporadic	11.042	0.133
Shorea curtisii	*Mutica*	Red meranti	1998	Mass	27.210	0.189
	Mutica	Red meranti	2002	Sporadic	24.349	0.169
	Mutica	Red meranti	2005	Mass	34.411	0.239

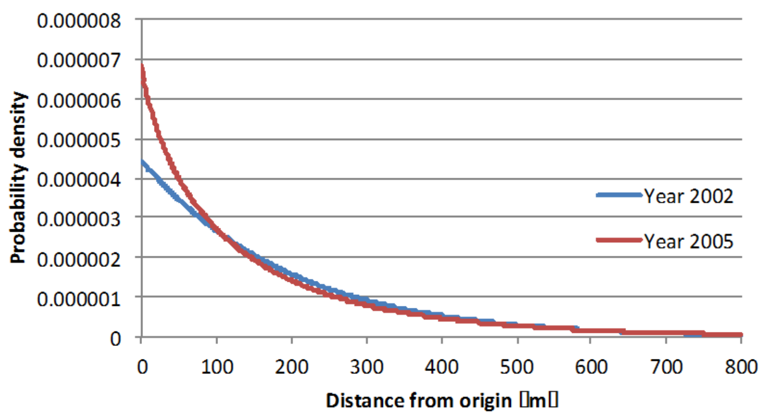
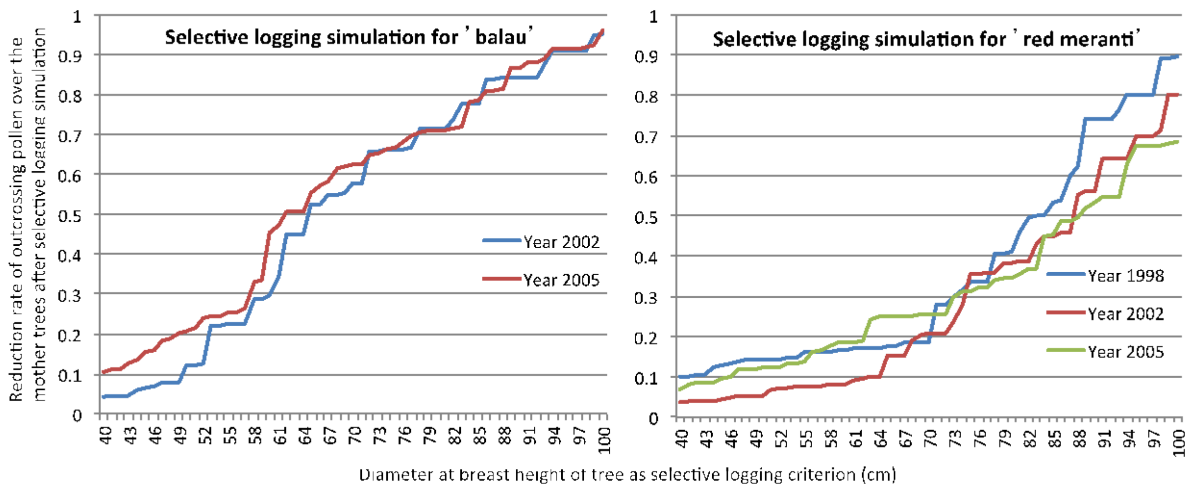


Fig. 1. Pollen dispersal pattern of *Shorea maxwelliana* recognized as 'balau' in two flowering events with different flowering maganitude (left).

Fig. 2. Reduction rate of outcrossing pollen over the mother trees after selective logging simulation with every 1cm increment of cutting limit from 40 to 100cm (below)



Digestibility of animal-based and plant-based diets in the tropical sea cucumber, *Holothuria scabra*

Since population of many sea cucumber species has been dwindling due to intensive fishery and trading in Southeast Asian countries, there is a need to artificially produce sea cucumbers by hatchery production and aquaculture. *Holothuria scabra*, commonly known as sandfish, has been most actively produced amongst tropical sea cucumbers, but the production efficacy remains low partly owing to the lack of information on its diet. *H. scabra* is a benthic deposit feeder, and it ingests a mixture of organic matters with sea sediment for feeding. It is hence difficult to determine what important nutrient sources in the sediment actually are. This study aimed to elucidate relative importance of animal- and plan-based diets for juvenile *H. scabra* by analyzing digestibility of different feed ingredients.

Apparent digestibility coefficient (ADC) of shrimp meal, mussel meal (animal-based), diatom and powdered seaweed (plant-based) was determined by tank rearing experiments. Compared to the plant-based diets, animal-based diets contained a higher fraction of organic matter and crude protein; on the other hand, the plant-based diets contained a higher fraction of crude carbohydrate. ADC of organic matter was significantly higher in the animal-based diets (77.1 – 86.2%) than in plant-based diets (32.3 – 55.1%). ADC of protein ($ADC_{protein}$) was significantly higher in shrimp meal, mussel meal and diatom (75.2 – 88.7%) than in seaweed (34.4%), indicating that animal diets are more reliable source of digestible proteins. ADC of carbohydrate (ADC_{carbo}) was generally lower than $ADC_{protein}$, and diatom and mussel meal (58.3 – 58.5%) had significantly higher ADC_{carbo} than shrimp meal and seaweed (28.0 – 31.6%) (Fig. 1). The high ADC_{carbo} in mussel meal may be attributable to its high content of glycogen that is readily digestible by animals unlike hard-digestible cellulose contained in a large amount in seaweed. Total assimilated nutrient (TAN) was estimated as the product of daily diet ingestion rate (IR) and ADC. The mean body weight of the experimental *H. scabra* was 10 g. TAN was largely affected by ADC since IR did not vary significantly amongst the diets. Shrimp meal had the highest TAN for organic matter (390 mg/day) and protein (347 mg/day) amongst the four diets, and diatom had the highest TAN for carbohydrate (247 mg/day) (Fig. 2).

H. scabra hatcheries commonly use diatoms and seaweeds as feed. This study indicates that there is a possibility that effective artificial feeds can be formulated by adding animal proteins to diatoms. High digestibility of animal-based diets also indicates that *H. scabra* is a good candidate for use in polyculture with finfish where feeds with high fish meal content are used.

(S. Watanabe, Z.G.A. Orozco [University of Tokyo], J.G. Sumbing [SEAFDEC/AQD], Ma. J.H. Lebata-Ramos [SEAFDEC/AQD])

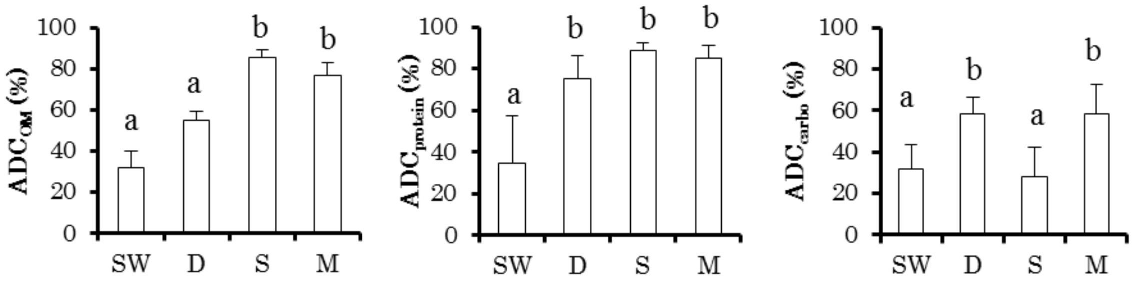


Fig. 1. Apparent digestibility coefficient of organic matter (ADC_{OM}), crude protein ($ADC_{protein}$) and crude carbohydrate (ADC_{carbo}) of seaweed (SW), diatom (D), shrimp meal (S) and mussel meal (M) in juvenile *Holothuria scabra*. ADC was obtained from the difference in nutrient contents between diet and feces. Different letters (i.e. a and b) indicate statistically significant difference ($p<0.05$).

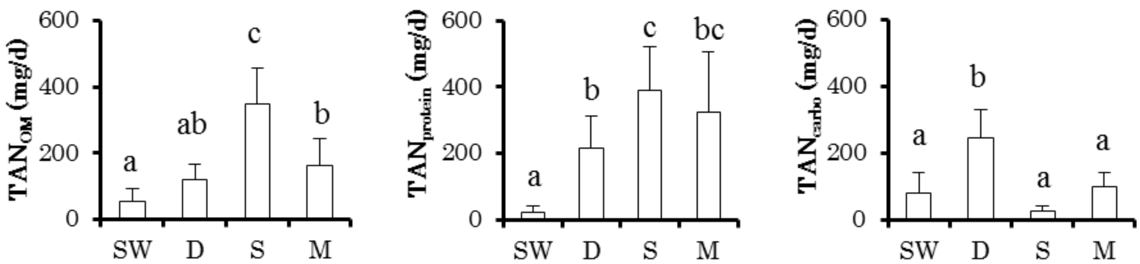


Fig. 2. Daily total assimilated nutrients (TAN) in juvenile *H. scabra* as obtained as the product of ADC and daily feed ingestion rate; assimilated organic matter (TAN_{OM}), crude protein ($TAN_{protein}$) and crude carbohydrate (TAN_{carbo}). The mean body weight of *H. scabra* was 10 g. Different letters (i.e. a, b and c) indicate statistically significant difference ($p<0.05$)

Limited Si-nutrient status of rice plants in relation to plant-available Si of soils, nitrogen fertilizer application, and rice-growing environment across Sub-Saharan Africa

Rice is a specific silica-accumulator among higher plants. The Si in rice enhances resistance to biotic and abiotic stresses. The booming demand for rice in Sub-Saharan Africa (SSA) requires rapid increases in rice production, and hence more Si supply will be needed from soils, irrigation water, and external inputs. However, there have been no Si management practices or any extensive surveys conducted to identify the nature and magnitude of the problems with plant Si nutrient status and Si availability in the soils for rice production in SSA. Therefore, an extensive survey is conducted for evaluating variability of Si concentration in rice straw in relation to soil properties, fertilizer management practices, and rice-growing environments across a wide range of local farmers' fields in SSA.

The Si concentration in straw ranges 1.7-8.4% among the harvest samples at 99 local farmers' fields in Benin, Ghana, Guinea, Kenya, Madagascar, Mozambique, and Nigeria, and the values in 68% of the fields are below the critical deficiency level of 5%* (Fig. 1; Fig. 2). The amounts of water-soluble Si in soils after 1-week anaerobic incubation at 40 °C sufficiently explain the variability in Si concentration in straw among the samples, and thus can be used for assessing the plant-available Si for wide-range of the SSA soils (Fig. 2). The plant-available Si and Si concentration in straw are both particularly low in the acidic soils of Highland and Humid Agro-ecological zones, mainly consisting of weathered Oxisols and Ultisols (Table 1). The mean Si values become lower with more unfavorable water conditions in the order of upland (3.4%) < rainfed lowland (4.3%) < irrigated lowland (5.3%) among different rice-growing environments (Fig. 3). There is a negative correlation between N application rate and Si concentration in straw (Fig. 3).

*The critical deficiency level of Si is referred to IRRI Handbook Series (Dobermann and Fairhurst, 2000)

The extensive dataset from local farmers' fields indicate that poor Si nutrient status of rice plants is widespread across SSA, which is largely attributable to limited plant-available Si in soils. The amounts of water-soluble Si after 1-week anaerobic incubation can be an appropriate index of plant-available Si in soils for rice fields in SSA. The application of Si management practices such as straw incorporation can be accelerated by matching vulnerable fields to biotic stresses and the Si-deficient factors in the current study. Further studies should demonstrate quantitative effect of improving Si nutrient status on rice productivity such as through the reduction of blast infection.

(Y. Tsujimoto, S. Muranaka, H. Asai, K. Saito [Africa Rice Center])

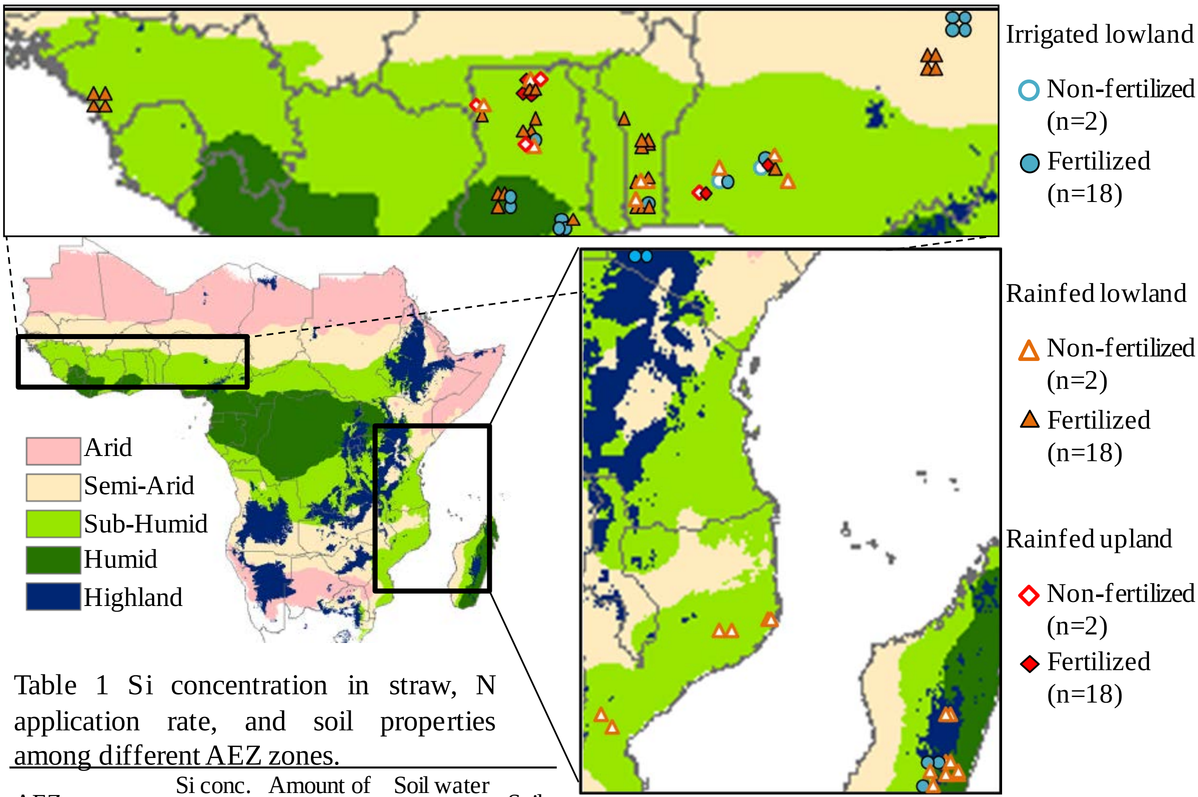


Fig. 1 Location of the 99 farmers fields in for plant and soil samples across SSA

'Fertilized' consist of 3 fields with organic materials and 61 fields with chemical fertilizer. A 5-class agro-ecological zone (AEZ) map is derived from Harvest Choice (.http://harvestchoice.org)

Table 1 Si concentration in straw, N application rate, and soil properties among different AEZ zones.

AEZ zone means	n	Si conc. in straw	Amount of N applied	Soil water soluble Si	Soil pH
		%	kg ha^{-1}	mg kg^{-1}	
Semi-Arid	8	4.9^{ab}	26^{a}	51.0^{a}	7.0^{a}
Sub-Humid	68	4.6^{a}	37^{a}	43.8^{a}	6.0^{b}
Humid	11	3.9^{b}	42^{a}	28.7^{ab}	5.7^{bc}
Highland	12	3.4^{b}	36^{a}	23.9^{b}	5.4^{c}
SSA total	99	4.4	36	40.3	6.0

Vlaues of the same alphabets do not differ at 5% (Tukey HSD)

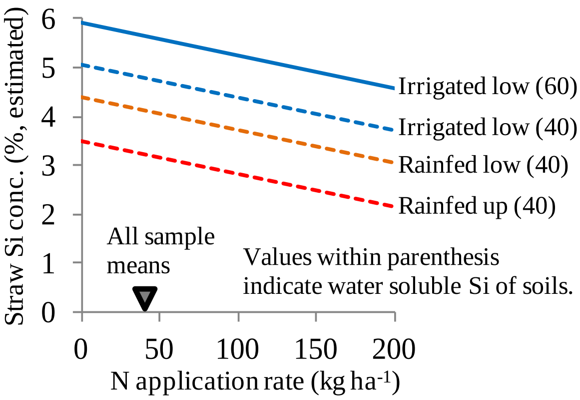


Fig. 3 Estimated Si concentration in straw against N application rate in different rice-growing environments and water soluble Si of soils.

The sensitive analysis was performed using multiple regression model with observed variables to explain the variability of Si concentration in straw (R^2=0.59).

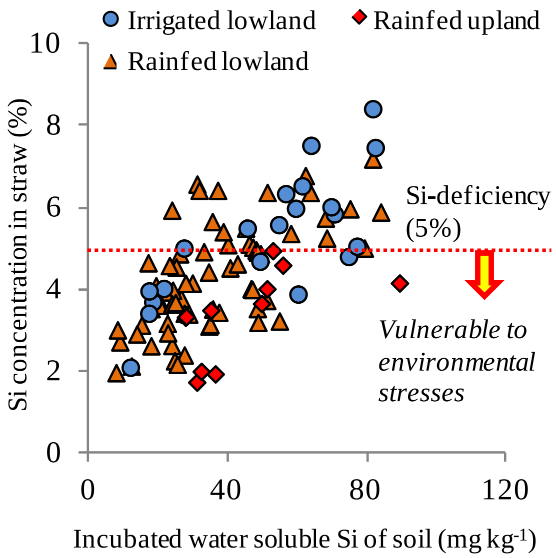


Fig. 2 Relationship between the amounts of water-soluble Si in soil and Si conc. in straw.