Sustainable use of biogas digester by applying unused biomass

A biogas digester (BD) is a simple and manageable farm equipment that collects biomass and produces gas through anaerobic respiration (Fig. 1). It was introduced as a Clean Development Mechanism (CDM) project in farming households in Vietnam's Mekong Delta. Pig manure is the main feedstock for BD; however, biogas production will diminish if the number of pigs decreases due to sale of mature pigs, disease outbreaks, or lowered profitability. When biogas shortage is prolonged, households become more inclined to stop using BDs. On the other hand, there is plenty of unused biomass like the fast-growing water hyacinth in canal networks and ponds. With the appropriate technology, applying unused biomass as feedstock will make BD use sustainable.

Annual monitoring was conducted on 435 households from 1 June 2013 to 31 May 2014, and results showed that 44 households did not use biogas for more than one day during this period. Cited as the main reason (55%) for the non-use of BD was the absence of pigs due to stoppage of pig raising activities, pig disease, and sale of mature pigs to market (Fig. 2). Can Tho City, where the CDM project is being implemented, is located at the center of the Mekong Delta and surrounded by tributary streams and canal networks. Plenty of productive and unused biomass like water hyacinth grow in these waterways and ponds. If the unused biomass is applied as feedstock for BD in case of pig shortages, BD use will be stabilized. Experiments applying unused biomass to BD were conducted to examine biogas production from selected materials. The experiments consisted of 4 materials: (1) pig manure (control), (2) duckweed (*Pistia stratiotes*), (3) water hyacinth (*Eichhornia crassipers*), and (4) grass weed, including *Oryza rufipogon Griff*. The size of BD was the same as the one used by households. After cutting these materials to 20-30cm, 2.7kg (dry matter weight) of each material was filled to each experimental BD every day continuously for 30 days and the biogas produced from BDs was measured for 60 days from the start of the experiment. The experiments were replicated 3 times. Results of the experiments showed that biogas production from water hyacinth and from both duckweed and grass weed was 70% and 90%, respectively, compared to the control (Fig. 3). As a verification study, one household that installed a BD was asked to apply only duckweed to the BD as feedstock for a year from the beginning. Results of this study showed that biogas from duckweed could be used as cooking fuel continuously, that it substituted with 2.4t of fuel wood, and that it contributed to 1.8tCO_2/year of GHG emission reductions (Fig. 4).

In summary, this technology contributes to sustainable BD use by bridging the gap in BD feedstock when livestock manure is in short supply. One important thing to consider, however, is that farm households that apply unused biomass as feedstock for BDs must have easy and continuous access to the resource. Although the collection time for unused biomass is shorter compared with fuel wood, it is longer compared with livestock manure. Unused biomass, therefore, should be applied as supplemental feedstock only when livestock manure is insufficient.

(T. Izumi, E. Matsubara)

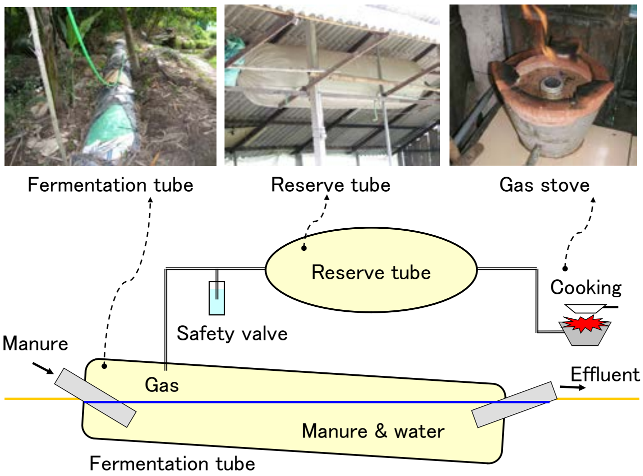

Fig. 1. Plastic-type biogas digester system

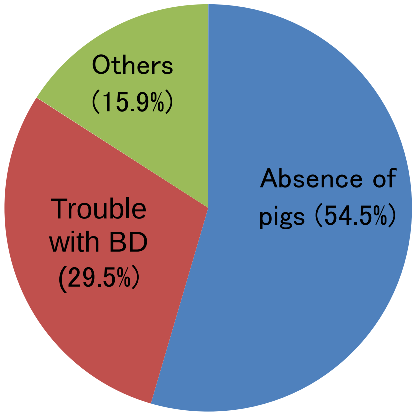

Fig. 2. Reasons for non-use of biogas

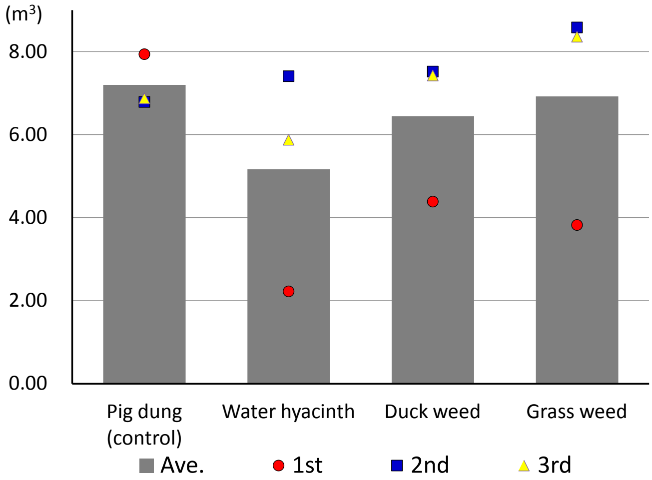

Fig. 3. Biogas production by applying unused biomass

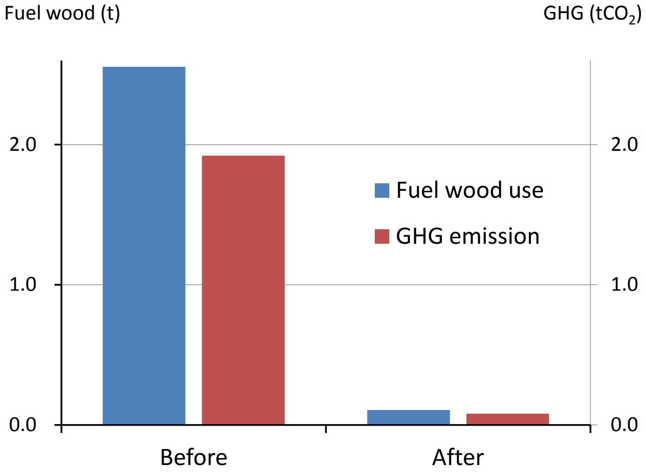

Fig. 4. Results of a verification study on a BD household applying only *Pistia stratiotes*. Graph shows significant drop in fuel wood use and GHG emissions over a one-year period.

Development of a near-isogenic line with early-morning flowering trait in the genetic background of Indica-type variety in the tropics

Rice spikelets are most susceptible to heat stress at flowering. Air temperature at 35^{o}C is the general threshold for heat-induced spikelet sterility (HISS) at flowering. Even a one-hour heat exposure to flowering spikelets can cause sterility. It is predicted that global warming will increase the risk of spikelet sterility in rice. Early-morning flowering (EMF) trait is considered effective in alleviating heat stress by shifting flower opening time (FOT) to earlier in the morning when it is cooler. In this study, we attempted to develop a near-isogenic line (NIL) with EMF trait in the genetic background of Indica-type variety, IR64.

Marker-assisted selection was employed to transfer the QTL for EMF (*qEMF3*) using the backcrossing approach. Eventually, the NIL with clear genetic background other than the QTL region was obtained (Fig. 1A). Under field condition, the developed NIL advanced peak (50%) FOT by 1.5-2.0h compared with the recurrent parent, IR64, both in the wet season and dry season (Fig. 1B). The NIL for EMF was further tested under elevated temperature regimes in environmentally controlled chambers. The times from dawn (zero hour) to 10% (T10), 50% (T50) and 90% (T90) FOT were calculated based on the R program. (T10, T50, and T90 means the starting, peak, and finishing FOT, respectively.) Under the given temperature regimes, T90 of NIL for EMF finished before the temperature reached 35^{o}C, while T10 of IR64 started after the temperature exceeded 35^{o}C (Fig. 2A). This result indicated that almost all flowered spikelets of NIL for EMF could escape from heat stress at flowering, but most of flowered spikelets of IR64 were exposed to heat stress. Spikelet sterility in IR64 and NIL for EMF was manually counted at maturity. The percentage of spikelet sterility was 55% in IR64 and 10% in NIL for EMF (Fig. 2B), indicating that NIL for EMF could significantly reduce heat-induced spikelet sterility at flowering. The NIL for EMF, therefore, is a novel breeding material that can be used for the development of heat-resilient rice to cope with future hotter climates.

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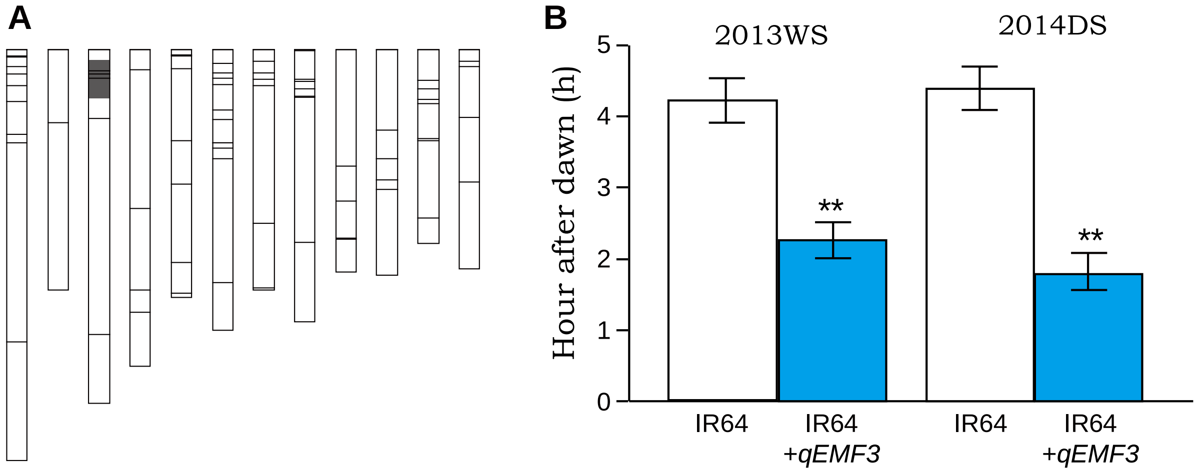

Fig. 1. Graphical genotype of IR64+*qEMF3* (A) and 50% of flower opening time in IR64 and IR64+*qEMF3* (B).

(A) Gray bar on the rice chromosome 3 is a locus of *qEMF3*. Other regions on chromosomes 1-12, indicated in white, refer to the IR64 genetic background. The horizontal lines on each chromosome refer to the positions of available SSR markers.

(B) Dawn was set as 0. Values indicate mean±SE of three or four replications. **Significant at 1% level by *t*-test. WS: wet season. DS: dry season.

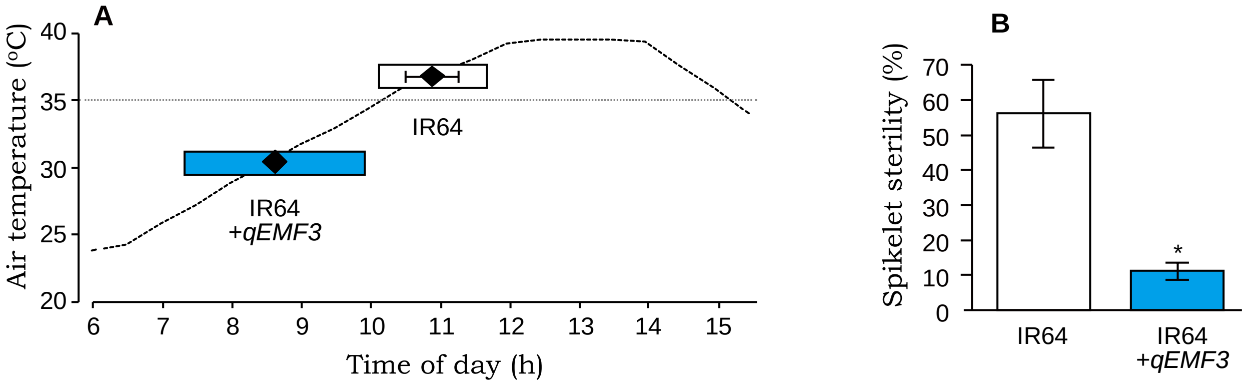

Fig. 2. The changes in time of day of flowering under elevated temperature conditions (A) and percentage of spikelet sterility at maturity (B).

(A) ◆ indicates 50% FOT±SE of three replications. The left and right sides of the horizontal bars indicate 10% FOT and 90% FOT, respectively. Horizontal broken line indicates the general threshold temperature (35°C) for HISS at flowering.

(B) *Significant at 5% level by *t*-test.

Heat tolerance and flower opening time of popular rice varieties in the tropics

Rice spikelets are most susceptible to heat stress at flowering, inducing spikelet sterility. Moreover, rice varieties face a higher risk of exposure to heat stress because of progressive global warming. Hence, improving heat tolerance and shifting flower opening time (FOT) to the cooler early morning period are among the recommended strategies to mitigate damage by heat stress at flowering. In this study, the heat tolerance and FOT of 23 popular varieties in the tropics were investigated.

The spikelets of 23 popular varieties were exposed to heat stress at 38^{o}C for 6 hours (0900h-1500h) at flowering. It was found that there is a wide genetic variation in heat tolerance (Table 1). Ciherang, a popular Indonesian variety, and Samba Mahsuri, a popular Indian variety, showed high heat tolerance comparable to that of N22, the heat tolerance check variety (Table 1). This result indicated that Ciherang and Samba Mahsuri are useful new genetic resources for heat tolerance at flowering. On the other hand, Fedearoz50, a popular variety in Latin America, showed moderate heat tolerance, whereas Sahel329 and Nerica L-19, popular varieties in West Africa, and KDML105, famous aromatic rice in Thailand, showed high heat susceptibility (low heat tolerance) similar to that of Morobrekan, the heat susceptibility check variety (Table 1). These results suggested that the heat resilience of Sahel329, Nerica L-19, and KDML105 must be improved. With regard to the FOT of the 23 popular varieties, no variety had early-morning flowering (EMF) trait when compared with near-isogenic line (NIL) for EMF trait (Fig. 1), clearly indicating transferring quantitative trait locus (QTL) for EMF to these popular varieties would be effective at increasing heat escape capability at flowering. Because NIL for EMF showed significant earlier FOT than *glaberrima* (which is known as an EMF variety to this day), NIL for EMF is considered a novel breeding material for improving heat resilience to cope with future hotter climates.

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Table 1. Heat tolerance of popular varieties

Variety	Countries grown	Fertility (%)		Tukey's test in heat stress
		Control (30°C)	Heat stress (38°C)	
Ciherang	Indonesia	93.6 ± 1.7	92.1 ± 1.5	a
Sambha Mahsuri	India	96.1 ± 2.6	88.1 ± 2.5	a
N22[1]	India	94.9 ± 1.8	88.4 ± 5.6	abc
Fedearoz50	Columbia, Costa Rica, Benezuela, Panama	92.9 ± 1.8	56.3 ± 6.3	d
Sahel329	Senegal, Mauritania	85.2 ± 1.2	22.7 ± 3.5	ef
Nerica L-19	Nigeria, Mali, Burkina Faso, Liberia, Sierra Leone, Cameroon, Togo	78.4 ± 5.8	25.7 ± 4.7	f
KDML105	Thailand	92.0 ± 2.3	13.8 ± 3.0	f
Moroberekan[2]	Côte d'Ivoire	92.7 ± 1.2	9.8 ± 4.8	f

Humidity was maintained at 60-70%. Different alphabet in Tukey's test indicates significant difference at 5% level. [1]Check variety for heat tolerance, [2]Check variety for heat susceptibility. Data not shown for the rest of 16 varieties.

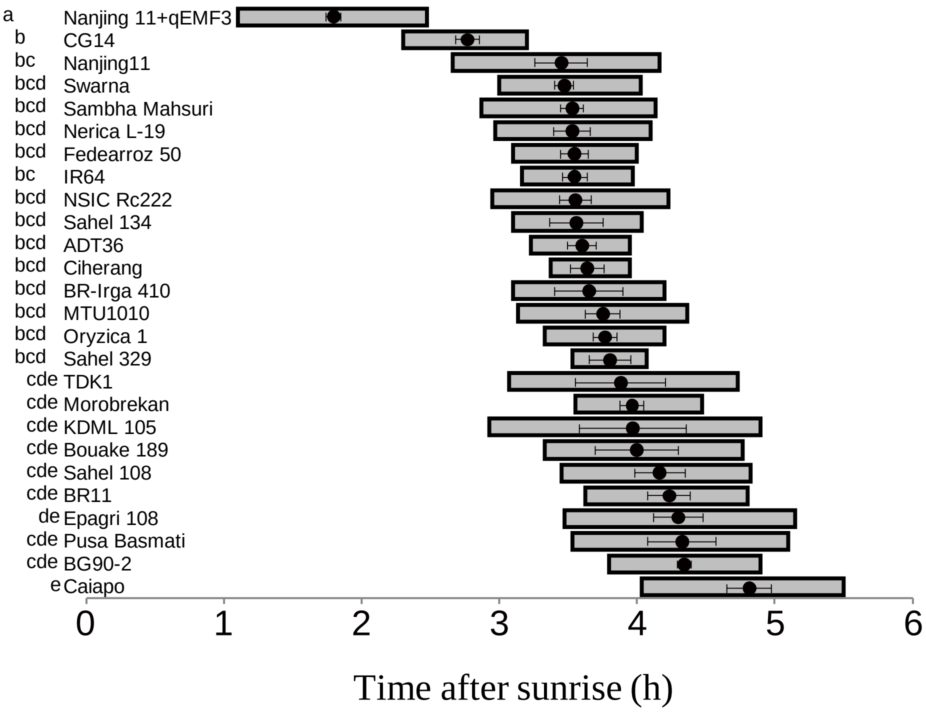


Fig. 1. Flower opening time of popular varieties, NIL for EMF (Nanjin11+*qEMF3*), and *glaberrima* (CG14). ◆ in each genotype indicates 50% FOT ± SE of at least three replications. The left and right sides of the horizontal bars in each genotype indicate 10% FOT and 90% FOT, respectively. Different alphabets in left side of each genotype indicate the significance at 5% level by Tukey's test.

A-04

Long–term global outlook of crop yields under climate change

A long–term outlook of crop yield is necessary for an economic evaluation of climate change on food supply and demand. If the temperature is lower than the optimum level, the crop yield will increase with rising temperature. Conversely, if the temperature exceeds the optimum level, the crop yield will decrease with rising temperature. The yield function, which considers the inverse U–shaped relationship between temperature and yield, is required for long–term outlook under climate change.

The purpose of this research is to analyze the effects of climate change on major crop yields using yield trend functions, incorporating parameters of yield for climate variables obtained from a crop model. The scenarios used in this research are those of the Representative Concentration Pathway (RCP) in the Fifth Assessment Report (AR5) of the Intergovernmental Panel on Climate Change (IPCC). There are four scenarios, i.e., RCP8.5, RCP6.0, RCP4.5, and RCP2.6, in descending order according to CO_2 concentration.

The parameters and functions for 46 crops of the crop model used for analyzing the Global Agro Ecological Zone of the FAO are presented in the report of the International Institute for Applied Systems Analysis (IIASA). The relationship between temperature and yield as indicated in Figure 1 is obtained from the parameters and the functions of the biomass production and photosynthetic rate of the model. These functions are smoothed using cubic spline interpolation.

The target crops are rice, wheat, maize, and soybeans, covering 126 countries or regions, the same number as when the world food model was developed. Data on crop yields were obtained from the FAO (data from 1961 to 2007).

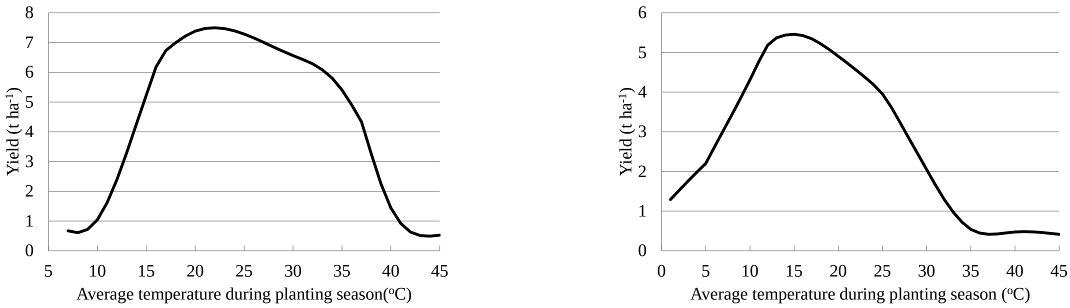
Logistic functions or linear functions with logarithmic time trends are estimated for each crop yield for each country as yield trend functions. Climate parameters of the crop model are incorporated into the trend function.

Climate data in this research are those of the Model of Interdisciplinary Research on Climate (MIROC5), which is a Global Circulation Model (GCM), and these are aggregated for large countries such as China based on the crop cultivation map of the United States Department of Agriculture (USDA).

Figure 2 depicts wheat yields in India and China. The baseline scenario assumes unchanged temperature, solar radiation, and rainfall values during simulation (period 2008-2050). Wheat yield in India is decreased by climate change under the RCP8.5 scenario. Climate change simulation results in China, on the other hand, show substantial fluctuation because the slope of the yield to temperature as indicated in Figure 1 (ii) is steeper than those of other crops.

Figure 3 presents differences in wheat yields between baseline and RCP6.0 scenario during the periods 2021–2030 and 2041–2050. These figures suggest that wheat yields in southern Asian and sub–Saharan African countries will decrease under the RCP6.0 scenario.

(J. Furuya, S. Kobayashi, Y. Yamamoto, M. Nishimori [NIAES])



(i) Indica rainfed rice

Planting days N=195(day), Harvest Index (HI)=0.38,
Leaf Area Index (LAI)=4.8,
Dry matter production rate on an overcast day bo=195(kg ha^{-1} day^{-1}),
Dry matter production rate on a clear day bc=375(kg ha^{-1} day^{-1}),

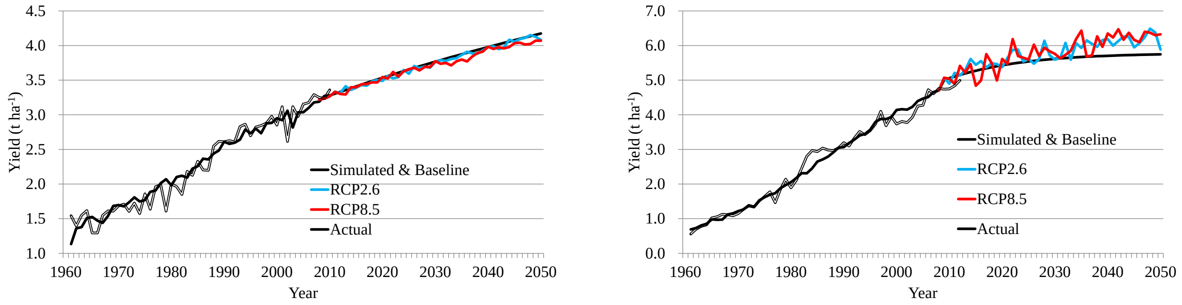
Solar radiation, Rg=16 (MJ m^{-2} day^{-1}) [Average in India]

(ii) Winter wheat

Planting days N=270(day), HI=0.2, LAI=5.5,
Dry matter production rate on an overcast day bo=190(kg ha^{-1} day^{-1}),
Dry matter production rate on a clear day bc=369(kg ha^{-1} day^{-1}),

Solar radiation Rg=14 (MJ m^{-2} day^{-1}) [Average in China]

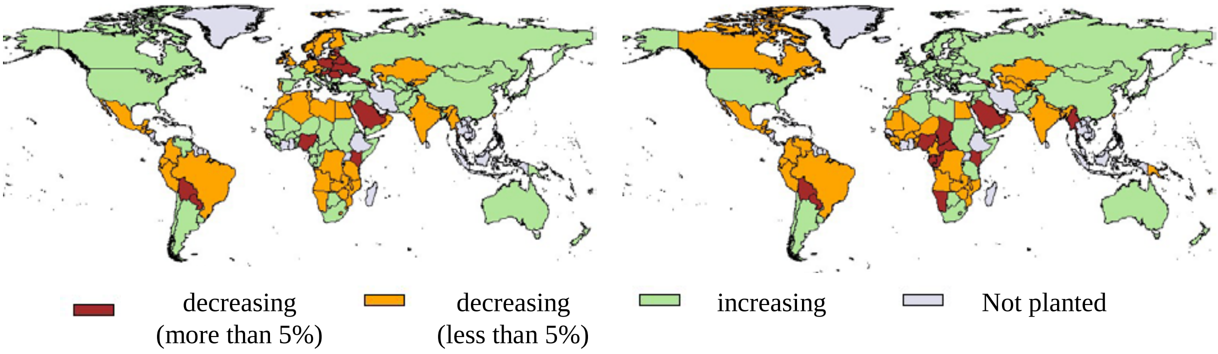
Fig. 1. Relationship between potential yield and temperature



(i) Rice in India

(ii) Wheat in China

Fig. 2. Trends in crop yield



(i) 2020s

(ii) 2040s

Fig. 3. Effects of climate change on wheat yield: differences between baseline and RCP6.0

Sorgoleone release determines the hydrophobic-BNI capacity in sorghum root systems

Nitrification and denitrification are the two most important processes that contribute to greenhouse gas emissions and the inefficient use of nitrogen. Suppressing soil nitrification through the release of nitrification inhibitors from roots is a plant function, termed 'Biological Nitrification Inhibition (BNI)'. Sorghum releases two categories of nitrification inhibitors from roots: hydrophilic BNIs and hydrophobic BNIs. Our earlier published work on sorghum mostly focused on characterizing hydrophilic BNI release. Here we report the characterization of hydrophobic-BNI release in sorghum. The functional role and contribution of sorgoleone release to hydrophobic-BNI function and the existence of genotypic variability for sorgoleone release is the focus of this investigation. Three sorghum genotypes (Hybridsorgo, IS 41245 and GDLP 35-5-5-3) were evaluated for their capacity to release sorgoleone in hydroponic, in soil culture, and under field environments. Sorgoleone released from roots is measured using a high performance liquid chromatograph (HPLC) and BNI activity is determined using a luminescent recombinant *Nitrosomonas europaea* assay.

Sorgoleone was found to be the dominant and major component of hydrophobic-BNI activity released from sorghum roots, and there were significant genotypic differences for sorgoleone release (Fig. 1). Sorgoleone release and BNI-activity release in sorghum roots are closely associated, i.e., 1 μg of sorgoleone released is equivalent to 1 ATU activity in the bioassay (Fig. 2). Sorgoleone genotypes release varying quantities of sorgoleone. GDLP 34-5-5-3 and Hybridsorgo have higher capacity for both sorgoleone release and BNI activity than IS41245. In soil culture, GDLP 34-5-5-3 released significantly higher quantities of sorgoleone into the rhizosphere, had higher BNI activity, and suppressed soil nitrification better than IS41245 (Fig. 3). Purified sorgoleone inhibited *Nitrosomonas* activity in the bioassay; when amended to soil, sorgoleone suppressed nitrification, improved NH_4^+ availability, and reduced NO_3^- formation in soils during a 60-day incubation study (Fig. 4). These results demonstrate genetic differences for sorgoleone release and its functional link to hydrophobic-BNI release and BNI capacity in sorghum.

Sorgoleone release contributes significantly to BNI capacity in sorghum. The significant genetic differences for sorgoleone release from sorghum roots suggest that there is potential for genetic improvement to improve sorgoleone release and BNI capacity in sorghum. Higher BNI capacity is critical to the development of low-nitrifying sorghum production systems and the results presented here suggest the feasibility of this approach.

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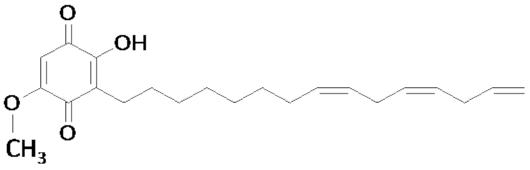

Fig. 1. Chemical structural formula of sorgoleone

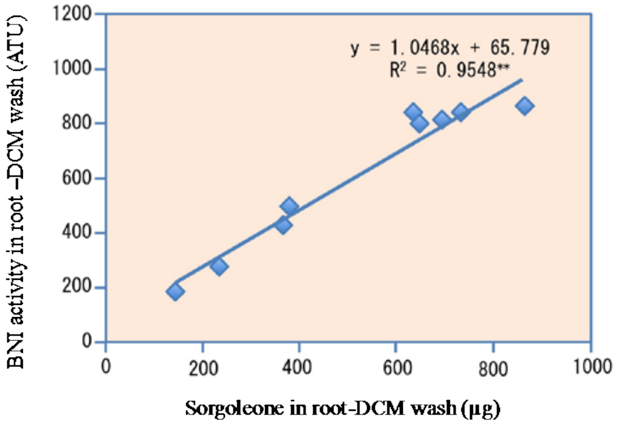

Fig. 2. The relationship between total sorgoleone concentration (μg) and BNI activity (ATU) in root–DCM wash of three sorghum genotypes

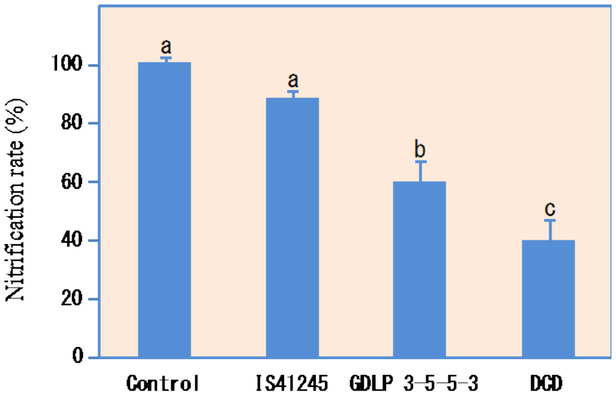

Fig. 3. Nitrification rate at 30-day incubation period along with NH_4^+ inoculation of rhizosphere soil collected from two sorghum genotypes (IS1245 and GDLP 34-5-5-3) grown up to heading stage in potted soil. Control pots were included with bare soil without plants but handled the same way like pots with plants. As positive control, soils taken from control treatments were also incubated with DCD addition at 25 ppm (a known synthetic inhibitor) as a reference.

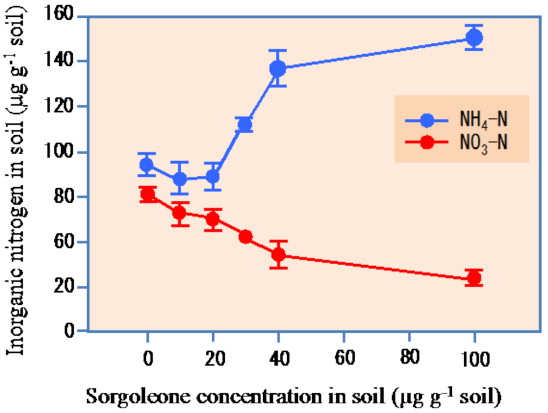

Fig. 4. Concentration of inorganic N (NO_3^- and NH_4^+) in soil samples incubated after adding different concentrations of sorgoleone (0, 10, 20, 30, 40, and 100 μg g^{-1} soil) for 60 days

Manual of soil fertility improvement technologies in lowland rice ecologies of Ghana

The impact of fertilizer application on crop production in Sub-Saharan Africa (SSA) is considered enormous as the region is very low in soil fertility. However, access to chemical fertilizers is difficult especially for small-scale SSA farmers who do not have sufficient financial resources in a market-oriented economy. This crucial issue underscores the urgent need for the farmers to increase agricultural productivity, which can be achieved through inexpensive and cost-effective techniques of improving soil fertility in rural areas.

With financial support from the Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan, JIRCAS carried out a study on technology development for improved soil fertility using indigenous resources that are accessible and acceptable to local farmers. The study, with rice being the target crop, is aimed at contributing to the goal of the Coalition for African Rice Development (CARD) to double rice production in SSA by 2018. Ghana was selected as the country of implementation because it has two major rice ecologies (rainfed lowland and irrigated lowland) and has good research counterpart institutions.

As one of the products of the study, a technical handbook, titled “Manual of soil fertility improvement technologies in lowland rice ecologies of Ghana,” was published. Written in English, this manual would greatly benefit extension workers and assist them in disseminating the technologies to rice farmers. A summary of the manual’s features is listed below.

1. The manual describes the application of indigenous organic matter as well as their composting and charring technologies, the application of phosphate rocks from neighboring Burkina Faso and its solubilizing technologies, and the enhancement of early rice growth using a minimum quantity of chemical fertilizer (Table 1).
2. The technologies mentioned in the manual were developed in consideration of available materials in each rice ecology and corresponding region. The technologies were evaluated for effectiveness and affordability to the rural communities in on-farm participatory studies.
3. Government officers as well as counterpart researchers in Ghana were actively involved in the editorial process, enhancing their sense of ownership of the manual and technologies. The foreword was written by the deputy minister of the Food and Agriculture (MoFA), Ghana.
4. This manual is compact enough to be carried around. It is printed on A5 size paper and contains only 44 pages, with visually descriptive text and plenty of visuals (photographs and illustrations).
5. The technologies adopted in the manual may be extended to other SSA countries having the same rice ecologies.

(Satoshi Tobita, Satoshi Nakamura, Monrawee Fukuda, Fujio Nagumo)

B-01

Table 1. Technology options adopted in the soil fertility manual

Options	Rice ecology (Agro-ecological zone)	
	Rainfed lowland (Guinea Savannah zone)	**Irrigated lowland (Equatorial Forest zone)**
Organic matter application	Rice straw base ❖ Direct application or composting ❖ If applicable, small quantity of chemical fertilizer shall be recommended.	Poultry manure base ❖ Prompt effect by direct application ❖ Direct application of rice straw and sawdust causes N starvation in this ecology.
Composting	Cow dung ＋ rice straw ❖ Not acceptable to some farmers	Poultry manure ＋ sawdust/rice straw ❖ Utilization of waste resources
Charring (Kuntan)	Soil physical/biological improvement, no direct effect on soil fertility improvement	
	Rice husks as material	Sawdust as material
Phosphate rock application	Phosphate rocks may appear in the Ghana market in the near future, depending on decision by stakeholders and policy-makers in Ghana. This option is applicable in neighboring PR-producing countries.	
	Direct application ❖ Very effective in all areas in the first year of application. Residual effects differed among fields.	Direct application ❖ Very effective in all areas in the first year of application, as well as having residual effects for at least 3 years.
	Burkina Faso PR is fine powder in texture, thus the spreading method, like mixing with mud, shall be considered.	
Dual application of organic matter and phosphate rock	Optimization of quantity and timing of application ❖ Rice straw shall be incorporated into soil just after harvesting to have better C/N ratio for the next season and to avoid unnecessary burning. ❖ Phosphate rock shall be applied at sowing or transplanting.	
Pretreatment	Early growth of rice is enhanced by pretreatment with a small quantity of water-soluble P fertilizer	
	Coating of fertilizer with rice seeds ❖ Compatible with direct sowing	Soaking of rice seedlings in fertilizer solution ❖ Compatible with transplanting
Technologies for the enhancement of phosphate rock solubility	Useful in upland environments where the solubility of PR is lower (for growing upland rice or upland crops).	
	(1) Incorporate PR in the composting process to make P-enriched compost	
	(2) Incorporate PR in the charring process, expecting calcination in relatively low temperature, to make P-enriched char	

B-01

Fig. 1. Charring of saw dust (Kumasi City)

Fig. 2. Demonstration in an on-farm field (at Ziong Village, a suburb of Tamale City)

A flexible high-throughput marker system to distinguish African rice (*Oryza glaberrima*) from Asian rice (*Oryza sativa*)

The African rice *Oryza glaberrima* is an important reservoir of genes for abiotic stress tolerance. To discover such tolerance genes and to exploit them in rice improvement, a flexible, high-throughput marker system is needed. Single nucleotide polymorphism (SNP) sites, where the genome sequence of two or more individuals differs by a single base, are increasingly becoming the marker of choice. Although the number of discovered SNPs has increased significantly over the past few years, most of these efforts have focused on variations within the Asian rice *Oryza sativa*. The aim of the present study was to detect a set of SNPs that differentiate between *O. sativa* and *O. glaberrima*, and to use a representative subset to develop a high-throughput PCR-based genotyping panel.

A genome-wide 44,000 SNP genotyping array identified a set of 9523 SNPs polymorphic between *O. glaberrima* and *O. sativa* subspecies *indica*, and 7444 SNPs between *O. glaberrima* and *O. sativa* subspecies *japonica* （Figure 1A）. From the above set, a subset of 1540 SNPs was selected in collaboration with partners within the Generation Challenge Program (GCP) for conversion into PCR-based markers, using the KASP (competitive-allele PCR) technology. A final set of 2015 SNPs were successfully converted to KASP markers, which are evenly distributed in the genome, with the exception of small gaps in chromosomes 4 and 10 (Figure 1B). The panel was validated in the ‘New Rice for Africa’ (NERICA) parents. Of the 2015 markers tested, 745 markers were polymorphs between CG14 and WAB56-104 (upland NERICA parents) and 752 between TOG5681 and IR64 (lowland NERICA parents) (Figure 2). Several subsets of these markers have been used successfully to map *O. glaberrima* introgressions in NERICA rice varieties and interspecific breeding lines.

This new genotyping panel is a cost-effective, gel-free genotyping platform that allows maximum flexibility for ‘pick-and-choose’ markers according to individual breeder’s needs. Presently, it is fully outsourced to service companies that can perform all steps, from DNA extraction to genotyping, which would extend the capacity of low-resource laboratories to perform molecular breeding using local rice varieties. In addition, the information for each SNP is publicly available allowing the rice breeding community to complement the set with their own subset of markers. JIRCAS is expanding its collaborative rice breeding network with applications ranging from parental surveys, development of QTL mapping populations, and marker-assisted introgressions of major stress tolerance genes like *OsPSTOL1*.

(M. Lorieux [CIAT], C. He [GCP], S. McCouch [Cornell University], MJ. Thomson [IRRI],
M. Wissuwa, J. Pariasca-Tanaka)

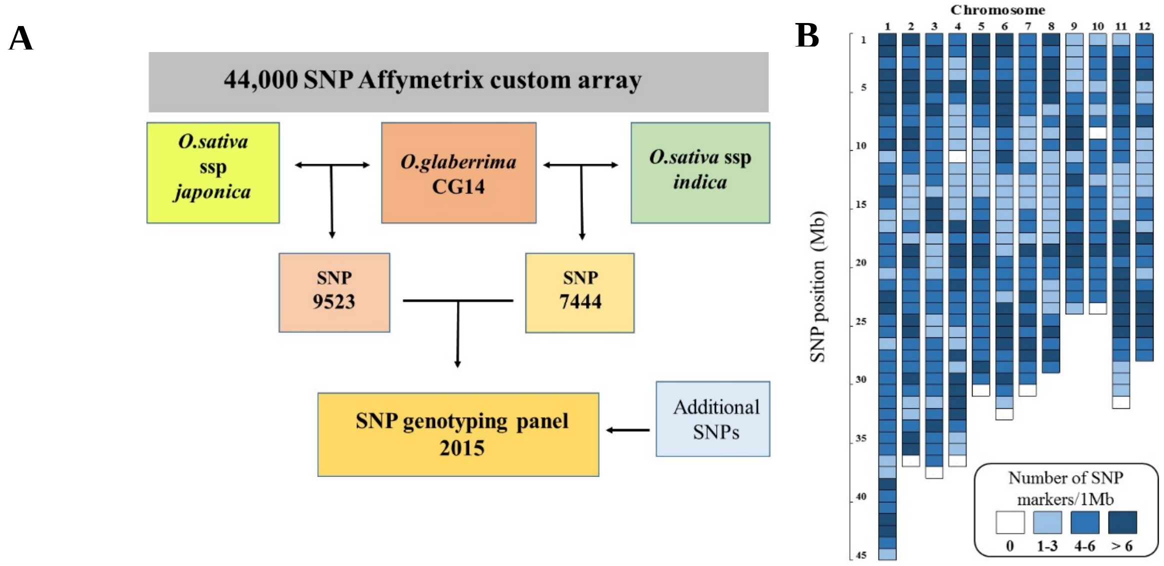

Fig. 1. Flow chart indicating the selection of polymorphic SNPs between *O. glaberrima* vs *O. sativa* ssp *japonica* and *indica* for conversion into PCR-based markers (A). Distribution of SNP markers along the rice genome. Color represents the number of markers per 1 Mb (B).

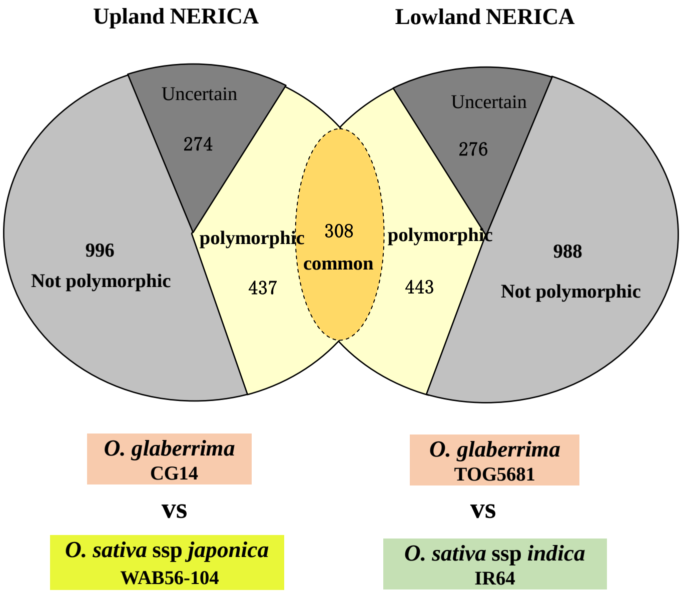

Fig. 2. Distribution of polymorphic SNP markers for crosses of *O. glaberrima* vs *O. sativa*. There are 745 and 751 polymorphic markers for upland and lowland NERICA varieties, respectively.

Integrated analysis of the effects of dehydration and cold on rice metabolites, phytohormones, and gene transcripts

Land plants must mount suitable responses to overcome the adverse effects of water stress caused by either drought or low-temperature conditions. Among external stresses, water stress is one of the most important limitations to crop productivity. Discoveries of useful genes for molecular breeding using metabolomics and transcriptomics promise to facilitate the improvement of crop yields under water stress conditions. It is important to identify plant metabolites and transcripts that respond to water stress to determine the essential steps in molecular processes related to the effective adaptation of plants to stress conditions. Metabolomic and transcriptomic data have provided much information on the metabolite, phytohormone, and transcript networks that control plant growth and development. Rice is important not only as a major crop but also as a model monocot.

In this study, we performed an integrated analysis of the metabolites, phytohormones, and gene transcripts in rice plants subjected to dehydration or cold treatments. Our aim was to comprehensively survey the molecular responses of rice to dehydration or cold stimuli. We used three types of MS systems: gas chromatography coupled with time-of-flight MS (GC-TOF-MS), capillary electrophoresis coupled with MS (CE-MS), and liquid chromatography coupled with MS (LC-MS). We identified and characterized representative dehydration-responsive and cold-responsive metabolites and phytohormones. We also performed a transcriptome analysis using a rice oligonucleotide microarray. We analyzed metabolite–gene and phytohormone–gene correlations and identified several genes encoding metabolic enzymes that might play key roles in the responses of rice plants to dehydration or cold. We compared the roles of the identified rice genes with those of their counterparts in Arabidopsis under dehydration or cold conditions.

An integrated analysis of metabolites and gene expression indicated that several genes encoding enzymes involved in starch degradation, sucrose metabolism, and the glyoxylate cycle are upregulated in rice plants exposed to dehydration or cold, and that these changes are correlated with the accumulation of glucose, fructose, and sucrose (Fig.1). In particular, high expression levels of genes encoding isocitrate lyase and malate synthase in the glyoxylate cycle correlate with increased glucose levels in rice, but not in Arabidopsis, under dehydration conditions, indicating that the regulation of the glyoxylate cycle may be involved in glucose accumulation under dehydration conditions in rice, but not in Arabidopsis. An integrated analysis of phytohormones and gene transcripts revealed an inverse relationship between abscisic acid (ABA)-signaling and cytokinin-signaling under cold and dehydration stresses; these stresses increase ABA signaling and decrease cytokinin signaling (Fig. 2). High levels of *OsNCED* transcripts correlate with ABA accumulation, and low levels of *CYP735A* transcripts correlate with decreased levels of a cytokinin precursor in rice. This reduced expression of *CYP735As* occurs in rice, but not in Arabidopsis. Therefore, transcriptional regulation of *CYP735As* might be involved in regulating cytokinin levels under dehydration and cold conditions in rice, but not in Arabidopsis.

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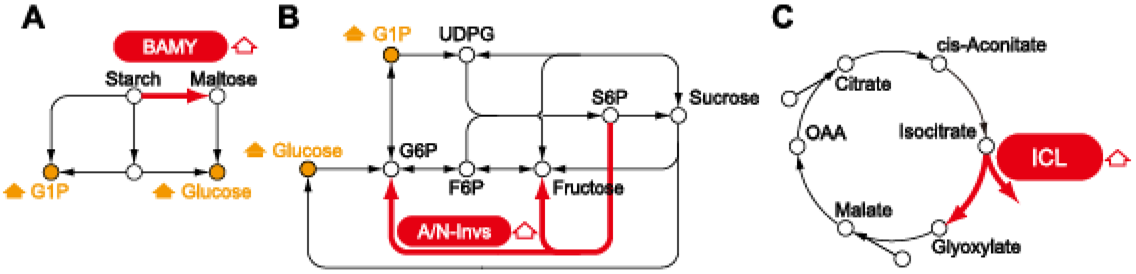

Fig. 1. Carbohydrate and amino acid metabolic pathways. (A) Starch degradation; (B) Sucrose metabolism; (C) Glyoxylate cycle: BAMY, β-amylase; A/N-invs, alkaline/neutral invertase; ICL, isocitrate lyase.

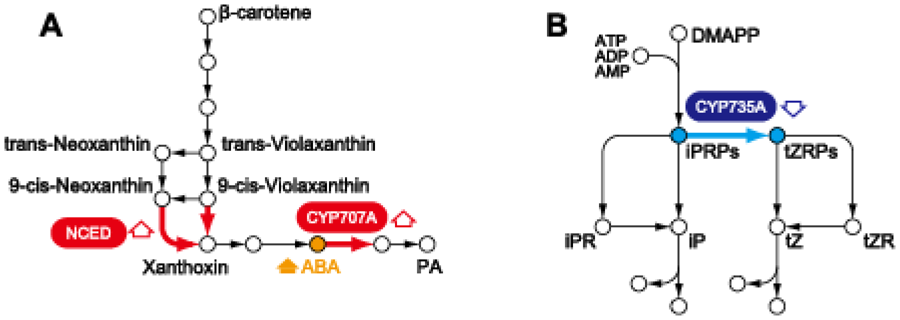

Fig. 2. Pathways for ABA and CK biosyntheses. (A) ABA biosynthesis; (B) CK biosynthesis. NCED, 9-cis-epoxycarotenoid dioxygenase.

Four AREB/ABF transcription factors function downstream of three SnRK2 protein kinases to regulate drought stress tolerance

Global climate change has increased the frequency and severity of drought, resulting in significant yield losses in staple crops worldwide. Drought is one of the major abiotic stresses that adversely affect plant growth, survival, distribution, and productivity. Under drought conditions, many genes involved in drought stress response are induced in a wide range of plant species. The plant hormone abscisic acid (ABA) plays a crucial role in coordinating the responses to reduced water availability as well as in multiple developmental processes. Endogenous ABA levels in plant cells are increased in response to drought stress, leading to expression of stress-responsive genes. In a model plant Arabidopsis, under drought stress conditions, ABA controls stress-responsive gene expression mainly through three bZIP transcription factors (TFs) -- AREB1/ABF2, AREB2/ABF4 and ABF3 -- which are activated by SNF1-related kinase 2s (SnRK2s) such as SRK2D/SnRK2.2, SRK2E/SnRK2.6/OST1, and SRK2I/SnRK2.3 (SRK2D/E/I). However, as the three AREB/ABFs are required, but not exclusive, for the SnRK2-mediated gene expression, transcriptional pathways regulated by SRK2D/E/I remain unknown.

In this report, we show that a Clade A bZIP transcription factor, ABF1, function as a homolog of AREB1/ABF2, AREB2/ABF4, and ABF3 in ABA-dependent gene expression in Arabidopsis. In spite of lower expression levels of ABF1 than those of the three AREB/ABFs, the *areb1 areb2 abf3 abf1* quadruple knockout mutant plants exhibited enhanced sensitivity to drought and reduced sensitivity to ABA in primary root growth compared with the *areb1 areb2 abf3* triple knockout mutant. Large-scale transcriptome analyses uncovered that expression of downstream genes of SRK2D/E/I, which include many functional genes in drought stress responses and tolerance such as TFs and LEA proteins, was mostly impaired in the quadruple mutant. These results indicate that the four AREB/ABFs are the predominant TFs downstream of SRK2D/E/I in ABA signaling in response to drought stress during vegetative growth.

Considering that many findings strengthen the view that ABA signaling has contributed to land colonization and adaptation to various environmental changes, the manipulation of genes involved in ABA signaling has the potential to improve crop productivity and quality under drought stress conditions. AREB/ABFs, which are key players in drought stress response, are well conserved in land plants, so that the four AREB/ABFs can be good candidates to engineer enhanced drought tolerance. Thus, engineering ABA signaling through manipulation of SnRK2-AREB/ABF-mediated transcriptional regulation would create a new path to face climate change through improvement of crop production.

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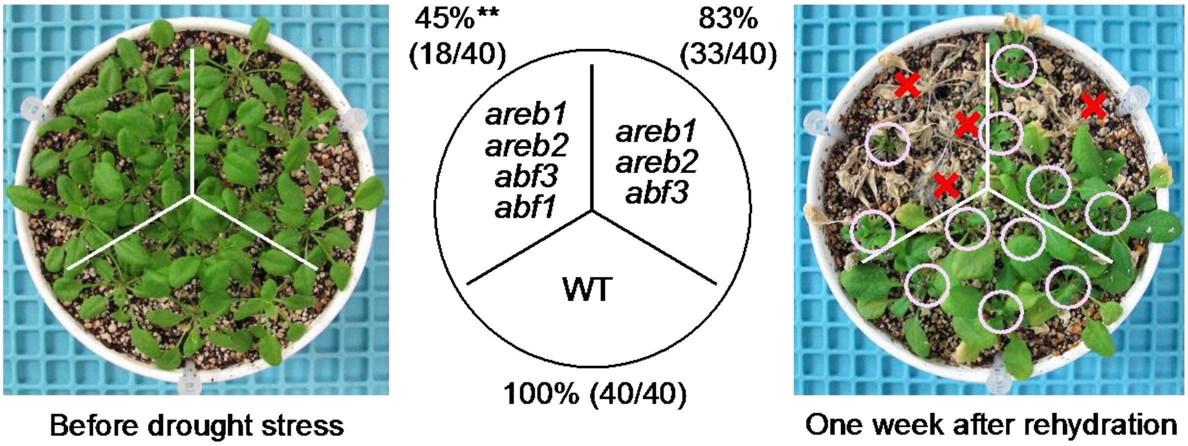

Fig. 1. The *areb1 areb2 abf3 abf1* quadruple knockout mutant displays enhanced sensitivity to drought compared with the *areb1 areb2 abf3* triple knockout mutant. Watering was withheld from 4-week-old plants for 11–12 d, and then

the plants were re-watered for 1 week before the photograph was taken. Circles and crosses indicate survival and dead plants, respectively. Wild-type (WT) and two kinds of mutant plants (n = 5 each) were grown in soil in a 9 cm pot. Survival rates were shown in the central column and calculated from the results from eight independent experiments (n=40). Representative result is shown. $^{**}P < 0.01$（Student's *t*-test, based on comparison with WT）

Fig. 2. Model showing the AREB/ABF-SnRK2 pathway that controls ABA-mediated transcriptional regulation in responses to drought stress in plants

Low night temperature inhibits fertilization and consequently reduces fruit set in ‘Monthong’ durian

Durian flowers mainly in January in Chanthaburi, the main production area in Thailand. Chanthaburi’s average minimum temperature for January is 20.7°C (10-year average); however, in 2014, the daily minimum temperature dropped to lower than 15°C for the first time in five years, with record-low temperatures (below 17 °C) lasting about a week. Usually, durian trees set fruits at 15-30%; however, the fruit set was quite poor that year especially for ‘Monthong’, the leading cultivar in Thailand. Empirical observations have shown that ‘low temperature’ during anthesis reduces fruit set, but the effect of low temperatures on the development of fruit set in durian is not fully understood because the trees grow very high in the fields where environmental factors, such as temperature, are difficult to control.

In this study, we developed a temperature controller that can be used in the orchard, and we examined the effect of night temperature on fruit set. Morphological development of ovules was also observed and its influence on fruit set was evaluated.

A polystyrene foam box equipped with Peltier devices was attached to a bearing branch to enclose one cluster (Fig. 1). The temperature inside the box was fixed at 15°C or 25°C during nighttime (2000 to 0800h) for 7 days after pollination (DAP). The box was detached after the 7th day. At 25°C, about 30% of the flowers set fruits at 28 DAP, whereas all flowers abscised by 21 DAP at 15°C (Fig. 2). Pollen tubes elongated within the styles in both 15°C and 25°C. However, at 15°C, 14.7% of ovules remained at the mature stage (Fig. 3A, Table1), which is the normal stage before accepting pollen-tube nucleus. No mature ovules were found at 25°C. On the other hand, 22.7% of ovules proceeded to the endosperm nuclei division stage at 25°C (Fig. 3B, Table 1). No endosperm-nuclei-division ovules were found at 15°C. Endosperm nuclei are the result of fertilization; therefore, it was considered that fertilization occurred at 25°C but did not occur at 15°C. The average length of the ovule at 15°C was considerably shorter than the one at 25°C (Table 1).

These findings suggest that night temperature of 15°C inhibits fertilization and consequently causes poor fruit set in ‘Monthong’ durian. ‘Monthong’ is the most important cultivar in Thailand; however, the fruit set is easily affected by night temperatures. To avoid risk caused by low temperature, planting other cultivars besides ‘Monthong’ or applying plant growth regulators to extend the flowering period is recommended. Adaptation of other cultivars that set fruits at low temperature conditions is also an effectual way for the stable production of durian. In this regard, the temperature controller we have developed can be a useful tool for examining cultivars that set fruit even at low-temperature conditions.

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S. Tongtao [Chanthaburi Horticultural Research Center])

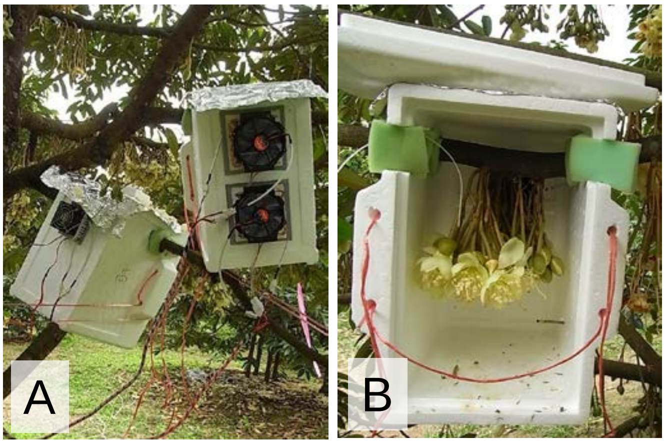

Fig. 1. Temperature controller set on a flower cluster. A: A polystyrene foam box equipped with Peltier devices was used as controller. B: Inside the controller (The lid was left open during daytime).

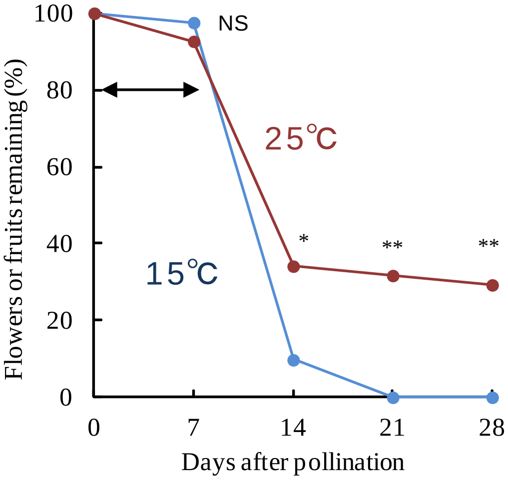

Fig. 2. Percentages of flowers or fruits remaining after pollination. Asterisks (* and **) indicate significant differences between the treatments based on Fisher’s exact test at *P*<0.05 and *P*<0.01, respectively. NS indicates a non-significant difference. Arrow shows the duration of temperature treatment.

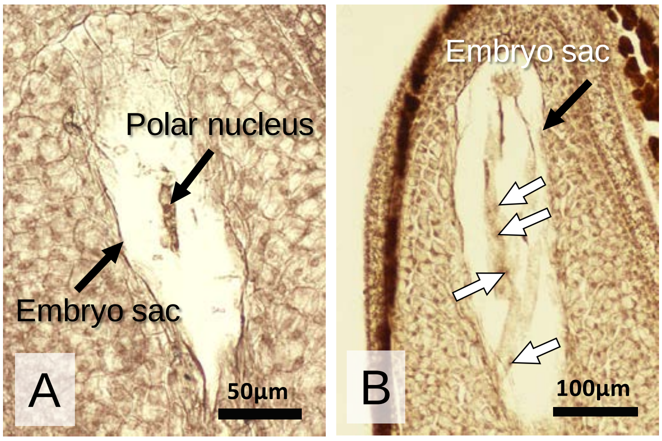

Fig. 3. Micrographs showing the morphological development of ovules at 7 days after pollination (DAP). A: Ovule at the mature stage containing an embryo sac with a fused polar nucleus (15°C). B: Ovule at the endosperm nuclei division stage (25°C). White arrows indicate endosperm nuclei.

Table 1. Average length of the ovules and the developmental stages at 7 DAP

Treatment	Number of ovules observed	Length of ovules (mm)	Stages of ovule development (%)			
			Mature	Endosperm nuclei division	Deformed	Degenerated
15 ˚C	21	1.19	14.3	0	66.7	19.0
25 ˚C	22	1.48	0	22.7	45.5	31.8

Variation at the *Pup1* locus within the genus *Oryza* predates domestication

The deficiency of phosphorus (P) in soil is a worldwide problem, and though there are many approaches to tackle this problem, the development of rice cultivars with enhanced P efficiency would represent a sustainable strategy to improve the livelihood of resource-poor farmers. Recently, the *Pup1* locus (Fig. 1), a major QTL for tolerance to P deficiency, was successfully narrowed down to a single-candidate gene, the protein kinase: P starvation tolerance (*OsPSTOL1*). The aim of this study was to search for novel *OsPSTOL1* alleles and to survey *Pup1* locus variation in Asian (*O. sativa*)- and African (*O. glaberrima*)-cultivated rice and their wild progenitors. This information would help in designing a suitable strategy for marker-assisted introgression of *Pup1/PSTOL1* into rice megavarieties.

A novel *OsPSTOL1* allele was detected in *O. glaberrima*. This allele has 35 base-pair changes (when aligned to Kasalath allele), but none of the functional domains were affected and it is expressed. Allele-specific markers were then developed for single PCR and/or duplex PCR system, which produce a band pattern clearly distinguishable on agarose gels (Fig. 2), and are therefore suitable for most marker laboratories throughout the world. Using these markers to survey allelic distribution of *PSTOL1* across the genus *Oryza* showed that the novel allele is common in accessions belonging to *O. glaberrima* and its ancestor *O. bartii*, but is not restricted to African rice as *O. sativa*, *O. rufipogon*, and *O. nivara* accessions do carry the *glaberrima* allele at low frequency (Fig. 1).

Using additional allele-specific markers across the entire *Pup1* locus revealed two main patterns in the Africa rice (*O. glaberrima* and *O. barthii*): the more typical ‘Africa pattern’ characterized by the novel *PSTOL1* allele and partial presence of the *Pup1*-specific INDEL region, but general absence of 90 kb of a region upstream of *PSTOL1* (pattern G, Fig. 1); and the less common pattern (K) with Kasalath alleles across most of *Pup1*. Within *O. sativa*, the Kasalath (K) and Nipponbare (N) patterns could be distinguished as described earlier, but in addition a mixed pattern (m) with partial presence of Kasalath, *O. glaberrima*, and novel alleles was detected. These three patterns were already present in *O. rufipogon* and *O. nivara*, the wild ancestors of *O. sativa*. Results suggested that *Pup1* locus variation was already a common feature within wild ancestors of cultivated Asian and African rice. Thus, divergence at *Pup1* appears to predate domestication of rice.

Since the function of other genes within the *Pup1* locus remains unclear, it would be desirable to transfer the entire *Pup1* region from Kasalath into recipient varieties during marker-assisted selection. Thus, we propose using two foreground markers (K46-K and K20-K) in breeding programs aimed at introgressing *Pup1*.

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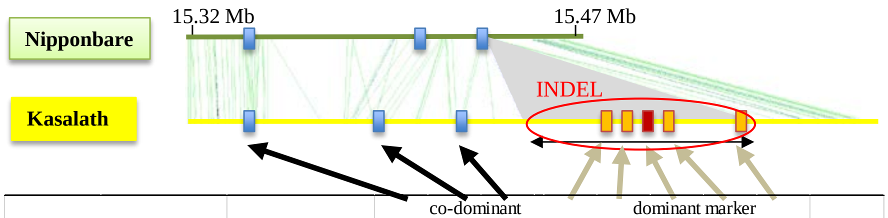
Chromosome 12 – *Pup1* locus



Oryza species		Pattern (type)	co-dominant K05	co-dominant K20-2	co-dominant K29-3	dominant marker K41	dominant marker K42	dominant marker **K46-K/G (*PSTOL1*)**	dominant marker K48	dominant marker K59	Number of accessions
African rice	cultivated **_O. glaberrima_**	CG14(G)	-	-	-	-	-	G	K	K	6
		CG14+(G+)	K/-	any	any	-	-	G	K	K	7
		Kasalath'(K)	-	K	K	K	K	K	K	K	1
	wild relative **_O. barthii_**	CG14(G)	-	-	-	-	-	G	K	K	4
		CG14+(G+)	K/-	any	any	-	-	G	K	K	5
		Kasalath''(K)	K	U	K	K	K	K	-	K	1
Asian rice	cultivated **_O. sativa_**	Nipponbare(N)	N	N	N	-	-	-	-	-	6
		CG14 and Mix(M)	U	K	U	K	-	G	U	-	7
		Kasalath(K)	K	K	K	K	K	K	K	K	5
	wild relative **_O. rufipogon_**	Nipponbare(N)	N	N	N	-	-	-	-	-	3
		CG14 and Mix(M)	N	N	N	K	-	G	K	-	1
		Kasalath'''(K)	N	K	K/N	K	K	K	K	K	5
	wild relative **_O. nivara_**	Nipponbare(N)	H	N	N	-	-	-	-	-	1
		Mix(M)	K	K	N	K	-	G/K	K	-	5
		Kasalath(K)	K	K	K	K	K	K	K	K	2
		Total									59

K	Kasalath	N	Nipponbare	G	*O. glaberrima*
U	novel allele	H	Heterozygous	-	no amplification
any	non-specific				

Fig. 1. Characterization of the *Pup1* locus in Nipponbare and Kasalath, and cultivated and wild rice genotypes. A main difference is the absence of a 90 kb INDEL region in Nipponbare containing *OsPSTOL1* and 20 other Kasalath-specific genes.

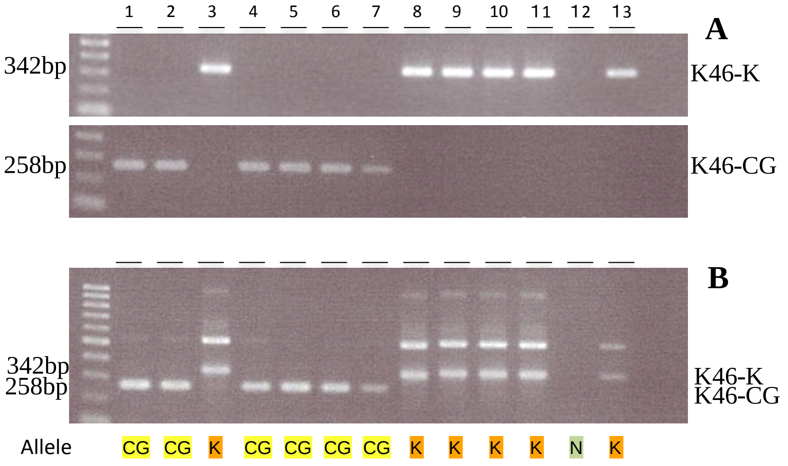


Fig. 2. Amplification of *OsPSTOL1* alleles using allele-specific markers for Kasalath and CG14 in single PCR (A), and duplex PCR system to detect both alleles in one reaction (B).
(1: CG14, 2: IRAT, 3: NERICA16, 4: WAB56-50, 5: NERICA1, 6: NERICA10, 7: WAB181-18, 8: IDSA, 9: IR12979, 10: WAB56-104, 11: IAC165, 12: Nipponbare, 13: Kasalath)

Identification of QTL for efficient root elongation under a wide range of nitrogen concentrations in a rice variety with an Indica-type genetic background

Because root is the sole organ for uptaking water and nutrients such as nitrogen from surrounding soils, improving root architecture and physiological functions are required for enhancing stability and production in rice. IR64, an elite Indica-type variety with high adaptability and high yield, has been widely accepted as a mega variety in rice production and breeding programs in many tropical regions. The International Rice Research Institute—Japan Collaborative Research Project has developed a total of 334 introgression lines (ILs) derived from ten high yielding donor varieties with the genetic background of IR64. Among them, a line designated as YTH183 showed stable and higher yield in tropical regions as well as in temperate regions compared with IR64. However, quantitative trait locus/loci (QTL) for the stable and higher yielding line have not been identified. Furthermore, QTL for root elongation efficiently under a wide range of nitrogen concentrations have not been identified in varieties with the genetic background of IR64. The aims of this study, therefore, were to map and characterize the QTL derived from YTH183 to understand the architecture and functions of roots in YTH183.

The longest root of YTH183 was significantly higher than that of IR64 seedlings hydroponically grown for 8 days in 5 μM NH_4Cl (Fig. 1). As a result, YTH183 had a QTL for root elongation, designated as *qRL6.4-YP5*. The longest root of near-isogenic line (NIL) for *qRL6.4-YP5* with the genetic background of IR64 was significantly higher than that of IR64 (Fig. 1). The QTL *qRL6.4-YP5* (R^2=0.37) was identified in the flanked region between RM6395 and RM8242 on the long-arm region of chromosome 6 (Fig. 2). Further characterization of *qRL6.4-YP5* was done using the NIL. Compared with IR64, total root length was always higher in the NIL and was enhanced in response to the increase in exogenous concentrations of nitrogen (Table 1). The length of the longest root was always higher in the NIL. It was enhanced under nitrogen concentrations of up to 50 μM NH_4Cl, but not in 500 μM NH_4Cl (Table 1). The effect of *qRL6.4-YP5* on root number was not proven in these conditions (Table 1).

To our knowledge, *qRL6.4-YP5* is the first promising QTL for efficient root elongation under a wide range of nitrogen concentrations in rice varieties with the genetic background of IR64. These achievements should help improve root architecture of rice for a stable and high yielding production system in tropical developing countries.

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Fig. 1. Typical phenotypes of seedlings grown for 8 days in 5 μM NH_4Cl.
YTH183 and NIL have positive allele of *qRL6.4-YP5*. Scale bar in individual pictures indicates 50 mm.

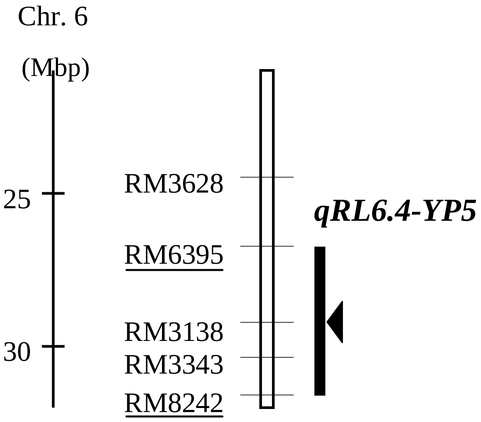

Fig. 2. Physical position of *qRL6.4-YP5* on the long-arm region of chromosome 6.
Closed vertical column indicates candidate region for *qRL6.4-YP5*. Closed triangle indicates the position of the peak *F* score.

Table 1. Elongation and development of roots in NIL for qRL6.4-YP5 under a wide range of nitrogen concentrations

Traits	Variety ･line	Nitrogen concentration							
		0 μM		5 μM		50 μM		500 μM	
Total root length (mm)	IR64	643		742		907		1108	
	NIL	801	**	864	**	1080	**	1276	**
Length of longest root (mm)	IR64	121		128		137		114	
	NIL	145	**	158	**	161	**	140	**
Root number ($plant^{-1}$)	IR64	7.6		8.0		11.0		16.6	
	NIL	8.0		7.8		10.6		15.6	

Plants were grown for 8 days.

NH_4Cl was the sole nitrogen source.

Asterisks (**) represent a significant difference between IR64 and NIL at P-value of 1% level (one-way ANOVA).

Economic benefits of various non-timber forestry products to Lao PDR's farm economy

Agriculture in Laos is primarily rain-dependent due to its tropical monsoon climate. Farmlands are often subjected to droughts and floods, rendering crop production unstable. Therefore, in addition to rice cultivation, Laotian farmers collect non-timber forestry products (NTFPs) in mountainous areas, thus providing a safety net to local residents in terms of livelihood support. To understand the actual utilization of NTFPs and its contribution to rural household economies, a study was conducted on all 140 households (104 valid responses) in a farming village in the northwestern part of Vientiane Province from July 2012 through June 2013, recording each day the types and quantities of NTFPs collected as well as their intended uses. To determine the economic values, a price table was created, with each NTFP's trade price multiplied by its trade value and then converted to a monetary amount.

Over 400 diverse types of NTFPs, including 289 plant-type products (such as mushrooms) in addition to 124 animal-type products, were utilized. Excluding the types of NTFPs categorized as textiles, resins, and medicines, over 90 percent of NTFPs were used by the collecting households and mostly consumed as food. Out of 24 mushroom-type NTFPs, which are easily influenced by the forest environment, nine types accounted for 90% of collected amounts. Of these, five types are collected during rainy season, whereas one type, *Lentinus polychrous* (Lao name: Hed bot), is collected during dry season (Fig. 1). Mushrooms are, therefore, valuable sources of food that are collected throughout the year.

NTFPs categorized as textiles, resins, and medicines were collected for sale, and of these, approximately eight tons (dry weight) of textiles were collected (Fig. 1). The majority of these NTFPs were summer cypress, from which flower clusters are collected to make products such as brooms. Summer cypress appears in fallow land after slashing and burning and can be collected in great quantity for the first three years. It is also a valuable source of income between periods of farming.

The economic value was estimated at 5,480,000 kip (broken down into 3,820,000 kip from plant types and 1,660,000 kip from animal types) (Fig. 1). This equates to approximately 2.4 tons of glutinous rice, enough to feed 9.6 people (at 250 kg/person/year) and contributing greatly to the rural economy.

This study is important towards understanding the subsistence strategies of area residents in Laos over a full year. It can also be used as a basis for implementing safeguards, including the protection of indigenous peoples/ area residents and the maintenance of species diversity, by institutions that implement rural development programs and forest preservation programs such as participatory forest management. Lastly, it provides concrete data on NTFPs, as identified in forestry strategies by Laotian government organizations such as the Agriculture and Forestry Office and the Ministry of Natural Resources and Environment.

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S. Xayalath [Forest Sciences Research Center], B. Khampumi [FSRC])

Table 1. Number of NTFPs, amount, economic value, purpose of collection, and main products based on the attributes of NTFPs collected

Category	Number of NTFPs	%1)	Amount collected: Weight (Kg)2)	Amount collected: Number of pieces	Economic value KIP	%1)	Intended use(%1)): Self-consumption	Intended use(%1)): Sale	Main products
NTFPs of plant origin	289	100	44,587	39,874	397,131,470	100			
Food	262	91	33,515	18,614	202,303,120	51	99	1	
Edible wild plant	133		11,481	105	62,205,920				*Azadirachta indica*, *Centella asiatica*
Flower	10		347	5	854,700				*Musa* spp.
Fruit	19		2,384	1,872	6,674,750				*Livistona saribus*, *Dialium indum*
Bud	68		14,719	16,632	76,082,950				*Schizostachyum blumei*, *Calamus tenus*
Root & Tuber	2		17		154,500				*Curcuma longa*
Spice	6		82		491,700				*Alpinia* spp.
Mushroo	24		4,485		55,838,600				*Lentinus polychrous*, *L. Squarrosulus*, *Schizophyllum comune*
Craft	13	4	228	21,143	68,722,200	17	93	7	*Calamus gracilis*
Fiber	4	1	7,891	114	119,961,650	30	9	91	*Thysanolaena maxima*, *Broussonetia papyrifera*
Resin	2	1	1,240		2,598,400	1	9	91	*Shorea obtusa*
Medicine	8	3	1,713	3	3,546,100	1	41	59	*Smilax glabra*, *Coscinium fenestratum*
Per household			429	383	<u>3,818,572</u>				
NTFPs of animal origin	124	100	5,331	5,543	172,372,200	100			
Mammals	21	17	133		49,816,400	29	97	3	*Callosciurus erythraeusm*, *Rattus norvegicus*
Birds	19	15		2,244	10,150,000	6	98	2	*Spilopelia chinensis*, *Ixobrychus cinnamomeus*
Fish & shelfish	33	27	4,155	2,544	80,807,200	47	99	1	*Cyprinidae* spp., *Clariidae* spp., *Synbranchidae* spp., *Viviparidae* spp.
Reptiles	5	4		97	2,210,000	1	97	3	*Colubridae* spp., *Varanidae* spp.
Amphibian	4	3	446	92	9,919,500	6	98	2	*Rana temporaria*, *Pelophylax esculentus*
Insects	42	34	597	566	19,469,100	11	94	6	*Gryllidae* spp., *Rhynchophoridae* spp.
Per household			51	53	<u>1,657,425</u>				

1) Percentage of each attribute in all NTFPs of plant and animal origins

2) Air-dry weight for craft, fiber resin, and medicen. Wet weight for others.

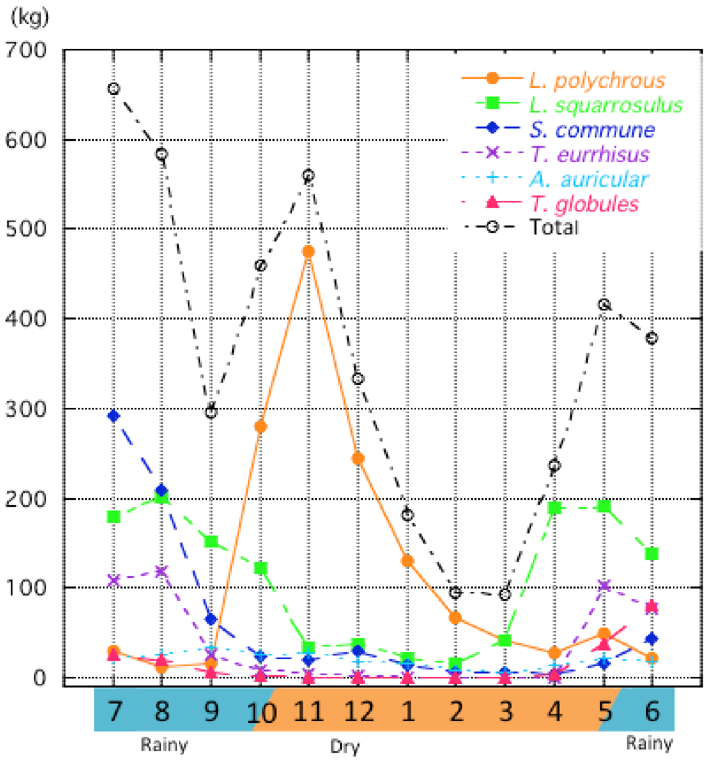


Fig. 1. Monthly collection for each type of mushroom

Resource management of a small-sized cyprinid population in an isolated small stream based on its ecological characteristics

The indigenous cyprinid *Rasbora rubrodorsalis* is widely distributed in agricultural water masses (e.g., irrigation canals) of various Indochinese countries including Laos. This species is small-sized (max. ca. 30 mm), occurs abundantly in remote rural areas of hilly/mountainous regions, and is an important food resource in the area. In recent years, however, settlements and habitat expansion of invasive alien fishes as well as agricultural exploitation / urbanization in such areas are becoming a concern, possibly causing the decline in species diversity/stock level of indigenous fishes. This situation necessitates the acquisition of ecological information conducive to the species' resource management.

The following ecological features and relevant findings were obtained in the present study.

1) The sex ratio of *Rasbora rubrodorsalis* is remarkably biased towards females (male : female = 0.43 : 1), and the females grow larger than males in size (figure omitted).
2) Mature female occurrence ratio increases with seasonal day-length extension (figure omitted), and breeding is more active during high-temperature period (March to October). However, mature females also occur even during low-temperature period (November to February), indicating that the species breed throughout the year (Fig. 2). Maturation sizes of females are > 20 mm SL and > 23 mm SL during high- and low-temperature periods, respectively (Fig. 2), and the maturation ages (in days) were estimated to be 50 and 80 days during high- and low-temperature periods, respectively (Fig. 3).
3) Longevities were estimated to be 150 days in females and 100 days in males (Fig. 3); therefore, plural generation alternations are considered to occur within a year.
4) Seasonal fishing control is considered not efficient for stock management of the species due to its short longevity. Although the upward migration of the species over the small waterfall located at the mid-stream is considered impossible because of limited swimming ability, stock provision from upstream to downstream areas is highly possible insofar as upstream breeding population is well conserved. Hence, continuous fishing in the downstream area concurrent with fishing prohibition in the upstream area is strongly recommended for the conservation of the breeding population and is contributory to both sustainable fisheries and species conservation (Fig. 4).

In addition to the above, environmental conservation is also indispensable for realizing the above-mentioned method for species conservation. Furthermore, considering the deterioration of genetic diversity as observed in the sympatric cyprinid *Esomus metallicus* and the ambassid *Parambassis siamensis* probably due to the geographical isolation, such deterioration may also be occurring in the *Rasbora rubrodorsalis* population. Genetic soundness, therefore, needs to be examined by micro-satellite DNA marker analysis.

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

Fig. 1. Laotian indigenous cyprinid *Rasbora rubrodorsalis* (adult, 24.3 mm SL)

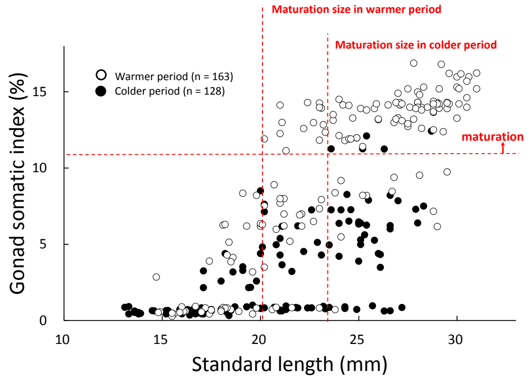


Fig. 2. Relationship between standard length and gonad somatic index in female *Rasbora rubrodorsalis* both in warmer and colder periods

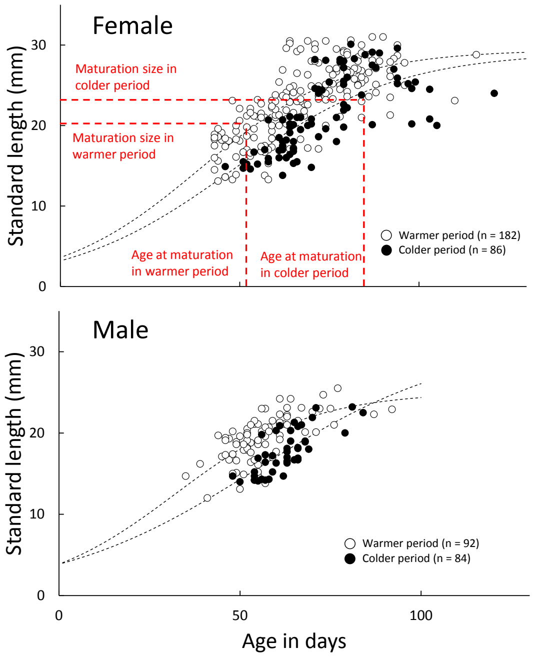


Fig. 3. Growth models of female and male *Rasbora rubrodorsalis* （fitted by Gompertz growth curves)

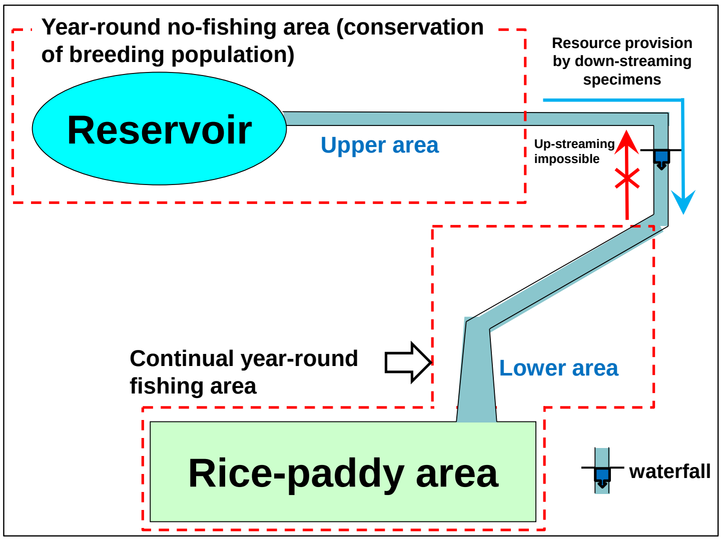


Fig. 4. Schematic drawing of the stream investigated in this study and the suggested year-round no-fishing area and continual fishing area for *Rasbora rubrodorsalis*

Direct Saccharification Technology From Lignocellulosic Biomass

Technology is important because of the high cost of obtaining fermentable sugars efficiently from cellulosic biomass. Many microorganisms capable of producing cellulose and hemicellulose-degrading enzymes have been reported and characterized. Currently, fungal cellulases are prepared and utilized to saccharify cellulosic biomass. It is known that the fungus *Trichoderma reesei* is able to produce high levels of secreted cellulases and several functionally distinct cellulase components. However, utilization of cellulases is an impediment to industrial application due to the high cost of enzymes.

On the other hand, *Clostridium thermocellum*, an anaerobic, thermophilic, spore-forming bacterium, is the most potent cellulose-degrading bacterium known to produce cellulosomes. The cellulosomes of *C. thermocellum* contain a surprisingly large variety of enzymes and show attractive enzymatic properties for the degradation of complex plant biomass. In a previous study, we demonstrated remarkable improvements in cellulolytic activity of cellulosomes from the hypercellulolytic *C. thermocellum* [1] in combination with a thermostable β-glucosidase from *Thermoanaerobacter brockii* (CglT) [2, 3].

In this research, we report on saccharification by *C. thermocellum* cultures supplemented with thermostable β-glucosidases, which we named biological simultaneous enzyme production and saccharification (BSES) [4]. BSES required no addition of cellulolytic enzymes. It can directly produce glucose from cellulosic materials due to supplementation of cellulose degrading cultures with CglT. Exclusive glucose accumulation of glucose occurred when *C. thermocellum* was cultured with a thermostable β-glucosidase under a high cellulose load. This approach may resolve a significant barrier to economical production of bio-based chemicals and fuels from lignocellulosic biomass.

[1] Tachaapaikoon C, et al: Biodegradation. 2012, 23:57-68.
[2] Waeonukul R, et al: Bioresour Technol. 2012, 107:352-357.
[3] Waeonukul R, et al: Bioresour Technol. 2013, 130:424-430.
[4] Prawitwong P, et al: Biotechnol Biofuels. 2013, 6:184.

(A.Kosugi)

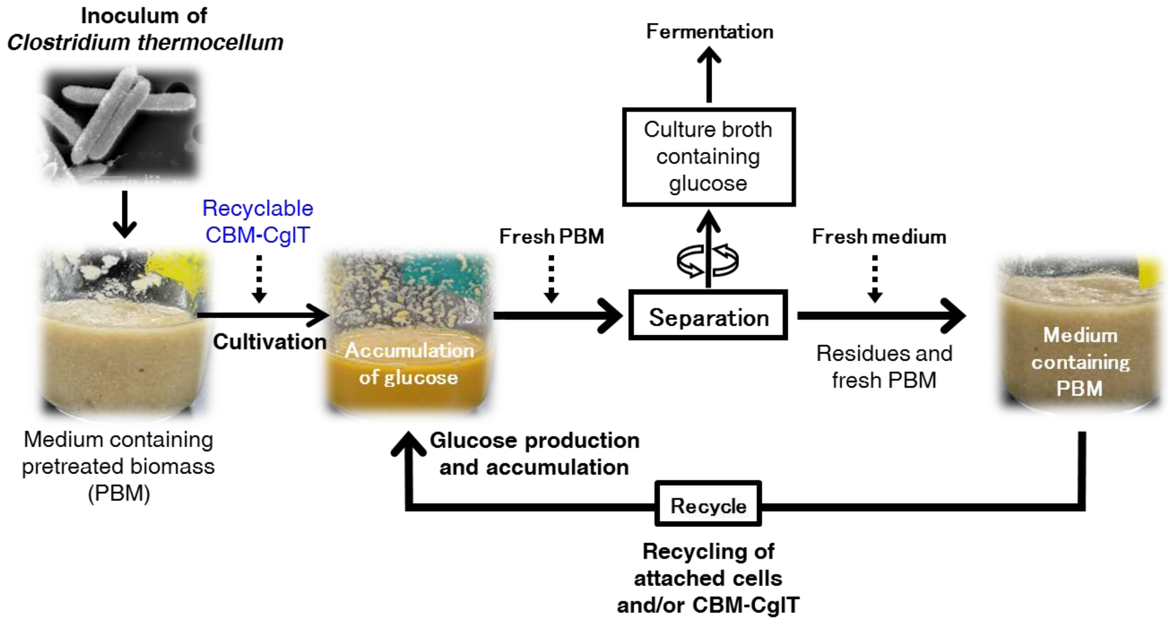


Fig. 1. Schematic of consecutive biological saccharification method by recycling of the hydrolyzed residue. Culture of *C. thermocellum* and supplementation of CBM3-CglT were only carried out the first biological saccharification round without further culture and addition of any enzymes [2]. To recover free cellulosomes and CBM-CglT, fresh pretreated cellulose substrates were added to the hydrolysis slurry, and then reabsorbed from the supernatant. A second round of biological saccharification was subsequently performed using the recovered enzymes by binding to fresh substrate and the hydrolysis residues containing *C. thermocellum* cells. Consecutive biological saccharification using these recycling procedures may be repeated several times.

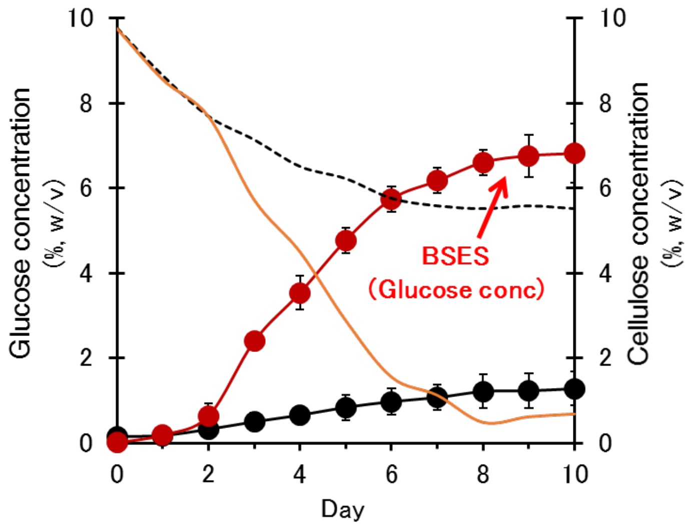


Fig. 2. Biological saccharification using *C. thermocellum* culture supplemented CglT. Cellulose hydrolysis ability and free sugars (glucose) accumulation was monitored under

cultivation of *C. thermocellum* with or without CglT (+CglT/–CglT), respectively. Solid and dotted lines indicated residual cellulose contents (w/v) in culture broth. Error bars represent ± SD (n=3).

Impacts of the oil palm development program in Indonesia on small scale farmers

Since 1977, the Indonesian government has been implementing an oil palm development scheme called the Nuclear Estate Smallholders (NES) program. The program aims to share the benefit of plantation development between palm oil companies and rural communities by allocating a part of the plantation to small scale farmers (hereinafter referred to as ‘plasma farmers’). Under the NES program, the company is also responsible for supporting the plasma farmers through various activities, such as providing soft loans and technical support as well as purchasing fresh fruit bunch (FFB) from farmers at a guaranteed price. Although there are significant differences in the level of performance from one NES case to another, it is difficult to evaluate because of a lack of indicators. Recently, the number of small scale farmers called ‘independent farmers,’ referring to those who don’t join any collaboration programs with companies, is increasing in Indonesia, especially in Sumatera Island. This study aimed to identify the effects of the NES program by comparing the FFB production of plasma farmers and independent farmers based on the survey results about farm household economies.

Tree age of oil palm strongly affects FFB yield. In general, the yield gradually decreases after achieving highest yield between the tree ages of 8 and 13 years. In the NES case of Company A in Riau Province, some plasma farmers were able to maintain or improve FFB yield even with trees aged 20 years or more (Fig. 1A). Such high yields in older trees were not observed in the NES case of Company B (Fig. 1B). From the graph, it can be seen that the net profit of plasma farmers in Company A was significantly higher than those of independent farmers (Fig. 2).

It must also be noted that the amount of fertilizer application by plasma farmers was higher than the amount applied by independent farmers. The amount of potassium fertilizer applied by independent farmers was particularly lower than the standard fertilizer application rate (Fig. 3). Applying the appropriate amount of fertilizer has contributed to better yield for plasma farmers.

While all plasma farmers planted high quality seedlings with certification provided by Company A, most independent farmers purchased seedlings without any quality assurance from local shops or nearby farmers (Fig. 4). Clearly, the quality of seedlings was another factor affecting FFB yield. Other factors that have contributed to higher yield by plasma farmers include continuous technical assistance, Company A’s attitude towards the plasma farmers (e.g., fulfillment of contracts), and Company A’s higher dependency on plasma farmers as a source of FFB for their palm oil mill.

The above findings can be used by local administrative agencies to formulate yield improvement policies that would benefit small scale oil palm farmers.

(T. Sugino)

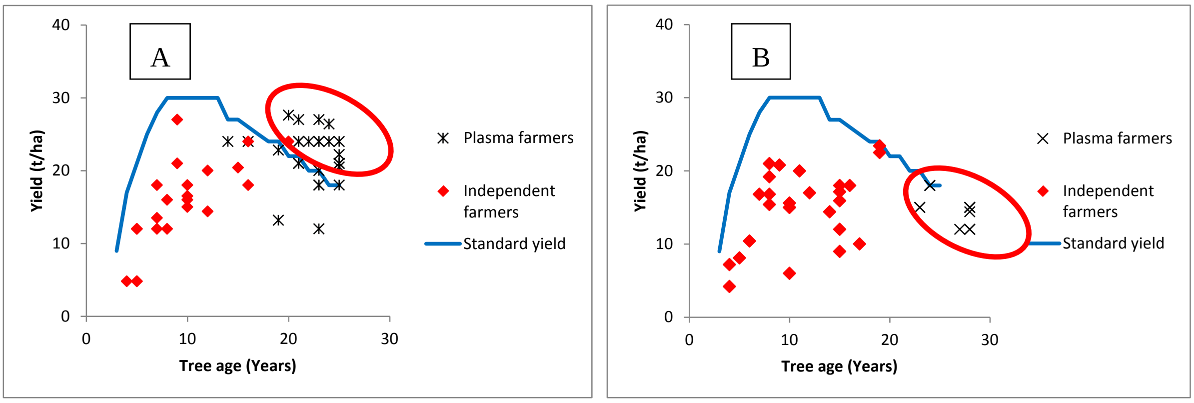


Fig. 1. Relationship between tree age and fresh fruit bunch (FFB) yield (left: Company A case study, right: Company B case study).

The standard yield shows the general relationship between tree age and FFB yield as reported by Adlin (1990). In the Company A case study, some of the plasma farmers maintained or improved FFB yield in spite of the higher tree age of 20 years or more (circled in red).

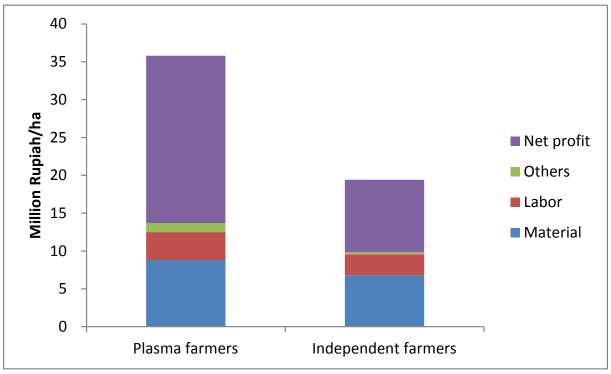
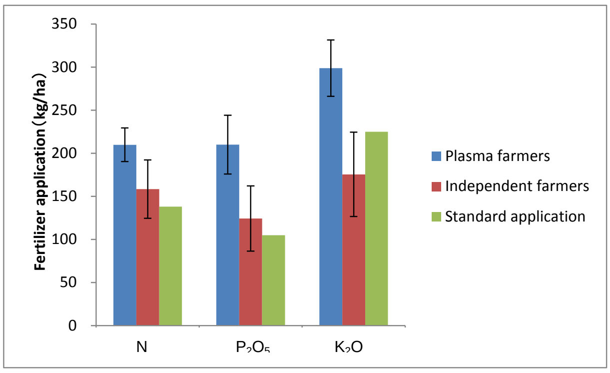


Fig. 2. FFB production cost and profit (Company A case study)

Cost and profit numbers were averaged for 26 plasma farmers and 22 independent farmers. Significant differences were observed in net profit (significant level: 1%), other cost (1%), labor cost (5%) and material cost (5%).



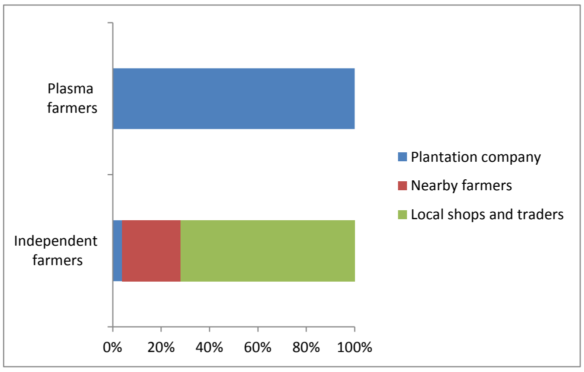


Fig. 3. Fertilizer application by farmers (Company A case study)

Percentages were averaged for 12 plasma farmers and 8 independent farmers. The error bars show the standard errors.

Fig. 4. Source of oil palm seedlings (Company A case study)

Number of respondents: 27 plasma farmers and 25 independent farmers

Methods to establish transfer zones of forest reproductive materials in Peninsular Malaysia

Uncontrolled transfer of forest reproductive materials (FRMs) has resulted to failures, sometimes taking many years to develop even after seemingly successful establishment. Failures, which should have been caused by less adaptation of transferred FRM to new environments, were often due to climatic, insect, or disease events that had much less impact on the native source. In uniform plantations, failures reduce the productivity of the new plantation, where better productivity is expected than in the previous plantation. Another problem arises in enrichment planting, which is commonly conducted in tropical rain forests in Southeast Asia. If transferred FRMs reach reproductive stage, they genetically contaminate the next generation through mating with native trees because enrichment planting is conducted in primary or secondary forests. Genetic contamination should not be ignored as the fitness of native forests in this region is affected when large volumes of transferred FRMs are introduced.

To avoid these problems, some methods have been proposed to establish FRM transfer zones. Provenance trials should provide the most reliable information for determining the limits of FRM movement and discerning which seed sources are best for planting locations. However, these trials have disadvantages and they are costly in terms of resources and time. If progeny test materials involve a few seed sources, then they can be used for assessing FRM transfer zones. Short-term common-garden studies, compared with field provenance trials, have the disadvantage of not evaluating seed sources during extreme climatic events and naturally occurring pest problems over time. However, they provide information about adaptation to environment expressed at the early stage of their growth. Although molecular markers are considered as neutral or nearly neutral against adaptation (selection), processes such as migration (movement of alleles among locations), population size, and genetic drift (random loss or fixation of alleles) affect the distribution of variation in molecular markers. There have been continuing discussions on how molecular markers are used for determining FRM transfer zones. Because the advantage of molecular markers are less time and labor, FRM transfer zones established by molecular markers should be considered tentative until confirmed by adaptive traits in a common environment (Fig. 1). Therefore, adaptive management should be introduced for FRM transfer zones based on molecular markers, which are going to be revised by the latest information on adaptive traits.

Because of the urgent necessity and the less time and labor it entails, we adopted the method of using molecular markers to establish FRM transfer zones. We analyzed the genetic structure of two important timber species in Peninsular Malaysia as examples. Genetic marker analysis has detected that the genetic diversity of *Neobalanocarpus heimii* (local name: chengal) was well explained when genetic diversity was separated as four different clusters. These four different clusters were localized at different regions in Peninsular Malaysia. When the localization of clusters was applied in determining the FRM transfer zone, four regions were recognized as FRM transfer zones. On the other hand, genetic marker analysis detected that the genetic diversity of *Shorea curtisii* (local name: seraya) was explained when genetic diversity was separated as three different clusters. These three different clusters, however, showed lower probability as regards cluster separation and showed ambiguous correspondence to localization in Peninsular Malaysia. This result supports the idea that strict regulation is not required for the species (Fig. 2). We must remember that the result was obtained using neutral genetic markers. If adaptive genetic variation is detected by long-term field provenance trial, medium-term progeny test, short-term common garden nursery and/or non-neutral genetic marker analysis in the future, then further revision of the regulation is required. Thus, FRM transfer zones should be determined for each important timber species in Peninsular Malaysia.

(N. Tani, N. Muhammad [Forest Research Institute Malaysia], S. L. Lee [FRIM], C. H. Ng [FRIM], L. H. Tnah [FRIM], K. K. S. Ng [FRIM], C. T. Lee [FRIM], N. F. Zakaria [FRIM], Y. Tsumura [University of Tsukuba])

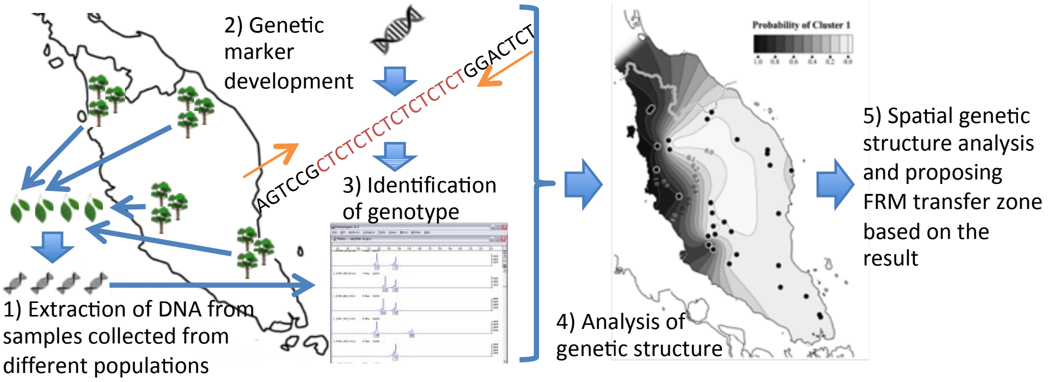


Fig. 1. Research flow showing the proposed forest reproductive material (FRM) transfer zones based on molecular markers

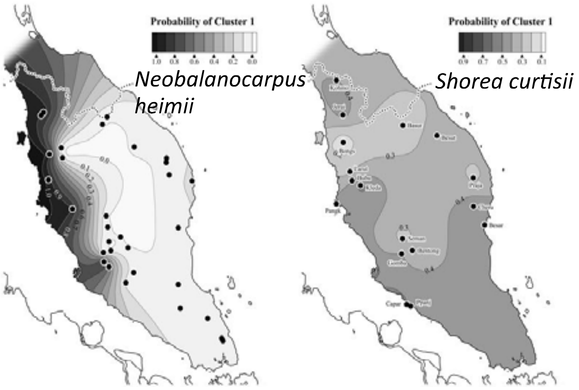


Fig. 2. Examples of two timber species, which showed distinct patterns of genetic structure, in Peninsular Malaysia

Extraction of *p*-hydroxybenzoic acid, a promising raw material for plastics, from oil palm biomass

Malaysia is one of the world's leading producers of palm oil. Huge amounts of residues like empty fruit bunches (EFBs), kernel shells (accumulated in mills), fronds and trunks (left at plantation fields) are generated from oil palm (*Elaeis guineensis* Jacq., Arecaceae). Low-molecular-weight phenolic compounds (LMPCs) extracted from subcritical water treatment of oil palm biomass has shown promise as a raw material for producing plastics, thereby promoting the efficient use of waste resources. In this study, the LMPC yield for each part of the oil palm was clarified.

An oil palm plant sample was divided into 11 parts for use as residue materials for extracting LMPC [e.g., *p*-hydroxybenzoic acid (PHBA)] (Fig. 1). We carried out subcritical water extraction of the oil palm trunk and the condition of transformation/extraction was optimized for a given combination of temperature and time. In contrast to supercritical water extraction, which causes pyrolysis, subcritical water extraction possesses a high hydrolytic ability. PHBA is a native constituent in oil palm, and its yield markedly increases with the degradation of ester linkages in high-molecular-weight secondary metabolites (Fig. 2A). Evaluations showed that the kernel shell had the highest PHBA yield. In comparison, the frond (including leaves, petiole, and rachis) was evaluated as superior, in consideration of the resource amount being up to six times that of EFB or trunk, although the PHBA yield from this part is not very high (Fig. 3).

Subcritical water treatment is an environmentally friendly method of extracting phenolic constituents because it does not require organic solvents, acids, alkalis, and so on. It can be performed if there is a pressure-tight container, which can withstand high temperatures of about 200–250˚C, and a temperature controller. Subcritical water extraction, therefore, has an advantage in terms of equipment cost and energy consumption. There is a huge amount of residue resources, particularly fronds; however, there are few utilization methods available at present. In this study, the potential of fronds was appreciated from LMPC yield data and resource amount for each part of the oil palm. The kernel shell, on the other hand, exhibited the highest PHBA and LMPC yields. Although the resource amount is less than that of fronds, it was classified accordingly as available raw material because palm kernel shells are regularly accumulated in palm oil mills as wastes.

(*F. Kawamura, R. Hashim [Universiti Sains Malaysia], O. Sulaiman [Universiti Sains Malaysia], N.S. Saary [Universiti Sains Malaysia]*)

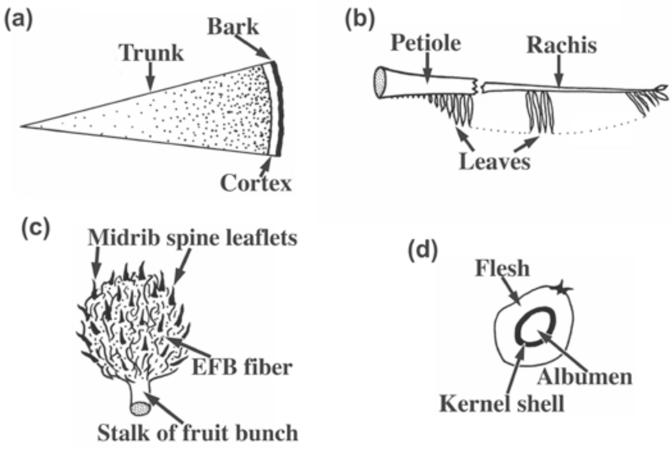


Fig. 1. Parts of the oil palm (a: cross-section of trunk, b: frond, c: empty fruit bunch, d: cross-section of fruit)

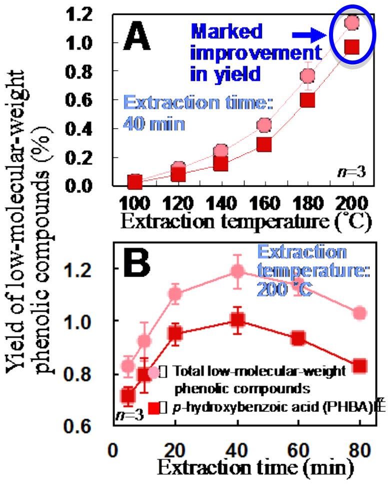


Fig. 2. Effect of temperature (A) and time (B) on the yields of low-molecular-weight phenolic compounds from oil palm trunk during subcritical water extraction

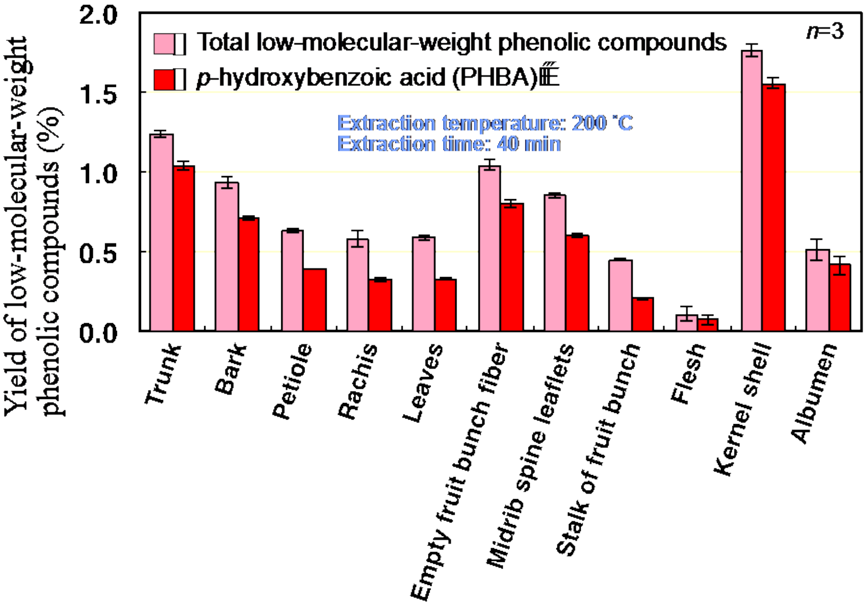


Fig. 3. Yields of low-molecular-weight phenolic compounds from each part of oil palm

Disincentive factors affecting stable aquaculture production of the blood cockle, *Anadara granosa,* in Malaysia

The blood cockle, *Anadara granosa*, is an important bivalve aquaculture species in Southeast Asian countries. They thrive where there is a widespread presence of mud flats, as in the west coast of Peninsular Malaysia, which provide suitable habitat and is a center for sowing aquaculture. Blood cockle spats are naturally abundant along the coasts and are exported to neighboring countries as aquaculture seeds. Recently, however, a huge reduction in the number of spats in aquaculture grounds in the west coast has been noted. Likewise, the farming process has been seriously affected by mass mortality. Therefore, some of the possible causes were investigated and countermeasures were formulated. In this study, we revealed the disincentive factors affecting blood cockle aquaculture and considered future measures for achieving stable production.

Regular monitoring was carried out in three sampling stations in blood cockle aquaculture grounds. Results showed failure of sexual maturation in Station 1 during the monitoring period (Fig. 1). In addition, surface sediments in Station 1 had been significantly reduced, according to an environmental survey carried out in November 2010 (Fig. 2). High organic loading in the aquaculture grounds was suggested as one failure factor for the phenomena. On the other hand, a case study of the mass mortality event in mid-February 2012 (more than 30% mortality in Selangor coast) revealed that the blood cockle was in its spawning season and that it was in failure condition due to reduced food availability, based on histological observations of the gonad and digestive ducts, respectively (Fig. 3). Moreover, these results suggested that the cockles failed to absorb nutrients as evidenced by the flattening of epithelial cells in their digestive glands (Fig. 4). Incidentally, mass mortality occurred during a week-long rainy period (over 30 mm/day for four days). Environmental changes associated with freshwater inflow, carrying with it high-suspended matter from rivers, may have weakened the blood cockle’s environmental resistance especially because they were spawning. Thus, it was inferred that reduced feeding opportunities and nutrient absorption disorders may have led to the blood cockle’s debilitated condition.

For a sustainable blood cockle aquaculture, it is important to lower the mortality rate in the aquaculture process and produce a stable supply of blood cockle spats. In the future, we need to establish water and sediment quality standards in aquaculture grounds as a fisheries management strategy and connect sound management approaches to stable production, with the Malaysian local government and fishermen playing leading roles in managing water and sediment quality. We intend to recommend this system to the Department of Fisheries in Malaysia for implementation and realization in the near future.

(T. Yurimoto, Faizul Mohd Kassim [FRI Malaysia], R. Fuseya [FRA Japan], Alias Bin Man [FRI Malaysia])

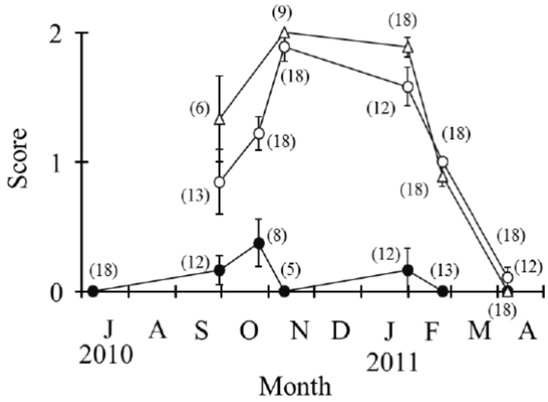


Fig. 1. Naked-eye observations of the visceral area covering the gonad of the blood cockle. Score 0: immature, score 1: developing, and score 2: mature. Number in parentheses refers to the number of individuals used for observation. The graph shows that there was no gonad development at Station 1 (●), whereas significant development was observed at Stations 2 and 3 (○ and △). Error bar indicates standard error.

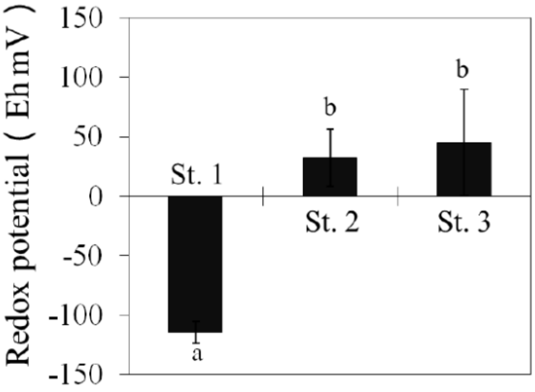


Fig. 2. Redox potentials in surface sediments around the blood cockle aquaculture grounds. Significant reduction was detected at Station 1 where sexual maturation of the blood cockle was not observed. Significant difference ($P < 0.01$) was observed between a and b. This survey was conducted in November 2010 when gonad development was observed in Stations 2 and 3. Error bar indicates standard error.

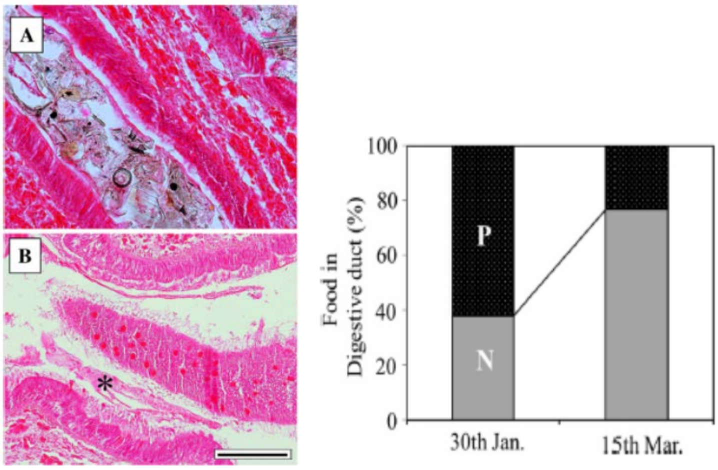


Fig. 3. Presence and nonpresence of food in the digestive duct of the blood cockle before and after the mass mortality event.
Photo A: digestive duct filled with food, Photo B: no food in the duct (asterisk shows the empty digestive duct.) Scale bar in photo: 100 µm. Graph data shows an increase in the number of individuals with empty digestive ducts after the mass mortality event.

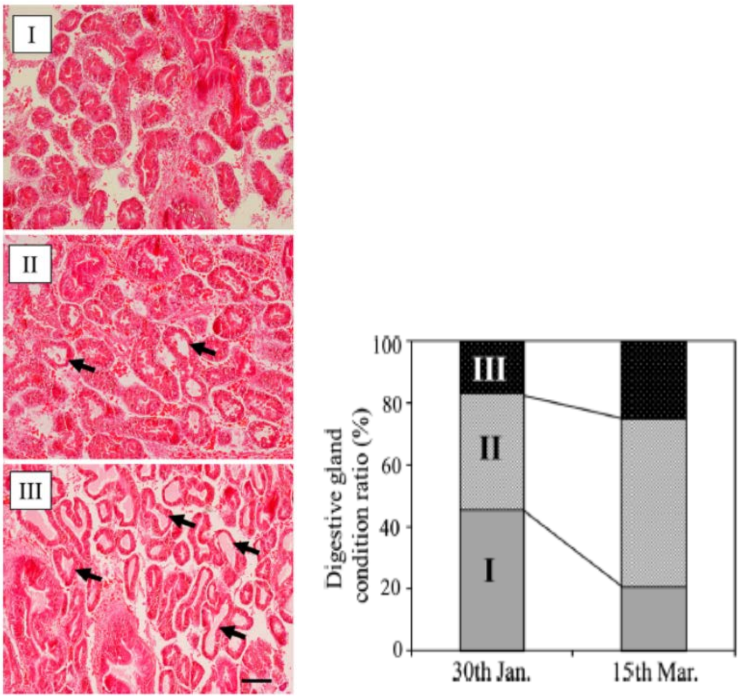


Fig. 4. Condition of epithelial cells in the digestive gland of the blood cockle before and after the mass mortality event
Photo I: good condition of epithelial cells, photo II: partial flattening of epithelial cells (arrows), photo III: substantial flattening of epithelial cells (arrows). Scale bar in photo: 100 µm. Graph data shows an increase in the number of individuals with flattened epithelial cells after the mass mortality event.

Production and trade of major crops in Myanmar

Myanmar has drawn attention as “the last frontier” after shifting to civilian rule and being relieved of economic sanctions in 2011. The agricultural sector is expected to grow rapidly as a result of an increase in investments, which had been very limited. Using official data published by the government of Myanmar, this study generated maps covering 14 administrative regions to grasp the spatial characters of agricultural production.

Maps showing paddy, oil crops (e.g., sesamum, sunflower, ground nut), pulses for food (e.g., green gram, black gram, pigeon pea), and several other crops were created (Table 1). Sown area, yield, and production quantity were displayed in maps subdivided into administrative regions (Fig. 1). In addition to these basic data, also generated were the estimated annual average rate of change of those items, the proportion of sown area in arable land, the contribution of yield change to production change, and the per capita supply of those items. Figure 2 shows a visualized time series data of sown area, yield, production quantity, domestic supply, and trade. Prices (e.g., market price, producer price, and export or import price) deflated by the consumer price index, or the GDP deflator, were juxtaposed in each graph. Graphs depicting domestic supply and trade included self-sufficiency rates, export rates, trade quantities, values, and prices. Trade values and prices in local currency, which were originally associated with “official exchange rate,” were revised with “parallel market exchange rate.” Furthermore, domestic market prices were added to the graphs of export or import price to show the gap between prices within and outside Myanmar.

The results of this study have been published by the Research Strategy Office and uploaded to JIRCAS’s Program D webpage. Although the report is written in English, some contents and descriptions are also written in Japanese. The spatial and temporal characteristics of agriculture in Myanmar can be grasped easily; for example, production quantities and deflated prices, such as market price, producer price, and export or import price, will help estimate the relationship between prices and supply responses. There are issues of reliability in the raw data; nevertheless, this report would be useful for gaining a rough perspective on the agricultural situation and could provide materials for further discussion. An update will be considered in 2016.

(E. Kusano, O. Koyama)

Table 1. Target crops

Commodity group	Commodity	
1 Paddy	1.1 Monsoon	
	1.2 Summer	
2 Oil crops	2.1 Groundnut	(1) Rain
		(2) Winter
	2.2 Sesamum	(1) Rain (Early)
		(2) Winter (Late)
		(3) Summer
	2.3 Sunflower	
	2.4 Oil palm	
3 Pulses	3.1 Black gram	
	3.2 Green gram	
	3.3 Pigeon pea	
	3.4 Chick pea	
	3.5 Other pulses	(1) Cow pea
		(2) Bocate bean
		(3) Krishna mung
3 Pulses	3.5 Other pulses	(4) Rice bean
		(5) Butter bean
		(6) Duffin bean
		(7) Lima bean
		(8) Sultani bean
		(9) Sultapya bean
		(10) Soy bean
		(11) Lablab bean
		(12) Garden pea
		(13) Lentil bean
4 Other crops	4.1 Wheat	
	4.2 Maize	(1) Seed
		(2) Cob
	4.3 Sugarcane	
	4.4 Tea	
	4.5 Coffee	

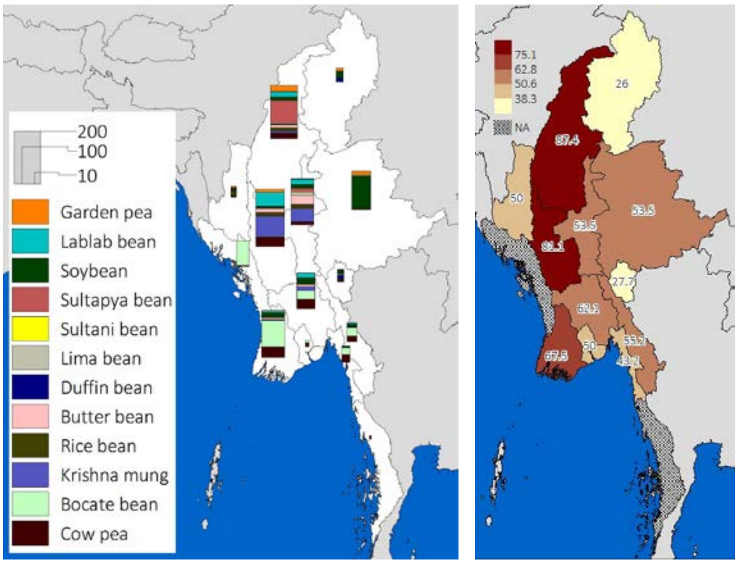


Fig. 1. Thematic maps
Left: Sown area of beans for food in 2010 (1,000ha).
Right: Contribution of the yield change in sesamum production during 2000–2009 (%)

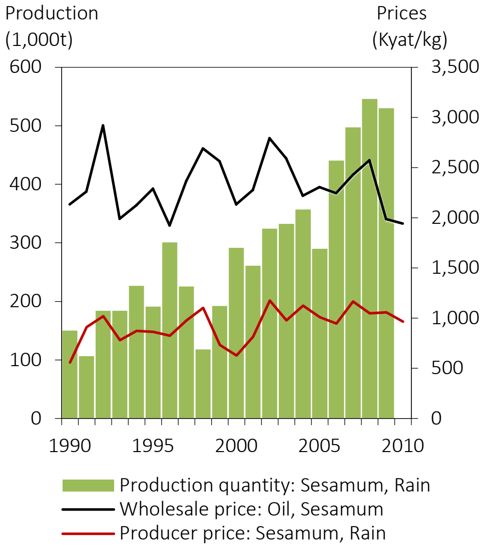


Fig. 2. Production quantity and prices (deflated to 2010 level).
1 kyat ≈ 0.001USD in 2010