Horse manure can replace half the amount of conventional chemical fertilizer application without any yield loss in West Java Highland

West Java Highland, which is underlain by soils derived from volcanic ash, is one of several production centers of temperate vegetables in Indonesia. Previous studies have found that vegetable farmers in this area tend to overdose on chemical fertilizer, thus organic matter application is considered an important option to improve soil fertility. However, the expansion of horticultural production in West Java Highland has caused a shortage of cow dung and chicken manure, which are popular forms of organic matter in the area. Although West Java Province has 14,000 horses (2014 data), horse manure use is very limited. This study, therefore, aimed to develop a technology to reduce chemical fertilizer application in West Java Highland without yield loss by applying horse manure, an underutilized organic resource in the area.

Horse manure is turned into compost by fermentation over a four-week period, with the manure turned over every week (Fig. 1). It contains around 0.7% nitrogen and 0.8% phosphate, which is equal to or higher than those of cow manure. Horse manure has lower C/N ratio than cow manure, which means that it is relatively easily decomposed in soil (Table 1). In field experiments at the Indonesian Vegetable Research Institute (IVEGRI), West Bandung, West Java, reducing chemical fertilizer application by half the standard dosage showed no significant yield difference if horse manure is also applied at an amount equal to 10t/ha (Fig. 2).

A field seminar was held at a village near IVEGRI, with seminar participants listening to explanations about chemical fertilizer reduction by manure application and receiving information leaflets written in the local language (Bahasa Indonesia) (Fig. 3). A questionnaire survey conducted after the seminar revealed that majority of farmers concerned (19 out of 30) were interested in technologies that reduce chemical fertilizer application.

In conclusion, it can be said that there is enough possibility for local vegetable farmers to apply this developed technology using local underutilized resources, such as horse manure, to reduce chemical fertilizer application. Horse dung production, which can be estimated from the number of horses in West Bandung, can meet the amount of horse manure necessary to enable the technology to be applied to all cabbage production areas in the region.

(T. Sugino, N. Sumarini [Indonesian Vegetable Research Institute], Suwandi [IVEGRI], R. Rosliani [IVEGRI], D. Setyorini [Indonesian Soil Research Institute], W. Hartatik [ISRI], R. Saraswati [ISRI])

A-01

Fig. 1. Horse manure production

Horse dung is piled (left) and fermented in a bamboo cage (right). (Photos courtesy of the Indonesian Soil Research Institute)

Table 1. Nutrient content of horse and cow manure (% wet weight)

	Water (%)	C[*2] (%)	N (%)	C/N	P_2O_5 (%)	K_2O (%)
Horse manure	77.6	5.93	0.67	8.9	0.77	0.74
Cow manure[*1]	66.3	8.93	0.65	13.8	0.18	0.81

*1 Katamine et al. (2000)

*2 Organic carbon in horse manure, total carbon in cow manure

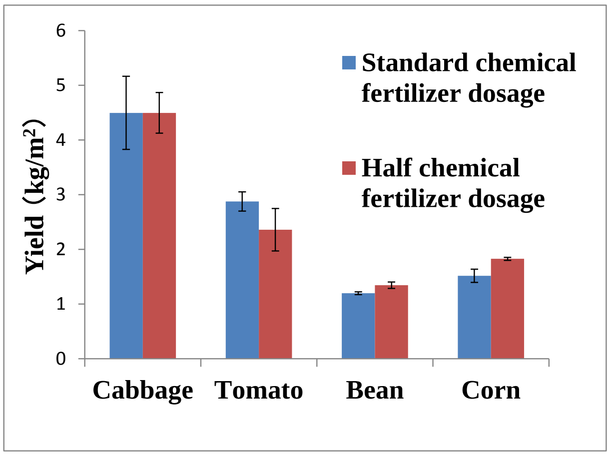

Fig. 2. Vegetable yields after chemical fertilizer application at standard and at half dosage rates in field experiments

Conditions: Horse manure 10t/ha, conventional tillage, average of three replications, error bars mean standard error

Cabbage and tomato: wet season in 2011

Bean: wet season in 2012

Corn: dry season in 2012

Fig. 3. Field seminar participants reading the information leaflet (inset) written in the local language

URL:http://www.jircas.affrc.go.jp/english/manual/horse_manure/horse_manure.pdf

Guideline for tackling the decrease in forest resources using the CDM programmes of activities

The decrease in forest resources is a pressing problem in Paraguay. One proposed solution to tackle this problem nationwide was to promote reforestation through the implementation of a Clean Development Mechanism (CDM) project. JIRCAS established Paraguay’s first CDM project, which was successfully conducted in Paraguarí Department in the eastern part of the country. In order to apply the results of the CDM project to other areas in Paraguay, JIRCAS conducted similar CDM projects in five departments under a Programme of Activities[1], which is a CDM support structure, and tried to form an extendable reforestation programme CDM with governmental bodies in Paraguay. However, specific methodologies and techniques for forming a reforestation programme CDM have yet to be established because there had been no previous registration or track record with the United Nations CDM Executive Board.

Consequently, JIRCAS developed a guideline describing the following: 1) the preparation of a Project Design Document, which is essential for forming the programme CDM; 2) the establishment of a coordinating and managing entity in Paraguay; and 3) the calculation of the volume of greenhouse gases (GHGs) absorbed by planted trees (Table 1).

The guideline also included JIRCAS’s recommendations for small-scale farmers on conducting reforestation activities appropriately and efficiently, such as the reduction of seedling weight by changing the planting method from “old-style pot type” to “tube type”, and the establishment of a reforestation promotion method consisting of a series of progressive reforestation activities (Fig. 1). Furthermore, to improve the livelihood of small-scale farmers, JIRCAS evaluated the profitability of eucalyptus planting and developed indicators for nurturing forests to ensure the profitability (Fig. 2). The feasibility of the reforestation promotion method has already been verified by officials of the National Forestry Institute of Paraguay.

The guideline is expected to be utilized for the preparation of Nationally Appropriate Mitigation Actions (NAMAs), which promote GHG emissions reduction in developing countries and whose national target for GHG emissions reduction in Paraguay was submitted for compulsory action to the 21st Conference of the Parties (COP21) of the United Nations Framework Convention on Climate Change (UNFCCC). To ensure the accuracy and reliability of GHG emission reduction calculations, they need to follow a series of actions such as measurement, report, and verification when they create a NAMA. Accordingly, this guideline, which is based on CDM methodologies and in line with the strict rules of the United Nations, has satisfied the appropriate standard.

It is possible for surrounding countries to utilize the guideline because Spanish, Portuguese, and English versions have also been prepared. This guideline can be viewed and downloaded from the JIRCAS website through http://www.jircas.affrc.go.jp/english/manual/manual_index.html

(M. Watanabe, S. Shiraki)

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[1] The CDM Programmes of Activities (the programmes CDM) has a property such that component project activities adapting the same technology and methodology during the project period can be added to the programmes CDM.

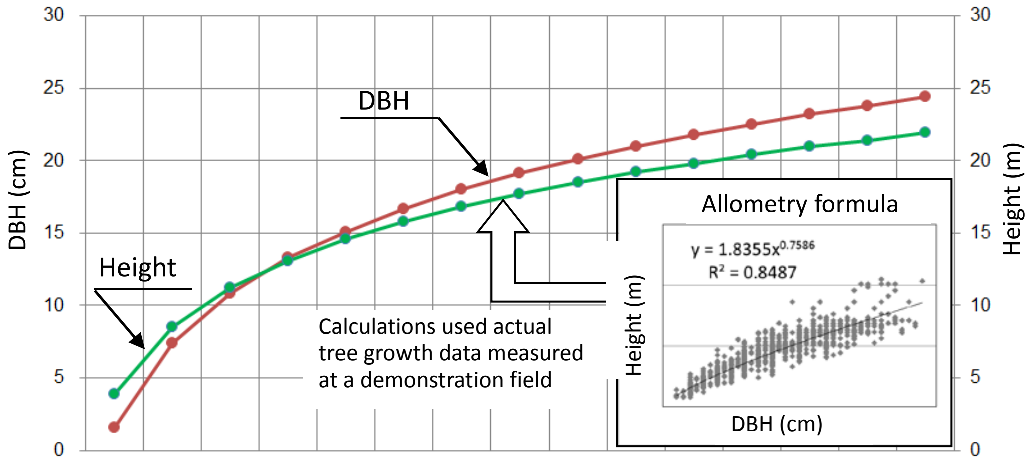
Table 1. The main structure and contents of the guideline

Introduction		Background and Objectives, Summary of activities, Structure and Content of Guideline and Use of Guideline
Chapter 1	Overview of the Reforestation Programme CDM	Overview of the Programme CDM, CDM flow, Role of the coordinating and managing entity, Contents and Organization of Project Design Document
Chapter 2	Efforts to form a Reforestation Programme CDM in Paraguay	Methodology and typology of the adopted CDM, Selection of Project boundaries, Formation of the Programme of Activities and Component of Project Activity (Establishment of Coordinating and Managing Entity, How to plan and implement the reforestation promotion method)
Chapter 3	Methods for Calculating Anthropogenic GHG removals	Applied CDM methodologies (AR-AMS0007ver03.1), Calculation of Anthropogenic GHG Removals
Chapter 4	Profitability Analysis	Profitability Analysis Procedures and Care Study

Box a) Pot type planting: Diameter 10cm, Height 13cm, and Volume 1000cm^3
Tube type planting: Diameter at the top (2.5cm) and bottom (1.0cm), Height 12cm, and Volume 60cm^3

* The change of planting type resulted to weight reduction, saving of production space, and improved distribution of seedlings.

Fig. 1. The Reforestation Promotion Method



Calculations used actual tree growth data measured at a demonstration field

Tree age (years)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
IRR (%)					10.8	11.7	13.6	14.3	14.4	14.2	13.8	13.4	13.0	17.6	16.7
NPV (US$)					14	40	112	161	196	215	219	213	205	748	704

Note) IRR that can guarantee profitability is 10% based on the interest rate for deposits in Paraguay Central Bank (10% in 2004)

IRR: Internal Rate of Return
NPV: Net Present Value
DBH: Diameter at Breast Height

Conditions for calculating IRR, etc:
- Reforestation interval 4m*2.5m (1,000 trees / hectare)
- Annual average growth volume 31m^3/ha
- No thinning, large-scale felling of trees after 5 years
- Price Reduction Rate = 10% of NPV

Fig. 2. Indicators for nurturing forests and desired IRR to ensure the profitability of eucalyptus planting

Forage intake of grazing sheep in Mongolian winter grassland is 20% higher than the UNDP index

Mongolia has been experiencing increases in livestock number in recent years, which in turn have led to an increased risk of climate-related disasters. To reduce the risks, it is necessary for the grassland to be managed appropriately using scientific data such as carrying capacity calculated from aboveground biomass and forage intake by animals. However, the method for calculating the index is not always clearly documented; therefore, the scope of application of the index is difficult to define. The forage intake of sheep was estimated to obtain basic information for calculating the carrying capacity of the Mongolian forest steppe and steppe grassland during autumn and the next spring.

Research data showed that aboveground biomass was lowest in February, and that the reduction rates of aboveground biomass in forest steppe and steppe from September 2011 to February 2012 were 70.9 and 82.6%, respectively. The dominant plant species were *Stipa* spp., *Cleistogenes squarossa*, and *Artemisia frigid* in the forest steppe, and *Stipa* spp., *Carex pediformis*, and *Agropyron cristtumn*. in the steppe. The body weight of sheep in forest steppe and steppe decreased by 13.6% and 8.7%, respectively, between November 2011 and February 2012 (Fig. 1). Daily fecal outputs in forest steppe and steppe were 0.475-0.665 and 0.467-0.550 kg DM, respectively. During the same period, fecal output in the forest steppe decreased significantly (Fig. 2, Table 1). Forage digestibilities determined from acid detergent lignin in pasture plants and in feces at forest steppe and steppe were 51.8-63.8 and 63.2-70.9%, respectively, and there were significant differences among seasons (Table 1). Forage intake calculated from the fecal output and forage digestibility at forest steppe and steppe were 1.10-1.89 kg DM/day (2.91-4.09% BW/day) and 1.30-1.73 kg DM/day (2.54-3.02% BW/day) (Table 1). A comparison of the forage intake during summer, autumn, winter, and spring showed experimental data values of 1.6, 1.8, 1.1, and 1.1 kg DM/day, respectively, which was 20% higher than the prevalent index data (which was determined by UNDP, 2007) for winter (February). Therefore, the carrying capacity in winter, as calculated from experimental data, is lower than the prevalent index.

In the future, it would be possible to extensively compare the carrying capacities by estimating the forage intakes on different regions and grazing environments. Forage digestibility is affected by seasonal differences and vegetation composition, and must be considered in the calculations. Also, it is recommended that feed intake expressed in terms of body weight (%BW/day) be calculated for the comparison.

(A. Uehara, S. Yamasaki, K. Shindo [NARO Institute of Livestock and Grassland Sciences], A. Erdenechimeg [Mongolian University of Life Sciences], G. Onontuul [MULS])

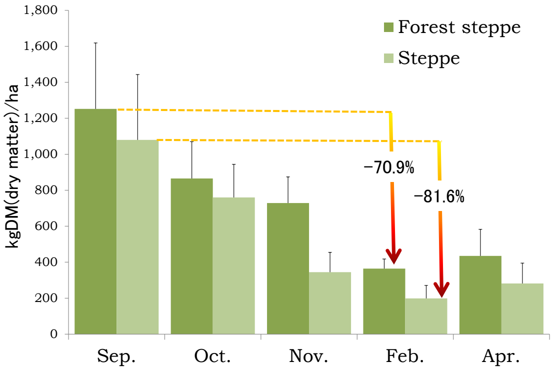


Fig. 1. Seasonal changes of aboveground biomass

Fig. 2. Fecal bag attached to the sheep

Table 1. Calculation of intake and other parameters measured in forest steppe and steppe

		2011			2012		ANOVA	
		Sep.	Oct.	Nov.	Feb.	Apr.	S.D.	P
Forest steppe								
Body weight	(kg)	41.8^{ab}	45.5^{a}	45.7^{a}	39.5^{b}	37.9^{b}	4.42	***
Fecal output	(kg DM/day)	0.557^{abc}	0.600^{ab}	0.665^{a}	0.500^{bc}	0.475^{c}	0.094	***
Digestibility	(% DM)	58.1^{bc}	51.8^{d}	63.8^{a}	62.2^{ab}	56.5^{c}	4.07	***
Intake	(kg DM/day)	1.33^{b}	1.25^{b}	1.89^{a}	1.34^{b}	1.10^{b}	0.33	***
	(% BW/day)	3.16^{b}	2.80^{b}	4.09^{a}	3.40^{ab}	2.91^{b}	0.62	***
Steppe								
Body weight	(kg)	53.8	57.5	56.4	51.5	51.0	5.66	*
Fecal output	(kg DM/day)	0.467	0.550	0.469	0.489	0.471	0.080	ns
Digestibility	(% DM)	70.5^{a}	68.2^{ab}	70.9^{a}	65.1^{bc}	63.2^{c}	2.89	***
Intake	(kg DM/day)	1.60^{ab}	1.73^{a}	1.64^{ab}	1.41^{ab}	1.30^{b}	0.30	**
	(% BW/day)	2.97	3.02	2.91	2.76	2.54	0.49	ns

Annual average temperature and precipitation in 2011-2012 were the same as normal. n=12
S.D.: Pooled standard deviation. ***: $P<0.001$, **: $P<0.01$, *: $P<0.05$, ns: $P>0.05$
$^{a, b, c}$: Means within the same row with different letters are significantly different ($P<0.05$)

Laura Lens Conservation and Management Manual

The Majuro Atoll, which contains the capital city of Majuro in the Republic of the Marshall Islands (RMI) in the Pacific Ocean, has a population of about 28,000. Freshwater in particular is a fragile resource because there are no rivers or lakes. People in the Majuro Atoll depend on Laura Island's freshwater lens (Laura Lens) for domestic and irrigational use. Laura is an island village situated on the western edge of Majuro Atoll, with an area of 1.8 km^2, an average altitude of a few meters, and a population of approximately 2,300. As a result of population growth in Majuro Atoll, water demand on the atoll is expected to rise and put increasing pressure on Laura Lens. Moreover, the presence of El Niño in the Pacific region often causes droughts in the RMI. There is also a concern that climate and meteorological changes caused by global warming may expand drought areas or cause them to shift. Thus, Laura Lens is in a critical state. Normal water-pumping rates can result in saltwater upconing (i.e., partial upward intrusion of saline water) at the Laura Lens boundary if monthly rainfall is reduced even slightly below normal levels.

In order to develop a method for conserving Laura Lens, a numerical simulation was performed using the SEAWAT model. To analyze water usage, numerical experiments were carried out in which monthly rainfall and daily pump discharge were set under varied boundary conditions. Saltwater upconing, which should not reach the shaft (lateral well), was simulated to obtain the sustainable daily pump discharge for Laura Lens. This manual, therefore, describes the "safe" daily pump discharge in accordance with the rainfall level, and recommends the dispersed pumping method (i.e., increasing the number of intake wells to reduce pumping pressure on one location) to maintain a healthy Laura Lens based on scientific findings.

To conserve the freshwater lens, it is important that the daily pumping discharge be reduced as the monthly rainfall decreases. It is thus desirable to devise a new groundwater intake system in which the wells are being pumped at low intensities. On February 3, 2016, a state of emergency was declared in the RMI because of the drought caused by El Niño. This manual is serving its purpose as it is being widely used by the Drought Committee, among others, in ensuring the sustainable use of a healthy Laura Lens by drought-affected people.

(K. Koda and T. Kobayashi)

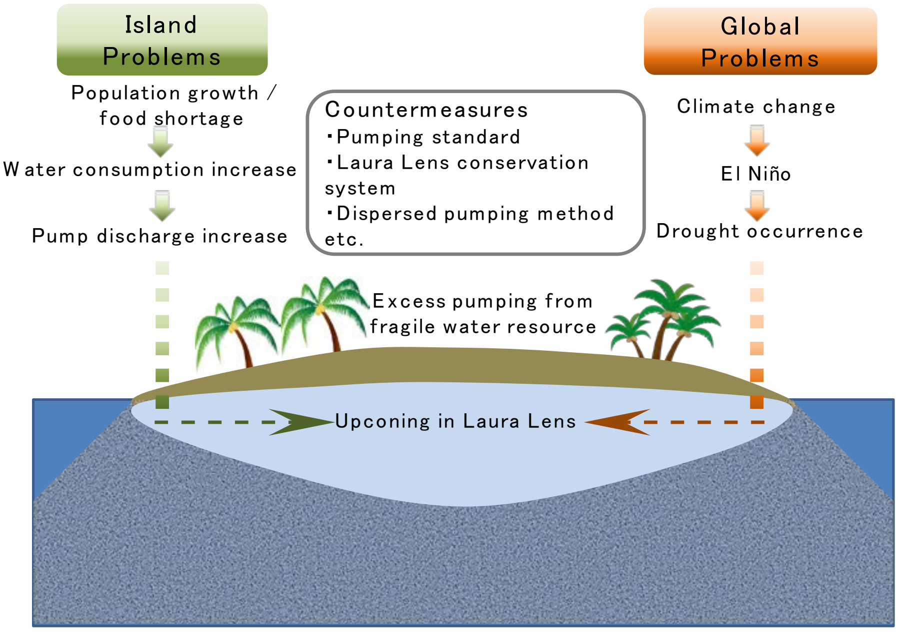
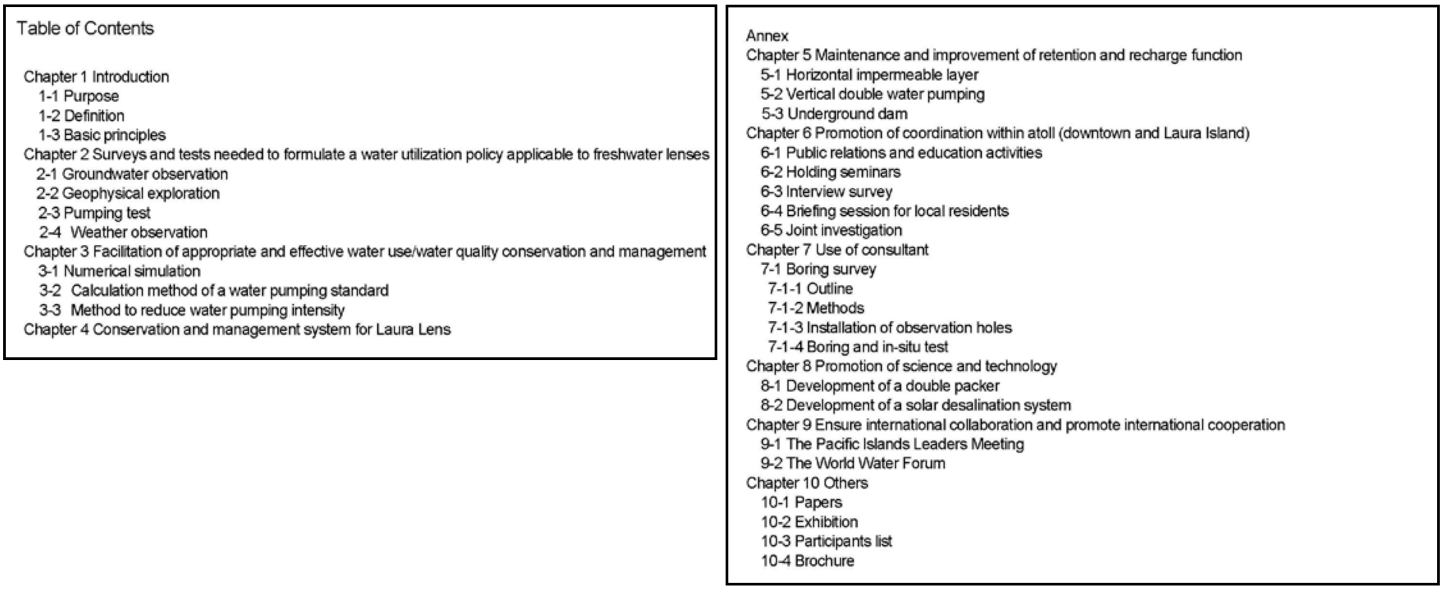


Fig. 1. Problems and Countermeasures in terms of Water Use

Fig. 2. Laura Lens Conservation and Management Manual



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Lowering of rhizosphere soil pH is a relevant factor on biological nitrification inhibition in sorghum

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the major food crops in the world. To date, most studies on biological nitrification inhibition (BNI) in sorghum have been performed on plants grown in hydroponic systems. To determine whether or not sorghum inhibits nitrification in fields, this current study was conducted in an area underlain by alfisols, which is a typical soil in semi-arid and sub-humid tropics, with the aim of clarifying the mechanism that results in the inhibition of soil nitrification in the field. Nitrification activity in the rhizosphere of sorghum, i.e., the soil (of a few millimeters thick) surrounding the roots, was measured and compared with those in the adjacent bulk soil.

Sweet sorghum (CSH 22SS, NTJ 2, 675x700, ICSV 25263, ICSV 25274, ICSV 93046) and grain sorghum (CSH 16, PVK 801, HTJH 3201) were cultivated in four alfisol fields in the semi-arid tropical region of India during the 2010 and 2011 rainy seasons. Soil samples were collected three times during the growing season. Nitrification activity in the rhizosphere soil was significantly lower than that in the bulk soil in 8 out of 12 samplings (Fig. 1a), while the pH (H_2O, 1:2) of the rhizosphere soil was significantly lower than that of the bulk soil in 10 out of 12 samplings (Fig. 1b). The nitrification activities and soil pH showed significant positive correlation for Alfisols 3 and 4 except for Alfisol 3 at 98 days after sowing, whereas the nitrification activity and soil pH had significant positive correlation for Alfisols 1 and 2 collected during the mid and late growth stages in the four fields (Fig. 2). Acidification of the soil by sulfuric acid decreased the nitrification activity to a comparable extent, as observed in the rhizosphere soils (Fig. 3).

These results indicate that acidification of soil around roots would be one of the main causes of nitrification inhibition by sorghum in the field. Although our study showed that acidification of soil would be the main driving force for nitrification inhibition in the rhizosphere, root exudates such as sorgoleone may also enhance BNI simultaneously. Due mainly to technical difficulties involving soil, which contains numerous and wide-ranging organic compounds, it remains unclear whether specific compounds exudated from plants inhibit nitrification in the soil-plant system. Further studies are necessary to clarify the contribution of root exudates to BNI activity in the field.

(T. Watanabe, S. P. Venkata [International Research Institutes for the Semi-Arid Tropics, ICRISAT], K. L. Sahrawat [ICRISAT], S. P. Wani [ICRISAT], O. Ito [United Nations University])

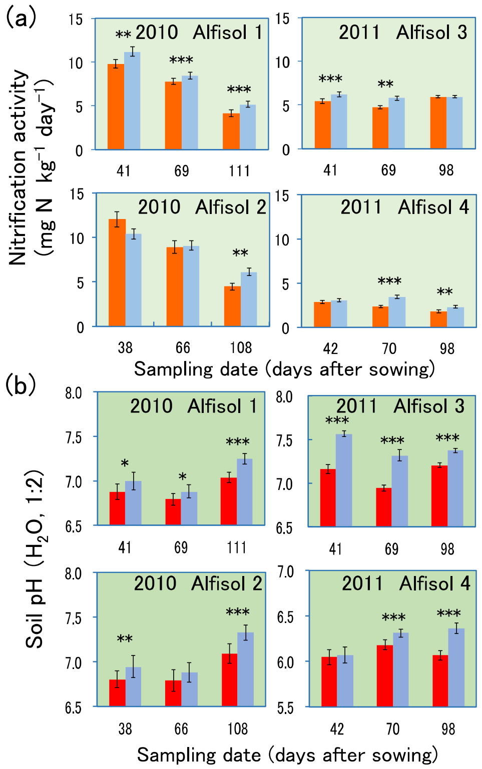


Fig. 1. Average nitrification activities (a) and average pH (b) in the rhizosphere soils (🟧, 🟥) and bulk soils (🟦, 🟦) in each sampling

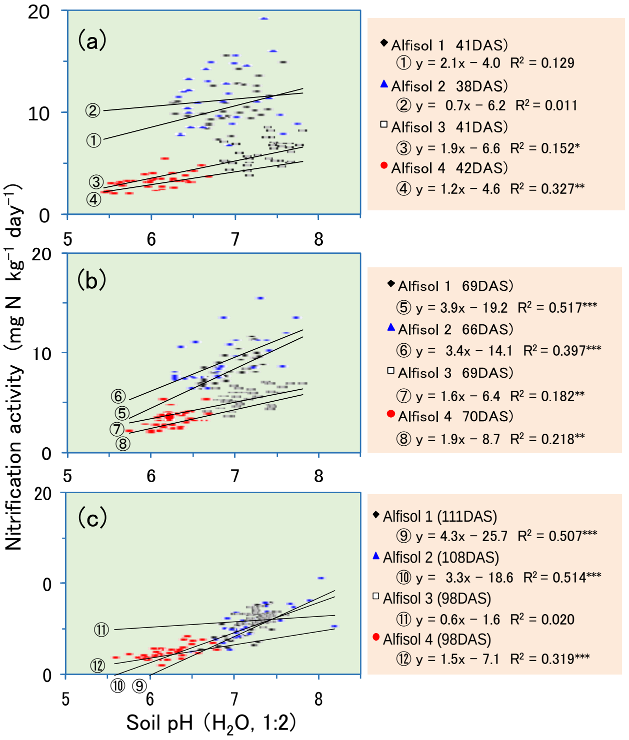


Fig. 2. Relationship between nitrification activity and soil pH (H_2O, 1:2) of rhizosphere soil in each sampling (early (a), middle (b), and late (c) stage of growth)

DAS: days after sowing

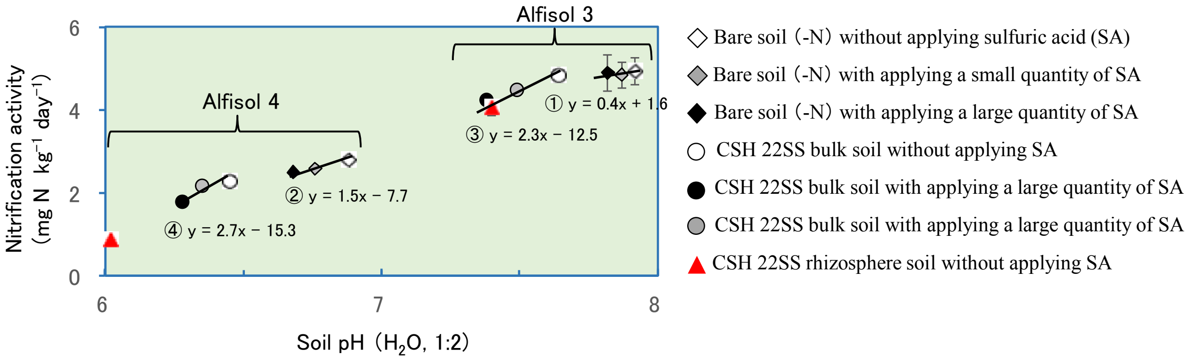


Fig. 3. Effects of soil pH modification on nitrification activity

Each nitrification activity of soils with modified soil pH by applying sulfuric acid was measured.

The release of biological nitrification inhibitors from sorghum root is regulated at the transcriptional level

Sorghum (*Sorghum bicolor*) roots release biological nitrification inhibitors (BNIs) to suppress soil-nitrifier activity and soil-nitrification. The presence of NH_4^+ in the rhizosphere stimulates the release of BNIs from roots and is hypothesized to be functionally linked to plasma membrane (PM) H^+-ATPase activity. However, whether the H^+-ATPase is regulated at the transcriptional level, and if so, which iso-forms of H^+-ATPases are involved in BNIs release are not known. Also, the stimulation of BNI release, whether it is due to NH_4^+ uptake or its assimilation in roots, is unclear, and it would be subsequently addressed by this study.

NH_4^+ concentrations up to 1.0 mM positively stimulated both PM H^+-ATPase activity and BNI release from sorghum roots; but at higher concentrations (>1.0 mM), NH_4^+ did not further increase BNI release and a decline in PM H^+-ATPase activity was observed (Fig. 1a, b). Vanadate, an inhibitor of H^+-ATPases, suppresses BNI release from intact sorghum plants (Fig. 1c). Twelve PM H^+-ATPase genes (iso-forms, designated as *SbA1 to SbA12*) were identified in sorghum genome; however, only five H^+-ATPase genes were stimulated by NH_4^+ in the rhizosphere. They have a similar expression pattern and is consistent with the observed variation in H^+-ATPase activity (Fig. 2). Methyl-ammonium (MeA), a non-metabolizable analogue of NH_4^+, had no significant effect on BNI release, H^+-ATPase activity, or the expression of H^+-ATPase genes (Fig. 3). These results suggest that the functional link between PM H^+-ATPase activity and BNI release is operational only at NH_4^+ concentrations of ≤1.0 mM in the rhizosphere. The variation in PM H^+-ATPase activity by NH_4^+ is due to transcriptional regulation of five iso-forms of H^+-ATPases. The stimulatory effect of NH_4^+ on BNI release is functionally associated with NH_4^+ assimilation and not from NH_4^+ uptake alone.

A mechanistic understanding of BNI release in sorghum helps in choosing suitable agro-ecological niche production systems where BNI function is expressed to its genetic potential for controlling soil nitrification. In addition, the use of slow-release N-fertilizers can allow soil ammonium levels ≤1.0 mM. This, coupled with the development of genetically modified crops with accelerated PM H^+-ATPase activity, can further improve BNI release from sorghum root systems to make production systems low-nitrifying and low-N_2O emitting with improved nitrogen-use efficiency, which in turn will be ultimately beneficial to human society and the environment.

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

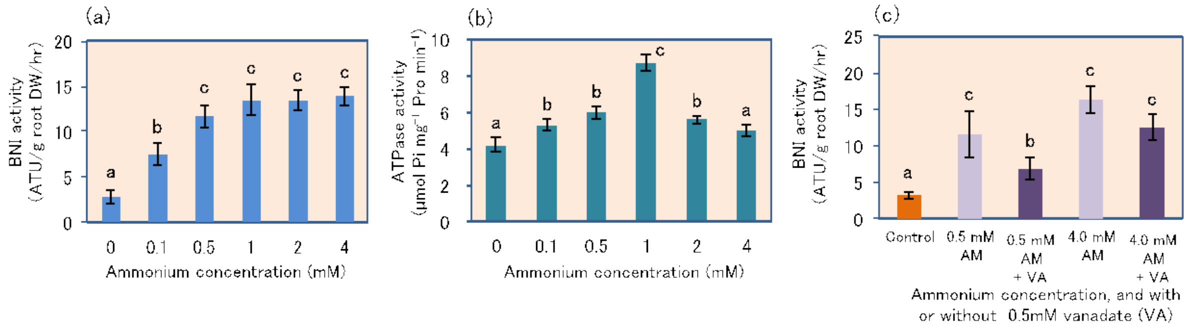


Fig. 1. The effects of ammonium (AM) on biological nitrification inhibitor (BNI) release from sorghum roots (a) and the plasma membrane (PM) H^+-ATPase activity in the roots (b), and the effect of vanadate (VA), an ATPase inhibitor, on BNI release from the roots (c)

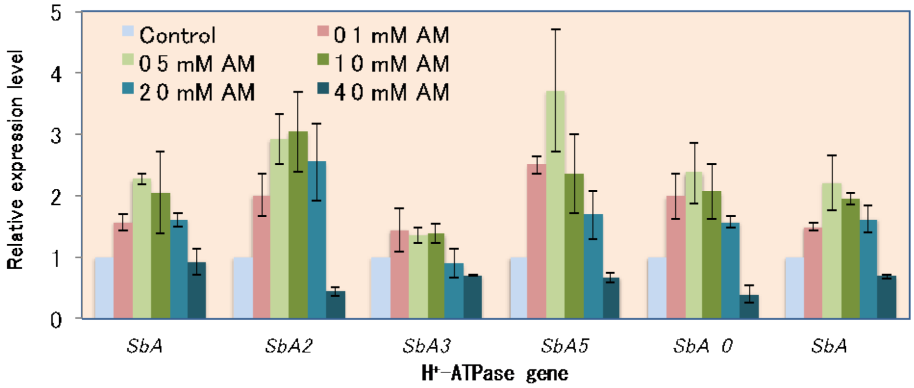


Fig. 2. The expression of six sorghum PM H^+-ATPase genes in response to NH_4^+ (AM) nutrition

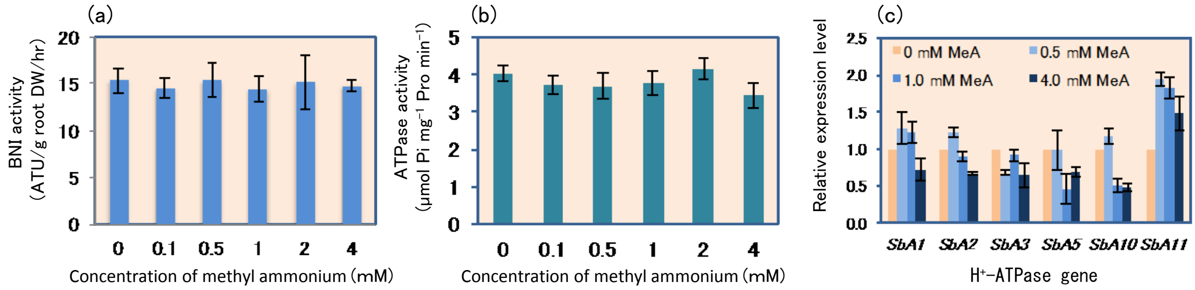


Fig. 3. The effect of methyl-ammonium (MeA), a non-metabolizable analogue to NH_4^+, on BNI release (a), the H^+-ATPase activity (b), and the expression of the H^+-ATPase genes in sorghum roots (c)

High-yielding and stable rice varieties in the flood plains of Upper Volta River in Ghana

Rice consumption is drastically increasing in West Africa; however, domestic production has not matched the increase in consumption. Moreover, the increasing cost of imported rice is putting the squeeze on the national budget. The utilization of fertile flood plains is thus being eyed as one solution to increase rice production in the region.

The fertility of flood plains changes drastically according to the distance from rivers. In addition, there is a large annual environmental variation in the areas where crops are grown.

We tried to screen suitable rice varieties for the flood plains. The experiments, which used 28 varieties, were conducted at 4 places (Fig. 1), each experiencing different flood conditions from 2012 to 2014 (Table 1). The result of cluster analysis showed the yield responses of four genotypes across three environments.

G-2 (Genotype-2) had the highest yield in E-3 (Environment-3) but showed lower yields in other environments (Fig. 1). A genotype showing high yield only in a particular environment is not adequate for flood plains because the environment undergoes large changes every year. On the other hand, G-3 showed moderate yields and was stable under different environments. Meanwhile, G-4 showed the highest yield in E-1 and relatively higher yields in other environments. Therefore, G-4 was considered the most favorable genotype in flood plains. We can see which varieties are favorable in flood plains by performing AMMI (additive main effect and multiplicative interaction) analysis. Varieties that are stable in any environment are plotted near the origin. The tested varieties, IRBL9-W[RL] (Japan-IRRI Project; https://www.jircas.affrc.go.jp/kankoubutsu/seika/seika2011/pdf/2011-10.pdf), in addition to some local varieties such as Amankwatia, Bodia, and Sakai, are among the top 20% in terms of yield (Fig. 2). IR42 (No.4) had the highest yield on average; however, it was not stable.

Our results showed that planting early-maturing varieties to avoid drought is not effective. We believe that this information will be useful in designing breeding strategies for rainfed rice in flood plains. We also hope that our results will contribute to achieving the goal of CARD (Coalition for African Rice Development), which targets rainfed lowlands for increased rice production.

(*M. Oda, Y. Tsujimoto, K. Katsura [Kyoto Univ.], K. Matsushima [Tokyo Univ. of Agriculture], B. Inusah [(Savanna Agricultural Research Center], W. Dogbe [SARC], J. Sakagami [Kagoshima Univ.]*)

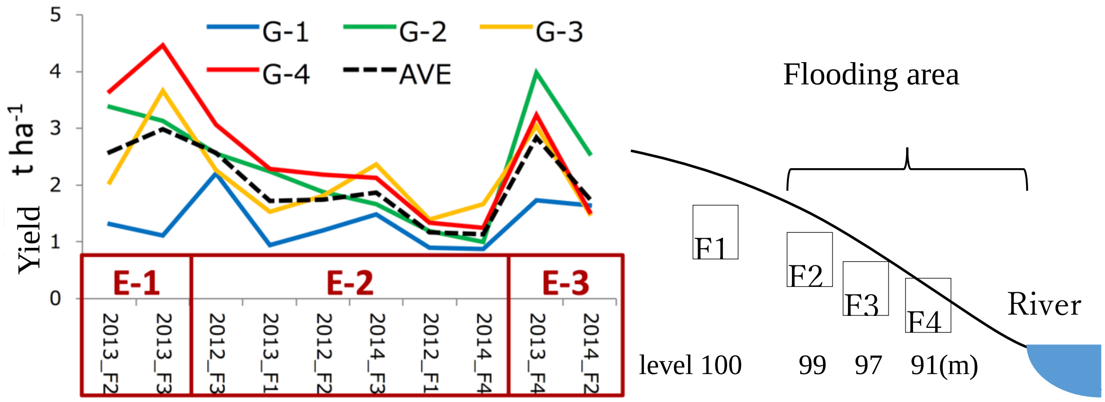


Fig. 1. Average yields of genotype groups across environment groups.
G: Genotype by cluster analysis, E:Environment group

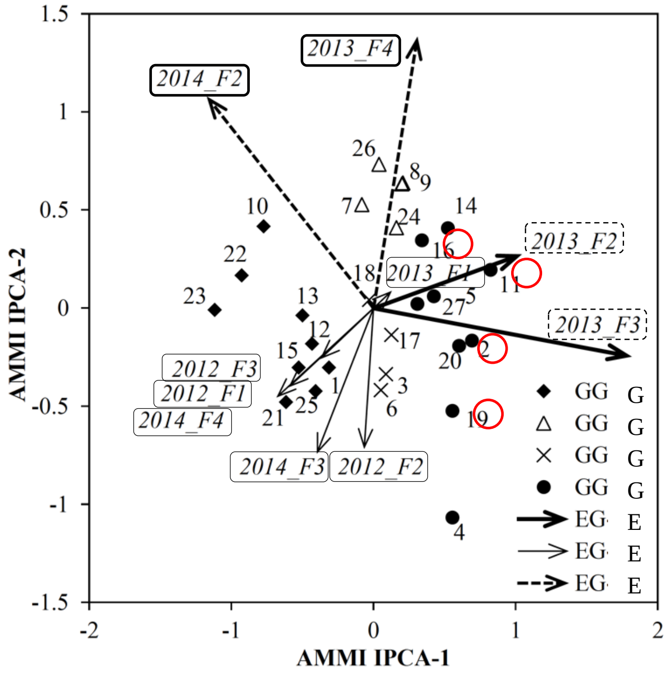


Fig. 2. Feature of each variety by AMMI analysis

Table 1. Average yields of the sampled varieties

No.	Genotype	Species	Yield	Character
G-1 (Very stable but low yield)				
1	CK40	*O. sativa*	1.91	Lowland
10	IR73020-19-2-B-3-2B	*O. sativa*	1.74	Submergence
12	N22	*O. sativa*	0.78	Upland
13	Nylon	*O. sativa*	1.38	Deepwater
15	Vandana	*O. sativa*	0.90	Upland
21	CG14	*O. glaberrima*	1.42	Lowland
22	Mala Noir IV	*O. glaberrima*	1.19	Deepwater
23	Yélé 1A	*O. glaberrima*	1.08	Deepwater
25	Séidou Bayebeli	*O. glaberrima*	1.66	Upland
G-2 (High yield in E-3)				
7	IR71700-247-1-1-2	*O. sativa*	2.12	Lowland
8	**IR72431-5B-18-B-10-1**	***O. sativa***	**2.66**	**Elongation**
9	IR73018-21-2-B-2-B	*O. sativa*	2.35	Submergence
24	Douboutou II	*O. glaberrima*	2.30	Lowland
26	Saligbeli	*O. glaberrima*	2.37	Deepwater
G-3 (Standard)				
3	IR07F323	*O. sativa*	2.01	AG + *Sub1*
6	IR67520-B-14-1-3-2-2	*O. sativa*	2.09	Submergence
17	Jasmin85	*O. sativa*	2.15	Lowland
18	Sikamo	*O. sativa*	2.25	Lowland
G-4 (High yield especially in E-1)				
2	IR07F297	*O. sativa*	2.39	AG + *Sub1*
4	**IR42**	***O. sativa***	**2.76**	**Irrigated**
5	IR11141-1-6-1-4	*O. sativa*	2.30	Elongation
11	**IRBL9-W[RL]**	***O. sativa***	**2.61**	***Sub1***
14	PSBRC80	*O. sativa*	2.18	Lowland
16	**Amankwatia**	***O. sativa***	**2.75**	**Lowland**
19	**Bodia**	***O. sativa***	**2.60**	**Lowland**
20	**Sakai**	***O. sativa***	**2.59**	**Lowland**
27	WAB1159-2-12-11-6-10	NERICA	2.44	Lowland

Varieties in red letters are in the top 20%（>2.5t）in terms of yield.

Improving the drought tolerance of a Brazilian soybean variety using *Agrobacterium*-mediated transformation

Brazil is the second-largest soybean producing country in the world. However, severe droughts have affected production in Brazil, thus the development of drought-tolerant soybean is required. For soybean, it has been difficult to produce transformants because the transformation efficiency is very low. Although transformation of Brazilian soybean varieties BR-16 using bio-ballistic had been reported, there is no report of *Agrobacterium*-mediated transformation of the Brazilian varieties. As the plants transformed by *Agrobacterium* generally have low copy numbers of transgenes, the establishment of an *Agrobacterium*-mediated transformation method will make it easier to obtain transformants stably expressing a gene of interest and allow the rapid fixation of inserted gene in transgenic plants.

We succeeded in improving the transformation efficiency of Brazilian soybean cultivars and were able to establish the transformation method using *Agrobacterium* (Fig. 1a-b). The transformation efficiency was 1.74% when we used the reporter *GUS (β-glucuronidase)* gene for the transformation. This efficiency can enable us to produce the transgenic soybean varieties at a practical level. We confirmed that the copy numbers of the transgene are low. Stress-inducible AREB (ABRE (ABA- responsive element)-binding factor) transcription factors play important roles in regulating stress responses and tolerances. We obtained transgenic events having *AREB1* stress-tolerance gene using *Agrobacterium* methods. The transgenic events showed drought tolerance in the greenhouse (Fig. 1c-e).

We are conducting an evaluation of transgenic soybean lines in the field. We expect to produce transgenic soybean varieties with high yield under drought conditions in the future. In addition, we expect to produce transgenic soybean varieties with various kinds of useful genes using the *Agrobacterium*-mediated transformation methods.

1) Kanamori N, et al. (2011) JIRCAS Working Report **71**:75-79.
2) Marinho JP*, Kanamori N*, et al. (2015) Plant Mol. Biol. Rep. (Marinho JP and Kanamori N contributed equally to this work)

(N. Kanamori, Y. Fujita, K. Nakashima, K. Yamaguchi-Shinozaki [The University of Tokyo], J. Marinho [Embrapa Soybean], A. Nepomuceno [Embrapa Soybean])

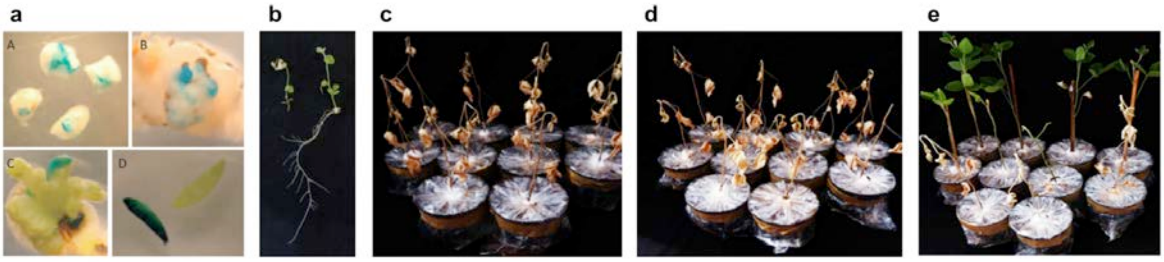


Fig. 1. Establishment of the transformation method applied to Brazilian soybean variety BR16 using *Agrobacterium*, and drought tolerance of a transgenic soybean expressing *AREB1* gene in the greenhouse.

a) GUS activity in the tissues of soybean after being infected by *Agrobacterium* carrying a binary vector with BAR and GUS genes. **A:** transient *gus* gene expression around the embryonic tip region after 5 days of co-cultivation; **B**: stable expression in meristems of embryonic tip cultured on shoot elongation medium for 1 week; **C:** stable expression in regenerated adventitious shoot cultured on shoot elongation medium for 2 weeks; **D:** leaflets with (left) GUS activity from a transgenic plant or without (right) from a non-transgenic plant. The figures were from Kanamori et al. (2011).

b) Root elongation of transgenic soybean plant. A differentiated shoot (left) developed roots (right) on a rooting medium. The figure was from Kanamori et al. (2011).

c-e) Survival rates of soybean plants of the genotypes 1Ea15 and 1Ea2939 transformed with 35S:AtAREB1 and non-transgenic BR 16 after 17 days of withholding irrigation followed by 7 days of rewatering. **c:** BR 16; **d:** 1Ea15; **e:** 1Ea2939. Figures **c**, **d**, and **e** were adapted from Marinho JP et al. (2015).

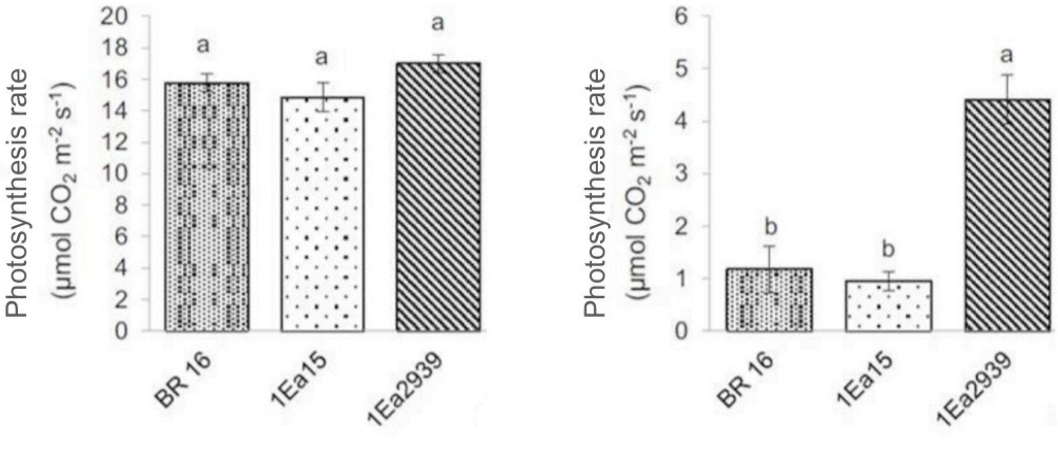


Fig. 2. Photosynthetic rate of soybean transgenic lines 1Ea15 and 1Ea2939 transformed with 35S:AtAREB1 and non-transgenic BR 16, grown under well-watered conditions (left) and under water deficit (right). Values represent mean± standard error; n=9 replicates. In each water condition, means followed by the same lowercase letters do not differ by the Tukey test ($p \leq 0.05$). The figures were adapted from Marinho JP et al. (2015).

Discovery of genes related to leaf senescence during long-term dry stress

Extreme weather events frequently occur around the world, causing crop damage. The damage caused by droughts is extensive and has become a serious global problem. Leaf senescence occurs as the late stage of leaf development and in response to environmental stress. Ethylene is a major plant hormone inducing leaf senescence. Recent studies have shown that abscisic acid (ABA) also induces leaf senescence. The elucidation of the mechanism of leaf senescence during long-term dry stress is important to enable stable food production under drought via development of crops. However, the detailed mechanisms of ABA-induced leaf senescence remain unclear. We focused on the A subfamily of stress-responsive NAC (SNAC-A) transcription factors, the expression of which is induced by abiotic stresses, particularly ABA.

Gene expression analysis revealed that seven SNAC-A genes (ANAC055, ANAC019, ANAC072/RD26, ANAC002/ATAF1, ANAC081/ATAF2, ANAC102 and ANAC032) were induced by long-term treatment with ABA and/or during age-dependent senescence. The SNAC-A septuple mutant clearly showed retardation of ABA-inducible leaf senescence. Microarray analysis indicated that SNAC-As induce ABA- and senescence-inducible genes. In addition, comparison of the expression profiles of the downstream genes of SNAC-As and ABA-responsive element (ABRE)-binding protein (AREB)/ABRE- binding factor (ABF) (AREB/ABFs) indicates that SNAC-As induce a different set of ABA-inducible genes from those mediated by AREB/ABFs. These results suggest that SNAC-As play crucial roles in ABA-induced leaf senescence signaling.

Our results indicate that the *SNAC-A* subfamily genes are mainly involved in ABA-induced leaf senescence. Controlling gene expression will enable researchers to regulate leaf senescence during stress responses, and thus contribute toward improving the yield of stress-tolerant crops.

1) Nakashima K et al. (2012) Biochimica et Biophysica Acta 1819: 97–103
2) Takasaki H et al. (2015) Plant J. 84: 1114-1123

(K. Nakashima, K. Maruyama, H. Takasaki [RIKEN, The University of Tokyo], T. Yoshida, K. Yamaguchi-Shinozaki [The University of Tokyo], F. Takahashi, M. Fujita, F. Myouga, K. Toyooka, K. Shinozaki [RIKEN]

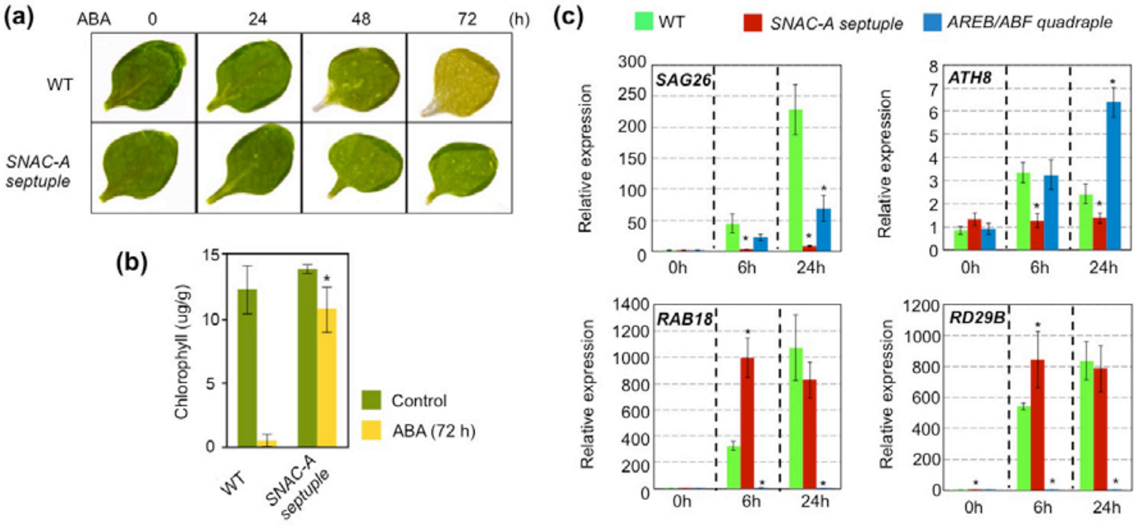


Fig. 1. Chlorophyll degradation and gene expression in ABA-treated wild-type and ABA-treated mutant leaves.

(a) Reduced sensitivity to ABA in chlorophyll degradation in ABA-treated wild-type (WT) and mutant (*SNAC-A septuple*) leaves. Each number indicates the time after 100 µM ABA treatment.

(b) The change in chlorophyll content in ABA-induced senescence. First rosette leaves of wild-type and mutant plants were grown on GM agar plates with or without treatment with 100 µM ABA for 72 h. Student's t-test: $*P < 0.05$. Each value represents mean±SD (n = 4).

(c) ABA-responsive gene expression in the SNAC-A septuple and AREB/ ABF quadruple mutants. Expression profiles of downstream genes of AREB/ABF and SNAC-A transcription factors. Two-week-old seedlings grown on GM agar plates were transferred to GM agar plated with 100 µM ABA for 6 and 24 h. Total RNA was isolated from seedlings.

The figures were adapted from Takasaki *et al*. (2015).

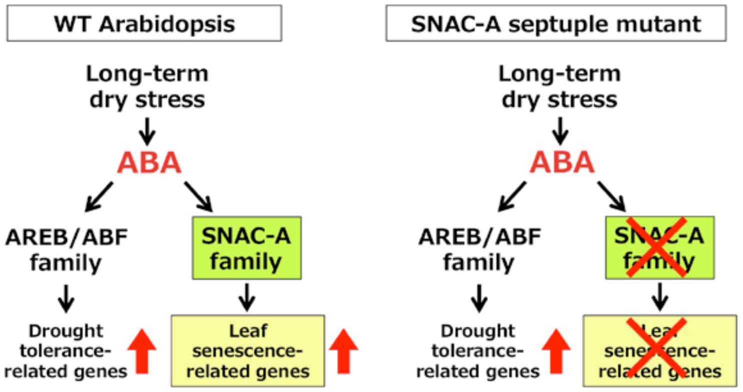


Fig. 2. Molecular mechanism of leaf senescence during long-term dry stress.

SNAC-As induce ABA-inducible genes related to senescence during stress, and SNAC-As induce a different set of ABA-inducible genes from those mediated by AREB/ABFs.

Map-based cloning of a salt tolerance gene *Ncl* and its utilization for improvement of salt tolerance in soybean

Soybean [*Glycine max* (L.) Merr.] is the world's primary crop source for protein and oil. Its cultivation is conducted in a wide range of environments and is exposed to many biotic and abiotic stresses that influence the sustainability of soybean production. Salt stress inhibits soybean growth and reduces grain yield. Genetic improvement of salt tolerance is essential for sustainable soybean production in saline areas. In this study, we isolated a quantitative trait locus (QTL) for salt tolerance using the map-based cloning strategy from a Brazilian soybean cultivar FT-Abyara to facilitate its use in soybean breeding.

To identify the gene that conditioned the salt tolerance QTL, we conducted fine mapping using 5,828 plants derived from F_9 residual heterozygous plants, and delimited the QTL to a 16.6-kb interval between markers SSR25.8 and CAPS42.4. Since only one predicted gene, *Glyma03g32900*, existed within the 16.6-kb region (Fig. 1), *Glyma03g32900* was thus determined as the causal gene underlying the salt tolerance QTL (*Ncl* locus). The salt-tolerant soybean lines showed higher expression of the salt tolerance gene *Ncl* in the root and resulted in lower accumulations of Na^+, K^+, and Cl^- in the shoot under salt stress. We transformed *Ncl* full-length cDNA driven with 35S promoter (*35S:Ncl*) into a Japanese soybean cultivar Kariyutaka using the *Agrobacterium*-mediated transformation method, and significantly enhanced its salt tolerance (Fig. 2). To determine the usefulness of *Ncl* in conventional soybean breeding, we conducted introgression of *Ncl* into a salt-sensitive variety Jackson through continuous backcross, followed by DNA marker-assisted selection (MAS) using primers Satt339, SSR222042, and SSR112166 in each generation, and finally produced an improved salt tolerance line (Fig. 3). This result demonstrated that the DNA markers around *Ncl* could be used for introgression of *Ncl* into a salt-sensitive cultivar for developing a soybean variety with high salt tolerance. To determine the effect of *Ncl* in salt stress field conditions, we evaluated the *Ncl* near isogenic lines (NILs) in a salt stress field condition. The field experiments, which were conducted over three years, showed that *Ncl* could increase soybean grain yield by 3.6–5.5 times in saline field conditions (Fig. 4).

Our study suggests that using *Ncl* in soybean breeding through transgenic or MAS would contribute to sustainable soybean production in saline-prone areas.

(D. T. Do, H. Chen, H. T. T. Vu, A. Hamwieh, T. Yamada [Hokkaido University], T. Sato [Tohoku University], Y. Yan [Xinjiang Academy of Agricultural Sciences, China], H. Cong [Xinjiang Academy of Agricultural Sciences, China], M. Shono, K. Suenaga, D. Xu)

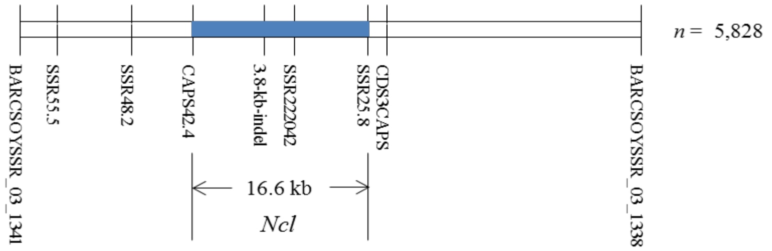


Fig. 1. Fine mapping (n = 5,828) delimits *Ncl* to a 16.6-kb region between markers SSR25.8 and CAPS42.4 on chromosome 3.

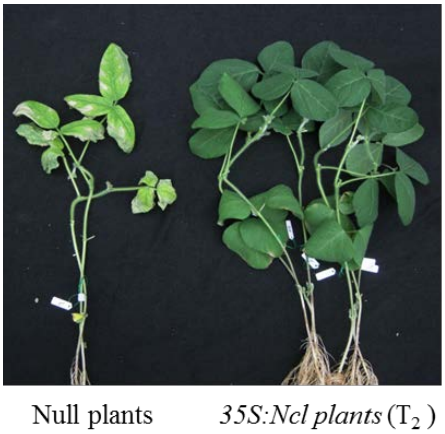


Fig. 2. Overexpression of *Ncl* in transgenic soybean lines enhanced its salt tolerance.

Fig. 3. Introgression of *Ncl* into a salt-sensitive variety "Jackson" by marker-assisted selection (MAS) produced an improved salt tolerance line.

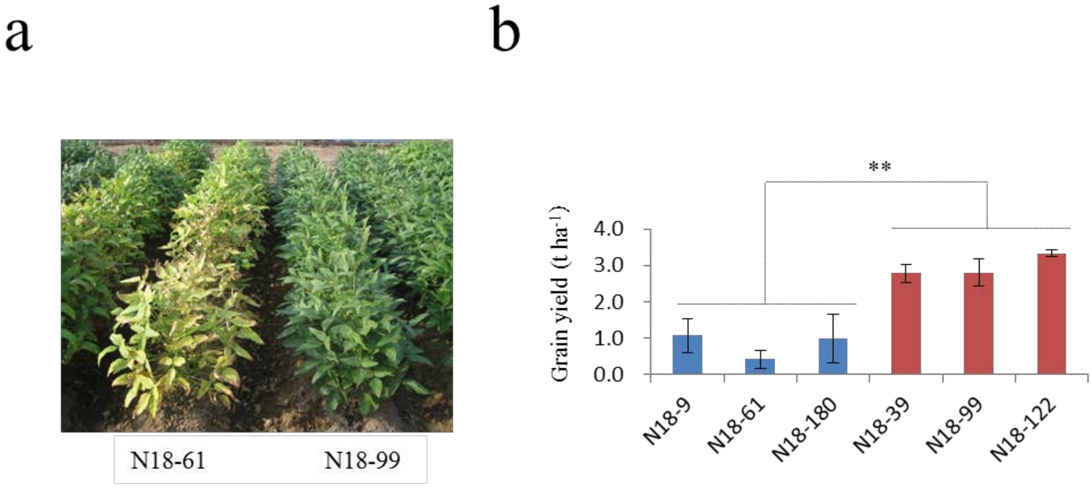


Fig. 4. Field performances of *Ncl* near isogenic lines (NILs) in a saline field condition in Miyagi Prefecture, Japan. (a) Top view of N18-61 and N18-99 grown in a salt stress field in 2012. (b) Grain yield results of the NILs in a salt stress field in 2012. N18-39, N18-99, and N18-122 are lines carrying the tolerant allele of *Ncl*, whereas N18-9, N18-61, N18-180 are lines that had the sensitive allele. Data are shown as mean ± s.d. from three replicates. **: Significant difference ($P < 0.01$) based on ANOVA (Tukey's multiple comparison test).

Development of SSR markers for diversity studies on Yam (*Dioscorea*) genetic resources

Yam (*Dioscorea* spp.) is a tuber crop widely cultivated in Africa, Asia, and South America. This traditional staple crop is very important for regional food security and income generation especially in West Africa (Fig. 1). However, yam breeding is constrained by the crop's inherent attributes, including a long growth cycle, being inconsistent or non-flowering, polyploidy, and high heterozygosity. The use of advanced genetic tools and genomic resources in this crop has remained slow and difficult mainly because very little is known about yam genetics. Moreover, the available yam genetic resources are poorly characterized, limiting the utility of the existing diversity in yam improvement programs. In this study, effective Simple Sequence Repeat markers (SSRs) were developed to demonstrate their effectiveness in estimating genetic diversity and in understanding the phylogenetic relationships of the genetic resources. The results of the study will serve as guide for implementing their use in yam breeding for the region.

A total of 90 SSRs were developed from an enriched genomic library of yellow Guinea yam (*D. cayenensis*). The number of SSRs developed in this study exceeds the total number of SSRs developed in the previous studies (67 SSRs in the seven reports). Cross-amplification revealed that 85 (94.4%) and 51 (56.7%) of these SSRs could be successfully transferred to the other two major cultivated species of *D. rotundata* and *D. alata*, respectively. A total of 30 markers were selected based on stable amplification in six important species, i.e., *D. cayanensis*, *D. rotundata*, *D. alata*, *D. dumetorum*, *D. esculenta* and *D. bulbifera*, for the analysis of phylogenetic relationships among *Dioscorea* species (Table 1). Among these 30, the six marked with * were considered to be useful marker sets for phylogenetic studies for their polymorphisms and high level of transferability over five species. Clustering based on these six SSRs among *D. cayenensis*, *D. rotundata*, *D. alata*, *D. dumetorum* and *D. bulbifera* detected groups consistently and reflected their taxonomic relationships as demonstrated by previous studies, e.g., *D. alata* and *D. bulbifera* were clearly separated, and *D. rotundata* and *D. cayenensis* were closely related to each other (Fig. 2).

These developed SSR markers can be used as handy markers not only for genetic diversity analysis but also for cultivar identification, gene/QTL mapping, and authentication of progenies obtained from genetic crosses particularly for the modestly equipped national agricultural research systems located in major yam growing regions.

(S. Yamanaka, H. Takagi, M. Tamiru [Iwate Biotechnology Research Center], R. Terauchi [Iwate Biotechnology Research Center])

Fig. 1. Yam germplasm field (left) and tubers sold in the market (right)

Table 1. SSR markers (among the six *Dioscorea* species) considered effective for phylogenetic studies

Marker	*D. cayenensis*	*D. rotundata*	*D. alata*	*D. dumetorum*	*D. esculenta*	*D. bulbifera*
YM002	++	++	++	−	−	+
YM003	++	++	++	−	−	−
YM005	++	++	++	−	−	−
YM006	++	++	−	−	−	−
YM009	++	++	++	−	−	−
YM010	++	++	++	−	−	−
YM011	++	++	−	−	+	−
YM012	++	++	++	−	−	−
YM013*	++	++	++	++	+	++
YM021	++	++	++	−	−	+
YM023	++	++	++	−	−	−
YM024	++	++	++	++	−	−
YM032	++	++	++	−	−	+
YM033	++	++	++	−	−	−
YM036	++	++	++	−	−	−
YM037	++	++	++	−	−	−
YM044	++	++	++	−	−	−
YM045	++	++	++	−	+	−
YM053*	++	++	++	+	+	+
YM055*	++	++	++	++	+	−
YM065	++	++	++	+	−	−
YM066*	++	++	++	++	−	+
YM071	++	++	++	++	−	−
YM074*	++	++	++	+	+	+
YM075	++	++	++	−	−	−
YM078	++	++	++	−	+	−
YM080*	++	++	++	++	+	++
YM084	++	++	−	−	−	−
YM087	++	++	++	−	−	−
YM089	++	++	++	−	−	−

++: amplified (polymorphic), +: amplified (monomorphic), -: no amplification
Symbols highlighted in yellow: showed species-specific polymorphisms
*: Markers showed amplification in > 5 species

Fig. 2. Dendrogram of yam genetic resources generated based on the genetic distances of six cross-amplified SSR markers. ■: *D. alata*, □ : *D. bulbifera*, ●: *D. rotundata*, ○:*D. cayenensis*, △: *D. dumetorum*

Evaluation of genetic diversity in quality-related traits of cowpea genetic resources for the development of an open access database

Cowpea [*Vigna unguiculata* (L.) Walp.], a traditionally important grain crop in sub-Saharan Africa, is an affordable source of protein and minerals as well as cash income, especially for small-scale farmers who have limited options for food and cash crops. It also retains tremendous potential for improving nutritional balance and livelihoods in the region. Africa has been experiencing rapid economic growth in recent years; thus, in addition to improving "quantity" in the production, more attention would be placed on "quality" to boost utilization and consumption, and to further promote the crop through "value addition". In this study, we sought possible strategies for value-addition using the rich genetic resources available, and we evaluated the genetic diversity especially in various grain quality-related traits and the relationships among these traits in cowpea.

Wide genetic variation and strong correlations among crude protein and Fe and Zn contents suggest the possibility of improving the concentrations of these nutritional factors simultaneously in 240 accessions evaluated (Table 1). Also, low associations among physical and nutritional properties of grain indicate the possibility of introgressing favorable traits utilizing identified genetic resources.

From these 240 accessions, a set of 20 accessions retaining the diversity in physical and basic nutritional properties were selected and further analyzed to create a detailed profile of the grain’s physical, nutritional/anti-nutritional, and functional properties (Table 2). Based on the observed narrow variation in amino acid (AA) composition, a reliable nitrogen-to-protein conversion factor of 5.45 was proposed for the estimation of crude protein content. We identified several improved breeding lines, such as IT93K-452-1, IT90K-277-2, and IT98K-205-8, with low concentrations of flatulence-causing oligosaccharides together with various favorable agronomic traits and nutrient contents. Also, TVu-12802 and TVu-467 were nominated as potential parental lines due to their high amounts of micronutrients and low phytic acid/Fe and phytic acid/Zn molar ratios.

The obtained results were organized into an open access "EDITS-Cowpea" database (http://www.jircas.go.jp/database/edits-cowpea) that enables all cowpea breeders and researchers to identify the potential germplasm resources to use to facilitate their work.

These findings suggest potential and possible uses of identified genetic resources with key quality-related traits which may stimulate breeding activities towards promotion and value-addition of the crop. Also, the developed open access database will enhance the utilization of cowpea’s wide genetic diversity. These outputs are expected to link the primal elements needed for grain quality improvement, and facilitate the development and deployment of cowpea varieties with improved nutrition and quality that meet the needs of farmers and consumers. This, in turn, should enhance cowpea consumption and production in the region.

(S. Muranaka, M. Shono, Y. Takagi, T. Myoda [Tokyo University of Agriculture])

Table 1. Phenotypic (upper diagonal) and genotypic (lower diagonal) correlations among major agronomic traits and physical and nutritional properties

	Dflow^{a}	Dharv^{b}	Byield^{c}	Gyield^{d}	CP^{e}	Fe	Zn	Mn	Cu	Gweight^{f}
Dflow		0.45 *	0.30 *	0.02	0.05	0.02	−0.01	0.08 *	−0.14 *	0.08
Dharv	0.44 *		0.10 *	0.09 *	−0.10 *	−0.13 *	−0.10 *	0.15 *	0.12 *	0.44 *
Byield	0.57 *	0.19 *		0.49 *	0.22 *	0.17 *	−0.01	−0.09 *	−0.15 *	0.04
Gyield	0.10	0.32 *	0.57 *		0.07	0.03	−0.23 *	−0.05	−0.01	0.17 *
CP	0.10	−0.15	0.25 *	−0.11		0.47 *	0.36 *	−0.02	0.07	−0.19 *
Fe	0.05	−0.22 *	0.16	−0.23 *	0.70 *		0.33 *	−0.03	0.04	−0.24 *
Zn	0.00	−0.25 *	−0.07	−0.38 *	0.70 *	0.68 *		0.05	0.13 *	−0.14 *
Mn	0.24 *	0.42 *	0.32 *	0.53 *	0.13	0.04	0.18		0.19 *	0.15
Cu	−0.30 *	0.17 *	−0.27 *	0.02	0.11	0.06	0.16	0.22		0.00
Gweight	0.13	0.53 *	0.06	0.35 *	−0.28 *	−0.39 *	−0.25 *	0.07	0.00	

* indicates *P<0.05*

[a] Dflow=Days to 50% flowring; [b] Dharv=Days to harvest; [c] Byield=Biomass yield;
[d] Gyield=Grain yield; [e] CP=Crude protein; [f] Gweight=100-grain weight

Table 2. Profile of the grain's physical, nutritional/ anti-nutritional and functional properties

Traits	Average	Highest	Lowest	S.D.
Grain size$^{a)}$				
100 seed wt (g)	11.7	18.7	4.0	2.9
Width (mm)	5.3	6.8	3.7	0.6
Length(mm)	7.2	9.7	4.8	0.9
Protein (%)$^{a,b)}$	20.4	24.1	17.0	1.3
Micronutrient (mg/kg)$^{a)}$				
Fe	53.1	66.3	41.4	5.0
Zn	39.6	47.3	32.1	2.9
Mn	25.3	39.4	14.7	3.8
Cu	4.8	7.3	3.4	0.7
Dietary fibre (g/100g)$^{c)}$				
Insolble	15.7	20.6	9.0	2.7
Solble	1.2	3.4	N.D.	0.9
Oligosaccharide (mg/g)$^{c)}$				
Stachyose	31.5	43.8	24.1	3.9
Sucrose	15.4	39.3	9.2	7.8
Raffinose	3.4	4.5	1.7	0.7
Phytic acid (mg/g)$^{c)}$	28.3	37.0	21.8	4.6
Polyphenol (mg/g)$^{c)}$	4.4	48.8	0.1	10.7
DPPH IC$_{50}$ (mg/g)$^{c)}$	416.7	1403.9	28.8	376.8
Cooking time (min)$^{c,d)}$	97.0	160.0	60.0	23.9

a) Data obtained in 240 genotypes

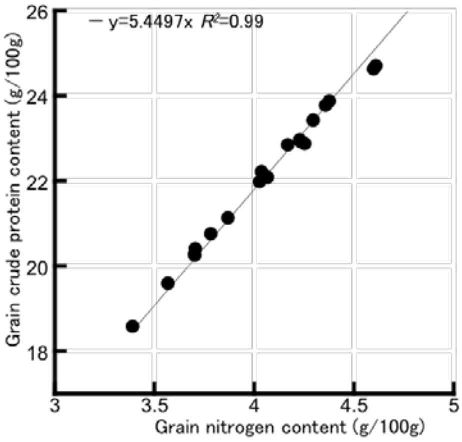
b) Calculated with N-P conversion factor of 5.45

c) Data obtained in selected 20 genotypes

d) Time to reach adequate hardness (2 - 4 N) as boiled bean

Fig. 1. Various cowpea grains

Fig. 2. Relationship between grain nitrogen and grain crude protein contents



JEC1, a new variety of *Erianthus* with higher mechanical harvesting efficiency

The enhancement of production and utilization of bioenergy crops as energy sources would play an important role in reducing CO_2 emissions, improving local energy production, and stimulating local economy. *Erianthus* (*E. arundinaceus*), a wild relative of sugarcane, is a perennial tall herb of Gramineae with C_4 photosynthesis. Because of its high biomass productivity, it is considered a good candidate as feedstock for bioenergy production in Japan especially in sub-tropical areas. To realize practical utilization of *Erianthus*, the development of new varieties that will enable low cost production is indispensable. Thus, in 2014, the Japan International Research Center for Agricultural Sciences (JIRCAS) and the National Agriculture and Food Research Organization (NARO) registered JES1 (Japanese *Erianthus* Seed 1) as its first *Erianthus* variety. JES1 is a variety that can be propagated by botanical seeds. Although it improves the propagation efficiency of *Erianthus*, the stools are relatively less uniform in their growth, which leads to lower mechanical harvesting efficiency. Since mechanical harvesting cost is a major portion of total costs in biomass production, we developed this new variety, JEC1 (Japanese *Erianthus* Clone 1). JEC1 can be propagated vegetatively and demonstrates improved mechanical harvesting efficiency over the previous variety, JES1.

JEC1 was selected from the open pollinated population of JW4, a Japanese *Erianthus* germplasm accession that exhibits late flowering and an erect growth habit. Vegetative propagation of JEC1 was done by stem cutting or rhizome division. Its heading was in early October, 10 days earlier than JES1, and the germination rate of florets was 9.4% in Kumamoto Prefecture (Table 1). The plant had a relatively prostrated growth habit compared with JES1 (Table 1, Fig. 1), and the dry matter yield in the 2nd year was 3.16t/10a, which was almost equivalent to JES1. The coefficient of variance of stalk number and the dry matter weight per stool in JEC1 were significantly lower than in JES1 (Table 2); as a result, a significantly higher machine harvesting efficiency of 9.3t/hr was achieved, compared with the efficiency of JES1 which was 7.3t/hr (Fig. 2). It should be noted that JEC1 produces fertile seeds in the southern part of Japan, therefore cultivation of this variety in Ogasawara and Nansei Islands is not recommended due to the risk of weed invasion. However, cultivation poses no problem for the northern part of Kyusyu mainland.

In Japan, the utilization of bioenergy crops is still under study phase. JEC1 is being considered for use as a pioneer material in technical development, empirical research, and pilot projects leading to the practical utilization of *Erianthus*.

(Y. Terajima, M. Gau [NARO], N. Uwatoko [NARO], A. Sugimoto)

Table 1. Major characteristics of JEC1 (Kumamoto Pref., NARO)

Characteristics1)	JEC12)	JES13)	KO2-erect3)	note
Plant type	5.1	3.0	5.5	(1 : erect - 9 : prostrate)
Leaf sheath hairiness	5.3	3.9	5.9	(1 : absent - 9 : very many)
Early growth (2nd year)	7.3	6.3	6.4	(1 : poor - 9 : very robust)
Plant length (cm)	418 a	396 a	371 b	2nd year (2013)
Culm diameter (mm)	14.8 a	14.2 a	14.3 a	2nd year (2013)
Stalk no. (no./a)	4751 a	4777 a	4959 a	2nd year (2013)
Dry matter yield (t/10a)	3.16 a	3.22 a	2.71 a	2nd year (2013)
Dry matter content (%)	42.6 a	39.1 a	50.4 b	2nd year (2013)
Ash content (%)	7.7 a	7.3 a	6.2 b	1st year (2012)
First heading date	10/8 a	10/18 b	9/30 c	2nd year (2013)
Germination rate 4) (%)	9.4	0.1	11.9	2nd year (2013)

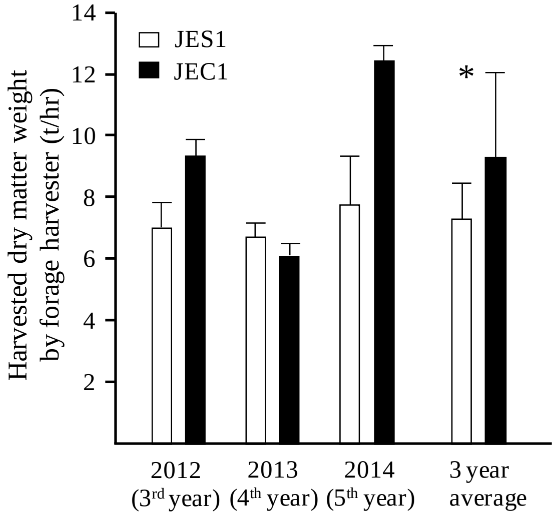
1) Different alphabets indicate significance at 5% level by Tukey's HSD.
2) Materials under test were propagated by tissue culture.
3) Check variety and clone
4) Calculated from the number of germinated florets over the total number of florets (Total floret number of JEC1, JES1, KO2-erect was 1451, 1352, and 1264, respectively.)

Fig. 1. JEC1 vs JES1

Table 2. Coefficient of variance in stalk number and dry matter weight per stool

Year	Variety1)		Stalk no.			Dry matter weight		
			no. /stool	CV		g /stool	CV	
1st year	JEC1		133	18		1630	27	
	JES1		86	38		1378	47	
2nd year	JEC1		89	46		5923	50	
	JES1		90	67		6031	80	
Two-way ANOVA2)		*df*	*F*	*P*		*F*	*P*	
Variety (JEC1 vs JES1)		1	9.34	0.016	*	31.70	0.008	**
Year (1st vs 2nd)		1	16.46	0.004	**	27.98	0.006	**
Variety x Year		1	0.03	0.875	*ns*	5.50	0.499	*ns*
Error		8						

1) Results from 1st and 2nd year data of the performance test field planted on June 2012.
2) Data were collected from 5 stools in each replication (3 rep.).
3) Results of two-way ANOVA for calculating the coefficient of variance of each trait. * and ** indicate the significance at 0.01<P<0.05 and P<0.01, respectively. ns = not significant.



1) Harvested dry matter weight per hour by forage harvester ‘‘CHAMPION 3000’’
2) Data were collected from 3rd, 4th, and 5th year experimental fields planted in June 2010.
3) The drivers of the forage harvester were different in 2012 and 2013, 2014.
4) * indicate the significance at 5% level between varieties by two-way ANOVA (variety and year as factors of variation).

Fig. 2. Mechanical harvesting efficiency of JEC1

Unmasking novel loci for internal phosphorus utilization efficiency in rice germplasm through Genome-Wide Association Analysis

Phosphorus (P) is an essential element needed for plant growth, but it is deficient in many cropping systems worldwide, leading to low grain yields. In addition, the depletion of non-renewable rock phosphate reserves and increase of P fertilizer prices have renewed interest in breeding P-efficient cultivars. Internal P utilization efficiency (PUE), or how efficiently the P that is taken up is utilized to accumulate either grain yield or vegetative biomass, is of prime interest because there has been no progress to date in breeding for high PUE.

We have characterized the genotypic variation for PUE present within the rice gene pool by using a hydroponic system that assured equal plant P uptake. This experiment was performed with a single dose of 800 µg P as the low-P treatment. All genotypes therefore had comparable P content and PUE was estimated as total biomass per amount of P available (P supplied and seed P).

Genotypes Mudgo, Yodanya, Santhi Sufaid, and DJ123 had higher estimated PUE values, while modern varieties such as IR64, Taichung, and Koshihikari had lower values. The loci controlling PUE were mapped via a Genome-Wide Association Study (GWAS), which included 292 diverse *Oryza sativa* accessions from 82 countries. The main loci associated with PUE were mapped on chromosomes 1 and 11 (Figure 1A). The highest PUE was associated with a minor *indica*-specific haplotype on chromosome 1 and a rare *aus*-specific haplotype on chromosome 11 (Figure 1A). Comparative variant and expression analysis for genes contained within the chromosome 1 haplotype identified two high priority candidate genes: *PUE1-7* and *PUE1-9* (Figure 1B). In terms of *PUE1-7*, there is no difference in gene expression pattern, while differences in coding regions between genotypes of contrasting haplotypes suggest that functional alterations for two predicted proteins are likely causative for the observed differences in PUE.

The loci reported here are the first identified for PUE in any crop that is not confounded by differential P uptake among genotypes. Importantly, modern rice varieties were found to lack haplotypes associated with superior PUE and would thus benefit from targeted introgressions of these loci from traditional donors to improve plant growth in phosphorus-limited cropping systems.

(K. Kondo, J. Pariasca-Tanaka, M. Wissuwa, TJ Rose [Southern Cross University])

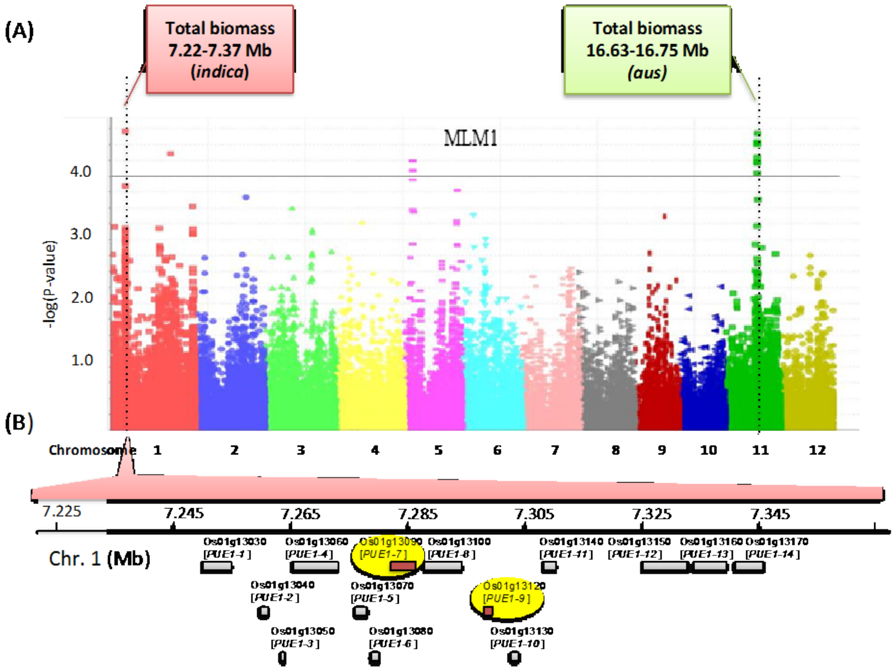


Fig. 1. Manhattan plots for PUE

(A) Dotted lines indicate two major QTL of PUE on chromosome1 and 11. They are involved in PUE for total biomass (shoot and root). The peak of chromosome 5 is associated with PUE for root biomass.

(B) *PUE* loci on chromosome 1 (7.247-7.343 Mb). The two candidate genes showed different gene expression patterns in low P conditions.

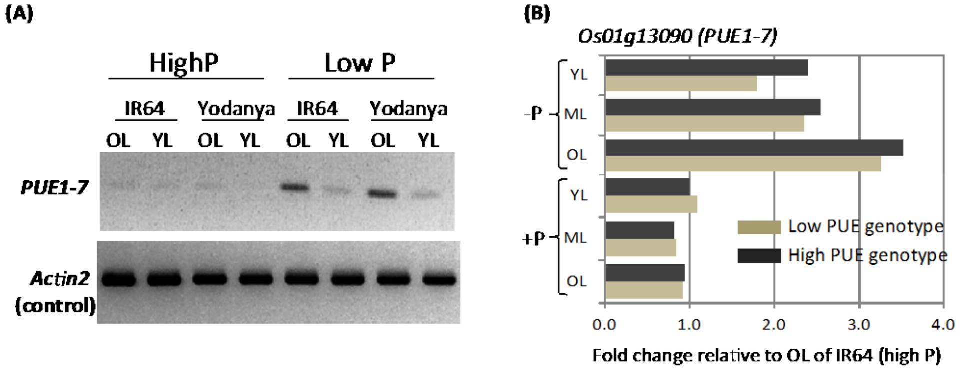


Fig. 2. Gene expression analysis for candidate gene (*PUE1-7*) of the PUE locus on chromosome 1

(A) Semi-quantitative RT-PCR (B) qPCR for candidate gene *PUE1-7*. Gene expression was compared in old leaves (OL, beginning to senesce), intermediate leaves (ML, typically the two leaves below the youngest fully expanded leaf) and young leaves (YL, >50% developed).

Co-differentiation of blast races and rice varieties in Japan

JIRCAS, through its project titled “Blast Research Network for Stable Rice Production,” has clarified the diversity of blast (*Pyricularia oryzae* Cavara) races and the genetic variation of resistance in rice (*Oryza sativa* L.) cultivars of Asia and Africa.

To develop a durable protection system against blast disease, which has caused serious damage to rice production in all rice cultivation areas of the world, the diversities of blast races and the variations of resistance in rice cultivars were examined. This is important for understanding and defining the relationships between the races and cultivars in order to provide basic information.

In Japan, we found multiple evidence of co-differentiation of blast races and rice cultivars, based on pathogenicity studies of blast isolates collected and genetic analysis for resistance of rice cultivars in each region.

The pathogenicity of 310 blast isolates were evaluated based on the reactions to 23 differential varieties, which harbor a single resistance gene in each genetic background and susceptible control, Lijiangxintuanheigu(LTH). It was then classified into three cluster groups, I, IIa and IIb. Blast isolates in group I were mainly virulent to resistance genes of *Pik* alleles, and were distributed with higher frequencies in Hokkaido, Tohoku, Kanto and Hokuriku, compared with the other regions. Those in group IIa did not show virulence to *Pik* alleles’ genes, and were distributed in all regions of Japan. Those in group IIb were not virulent against *Pik* alleles’ genes and *Pii*, *Pi3* and *Pi5*(t), and the distributions were limited to Hokurikiku, Tokai, Chugoku/Shikoku and Kyushu.

A total of 350 rice accessions were classified into four cluster groups, A1, A2, B1 and B2, based on the resistance reactions to blast isolates from Japan and the Philippines. A1 was the most susceptible among the four groups, and included differential varieties and LTH only. The most resistant group was B2 followed by B1 and A2. Many accessions of group B1 were expected to harbor the *Pik* alleles’ genes in the genetic backgrounds, and these were cultivated with higher frequencies in Hokkaido and Tohoku than in other regions. The highest resistance group B2 was found with high frequency in Kanto region.

Blast races and resistance of rice accessions varied in each region of Japan, and the differentiations of blast races corresponded with the variations of rice varieties in each region. In particular, the relationships between virulent blast races for *Pik* alleles’ genes and rice accessions with *Pik* alleles’ genes were found in Hokkaido and Tohoku regions. These results indicated that blast races were differentiated on the basis of resistance genes in rice varieties.

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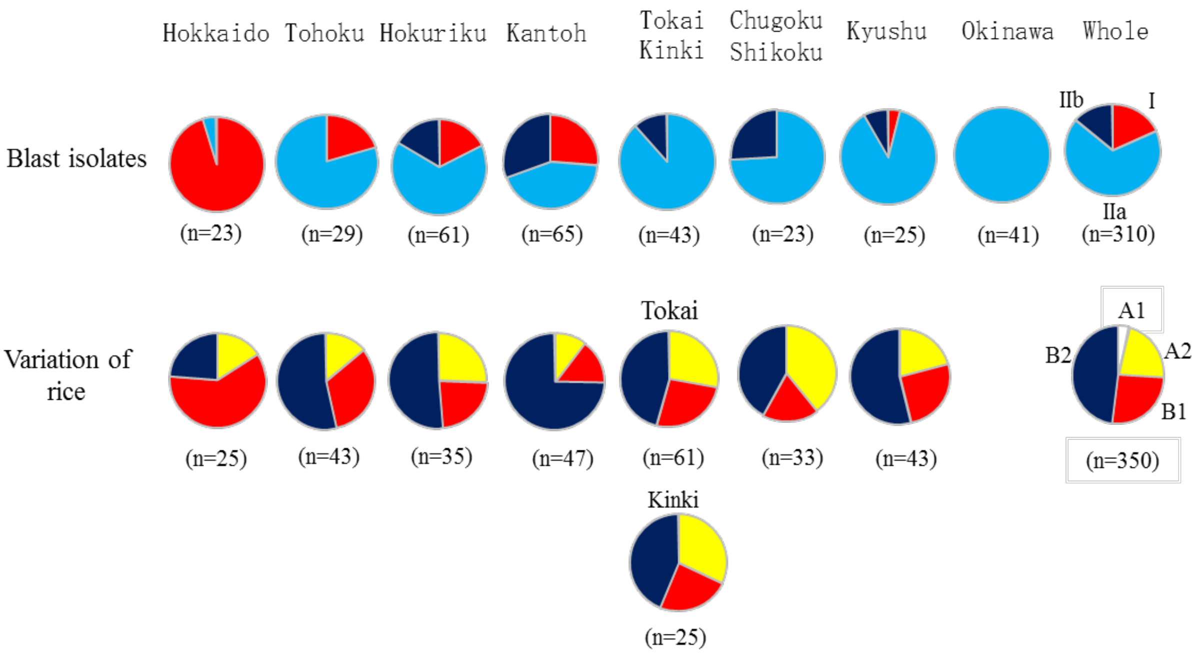


Fig. 1. Genetic variation of blast races and resistance in rice cultivars in each region of Japan. Virulent blast isolates for resistance genes of *Pik* alleles, which were classified into cluster group I (red color), were dominant in Hokkaido and Tohoku regions. These *Pik* alleles' genes were included mainly in the rice cultivar's group blast isolates (BI), and higher frequencies were shown in Hokkaido and Tohoku compared with the other regions.

These data on blast races and rice accessions were modified from Kawasaki-Tanaka et al. (2016, Plant Disease 100: 816-823) and Kawasaki-Tanaka and Fukuta (2014, Breeding Science 64: 183–192), respectively.

New sugarcane varieties using wild sugarcane and collaboratively bred in Thailand

Along with an increasing world population, tight food and energy supplies have become a problem. In areas where agricultural productivity is low, urgent measures are required to increase crop productivity in the fields and to enhance food and energy production. Sugarcane was among those identified as important candidate crops because it can produce food and energy from its sugar and fiber. Therefore, we tried to develop new sugarcane varieties that provide high yields of both sugar and fiber in Northeast Thailand, where sugarcane productivity is low because of severe drought, infertile soil, and sugarcane white leaf disease. Sugar is a food component and can also be a source of bio-ethanol, whereas electricity can be generated from bagasse, a byproduct of sugarcane fiber.

Interspecific hybridization between commercial cultivars of sugarcane and *Saccharum spontaneum* (wild sugarcane) clones, which were collected from all over Thailand, was carried out and F_1 populations were obtained. F_1 hybrids were crossed with commercial cultivars of sugarcane and BC_1 populations were obtained. From the BC_1 population, TPJ03-452, TPJ04-713, and TPJ04-768 were selected and registered as new varieties of sugarcane by the Department of Agriculture, Thailand on February 5, 2015 (Table 1). The sugar yields of TPJ03-452 and TPJ04-768 were at a comparable level with those of commercial cultivars, Khonkaen3 and K88-92, but the fiber yields were higher than those of Khonkaen3 and K88-92. Total fiber yield of TPJ03-452 in three years was about 1.9 times more than that of Khonkaen3 and total fiber yield of TPJ04-768 in two years was about 1.6 times more than that of Khonkaen3 (Table 2). Cane yield, sugar yield, and fiber yield of the first ratoon cane of Khonkaen3 decreased a lot from those of the planting cane but in the case of TPJ04-768, the decrease was less than those by Khonkaen3 (Table 2). TPJ03-452 and TPJ04-768 had thinner stalks and higher fiber content than Khonkaen3 and K88-92. TPJ04-768 had longer stalks than Khonkaen3 (Table 3 and Fig. 1).

Ratooning is a labor-saving and a low cost method of cultivating sugarcane. TPJ04-768 can produce sugar at a comparable level to commercial cultivars, and the decrease of cane yield from the planting cane to the ratoon cane is small. Multiple ratoon cultivation of this variety is expected in Northeast Thailand, which has a long and severe dry season. It will be supplied to farmers and sugar mills for field tests in order to become a recommended variety. A harvester will be needed because the stalks are thin and the leaves are difficult to remove. However, unlike commercial cultivars, these new varieties have high fiber content, thus new methods may need to be developed to produce sugar and ethanol.

(S. Ando, Y. Terajima, S. Tagane, M. Sato, K. Ishiki, M. Matsuoka, Y. Takagi, A. Sugimoto, W. Ponragdee [Khon Kaen Field Crops Research Center, Department of Agriculture Thailand (KKFCRC)], T. Sansayawichai [KKFCRC], A. Tippayawat [KKFCRC])

Table 1. History of new sugarcane varieties

Name of variety	Mother plant	Father plant
TPJ03-452	Uthong1	F_1 interspecific hybrid (K84-200 x *S. spontaneum*)
TPJ04-713	CP72-5028	F_1 interspecific hybrid (88-2-401 x *S. spontaneum*)
TPJ04-768	94-2-128	F_1 interspecific hybrid (88-2-401 x *S. spontaneum*)

Except for *S. spontaneum*, names of commercial cultivars or lines are indicated.

Table 2. Millable cane yield, sugar yield, and fiber yield of new sugarcane varieties per hectare

Name of variety	Millable cane yield (t/ha)				Sugar yield (t/ha)				Fiber yield (t/ha)			
	1st year	2nd year	3rd year		1st year	2nd year	3rd year		1st year	2nd year	3rd year	
TPJ03-452	105.1	76.0 (72)	58.7 (56)	a	10.7	10.4 (97)	4.6 (43)	a	19.7	12.1 (62)	9.4 (48)	a
Khonkaen3	91.4	64.9 (71)	36.5 (40)	a	13.2	9.7 (73)	4.4 (33)	a	10.8	7.8 (72)	3.4 (32)	b
K88-92	92.9	58.9 (63)	39.0 (42)	a	10.9	7.9 (73)	4.0 (37)	a	9.7	5.5 (56)	3.6 (37)	b
TPJ04-713	76.8	77.2 (101)		a	6.6	6.8 (103)		a	9.6	9.3 (97)		ab
TPJ04-768	77.1	79.5 (103)		a	8.9	10.1 (113)		a	13.3	11.9 (89)		a
Khonkaen3	84.0	61.9 (74)		a	12.2	8.6 (70)		a	8.8	7.0 (80)		b

Results at the Tha Phra branch of Khon Kaen Field Crops Research Center (KKFCRC) are shown in the upper table. Sugarcane was planted in March 2008 with two replications; the planting cane of the first year was harvested in February 2009, the first ratoon cane of the second year was harvested in January 2010, and the second ratoon cane of the third year was harvested in December 2010. Results at the KKFCRC were shown in the lower table. Sugarcane was planted in May 2013 with four replications; the planting cane of the first year was harvested in March 2014, and the first ratoon cane of the second year was harvested in March 2015. Sugar yield (Commercial cane sugar (CCS) yield) = millable cane yield x CCS (%) /100. CCS (%): % of calculated recoverable sugar from millable cane. Fiber yield = millable cane yield x fiber content (%) /100. Figure in parentheses indicates the relative yield of the second or the third year to the yield of the first year. The varieties with same letter in the column are not significantly different according to the Tukey's method at P = 0.05 in two-way analysis of variance among varieties and years.

Table 3. Characteristics of new sugarcane varieties

Name of variety	No. of millable cane (/ha)		Length of millable cane (cm)		Diameter of millable cane (cm)		Brix (%)		CCS (%)		Fiber (%)	
TPJ03-452	66026	a	293	a	2.18	c	20.4	a	13.9	a	15.8	a
Khonkaen3	61058	a	172	a	2.62	b	23.2	a	14.4	a	12.2	ab
K88-92	49519	a	223	a	3.13	a	19.5	a	13.5	a	9.3	b
TPJ04-713	62179	a	269	b	2.63	a	17.5	b	9.0	b	12.1	b
TPJ04-768	51282	ab	342	a	2.22	b	22.4	a	12.7	a	15.0	a
Khonkaen3	42468	b	240	b	2.84	a	22.9	a	14.0	a	11.3	b

Results at the harvest of the first ratoon cane of the second year in the field experiments of Table 2 are shown in Table 3. Brix (%): weight % of soluble matter. CCS (%): % of calculated recoverable sugar from millable cane. The varieties with same letter in the column are not significantly different according to the Tukey's method at P = 0.05 in one-way analysis of variance.

Fig. 1. TPJ03-452 (left) and TPJ04-768 (right) planting canes. Photos taken in October 2013.

Movement ability of vector insects of sugarcane white leaf disease

Sugarcane White Leaf Disease (SCWLD) is a dominant limiting factor of sugar production in Thailand, the world's second largest sugar-exporting country. This disease is transmitted by the planting of infected seed-cane and the migration of two insect species, *Matsumuratettix hiroglyphicus* and *Yamatotettix flavovittatus* (Fig. 1). A good knowledge of the movement ability of vector insects is thus required to establish control techniques. However, it is difficult to estimate the insects' movement ability by visual observation because the vectors are tiny species and they hide at the lower part of the sugarcane stalk. Therefore, we carried out mark-recapture experiments at a sugarcane field to evaluate the movement ability of the vector insects.

We made sticky plastic traps based on the vector insects' color preferences. The traps were distributed in a stellate pattern, radiating from a common center to 50m outwards (Fig. 2). The marked vectors were released from the center, and we counted the number of trapped specimens every two days. We stopped the experiment after 20 days because no vector insects were trapped by the 20th day. The experiment was replicated three times for *M. hiroglyphicus* and the number of released specimens were 1980, 1200 and 800, respectively. In the case of *Y. flavovittatus*, the experiments were replicated two times and the number of released specimens was 2700 and 2100, respectively. On the first experiment, the specimens were trapped most frequently at the nearest trap (for both species) (Fig. 3, left). On the other hand, the percentage of trapped *M. hiroglyphicus* at the farthest trap from the release point was only 3.9%. The trapping percentages of the vectors calculated from the cumulative total of all replications were about 10% for *M. hiroglyphicus* and about 13% for *Y. flavovittatus*. These values were deemed sufficient for evaluation of movement abilities. The probability distribution of the reached distance by each species on the 20th day was calculated (Fig. 3, right). We corrected the underestimation caused by trapping and moving out of the trapping range using Yamamura's method (Yamamura, 2003). Based on the probability distance, the estimated average movement distance after 20 days was 162.1m for *M. hiroglyphicus* and 387.5m for *Y. flavovittatus*.

In SCWLD-infested areas, the risk of disease invasion to newly established healthy seed-cane production fields by the vector insects is high. Our results suggest that if we establish a large field and treat it with pesticides, the infection risk inside the field will be low because the movement ability of the vector insects is not high. It means that we probably can produce healthy seed-cane in a SCWLD-infested area. Our results will also be helpful in establishing control techniques when SCWLD occurs in Japan. It must be noted, though, that the specimens in our experiments were forced to move at least once because they were released from plastic boxes that did not have food. Thus, our estimated movement distance values were possibly overestimated compared with actual values using wild vector insects.

(Y. Kobori, S. Ando, M.M. Thein, [Khon Kaen University] Y. Hanboonsong [Khon Kaen University])

Fig. 1. The vector insects of sugarcane white leaf disease.
Left: *Matsumuratettix hiroglyphicus* (body length: 4mm)
Right: *Yamatotettix flavovittatus* (body length: 5-6mm)

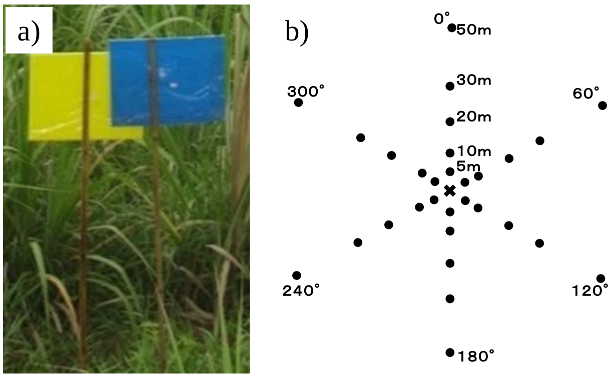
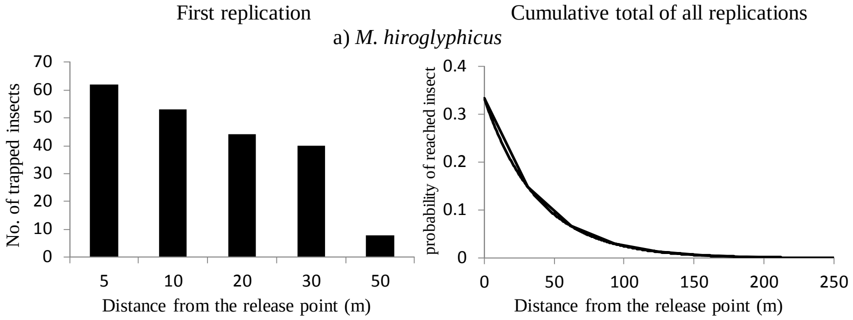
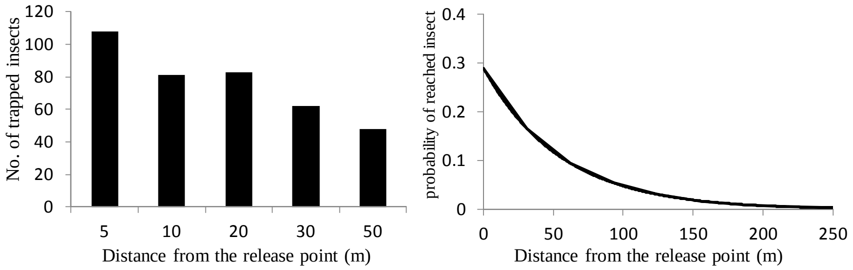


Fig. 2. Details of the sticky trap and the layout.
a) One set of sticky traps made from blue and yellow plastic plates (about 40 x 50cm).
b) The traps were distributed in a stellate pattern at 5, 10, 20, 30, and 50-m intervals from the center. The height was same as the plant height.



a) *M. hiroglyphicus*



b) *Y. flavovittatus*

Fig. 3. Frequency-distance graphs of trapped vector insects from the release point (left) and the probability distribution of reached distance (right).
Left: Results from the first replication. 1,980 *M. hiroglyphicus* and 2,700 *Y. flavovittatus* specimens were released.
Right: The probability distributions were calculated using a cumulative total of 20 days. The underestimation was corrected. The data from all replications were pulled.

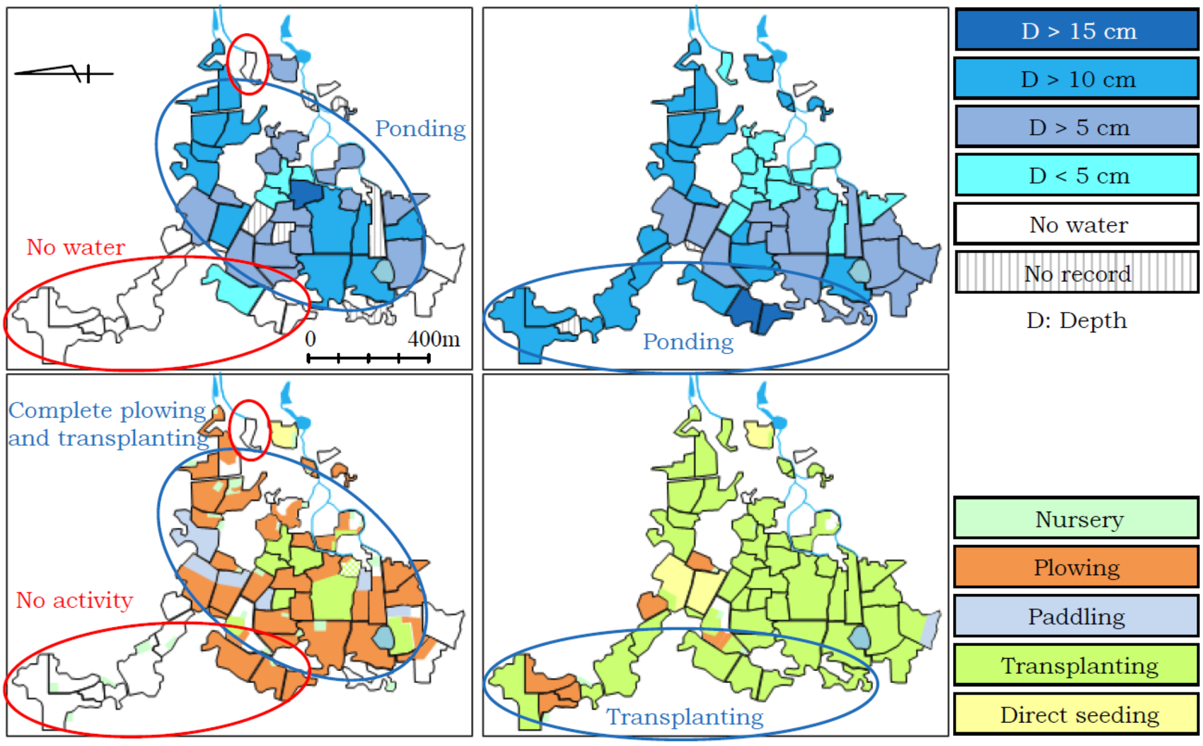
Late transplanting caused by water shortage leads to yield reduction in plot-to-plot fields in Central Laos

Irrigation systems in the semi-mountainous areas of Laos (officially the Lao People's Democratic Republic) are not well-developed. Most farmers do not have direct access to irrigation water, thus they depend mainly on plot-to-plot irrigation and rainwater. Fields in the upper parts of the system have priority, and there is low flexibility in water use especially in the lower parts, including the target area at N Village in the northwestern part of Vientiane Province. This study aims to clarify the location of low productivity fields, identify the constraints to crop yield, and propose measures to increase yield, in the hope of making a contribution to improve lowland rice productivity.

As noted during the 2013 crop season, transplanting of rainy season rice started at the beginning of July and was completed in mid-August. However, even as transplanting went on in the upper and middle parts of the lowland area in mid-July, the lower fields still had not received water and had not even began plowing. In such fields, transplanting started from the beginning of August (Fig. 1). After transplanting, there was no serious water shortage in the entire lowland field areas. Fields with yields greater than 4.0 t ha^{-1} were mainly located in the upper and middle parts; in contrast, fields with low yields (less than 2.0 t ha^{-1}) were located in the lower part of the area. A relational analysis of the yield at 137 plots showed that grain yield was significantly higher in fields that had early ponding (before 20 July) and early transplanting (in July) than in fields that had late ponding (after 21 July) and late transplanting (in August) (Table 1). There was no correlation between soil fertility and rice grain yield (total nitrogen: R^2=0.004, available phosphorus: R^2=0.08). Field experiments also indicated that grain yield in early transplanted field (in July) was higher than in late transplanted field (unpublished data). The results suggested that late transplanting caused by water shortage led to yield reduction in the lower fields of the plot-to-plot irrigated area. In order to increase grain yield in the lower fields, transplanting should be completed by the middle of July, and early irrigation or increasing irrigation amount in the upper fields are necessary to accelerate water supply to the lower fields.

In order to share water resources to the lower fields, a consensus on water allocation among villagers is essential. The head of N village or a local government staff should play the role of coordinator. Farmers' working schedules should also be arranged to avoid labor conflicts caused by the change in transplanting time. In addition, water resource development and irrigation facility improvements are necessary to increase the capacity according to expanding water demand from the mid-June to early July. Irrigation facilities are not developed in 73% of agricultural lands in the five countries of Indochina, which means that most fields are irrigated plot-to-plot. The results of this research, therefore, can contribute to such plot-to-plot fields.

(H. Ikeura, S. Phongchanmixay [Agricultural Research Center, National Agriculture and Forestry Research Institute, Laos], S. Phonsagon [Institute Technology Bandung], S. Inkhamseng [National University of Laos])



14 July 2013 04 August 2013

Fig. 1. Surface water depth (upper) and practiced farming activities (lower) in each field

Table 1. Relationship between grain yield and start times of ponding/ transplanting

Classification		N*	Avg. grain yield t ha^{-1}	Note
Start time of ponding	Before 20 Jul	108	3.87 ^{a}	Significant difference between a and b at $p < 0.05$ according to t test
	After 21 Aug	29	2.22 ^{b}	
Start time of transplanting	Before 14 Jul	28	4.20 ^{a}	Significant difference between a and b at $p < 0.05$ according Tukey-HSD test
	15 Jul – 28 Jul	64	3.68 ^{a}	
	29 Jul – 11 Aug	45	2.88 ^{b}	

* Sampling quadrats (1 m × 1 m) were installed in 47 field blocks as shown in Fig. 1. Three large field blocks were divided into two parts, and three quadrats were installed for each (in total 150 plots in 50 blocks). The relationship between yield and times of field ponding and transplanting were analyzed. The 13 samples (4 plots harvested by farmers before sampling and 9 plots in direct seeding field) were excluded from the analysis (n = 137).

Techniques for collecting black soldier fly eggs year-round for use as a promising feed material for fish culture

In rural areas of Laos, fish culture development is strongly encouraged for stabilizing food self-sufficiency as well as animal protein supply. However, feed cost occupies a large portion of total cost in fish culture operations and needs to be reduced. The black soldier fly (BSF, *Hermetia illucens*) is distributed over Laos, and its larvae (Fig. 1) could be a promising feed material for fish culture because of its high protein content. By using the larvae, the feed cost for fish culture is expected to be largely reduced because they can be reared with food residues and livestock manure. Although people in Laos commonly consume a wide range of insects as food, BSF is not included, which means that it has exploitable potential. This study, therefore, aimed at the development of a year-round egg collection technique that can be applied by small-scale farmers. The following findings were obtained:

1. The larvae of BSF contain considerable nutrients (e.g., protein and fat) (Table 1) and are valuable as feed material for fish culture.
2. BSF adults are attracted to outdoor oviposition traps throughout the year; however, the oviposition incidence has large fluctuations, thus periods with scarce oviposition were observed during seasonal occurrence (Fig. 2).
3. Although some reports mentioned that around 1,000 newly-emerged BSF adults must be released in a large net cage (about 2 m on all sides) to obtain fertilized eggs of BSF, it was also observed that oviposition can occur in a smaller-scale system, with 100 adults in a small net cage (27 cm on all sides) as performed in the present study (Fig. 1).
4. Although previous reports mentioned that sunlight and/or a large incandescent lamp, in addition to the large cage, were necessary for oviposition of BSF, the oviposition was observed using other smaller illuminants, e.g., a 40W fluorescent light or a 20W LED light, like in the present study (Table 2). The pre-oviposition periods were 4–5 days, and the oviposition periods lasted approximately 7 days under artificial lights and about 9 days under sunlight/artificial light. The fertilization rates under the latter were relatively higher.
5. Stable production of BSF larvae that is low-cost and space-saving became more feasible with application of the above-mentioned system.

In addition to the above findings, the following observations were noted:

1. Fruit rinds (e.g., water melon) are efficient as attractants, resulting to an influx of incoming BSF adults to the oviposition traps.
2. It is necessary to clarify the number of ovoposition events/ mating frequency of BSF adults during its lifetime to improve the collection of fertilized eggs.
3. The mechanism of enhancing egg fertilization under the sunlight should be studied.
4. This system is applicable not only in Laos but also in other areas / countries.

Fig. 1. A female BSF (upper left), larvae (upper right) and adult BSF in a rearing and egg collection cage made of polyethylene (lower photo)

Table 1. Crude protein, crude fat, and ash contents (% dry weight) in black soldier fly (BSF) larvae and in general commercial feed for fish culture in Lao PDR

Constituent	Content (%)	
	BSF	Fish feed*
Crude protein	67.1	32.0
Crude fat	6.9	4.0
Ash	6.5	–
Others	19.5	74.0

*manufactured by Centago, Thailand

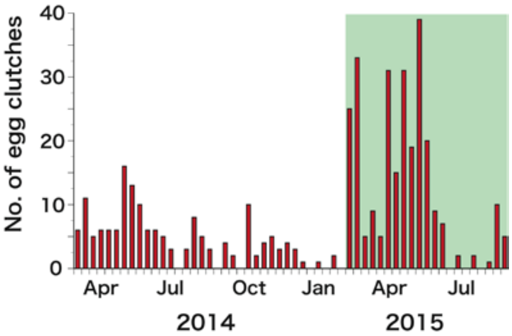


Fig. 2. The number of egg clutches collected at outdoor traps in Laos. (The part shaded in green showed a marked improvement.)

Table 2. Life-history parameters of adult *Hermetia illucens* under different light sources

Life-history parameter	Supplemental light source (mean ± SE)		
	LED lamp only	LED lamp + 2 h sunlight[1]	
Pre-oviposition period (days)	4.6 ± 0.3	4.4 ± 0.3	
Oviposition period (days)	7.6 ± 0.8	9.4 ± 0.8	
No. of clutches/female	0.43 ± 0.04	0.39 ± 0.04	
No. of hatched clutches/female	0.05 ± 0.03	0.15 ± 0.03	*
Hatchability (%)[2]	11.2±9.1	39.5±6.3	*
No. of eggs/female	289.0 ± 27.0	240.2 ± 31.6	
No. of hatched eggs/female	43.7 ± 35.8	84.4 ± 19.0	
Male longevity (days)	12.8 ± 0.2	14.1 ± 0.3	*
Female longevity (days)	12.3 ± 0.2	12.7 ± 0.2	

*Difference was significant between light sources (ANOVA, $p<0.05$)

[1] Exposed to sunlight between 1000 and 1200 for the first 15 days after emergence as adults (14 out of 15 days had clear weather).

[2] Number of hatched clutches/total number of clutches

Specific tree species preferred for use as firewood in Central Laos

The villagers of Laos harvest non-timber forest products (NTFPs) from forests for daily use. One of the many NTFPs obtained from forests is firewood, their main fuel source. Based on a survey in N village, each farming household consumes approximately 1.94 tons per year or a total of 272 tons per year for the village’s 140 households. This is equivalent to 16 hectares of forest area. Villagers prefer two species in particular, and this has had a major influence in decreasing the number of forest trees in central Laos.

Firewood harvesting areas at the target villages in Vientiane Capital were also surveyed. The areas are about 30-60 minutes by foot from the village center and the villagers largely focus on hilly areas where timber harvesting is permitted. A single harvest requires about three to four hours. Besides leftovers from slash-and-burn operations, firewood also comes from living trees that have been cut down (Fig. 1).

Firewood is used mostly as cooking fuel and for adjusting feed for livestock (pigs, chickens). The difference in usage per household is small. On average, a household uses about 1.94 tons (dry weight) per year, thus the amount of firewood consumed by the whole village’s 140 households comes to about 272 tons per year.

A survey was also conducted to calculate the vegetation of firewood harvesting areas (5-year fallow) and forest areas using the biomass estimation equation for slash-and-burn fallow forests. Results showed that the village’s yearly consumption is equivalent to 16 hectares of felled trees. Areas where firewood harvesting is permitted total to about 800 hectares.

About 50 tree species, including bamboo, are used as firewood. Of those consumed, people prefer the two species, *Cratoxylum* sp. (Hypericaceae), which is preferred by survey respondents, and *Peltphorum dasyrachis* (Fabaceaae) (Fig. 2). People prefer these two species because of their burning efficiency and heat retention qualities. The potential yield of *Cratoxylum* and *Peltphorum* is 0.12 and 0.28 ton per hectare, respectively. The demand for these two particular species is especially high, thus planting these tree species will expectedly reduce the amount of labor for harvesting firewood and ensure a stable fuel supply.

Projects in the forestry field, like those implemented under REDD+, are aimed at obtaining basic information upon investigation of the amount of firewood taken from forests. Because forest vegetation changes depending on the region or fallow length, carrying out a vegetation survey of the applicable region is necessary in order to estimate forest area from firewood consumption information.

Due to the high demand for these tree species, setting up a community forest where members can harvest firewood is suggested. In particular, ‘white’ charcoal produced by burning *Cratoxylum* fetches a higher price than black charcoal, thus higher added value can be expected if this species is selected for planting. Furthermore, community forestry of *Cratoxylum* sp. is expected to not only positively affect labor hours but also improve everyday rural life.

(K. Kimura, R. Yoneda, Bounpasakxay Khamphumi [Forest Science Research Center], Singkone Xayalath [FSRC])

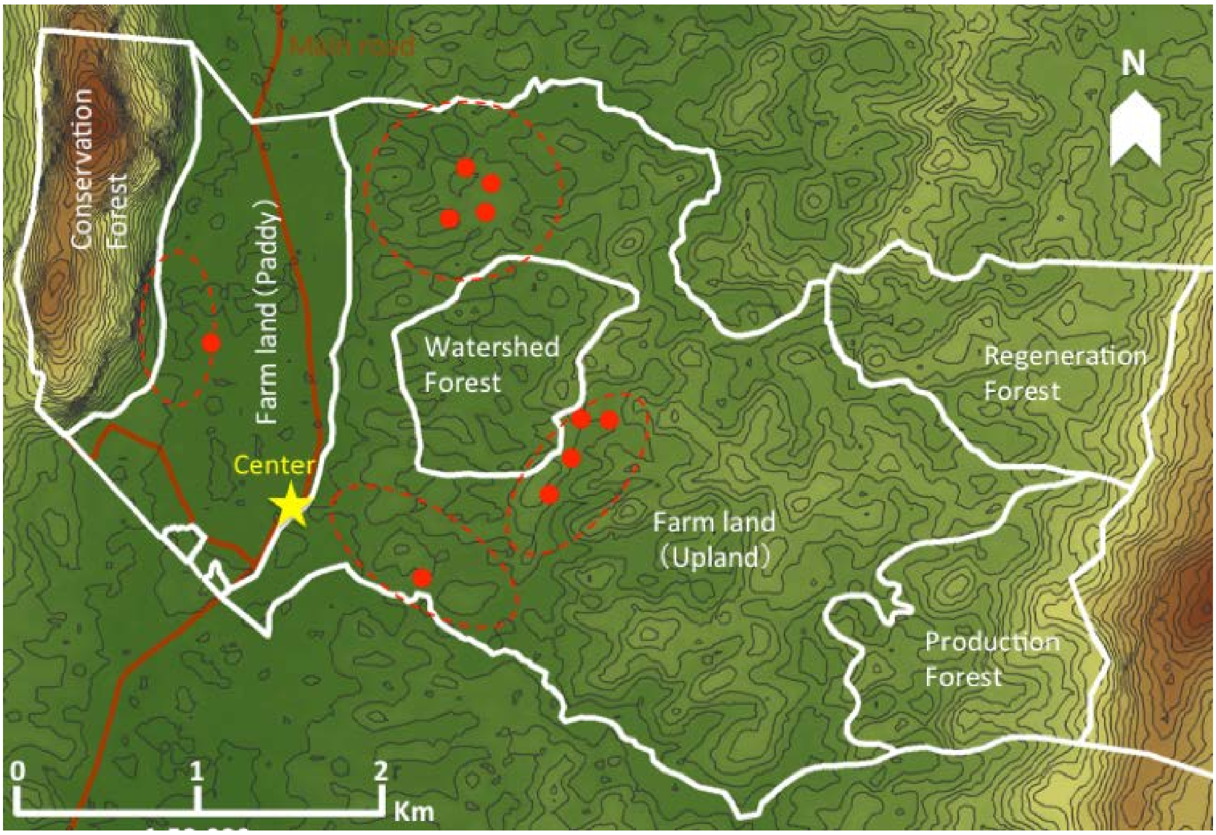


Fig. 1. Firewood harvest locations of target survey villages in Vientiane Capital.
Red dashed lines are harvesting lands as indicated by farmers. Red dots are actual 2013 harvesting sites.
Contour interval is 20m.

Fig. 2. Villagers prefer these tree species for firewood use（Left: *Cratoxylum sp*., Right: *P. dasyrachis*）

A Laotian tree database including useful varieties in secondary forests after slash-and-burn agriculture

The inhabitants of rural Laotian villages harvest various non-timber forest products (NTFPs) from their forests, hence basic information on these plants is necessary for the preservation and management of the forests as well as for dendrology (study of woody plants) and NTFP studies. However, in Laos, where national development began late, there has been no progress as regards the maintenance of illustrated plant references and related materials. *Forests and Trees of the Central Highlands of Xieng Khouang, Lao PDR* (Lehmann, L., Greijmans, M. & Shenman, D., 2003), despite its information being limited to the arboreal varieties of only a single region, is one of the precious few such volumes about Laos. Unfortunately, it is out of print and there are no existing plans for a second edition, which means that there are currently (as of 2015) no illustrated reference books available at places like bookstores. In order to assist forest investigations by researchers, forest preservation administration officers, and NGO personnel, among others, we began constructing a database of trees and shrubs as foundational information for the maintenance of harvested leaf specimens.

We prepared leaf specimens taken from over 300 tree samples gathered in post-slash-and-burn second-growth forests (fallow forests) in the investigated central Laotian villages. We have finished identifying 120 of them (Table 1) and assigned code numbers to the identified samples so we can look them up. The samples were then collected and maintained for browsing at the leaf sample repository in Laos Forest Research Center.

In samples captured with a CMOS sensor, information necessary for identification, such as the presence or absence of fine hairs, is lost from the image. In order to make a detailed observation of harvested leaf specimens on a computer screen, high-resolution images were captured using a CCD sensor. At present, we are processing approximately 70 high-resolution images.

The specimen images can be browsed on the newly-created internet database titled “Specimen Trees of Secondary Forests in Lao PDR.” In this database, local names, scientific names (genus and species), usage (in Laos), photographs, specimen images, specimen collection locations, specimen codes, and links to existing databases aside from JIRCAS are included. It is also possible to look up the local names in Laotian (Figure 1). Furthermore, we have linked our data to the GBIF (Global Biodiversity Information Facility) and the EoL (Encyclopedia of Life) databases.

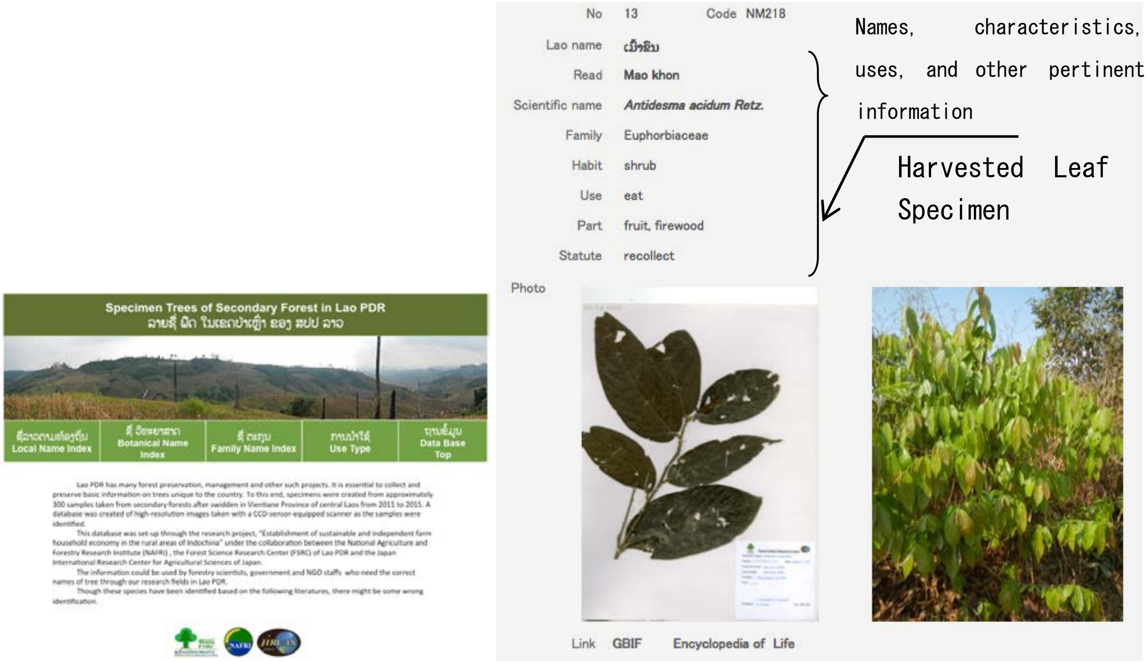
Several forest-related projects are underway in Laos, and we expect that our results will be useful to the project staff, the Laotian government and universities, and so on. In particular, several REDD+ projects are in preparation within Laos, and they are expected to make use of this database.

We will continue adding new information to the published databases in order to hasten the identification of as yet unidentified tree varieties. Moreover, because our focus has been on specimens collected from the slash-and-burn fallow forests of central Laos, it will hereafter be necessary to compile specimens and local names from other Laotian regions.

(K. Kimura, Singkone Xayalath [Forest Science Research Center], Bounpasakxay Khamphumi [FSRC])

Table 1. Compiled database showing some of the trees appearing after slash-and-burn agriculture

Local Name	Local Name	Scientific Name	Family Name
ຄັບ	khup	*Maesa ramentacea* Wallich	Loganiaceae
ຕອງຕາແອນ	Tong ta van	*Mallotus paniculatus* Mull.	Euphorbiaceae
ສົ້ມພົດ	Som phot	*Rhus chinensis* Mill.	Anacardiaceae
ເໝືອດ	Muat	*Aporosa villosa* (Lindl). Baillon	Euphorbiaceae
ຕິ້ວຂົນ (ໜາມ)	Tiew khon (Nam)	*Cratoxylum maingayi* Dyer	Hypericaceae
ນ້ຳກ້ຽງ	Nam kieng	*Gluta usitata* (Wall.) Ding Hou	Anacardiaceae
ກະເບົາ	Ka bao	*Hydnocarpus ilicifolia* King	Achariaceae
ນົມຍານ	Nom nhan	*Barringtonia annamica* Gagnep.	Lecythidaceae
ໝາກກໍ່	Mark Kor	*Castanopsis* sp.	Fagaceae
ສະຄາມ	Sa kham	*Peltophorum dasyrrhachis (Miq.)*	Fabaceae



Specimen Trees of Secondary Forest in Lao PDR
ລາຍຊື່ ພືດ ໃນເຂດປ່າເຫຼົ່າ ຂອງ ສປປ ລາວ

Lao PDR has many forest preservation, management and other such projects. It is essential to collect and preserve basic information on trees unique to the country. To this end, specimens were created from approximately 300 samples taken from secondary forests after swidden in Vientiane Province of central Laos from 2011 to 2015. A database was created of high-resolution images taken with a CCD-sensor-equipped scanner as the samples were identified.

This database was set-up through the research project, "Establishment of sustainable and independent farm household economy in the rural areas of Indochina" under the collaboration between the National Agriculture and Forestry Research Institute (NAFRI), the Forest Science Research Center (FSRC) of Lao PDR and the Japan International Research Center for Agricultural Sciences of Japan.

The information could be used by forestry scientists, government and NGO staffs who need the correct names of tree through our research fields in Lao PDR.

Though these species have been identified based on the following literatures, there might be some wrong identification.

No 13 Code NM218
Lao name ເມົ້າຂົນ
Read Mao khon
Scientific name *Antidesma acidum Retz.*
Family Euphorbiaceae
Habit shrub
Use eat
Part fruit, firewood
Statute recollect
Photo

Names, characteristics, uses, and other pertinent information

Harvested Leaf Specimen

Fig. 1. Database of trees appearing in slash-and-burn secondary forests (left: lookup screen, right: tree classification information screen)
URL: **http://www.jircas.go.jp/database/secondarytreelaos/**

Improving the productivity of bean sprouts with slightly acidic electrolyzed water

Sprouts are among the most popular and favorite vegetables in Japan and other countries. They are easy to produce and contain many nutrients that benefit human health. During the germination of seeds and the growth of sprouts, watering at a regular time is needed; however, if the surrounding environment is humid and warm, it will provide a suitable condition for microbial reproduction. Once high microbial populations exist on the sprouts, it will have a negative impact on the shelf life of sprouts and ultimately on human health. Sprouts are often used as ready-to-eat vegetables, thus measures should be taken to control microbial contamination on the sprouts, and a successful seed decontamination treatment must be performed to inactivate microbial pathogens while preserving seed viability, germination, and vigor.

Slightly acidic electrolyzed water (SAEW), which has a near-neutral pH and contains available chlorine concentration (ACC), can be generated by electrolyzing dilute hydrochloric acid. SAEW has many advantages: 1) It is a kind of high-efficiency disinfection agent; 2) It is converted to normal water after use, so it is environmentally friendly; 3) It physically kills microorganisms, and prevents microorganisms from acquiring resistance; and 4) It functions at room temperature, so SAEW does not result in changes in ingredients, texture, scent, or flavor which often occur after heat treatment. In this study, SAEW was applied in producing bean sprouts and the effects of SAEW on germination, sprout growth, and physiological activity of bean seeds were evaluated.

Results demonstrated that SAEW with available chlorine concentration (ACC) of around 10 mg/L could greatly improve germination percentage (Fig. 1). Sprout length of soybean seeds soaked and then watered during germination period by SAEW was significantly longer than that of tap water (TW) control ($P<0.05$). Also, using SAEW instead of TW in either soaking or germination period could enhance sprout length (Fig. 2).

Results also indicated that soybean soaked in SAEW prior to germination greatly affected the later growth of sprouts. The length of soybean sprouts after five days of germination treated by TW or SAEW with different pH and ACC are shown in Figure 3. Furthermore, both pH and ACC of SAEW can affect the growth of soybean sprouts. Figure 4 shows the effect of SAEW on the length of mungbean sprouts. Mungbean sprouts treated with SAEW of ACC 10, 20 and 30 mg/L grew longer than TW control by 23.87, 25.81 and 23.04 %, respectively, after soaking procedure.

In Japan, SAEW has been an authorized food additive since 2002 and a specified agricultural chemical since 2014 because of its proven biological safety and effectiveness as a bactericide even at a low ACC of 10–30 mg/L and pH of 5.0–6.5. SAEW shows promise as a kind of disinfectant for seed sprouts, not only by reducing microorganism populations and prolonging shelf life but also by promoting sprout growth.

(E. Tatsumi, S. Nirasawa, H. Liu [China Agricultural University], R. Liu [CAU])

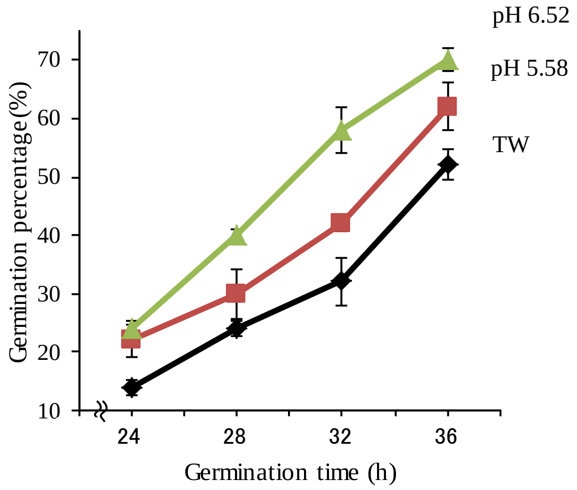


Fig. 1. Effect of slightly acidic electrolyzed water (ACC 10 mg/L) on the germination percentage of soybean seeds

TW: Tap water (pH 7.35 and ACC not detected)

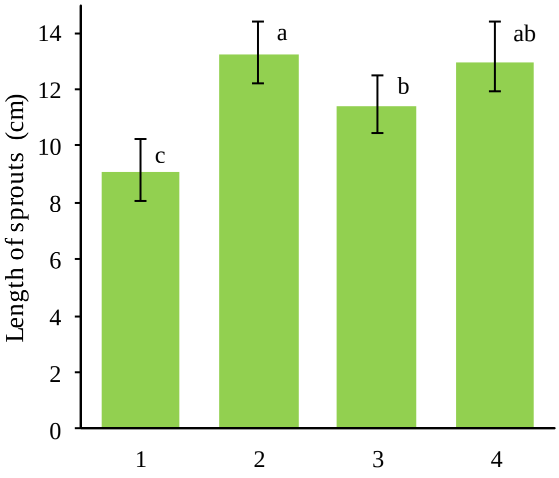


Fig. 2. The length of soybean sprouts treated by slightly acidic electrolyzed water (SAEW) after five days of germination

1-soaked and watered during germination by TW

2-soaked and watered by SAEW

3-soaked in SAEW and watered by TW

4-soaked in TW and watered by SAEW

Different letters mean statistically significant difference (P<0.05).

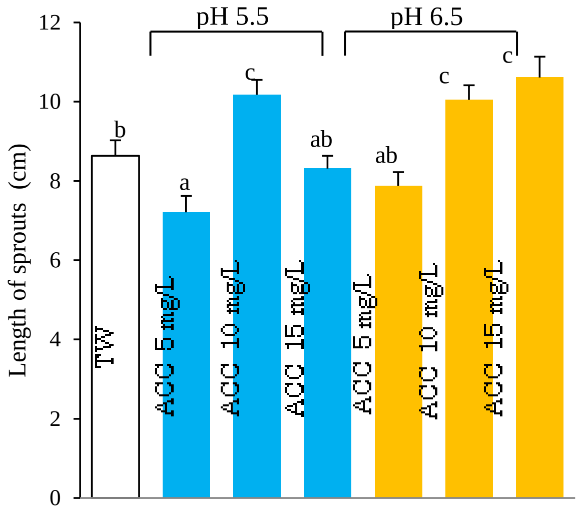


Fig. 3. The length of soybean sprouts treated by TW and SAEW after five days of germination

Different letters mean statistically significant difference (P<0.05).

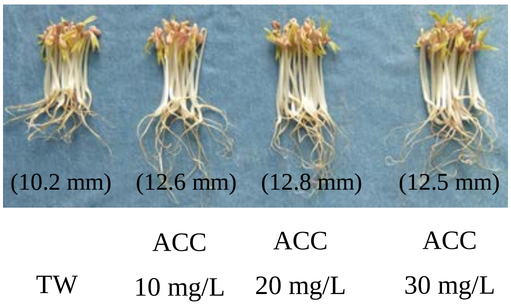


Fig. 4. The effect of SAEW on mungbean sprouts after 108 hours of germination

The length of mungbean sprouts is written in parentheses.

Construction of “The Traditional Fermented Foods of Thailand” Database

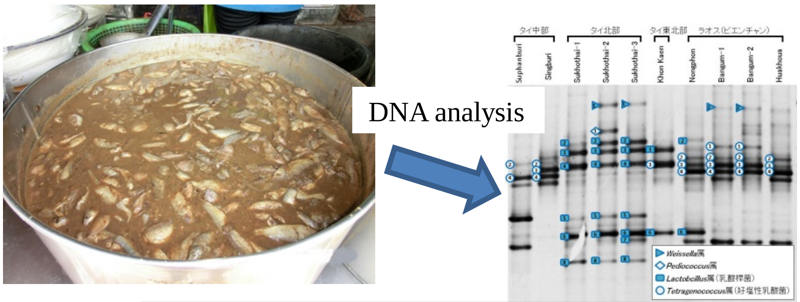
Various fermented foods that are produced and utilized in Thailand have been influenced by its neighboring countries. This research, therefore, looked at the possibility of adding more economic value to local food resources by employing traditional production technologies, specific fermentation microorganisms, and characteristic components. A review of existing literature revealed that only a few materials containing comprehensive information on traditional fermented foods of Southeast Asia have been collected. Kasetsart University of Thailand had once published such a book, entitled “The traditional fermented foods of Thailand” (Bhithakpol et al., 1995), but it is difficult to obtain today. With this in mind, JIRCAS constructed and opened an electronic database on traditional fermented foods of Thailand on the web, with recently obtained information from the results of microbiological studies in JIRCAS.

The database (https://www.jircas.affrc.go.jp/DB/DB11/) is composed of articles featuring 86 kinds of foods (26 fishery products, 8 animal products, 17 fruits, 23 vegetables, 6 rice products, 4 soybean products, and 2 others). Each article contains the local names in Thailand, ingredients, fermentation process, microorganisms, conditions, ways of eating, general nutritional information and pictures. They also show recent information on microorganisms, such as lactic acid bacteria in fermented fish, which have been identified by JIRCAS researchers. The articles have been organized by material and are easy to search.

Various traditional foods in East and Southeast Asia have many common characteristics and employ similar production technologies. By sharing information, it is expected that the production practices and quality of food in each of the countries are improved, and that newly designed food products would be developed in the future based on traditional technologies.

(K. Nakahara, J. Marui, W. Panthavee [Kasetsart University], G. Trakoontivakorn , [KU] P. Tangkanakul [KU])

Fig. 1. An example of fermented food in East and SE Asia (fermented rice noodle)



Microorganisms
Bacillus subtilis, Bacillus licheniformis, Micrococcus sp., Pediococcus sp., Staphylococcus epidermidis, Staphylococcus sp., Tetragenococcus halophilus, Tetragenococcus muriaticus, Lactobacillus acidipiscis

Fig. 2. Newly identified lactic acid bacteria from fermented fish

Chemical composition

Moisture (%)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	NaCl (%)	Total invert sugar (%)	Acidity as lactic acid (%)	pH	aw
69.3	5.6	1 1	0.9	0.7	0	0.1	0.2	4.0	0.92

Fig. 3. A sample database page (General nutritional information for fermented rice noodle)

THE TRADITIONAL FERMENTED FOODS OF THAILAND

Fermented Food play in an important role in the Thai diet and in the life of Thai people. Most Thai eat a fermented product in one form or another every day. As a country with a seasonal tropical climate Thailand is very dry except for the 3-4 months of the rainy season. Therefore, little food can be obtained for several months of the year when there is a shortage of water and before the crops are ready for harvesting, making food storage and preservation important factors in maintaining food supplies in rural areas.

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Fig. 4. Database homepage

Discrimination of old oil palm trunks to maximize production of fermentable sugars in sap: A promising source of sugars for biofuels and biomaterials

Oil palm (*Elaeis guineensis*) is widely planted for its edible oil in tropical countries such as Malaysia and Indonesia. In general, the palm starts bearing oil-contained fruits 2.5 years after planting, but its productivity becomes lower after 20-25 years, making it necessary to cut the old palms and replant new seedlings at plantation sites.

In Malaysia, an estimated 120,000 hectares of oil palm were replanted annually (from 2006 to 2010) to maintain oil productivity (1, 2). When replanting, old palms are cut and most of them are discarded or burnt at the plantation site. Efficient ways of utilizing oil palm trunks (OPT) are desired for an ideal oil palm plantation and a sustainable palm oil industry, thus we investigated the amount and composition of sugars in the sap squeezed from felled trunks together with moisture content. We also examined the effects of storage of the felled trunks on sugars in the sap (1,2).

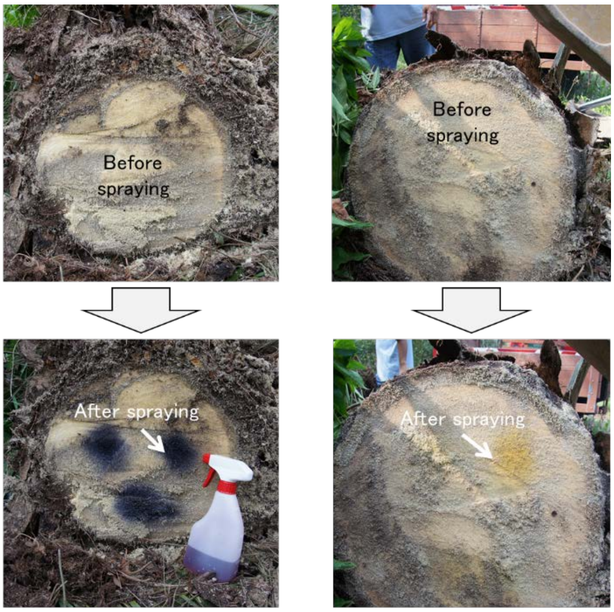
Free sugar content in OPT sap is at maximum level at 30-60 days of storage after logging, thus the sap should be squeezed during this period to obtain the highest sugar concentration for further utilization (2). In addition, there was an accumulation of free sugars depending on starch concentration in OPT, as observed by an increase in sugar content in OPT with high starch content, though it decreases after a certain period of storage (3). Thus, starch concentration in OPT is useful as a key indicator to discriminate OPTs that exhibit increasable fermentable sugars in the oil palm sap during storage of the trunks after logging. On the other hand, it may be difficult to discriminate an OPT that has high starch content from its appearance.

In this study, we reported a method to discriminate a promising OPT among felled OPTs using iodine solution. To easily determine the starch content in felled OPT, iodine solution was sprayed to its cross section. When iodine solution was sprayed to the cross section of OPT containing high starch content, a dark blue color immediately appeared on the surface of the cross section. In contrast, when felled OPT containing low starch content was sprayed with iodine, the color of the cross section did not change.

To confirm whether OPT with high starch content increases its free sugars in the sap during storage, the sugar concentration in sap was compared to sap from OPT with low starch content during storage. Results have shown that the increase of free sugar in sap was observed only in OPTs with high starch content, indicating that this phenomenon depends on starch content in OPT. This method, in which iodine solution is sprayed on OPT cross-section, is useful in the discrimination of promising OPTs that exhibit increasable free sugar content during storage.

(1) Kosugi et al. (2010), J Biosci Bioeng. 110:322-325. doi: 10.1016/j.jbiosc.2010.03.001.
(2) Yamada et al. (2010), Biomass Bioenergy 34:1608-1613
(3) Abdul Hamid et al. (2015), Int. J. Green Energy, doi.org/10.1080/15435075.2014.910786

(A. *Kosugi, T. Arai, S. Nirasawa, Z. A. Abdul Hamid [Universiti Sains Malaysia] ・O. Sulaiman [USM] ・R. Hashim [USM])*



A. Felled OPT (high starch content)

B. Felled OPT (low starch content)

Fig. 1. Spray tests using iodine solution for felled oil palm trunk (OPT). OPT was obtained from a commercial oil palm plantation in Selangor, Malaysia. The starch content of OPT was measured using a total starch assay kit before the spray tests were carried out. The starch concentration of OPT containing high and low starch contents was 46.8% (w/w dry) and 7.1% (w/w dry), respectively.

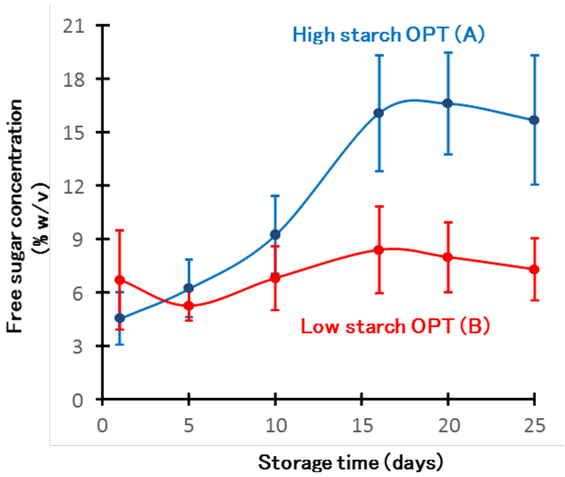


Fig. 2. Storage tests using high starch OPT (A) and low starch OPT (B). Free sugar was measured by high-performance liquid chromatography (HPLC) using squeezing sap of trunk samples obtained from each storage time. The total amount of glucose, fructose, and sucrose shows as free sugar in the sap.

Evaluation of the economic viability of ethanol production from palm sap with low sugar concentration

Some palm saps extracted from old oil palm trunks have low sugar content due to differences in species or in plant physiology. Here, we condensed palm sap with low sugar content by flat membrane filtration, then fermented the condensed palm sap at a high temperature using the thermotolerant yeast, *Kluyveromyces marxianus*. The input energy required to concentrate the palm sap and the output energy that could be generated from the ethanol were calculated. The condensation of sugar in sap from palm trunk required for an economically viable ethanol production was evaluated.

Experiment results showed that when palm sap squeezed from oil palm trunk (MC 80%, 30kg) had 3.1% sugar content, the energy required to condense up to 9.6% by flat membrane filtration was 10.9MJ (Fig.1). The energy required for squeezing and fermentation was 5.8MJ and 0.85MJ, respectively. Total input energy for ethanol production was 17.6MJ when the energy for condensation (10.9MJ) was added (Fig 1).

When ethanol production was conducted using thermotolerant yeast, ethanol (0.0454kg/L and 0.32L) was produced from 9.6% sugar content in palm sap. The output energy from produced ethanol was 6.7MJ (Fig.2). Consequently, the energy balance between input and output was calculated and plotted. When sugar concentration in palm sap reached 6.1% or more, output energy became higher than input energy in palm sap (i.e., output energy turned to positive based on the plot between input and output energy) (Fig.3). The energy required for sugar condensation from 3.1% to 9.6% was in proportion to sugar concentration in palm sap.

A simple method to distinguish high-sugar trunk from low-sugar trunk is reported in another research highlight report. This result only provides information to get appropriate sugar content in palm sap before ethanol production. Even if sugar in palm sap is less than 6.1%, it is still possible to use heat energy from the residue after squeezing the sap. The output energy, however, is reduced when fermentation efficiency is less than 90%.

(Y. Murata, T. Arai, A. Kosugi)

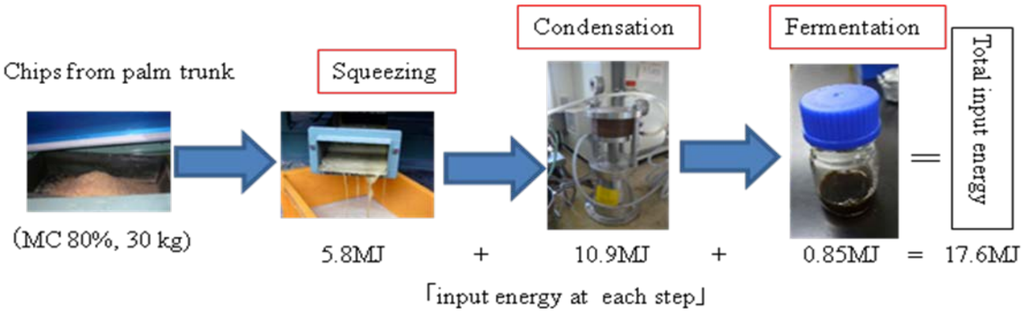


Fig. 1. Total energy required for squeezing, condensation, and fermentation from chips of oil palm trunk (17.7MJ)

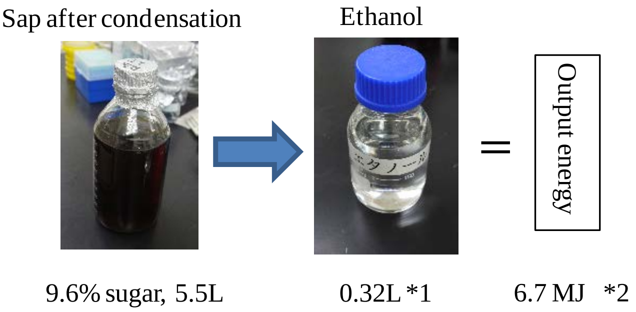


Fig. 2. Calories in ethanol from fermentation of palm sap (Output energy)
*1. Ethanol (L) produced from palm sap 5.5L was calculated from following equation: (0.0454kg/L x 5.5L)/0.789kg/L *2 calories of ethanol = 21.2MJ x 0.32L

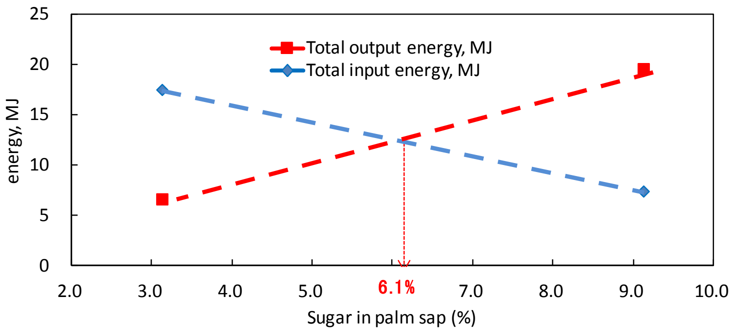


Fig. 3. The energy balance turns positive when the sugar in palm sap is more than 6.1%.

[Others]

Title: The development of biofuel and biomaterial production from biomass in Southeast Asia

Program : Technology development for income and livelihood improvement of the rural population in developing regions

Budget : Subsidy [Asia biomass]

Term: 2013 (2011-2015)

Researchers: Yoshinori Murata, Takamitsu Arai, Akihiko Kosugi

References: 1) Y. Murata, et al. (2015) AIMS Journal 3(2):201-213

Characterization of oil-palm trunk residue degradation enzymes from the isolated fungus, *Penicillum rolfsii*

Oil palm (*Elaeis guineensis*) used in palm oil production must be replanted at 20 to 25-year intervals in order to maintain oil productivity. Consequently, the felled palm trunks represent one of the most important biomass resources in Malaysia and Indonesia. Oil-palm trunk biomass consists of a complex network of cellulose, hemicellulose, and lignin, the major constituents of which are celluloses and hemicelluloses.

To utilize the felled palm trunks specifically for bioethanol and bioplastic production, and to exhibit the advantages of hydrolysis compared to using commercial enzymes, we characterized the crude ligno-cellulolytic enzyme of the fungal isolate *P. rolfsii* c3-2(1) IBRL utilizing oil-palm trunk residues.

We were able to show that the mesophilic fungus *P. rolfsii* c3-2(1) IBRL produces high activity enzymes (P. rolfsii) including xylanase, laminarinase, and arabinase. P. rolfsii displayed higher thermal stability compared with commercial enzymes, Celluclast 1.5 L and Acellerase 1500 (Fig.1). The effects of isolated lignin residual on biomass saccharification revealed that P. rolfsii possesses weak 'lignin-binding' enzymes that may contribute to their higher hydrolysis efficiency on oil-palm trunk residues (Fig. 2). The hydrolysis efficiency of P. rolfsii is 1- to 1.5-fold higher than that of commercial enzymes following 48–72 h of biomass saccharification (Fig. 3). These findings suggest that *P. rolfsii* c3-2(1) IBRL is a fungal strain isolate that can potentially be used as a microbial factory for ligno-cellulolytic enzyme production. Furthermore, *P. rolfsii* c3-2(1) IBRL may represent an alternative for biomass utilization, such as oil-palm trunk residues. The high performance of ligno-cellulolytic enzymes produced by *P. rolfsii* c3-2(1) IBRL deserves significant attention as an alternative to other commercial enzymes for the production of second-generation biofuels and bioplastic.

(T. Arai, K. Lee [Universiti Sains Malaysia], D. Ibrahim [USM], Y. Murata, A. Kosugi)

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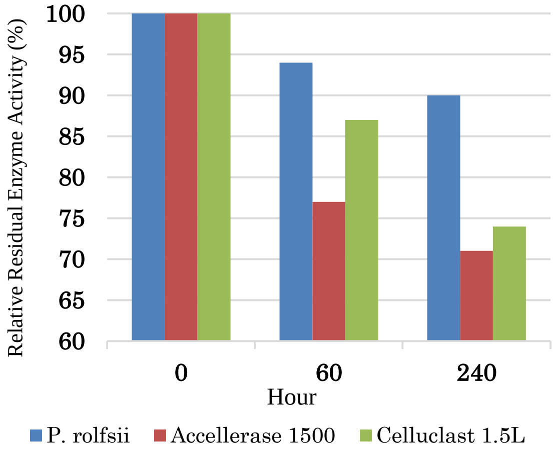

Fig. 1. Residual activity expressed as a percentage of the maximum oil-palm trunk residue activity by P. rolfsii c3-2(1)

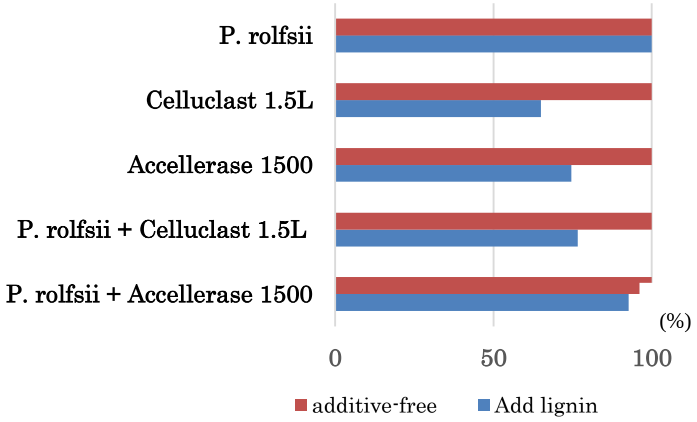

Fig 2. Absorption of P. rolfsii c3-2(1) IBRL enzymes and commercial enzymes on Klason lignin residues after 1.5 h at 4°C

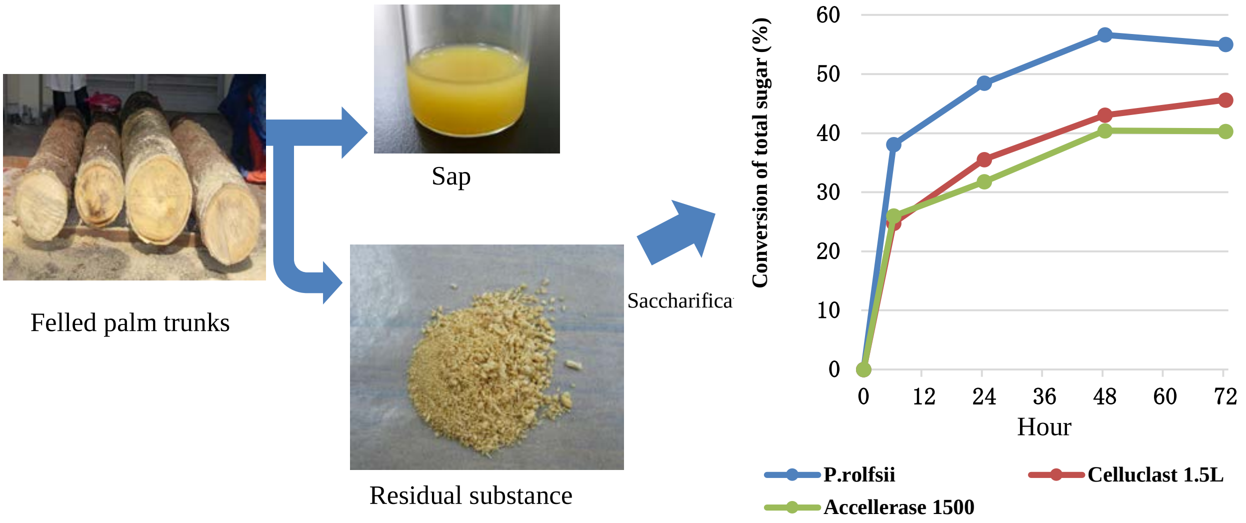

Fig. 3. Time course for the hydrolysis of oil-palm trunk residues using P. rolfsii c3-2(1) IBRL enzyme and commercial enzymes based on the hydrolysis of total sugar conversion (%)

Identification of factors that promote CSR activities by Indonesian palm oil companies

Since 1977, the Indonesian Government has been implementing the oil palm estate development program called the Nucleus Estate Smallholders (NES), whose major objective is to promote benefit-sharing between estate companies and rural communities. The NES is a kind of land allocation agreement between small-scale farmers and estate companies. When companies develop new oil palm estates, they are obliged to allocate a part of the estate to small scale farmers, called “plasma farmers.” In addition to the NES program, palm oil companies have also taken an increased interest in Corporate Social Responsibility (CSR). Although the primary objective of NES is profit making, it is believed that NES also promotes CSR activities by estate companies because collaborations with local communities under the NES program lead to a better understanding of rural societies by the companies. This study aimed to identify the factors that promote CSR activities by Indonesian palm oil companies through an analysis of questionnaire survey results answered by 132 member companies of the Indonesian Palm Oil Association (GAPKI), which is the sole association of Indonesian palm oil producers.

The results of the questionnaire survey showed that all but two respondents were aware that their companies have been implementing CSR activities. Out of 16 CSR activities that were listed, “Infrastructure development” was the most common, followed by “Education support”, “Having CSR staff” and “Environment conservation” (Fig. 1). According to the basic procedures of the NES scheme as stipulated by the Indonesian Ministry of Agriculture, estate companies are required to provide support activities such as “Technical support for crop production”, “Purchasing products at a guaranteed price”, and “Infrastructure development” to plasma farmers. However, among companies that implemented the NES scheme, only around 70% answered that they already provide these three support programs to plasma farmers (Fig. 2). The results of regression analysis performed on data from the questionnaire survey concluded that the performance of the NES scheme, the size of the company, and the perception of relationships with local communities are major driving forces that stimulate participation in CSR activities (Table 1). A previous study found that the NES scheme can improve the fresh fruit bunch yield of plasma farmers by promoting appropriate fertilizer use and providing high quality seedlings (JIRCAS Research Highlights in 2014). The results of the present study indicated that the NES scheme can also promote CSR activities by palm oil companies.

When the NES scheme was launched in 1977, the government strongly supported it with subsidies. However, recent policies have resulted in decreased direct support for the scheme. The findings of this study can be used as evidence for the government to create a more supportive environment to promote the NES scheme.

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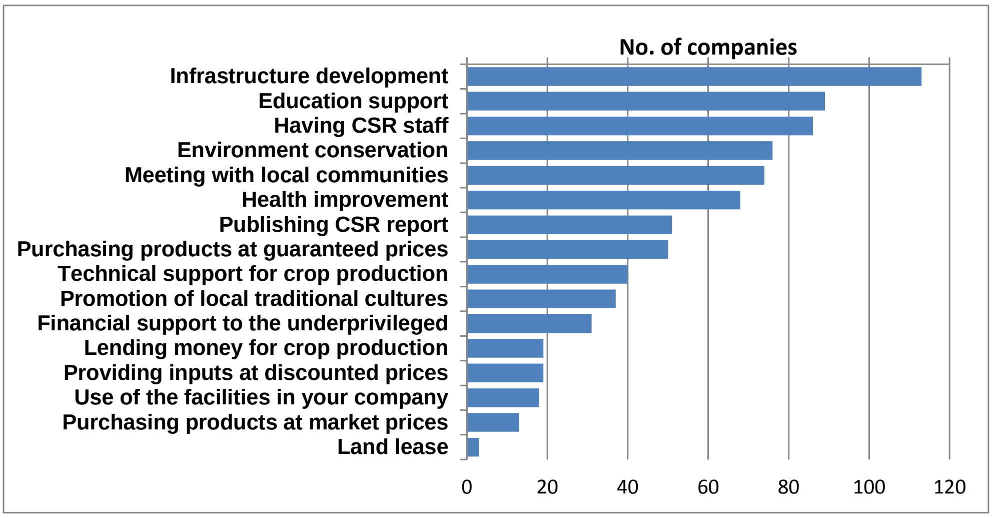

Fig. 1. Number of companies devoted to CSR activities (n=132, Multiple answers allowed)

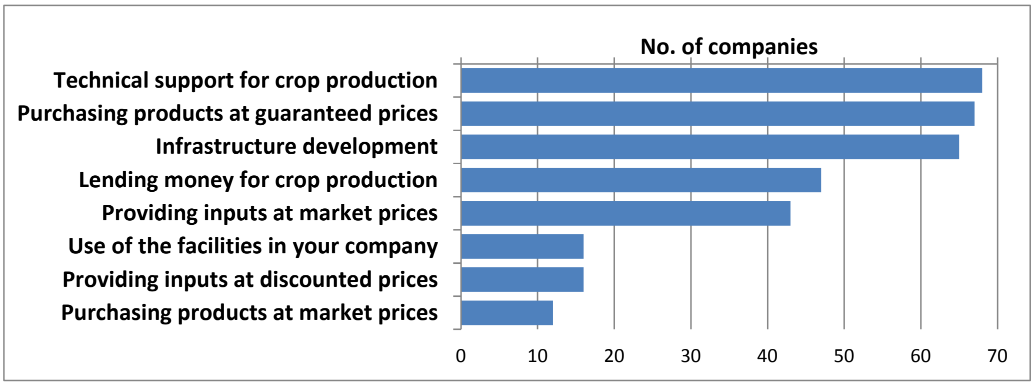

Fig. 2. Implementation of eight programs in the NES scheme (n=91, Multiple answers allowed)

Table 1. Summary results of regression analysis (n=115)

	Standardized partial regression coefficient (P value)
X_1	0.2973 *** (0.0001)
X_2	0.4653 *** (0.0000)
X_3	0.2896 *** (0.0001)
PS	0.0744 (0.3113)
RF	-0.0615 (0.4551)
PD	-0.1235 (0.1363)
RG	-0.0906 (0.2494)
Constant	6.9594
Adjusted R^2	0.4341
P value	(0.0000) ***
AIC	229.4

***: (P<0.01)

$Y = f(X_1, X_2, X_3, PS, RF, PD, RG)$

where

Y: The degree of CSR activities

X_1: The size of the company evaluated by FFB production in metric tons

X_2: A composite variable representing the company's efforts in the NES scheme [a)]

X_3: A composite variable representing the extent to which the company thinks each activity/concept will be effective in establishing better relationships with local communities [b)]

PS: The share of plasma farmers in terms of farmland area in the company

RF: Annual rainfall (mm)

PD: Population density in the province

RG: Regional Gross Domestic Product per capita in the province

a) The score of each sample on the first principal component of Primary Component Analysis (PCA) about variables of the programs in the NES scheme

b) The score of each sample on the first principal component of PCA about variables of component regarding the activities/concepts of the companies for contributing to establish better relationships with local communities

Maintenance of outbred seraya seed production by selectively excluding inbred seeds in natural hill dipterocarp forests

Maintaining forest regeneration is essential for sustainable forest management when timber and other forest materials are extracted. Nearly all unprotected forests in the humid and sub-humid tropical regions should be regarded as disturbed rather than truly primary forests because timber has been selectively logged from them. It has been widely believed that forests have sufficient resilience to recover from selective logging, and selective logging regimes have been widely applied in management programs for tropical forests. However, selective logging may threaten the pollination and sexual reproduction systems of tropical tree species, and hence ultimately the regeneration of healthy cohorts of seeds, seedlings, and saplings of timber trees required for sustainable forest management. Consequently, outcrossing restrictions can markedly increase the proportions of unhealthy offspring through inbreeding depression. Generally, pollination and subsequent outcross mating are susceptible to reductions in population density for tropical tree species, which depend on weak flyer insects for pollination. In particular, tree species of the family Dipterocarpaceae are widely distributed and dominant in Southeast Asian tropical rain forests.

The pollination of tree species belonging to section *Mutica* of genus *Shorea* (Dipterocarpaceae), including our study species, *Shorea curtisii* (seraya in local language), depends mainly on weak flyer insects such as thrips. This in turn leads to lower mature seed production and decreased outcross mating, which have been reported to be associated with low population density. It has also been unveiled how tropical forest species maintain outcrossing at a high level. Outcross mating is maintained not only by the relative amount of self vs. outcross-pollen landing on the stigma but also by some biological processes, such as partial self-incompatibility and inbreeding depression, which can reduce the amount of self-mating. Therefore, we revised our modeling of pollen dispersal and male fecundity (See Tani et al. 2011 in JIRCAS Research Highlights) by incorporating a parameter expressing the biological processes to exclude self-mating, namely partial self-incompatibility and/or inbreeding depression until seed maturation, and simultaneously estimated the parameters of pollen dispersal, male fecundity, and the biological process by hierarchical Bayesian method. We compared parameters expressing the biological processes to exclude self-mating between natural and selectively logged forests. The estimated parameter showed that fertilization of the ovules of self-fertile mother trees was not restricted and that self-fertilized seeds grew into maturity in the selectively logged plot. In contrast, the estimated parameter showed that higher outcrossing in the natural forest was caused by the exclusion of large amounts of self-pollen due mainly to biological processes. Mother trees with higher amounts of total pollen indicated exclusion due to biological processes during seed maturation. These mother trees also showed large female fecundity, which implied that the higher fecund trees tend to exclude self-pollen and/or abort immature selfed seeds.

(N. Tani, Norwati. M., S. L. Lee, C. T. Lee, [Forest Research Institute Malaysia], Y. Tsumura [University of Tsukuba])

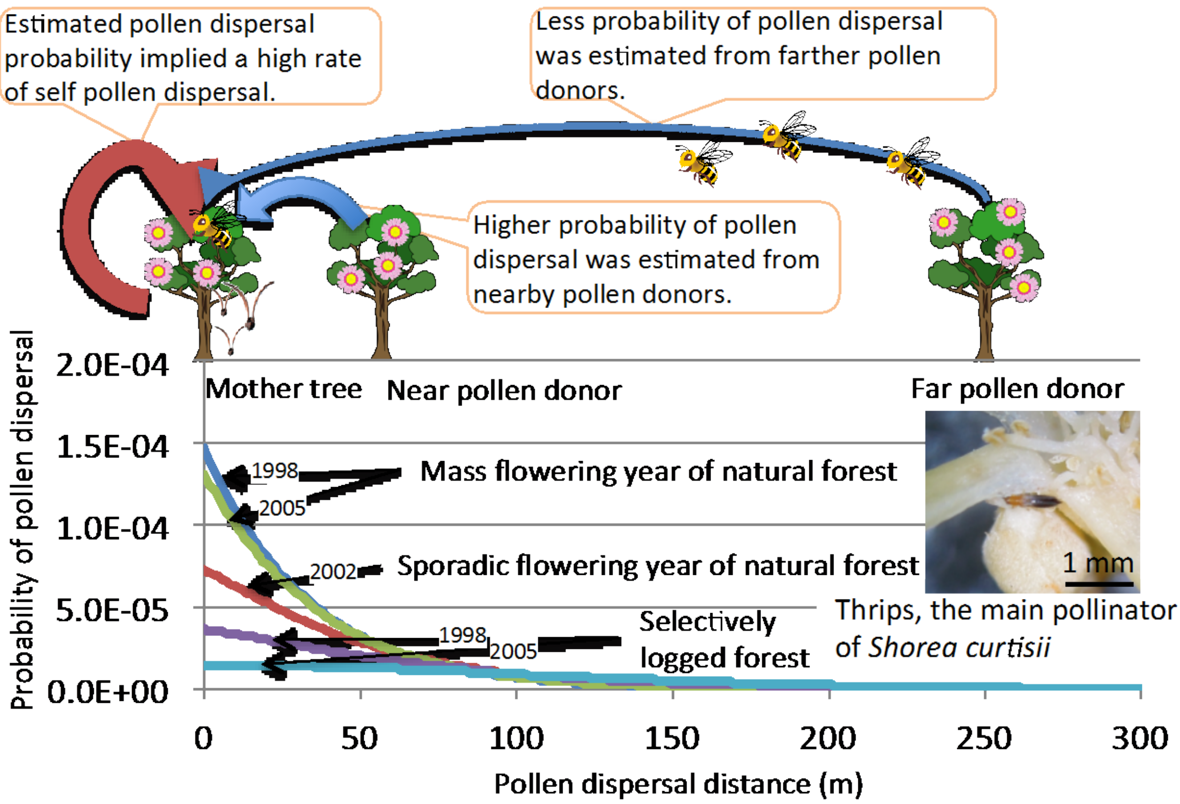

Fig. 1. Probability of pollen dispersal with distance between mother tree and pollen donors.

Pollen dispersal probability was estimated using paternity of 1,492 seeds collected in three synchronized flowering years at a natural forest and paternity of 728 seeds collected in two synchronized flowering years at a selectively logged forest. Self-pollen should be dispersed to each mother tree with higher probability even in the natural forest.

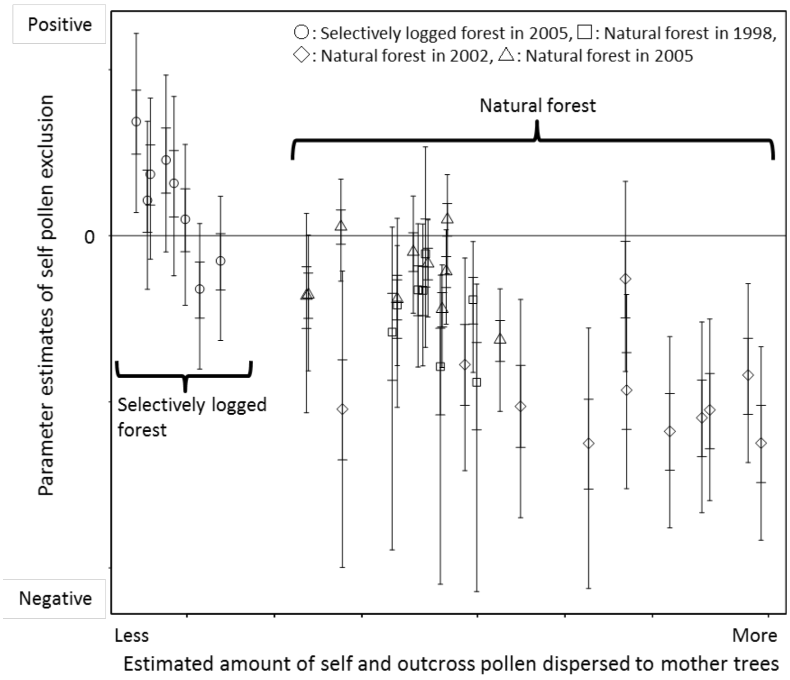

Fig. 2. Relationship between parameter estimates of self-pollen exclusion to total pollen amount dispersed to each mother tree.

The short and long ranges of vertical bar indicate 50% and 95% Bayesian credibility intervals, respectively. When the bar does not cross over zero and is in a negative area, self-pollen and/or selfed seeds were excluded by the biological processes.

Improving the production of giant tiger prawn using an unidentified species of *Chaetomorpha* having euryhaline nature

For three decades, giant tiger prawn (*Penaeus monodon* Fabricius) has been an important aquatic export product of Southeast Asian countries, providing income support to shrimp aquaculturists. However, giant tiger prawn production has recently been decreasing due to pond eutrophication and/or shrimp diseases caused by high-density and intensive aquaculture systems. We have been developing a closed co-culture system incorporating giant tiger prawn and unexploited benthos, *Chaetomorpha* sp., under an international collaboration project between King Mongkut's Institute of Technology Ladkrabang (KMITL) and Japan International Research Center for Agricultural Sciences (JIRCAS).

Chaetomorpha species (Fig. 1) found at the coast of central Thailand is considered to be an unidentified species from the results of morphological observation, ecological monitoring, and phylogenetic molecular sequencing analysis. Field surveys revealed this alga to be abundant throughout the year in stagnant coastal ponds and irrigation channels with salinity of 3.4–90 psu. Its highest mean specific growth rate of approximately 60% day^{-1} (2 mm particle became 20 cm within one week) was observed in laboratory experimental trials at salinities of 20–30 psu at 30 °C (Fig. 2). This seaweed contained 20.4% protein and 64.8% carbohydrates, and giant tiger prawn preferably grazed this alga even when provided artificial feed pellets. When giant tiger prawn was co-cultured with *Chaetomorpha* sp., shrimp growth and feed conversion ratio (FCR*) improved by approximately 57% and 39%, respectively, compared with monocultured prawn (Table 1).

It is expected that this alga can be easily applied to intensive shrimp aquaculture ponds worldwide due to its wide tolerance to salinity. However, experimental data at earthen pond level is needed for practical use of this co-culture system. An analysis of consumers' preferences, such as shrimp color, taste, etc., also needs to be undertaken in anticipation of product distribution.

* An index indicating the feed quantity necessary to increase the specific weight of fisheries animals. Lower value shows greater efficiency.

FCR = feed given (dry weight) / weight increase in fisheries animals (wet weight)

(I. Tsutsui, D. Aue-mneoy [King Mongkut's Institute of Technology Ladkrabang])

Fig. 1. *Chaetomorpha* sp. and giant tiger prawn. Inset photo (upper left corner) shows microscopic photograph of *Chaetomorpha* sp.

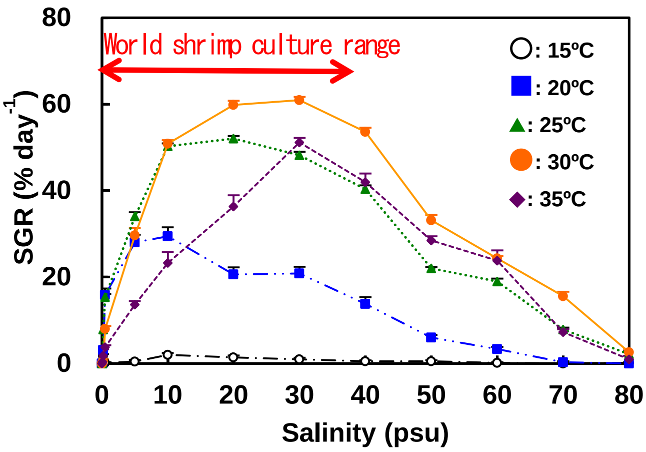

Fig. 2. Mean specific growth rates (SGR) of *Chaetomorpha* sp. under different salinities and water temperatures

Table 1. Growth performance, SGR, and FCR of giant tiger prawn juveniles in a monoculture and in co-culture with *Chaetomorpha* sp.

	Monoculture	Co-culture
Mean initial weight of individual shrimp (g)	0.39 ± 0.03 ^{a}	0.39 ± 0.03 ^{a}
Mean terminal weight of individual shrimp (g)	7.15 ± 1.28 ^{a}	11.20 ± 0.65 ^{b}
Final weight difference compared with control (%)	100	157
SGR of shrimp (% day^{-1})	4.14 ± 0.27 ^{a}	4.79 ± 0.08 ^{b}
FCR of shrimp	2.39 ± 0.28 ^{a}	1.46 ± 0.62 ^{b}
FCR reduction rate compared with control (%)	100	61

Values are shown as mean ± standard deviation from triplicate data. Different superscript labels within the same row indicate significant difference between means (Tukey-Kramer HSD test, $p < 0.05$).