Manual for growth promotion of dry season vegetables utilizing limited water resources

The Republic of Niger is situated in an arid to semi-arid area at the southern end of Sahara desert; hence, water is a precious resource. However, these water resources are not fully utilized even though there are a number of seasonal streams and ponds. According to the Water Resources Development Master Plan (1999) report by the Ministry of Water Resources, Environment & Desertification Control, the country has more than 1,000 ponds of which 175 are distributed as permanent ponds. However, these ponds rarely undergo water utilization surveys and there is no clear strategy for its long-term development and use.

As a result, water utilization for dry season vegetable cultivation can be described as underdeveloped even as increased usage is expected in the future. For this reason, the authors recognized the need for the development and diffusion of cultivation methods to effectively promote cultivation of dry season vegetables despite limited water resources.

A questionnaire survey targeting farmers from 37 villages was conducted to identify the constraining factors associated with the growth promotion of dry season vegetables. Three major constraints were discovered from the questionnaire survey, namely: (1) crop damages from feeding livestock, (2) difficulties in acquiring agricultural materials and equipment, and (3) crop damages from diseases and harmful insects. In addition, the survey group also found two more problems from the survey result, specifically: (1) the lack of seedling-rearing techniques, and (2) insufficient organizational efforts. Based on these findings, countermeasures were formulated, including: (1) support for the installation of fences to prevent feeding damages, (2) support for the introduction of purchase system for agricultural materials and equipment, (3) support for technical training on vegetable cultivation, and (4) organizational support (Fig.1). An experimental study of the target sites was subsequently conducted (Fig. 2).

This technical manual summarizes the results of an experimental study performed targeting the periphery of natural ponds which have not been fully utilized. Nevertheless, its contents can be applied not only to natural ponds but also to sites with water sources originating from dams, flood control basins and water wells.

Also, this manual was compiled hoping that it finds a high degree of utilization in the field. The contents were edited with inputs from a technical committee composed of interested departments and agencies of the Ministry of Agriculture in Niger. Concurrently, a repeat peer review in French was also conducted so that local agricultural extension workers can understand and for the manual to gain approval from the Department of Survey and Planning, Ministry of Agriculture.

Lastly, this manual is intended for use by those who provide support and guidance to farmers including extension workers and farmer-trainers employed by non-governmental and international organizations, with the aim of promoting vegetable farming in the dry season. It is necessary to properly implement the contents of this manual to effectively utilize the natural ponds and to promote the growth of dry season vegetables. However, prudence must

be exercised when following the instructions contained herein. It must be in accordance with actual site conditions because some of the contents may have already been implemented or the manual may prove insufficient due to reasons that are particularly site-specific.

A total of 500 French-version copies of the manual have been distributed by the Ministry of Agriculture and relevant authorities to field agencies during a publication seminar; thus, providing tangible support to the vegetable cultivation domain and seminar participants.

(K. Oosuga, H. Dan, J. Yasuhisa, T. Shinohara, M. Charles, N. Kawano, H. Oomae)

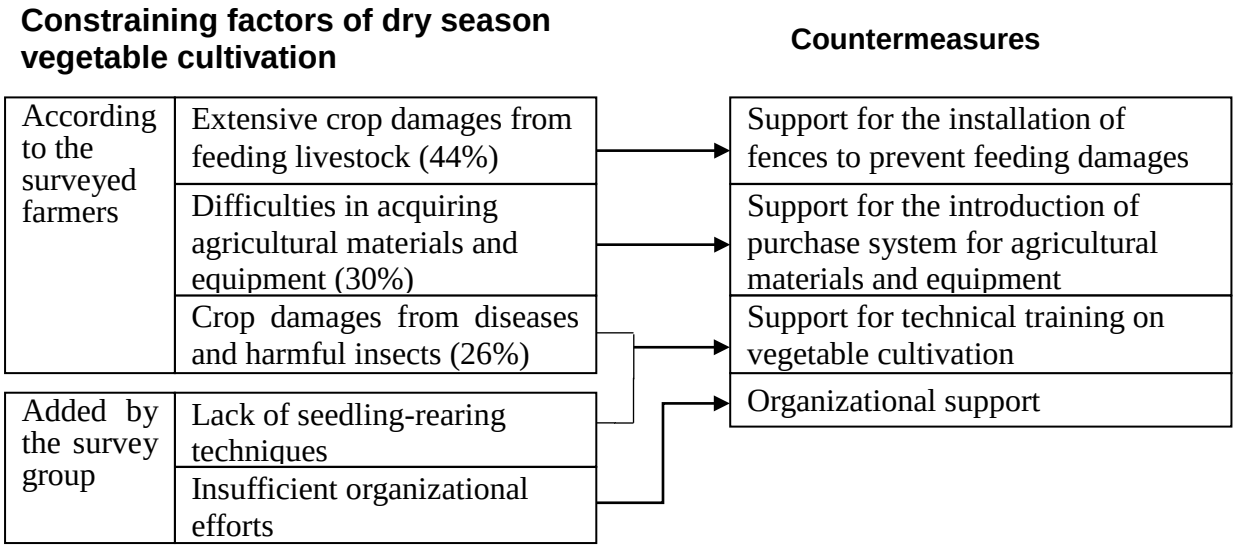

Fig. 1. Dry season constraints on vegetable cultivation and the proposed countermeasures

Fig. 2. Efforts undertaken at experimental study areas

a) (left photo) Provision of assistance to organize water resource users. As for the selection of union executives, voting in a secret ballot was adopted so people can select from among multiple candidates and vote anonymously.

b) (middle photo) With the farmers' consent and support, fences were installed to prevent crop damages from feeding livestock .Workers learned building fences, from design to construction, via on-the-job trainings.

c) (right photo) Implementation of improved farming techniques. Vegetable cultivation training on diversified seedling-rearing techniques, crop disease and pest control measures and water irrigation techniques was provided.

Impacts of climate changes through variations of evapotranspiration on rice market and production capacity in the lower Mekong River Basin region

Supply of water to farms will be varied by climate changes. On the other hand, per capita demand of rice will decrease in the lower Mekong River Basin, where two major rice exporting countries are situated and remarkable economic growth is taking place. Thus, supply and demand analyses of agricultural products are important in this region where fluctuating water supply and a decrease in per capita rice consumption is anticipated. In this study, the impacts of water supply fluctuations on the rice market are analyzed by using supply and demand models of rice including water variables. The results of this study will help in the preparation of agricultural policies and plans for the lower Mekong River Basin.

A supply and demand model of rice in the lower Mekong River countries was used for the analysis. The structure and the data of the model are written down in Furuya *et al*. (2010), and it can evaluate the impacts of climate change through variations in evapotranspiration.

The following two simulations were conducted: 1) base-line, 2) CC_B2 (Climate Change for scenario B2). Base-line simulations assume that the evapotranspiration in each province or region after 2000 is the average during 1995-1999 and that the population and gross domestic product (GDP) of each country follow B2 scenario of the Intergovernmental Panel on Climate Change (IPCC). The B2 scenario refers to the socio-economic scenario which prioritizes environment over economy and region over globe, with population and GDP projections deemed intermediate in the four scenarios. CC_B2 simulations assume that the evapotranspiration in each province or region follows the B2 scenario and population and GDP are same as those of the base-line scenario. The evapotranspiration of CC_B2 to that of base-line in rainy season will increase in Laos and northeast Thailand at trans-planting season and will decrease in all regions at flowering season.

Based on model simulations, climate change will decrease wet season rice production in Cambodia (Fig. 1) and will decrease dry season rice production in Mekong Delta region (Fig. 2). Furthermore, climate change will increase farm prices of rice in Cambodia, Thailand, and Viet Nam (Fig. 3), and these price spikes will weigh on the living costs of consumers.

The gaps between the forecasted planted area of rice and irrigation area of the basin development plan of the Mekong River Commission in 2030 were investigated for each province and region. The results suggest that planted areas of dry season rice on the west side of the Mekong River Basin and Mekong Delta region will reach the upper limit of the irrigation area for rice cultivation as shown by the yellow-colored regions (Fig. 4).

The results of this study (specifically, forecasting rice productions and farm price of rice, etc. for each province and region) will contribute to making a feasible agricultural production plan for the lower Mekong River Basin countries.

(*J. Furuya and S. Kobayashi*)

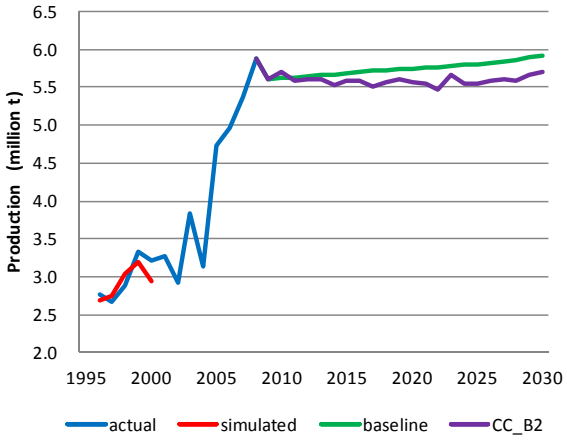

Fig. 1. Wet season rice production in Cambodia

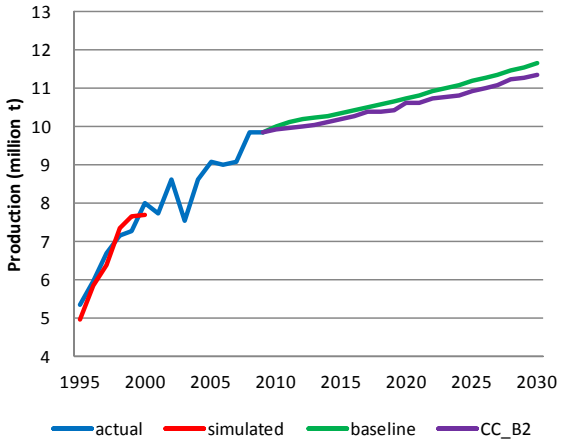

Fig. 2. Spring season rice production in Mekong Delta

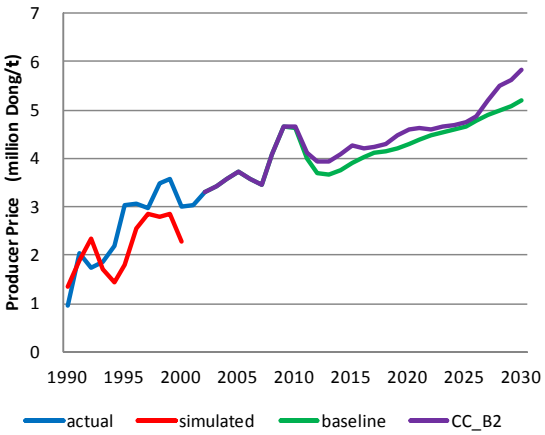

Fig. 3. Farm price in Viet Nam

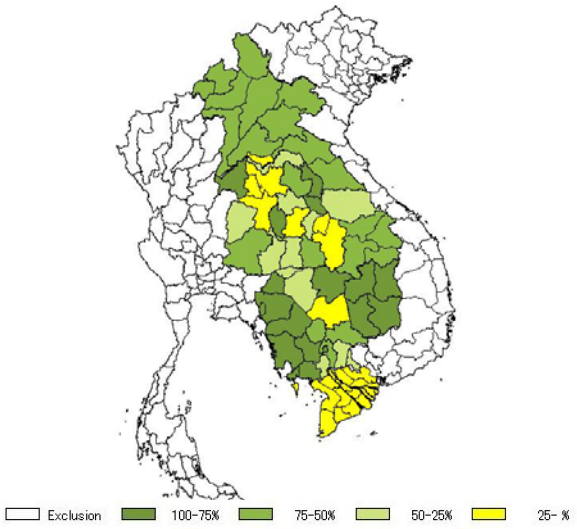

Fig. 4. Rice production capacity

Note: “Simulated” in Figures 1-3 shows estimation results of the model.

References

Furuya J, *et al*. (2010), Development of Supply and Demand Models of Rice in Lower Mekong River Basin Countries: REMEW-Mekong, JIRCAS Working Report 68.

Near-isogenic lines for days to heading with an Indica-type variety IR64 genetic background

IR64, recognized globally as a high quality rice variety, was first released by the International Rice Research Institute (IRRI) in 1985. To increase yield potential and broaden the adaptability of IR64, a set of near-isogenic lines (NILs) of IR64 with various days to heading (DTH) were developed using Japonica-type high-yielding varieties including new plant type varieties as donor parents.

A total of five NILs were developed through marker-assisted selection. Three NILs having quantitative trait loci (QTLs) from IR65600-87-2-2-3 (IR64-NIL7) and Hoshiaoba (IR64-NIL10 and IR64-NIL11) showed earlier heading by 5 days than IR64, while two NILs from IR65598-112-2 (IR64-NIL8) and IR69125-25-3-1-1 (IR64-NIL9) showed later heading by 10 days (Table 1). Three QTLs for short DTH were detected on chromosomes 8, 6 and 11, while two QTLs for long DTH were detected on chromosomes 6 and 11 (Fig. 1 and Table 1).

These lines are the first set of NILs carrying various QTLs for DTH suitable for tropical conditions. These NILs can be used to understand the genetic basis of DTH and the effects of a single QTL/gene by testing it under different environmental conditions. Furthermore, early heading NILs could be useful for avoiding abiotic stresses at the late growth stage, while late heading NILs could be useful as breeding materials to develop high yielding lines.

(T. Ishimaru, N. Kobayashi [NICS], D. Fujita [NICS/JSPS], Y. Fukuta)

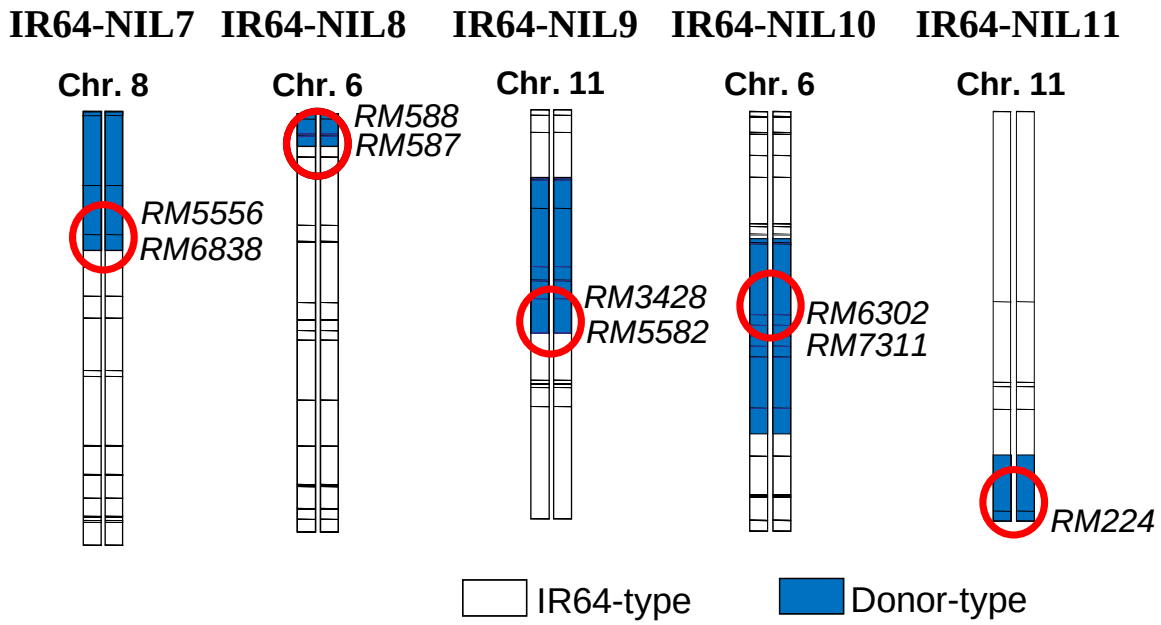

Fig. 1. Graphical genotypes of IR64-NILs' chromosomes which contain QTL regions for DTH (indicated by red circles) derived from donor parents

Table 1. Agronomic characteristics of near-isogenic lines of IR64 for days to heading

Variety/Line	QTL	Donor parent	Agronomic trait (average ± standard deviation)					
			Days to heading	Culm length (cm)	Panicle length (cm)	Leaf length (cm)	Leaf width (cm)	Panicle number
IR64	–	–	85.4±1.2	78.6±3.0	25.2±1.5	38.2±5.3	1.29±0.1	18.0±5.3
IR64-NIL7	*qDTH8*[yp1]	IR65600-87-2-2-3	80.5±2.2*	71.8±2.0*	22.3±1.9*	36.1±4.6	1.22±0.0	21.1±5.1
IR64-NIL8	*qDTH6*[yp3]	IR65598-112-2	95.6±2.3*	85.6±1.8*	26.6±1.7	42.8±4.5	1.41±0.1*	18.5±5.6
IR64-NIL9	*qDTH11*[yp6]	IR69125-25-3-1-1	93.4±2.5*	83.6±4.1*	25.7±1.6	40.7±3.0	1.38±0.1*	26.6±8.5*
IR64-NIL10	*qDTH6*[yp7]	Hoshiaoba	80.3±2.5*	76.4±6.0	26.7±2.1	43.1±5.1	1.30±0.0	18.6±3.3
IR64-NIL11	*qDTH11*[yp7]	Hoshiaoba	81.9±2.2*	77.9±2.6	26.8±2.1	41.9±6.2	1.36±0.1	18.8±5.3

Data was obtained in the IRRI experimental field (Los Banos, Philippines) during 2010 dry season (Jan – May) except for days to heading during 2010 wet season (Jun – Nov). Asterisks indicate significant difference with IR64 at the 5% level according to Dunnett's test.

Manual for Improving Rice Production in Africa

In Western African countries such as Nigeria and Ghana, a particular way of rice cultivation called the "Sawah" system, is being practiced on some rice fields. The "Sawah" system is technically defined as rice cultivation on a bunded, (i.e. leveed or embanked) well-leveled rice field with an inlet for irrigation and an outlet for drainage. Based on observations, the system reported remarkable results comparable to traditional rice cultivation (i.e. on rice paddies without levees).

JIRCAS saw the need to develop the inland-valley areas where grass-roots support can be readily harnessed and developed for the local farmers to become skilled practitioners of the "Sawah" system. JIRCAS started the study through the Development of Improved Infrastructure and Technologies for Rice Production in Africa (DIITRPA) program in 2008, with financial support provided by the Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan. The project was carried out by doing the following: (a) manual construction of the levee or embankment, (b) leveling the land using a power tiller, and (c) delivering irrigation water through manmade canals.

As one of the outputs of the study, JIRCAS published a technical manual, an index of which is listed on Table 1. It includes many findings acquired through four years of validation studies in Ghana and Ethiopia, including: (a) site selection, (b) organizing farmers' groups, (c) appropriate use of power-tiller or oxen (Fig. 1), (d) constructing small-scale irrigation facilities, irrigation canals and levees, (e) leveling, puddling and transplanting (Fig. 2), (f) adequate weeding and fertilizer application, and (g) post-harvesting techniques, among others.

On JIRCAS' recommendation, many charts and illustrations were used to make the manual easier to comprehend and help the target readers, particularly extension officials (EOs) and farmers, digest the contents easily. Any rice producer who uses the manual is expected to easily understand the steps to undertake on the first year and the following years (Fig. 3). Usually, the first year is most crucial as it is the time when land is initially developed for rice cultivation, with lighter work expected for the succeeding years.

Some difficulties were encountered during validation studies in Ghana due to the area's natural conditions (i.e. topography and precipitation). To address the issue, JIRCAS recommended and shared the cost of constructing canals on a case-to-case basis to enable conveyance of irrigation water to the field. The availability of several types of irrigation facilities such as: (1) dike and weir type, (2) canal type and (3) water-harvesting type in the capital city of Kumasi in the Ashanti Region allowed the study to overcome such difficulties. The validation studies showed that the proposed techniques are effective on paddy fields of bunded and leveled conditions similar to the traditionally-practiced paddy fields in Japan.

In addition, a guide for rehabilitating constructed irrigation facilities such as weirs and canals was also included in the technical manual for the first time—an invaluable information that was never contained in previous manuals .

JIRCAS recognized the importance to have the manual published in local language in order to reach the most number of its intended users. The first draft was compiled in 2009 and delivered to EOs as well as farmers within JIRCAS experimental plots to solicit comments and suggestions. The draft manual was then revised after further consultation with government officers, EOs and researchers.

In Ashanti Region, Ghana where the validation study by JIRCAS was conducted, some farmers have already enjoyed a twofold increase of rice yield to 4.2 ton/ha whereas traditional practice produced only 2.0 ton/ha (based on the Afari site, as reported by the Ministry of Food and Agriculture). Similar achievement can be expected in rain-fed inland valleys in Africa (around 4.5 million hectares, according to estimates by the Coalition for African Rice Development or CARD) if farmers practice rice cultivation with the aid of the manual.

The role of EOs is very important as they could serve as catalysts in achieving the goal of increasing rice yield through effective transfer of technological information to the farmers. Thus, enhancing their roles and getting them more involved would be greatly beneficial to the program. Mechanization, however, is not as easily achievable in the short term (i.e. within the next few years) because power tillers are operated continually in a particular site and these machines require regular maintenance. Maintenance of these machines entail procurement of spare parts that may not be readily available, not to mention the need to have a standby mechanic and blacksmith for occasional repairs.

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Fig. 1. Land leveling by a power tiller (Ghana)

Fig. 2. Transplanting (Ghana)

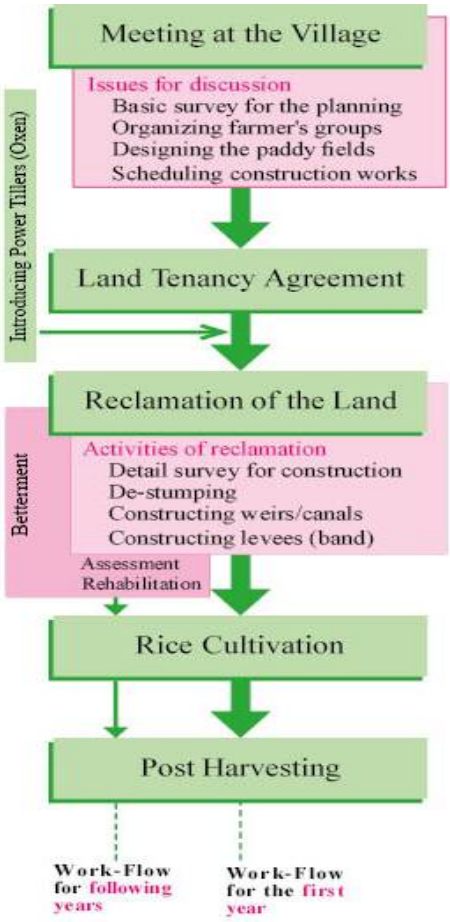

Fig. 3. Flow of Reclamation

Table 1: Chapters and Sections of Manual for Improving Rice Production in Africa.

Chapters	Sections
1. Introduction	(1) Background (2)Why JIRCAS Started the Study (3) The Study (4) Environmental Condition
2. Site Selection	(1) Feasibility (2) Planning
3. Farmers Organization	(1) Establishing Farmers' Organization (2) Advantages and Disadvantages of Group Activities, (3) Points of Concern for Group Activities (4) Land Tenancy
4. Land Development	(1) Clearing (2) Canal Construction (3) Effects of Slope Canal (4) Process of Canal Construction (5) Canal Construction Material (6) Plot-to-plot Irrigation (7) Drainage Canal (8) Intake (9) Division Work (10) Pond (11) Land Preparation (12) Maintenance of Irrigation Facilities (13) Repair of Broken Down Irrigation Facilities
5. Rice Farming	(1) Basic Knowledge of Rice Cultivation (2) Cropping Calendar (3) Paddy Field Preparation (4) Vegetative Stage (5) Reproductive Stage (6) Ripening Stage (7) Harvest (8) Post Harvest (9) Basic Rice Cultivation Problems
6. Power Tiller	(1) Advantages of Using the Power Tiller in Rice Cultivation (2) Operation of the Power Tiller (3) Maintenance of the Power Tiller (4) Common Usage of the Power Tiller by Farmer-Based Organization
7. ANNEXES	Land Tenancy Agreement, Power Tiller Lending Agreement, etc.

Indigenous organic resources for improving soil fertility in rice systems in Sub-Saharan Africa

Low soil fertility in Sub-Saharan Africa (SSA) has resulted in a decrease in rice production. It was also found that small-scale farmers do not have enough purchasing power to afford sufficient amounts of commercial inorganic fertilizers to replenish soil fertility. Thus, to increase agricultural productivity without spending much on fertilizers, easily obtainable and low-cost indigenous resources were examined. This study was aimed to quantify the abundance of various indigenous organic resources, estimate the amount of fertilizer equivalent, and map the distribution of resources in Ghana, SSA. The survey was conducted to investigate the availability of indigenous organic resources for use in agriculture and determine the amounts of plant- and animal-derived nitrogen (N), phosphate (P), and potassium (K). Moreover, this study was aimed to improve rice productivity pursuant to the goal of the Coalition for African Rice Development (CARD).

It was found that various indigenous resources from agricultural wastes such as rice straws and rice husks could be used for rice systems in Ghana. These residues were abundant in Northern, Volta, Upper East, Western, and Eastern regions where large amount of rice was produced (Fig. 1). Total rice straw and husk produced as waste in Ghanaian rice systems was 430,000 tons. These contained N, P, and K elements with nutrient equivalent (in tons) of 2,530 N, 990 P_2O_5, and 5,460 K_2O, respectively (Table 1).

Various livestock excreta including dung and urine were also abundant, particularly from cows followed by excreta from goats. Amount of excreta differed among regions. Cow and pig excreta were largely produced in Northern, Upper East, and Upper Western regions. Poultry manure was ample in Ashanti, Greater Accra, and in other municipalities and cities (Fig. 2). Livestock excreta produced in Ghana were calculated to contain 80,500 N, 44,500 P_2O_5, and 59,200 K_2O (total nutrient equivalent, in tons). Phosphate was found in all livestock dung (Fig. 3). (It should be noted that the calculated composition of fertilizer in each resource was based on previous documents and statistics data of the Ministry of Food and Agriculture of Ghana published in 2007.)

The estimated quantity of indigenous organic resources differed among regions; thus, resources should be optimized for use in the host areas. For example, in the Northern region where rice cultivation is most prominent, adding rice straw into the rice cultivation system would cover approximately 20 percent of N and P, and most K requirement of the soil compared to applied chemical fertilizers. Also, based on total estimated organic resources from livestock, if only 20 percent of these resources were utilized, it could replace the requirement for chemical fertilizer in rice cultivation system for the entire Northern region. Furthermore, development of composting techniques is required for sustainable and effective application of these organic resources. Meanwhile, it has been noted that grazing livestock excreta (dung and urine) were difficult to manage; hence, effective methods for its collection and transportation from the sources should be investigated prior to its use. In addition to the

aforementioned observed resources in Ghana, other organic resources such as human excreta, sawdust, and oil palm shells are also available and may prove useful. Therefore, further studies on the abundance and usefulness of these resources should be considered.

(S. Tobita, R. N. Issaka [Soil Research Institute, Ghana], M. M. Buri [Soil Research Institute, Ghana], M. Fukuda, and S. Nakamura)

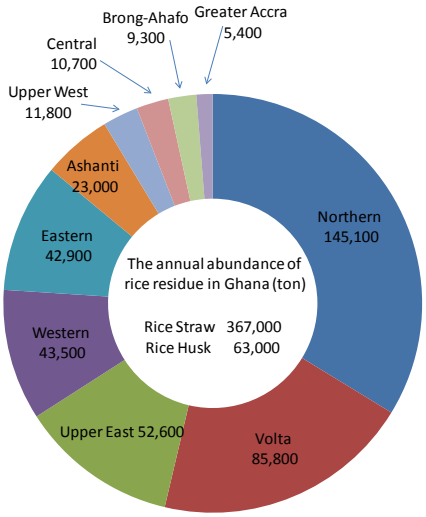

Fig. 1. Annual abundance of rice residues in Ghana (Issaka *et al.*, 2011)

Table 1. Nutrient equivalent (in kt y^{-1}) of organic residues in Ghana

			N	P_2O_5	K_2O
Plant Souces	Rice	Straw	1.8	0.6	5.1
		Husk	0.7	0.4	0.3
Sum			2.5	1.0	5.5
	Cow	Dung	20.7	31.6	8.9
		Urine	21.1	0.2	23.6
	Pig	Dung	2.7	2.2	2.1
		Urine	0.4	0.1	1.1
Animal Souces	Chicken	Dung	5.8	3.1	2.9
		Urine			
	Sheep	Dung	8.8	3.2	3.2
		Urine	4.6	0.2	6.1
	Goat	Dung	10.8	3.9	3.9
		Urine	5.6	0.2	7.5
Sum			80.5	44.5	59.2
		Total	83.0	45.5	64.6

Nutrient equivalent were calculated and based on various sources (from Dobermann and Fairhurst (2002), Buri *et al.*, (2004), MaCalla (1975), Mahimairaja *et al.* (2008)).

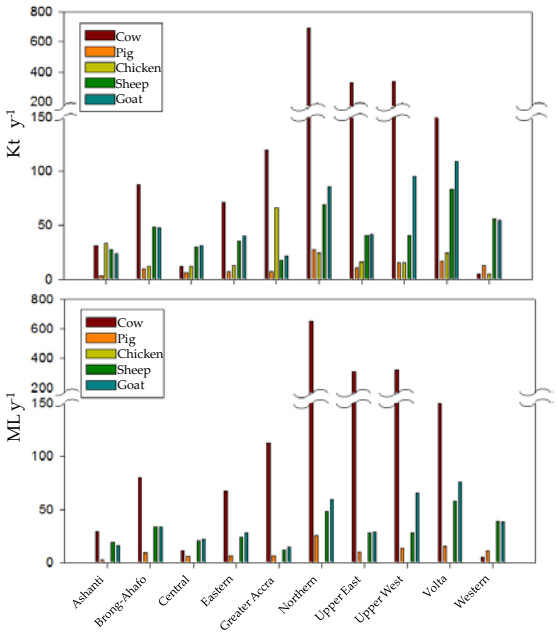

Fig. 2. Quantities of dung and urine excreted by five most common livestock in various regions

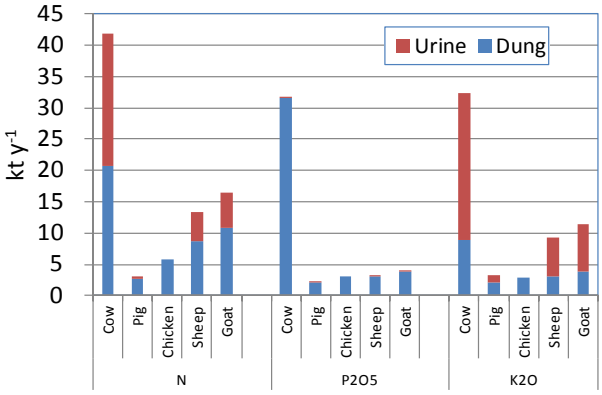

Fig. 3. Nutrient equivalent (N, P_2O_5, K_2O) of livestock excreta in Ghana

Simple and rapid measurements of photosynthetic activity in submerged rice by measuring chlorophyll fluorescence emissions

Submerged rice plants experience low oxygen and irradiance underwater, causing severe visible damages and photosynthesis inhibition. In recent years, the measurement of chlorophyll fluorescence emissions using a Pulse Amplitude Modulation (PAM) instrument has become a powerful tool for quick and non-invasive evaluation of the photosynthetic activity and photoinhibition in plants. However, measurements of chlorophyll fluorescence in rice underwater have not been developed. Previous studies on photosynthetic activity of submerged rice plants technically involved only numerical values measured above water (i.e. aerobic conditions) as measurements were taken after the removal of plants from submergence instead of measuring it while submerged. By doing so, it discounted the effects of environmental changes from anaerobic to aerobic conditions. The objective of this study is to develop a method for evaluating photodamage directly under water by analyzing chlorophyll fluorescence in leaves using a portable chlorophyll fluorescence meter with a waterproof probe.

Two rice (*Oryza sativa* L.) cultivars differing in their response to submergence were compared, namely: (1) a tolerance cultivar IR 67520-B-14-1-3-2-2 (IR67520), and (2) a susceptible cultivar IR72442-6B-3-2-1-1 (IR72442). Twenty-three-day-old seedlings were submerged in 80-cm-deep water for 14 days. The maximal quantum yield of PSII (Fv/Fm) of the dark-adapted leaves was measured using a portable chlorophyll fluorometer (OS5p; Opti Sciences Inc., USA) with the probe wrapped tightly with polyolefin to become waterproof (Fig.1A). Dark-adaptation leaf clips were mounted on the leaves before readings were taken (Figs. 1B and 1C). During submergence, Fv/Fm of the submerged leaf decreased earlier in IR72442 than in IR67520 compared to control plant (Fig.2). Fv/Fm showed a significant positive correlation with chlorophyll content during submergence (Fig. 3). At 14 days after submergence, the water in the tank was lowered to a 4-cm depth. Although Fv/Fm of the newly developed leaves during submergence in submerged IR67520 increased substantially from 2 days post-submergence, IR72442's decreased because of leaf chlorosis and all plants eventually died (data not shown). Therefore, it can be inferred that the tolerance cultivar coped with submergence by inhibiting photodamage and maintaining high chlorophyll content in the leaves.

The chlorophyll fluorescence meter with a waterproof probe can be utilized for the measurement of photochemistry reactions in rice leaves under floodwater. In our method, Fv/Fm can be measured in less than one minute per plant in air and underwater, with the results expected to become valuable screening tools for research and breeding programs for the improvement of submergence resistance in rice.

(J-I. Sakagami)

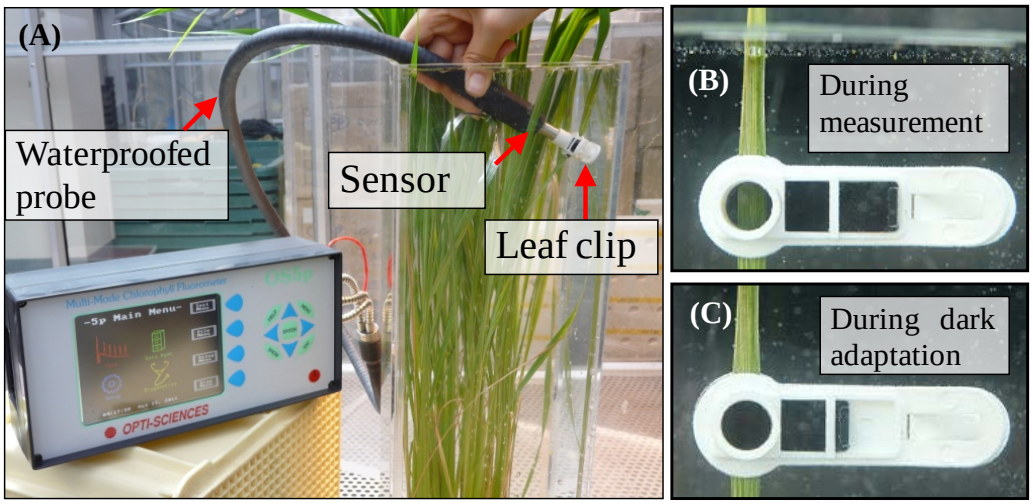

Fig.1. The measurement system of chlorophyll fluorescence in rice leaves underwater.
(A) The portable chlorophyll fluorescence meter with a waterproof probe. (B) The leaf clip for dark adaptation can be attached to the leaf underwater. (C) Dark adaptation can be accomplished by closing the dark slide of the clip.

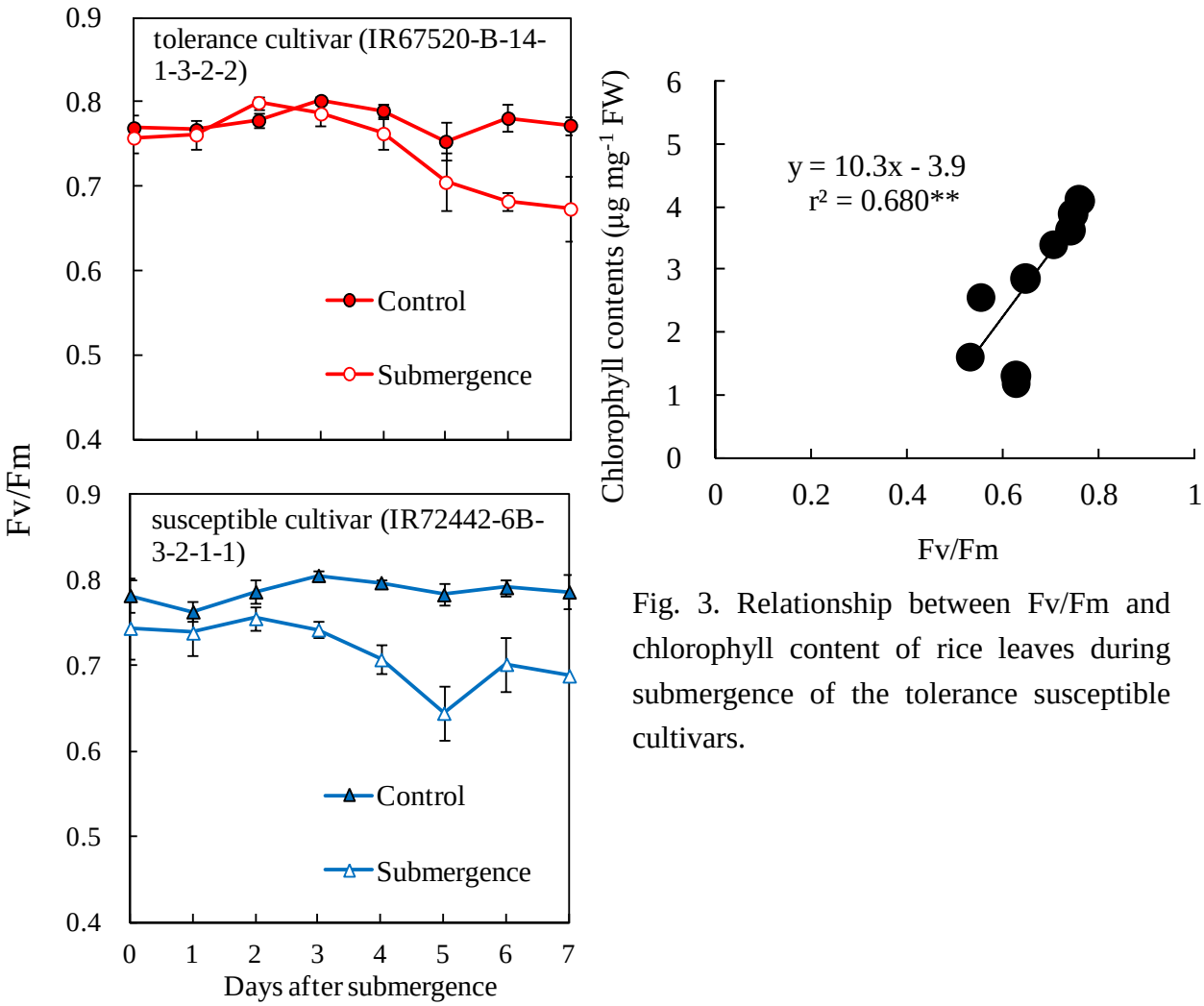

Fig. 3. Relationship between Fv/Fm and chlorophyll content of rice leaves during submergence of the tolerance susceptible cultivars.

Fig.2. Changes of maximal quantum yield of PSII photochemistry (Fv/Fm) in rice leaves during submergence.

Identification of cis-acting promoter elements in cold- and dehydration-induced transcriptional pathways in rice, soybean and Arabidopsis

Low temperature and dehydration affect plant growth and productivity. Many genes respond to both stressors at the transcriptional level, and their gene products function in terms of stress tolerance and response. These genes include key metabolic enzymes, late embryogenesis-abundant (LEA) proteins, detoxification enzymes, chaperones, protein kinases, and transcription factors. The cis-acting elements that function in stress-responsive gene expression have been analyzed to elucidate the molecular mechanisms of gene expression in response to these stresses. The dehydration-responsive element (DRE) containing the core sequence A/GCCGAC is a cis-acting element that regulates cold- and dehydration-responsive gene expression in Arabidopsis (*Arabidopsis thaliana*). The abscisic acid (ABA)-responsive element (ABRE) containing the core sequence ACGTGG/T is a cis-acting element that regulates dehydration- and high salinity-responsive gene expression in Arabidopsis and rice (*Oryza sativa*). ABA-responsive gene expression requires multiple ABREs or an ABRE with a coupling element as a functional promoter.

In this study, oligo microarrays were used to identify cold- and dehydration-responsive genes in rice, soybean and Arabidopsis. The observed frequencies of all ($4^6 = 4096$) hexamer sequences in cold- and dehydration-inducible promoters were compared with standardized promoters to estimate conserved sequences and to determine representative cold- and dehydration-responsive transcriptional pathways in rice, soybean and Arabidopsis.

Microarray analyses were performed using the three species (rice, soybean and Arabidopsis) and the characteristics of identified cold- and dehydration-responsible genes were compared. Transcription profiles of the cold- and dehydration-responsive genes were similar among these three species, showing representative up-regulated (dehydrin/LEA) and down-regulated (photosynthesis-related) genes. All ($4^6 = 4096$) hexamer sequences in the promoters of the three species were investigated, revealing the frequency of conserved sequences in cold- and dehydration-inducible promoters. A core sequence of the ABRE was the most conserved in dehydration-inducible promoters of all three species, suggesting that transcriptional regulation for dehydration-inducible genes is similar among these three species with the ABRE-dependent transcriptional pathway. In contrast, the highly conserved sequences in cold-inducible promoters of Arabidopsis are different from those of rice and soybean. DRE is the most conserved sequence in cold-inducible promoters of Arabidopsis, but not in those of rice and soybean. The novel sequence and ABRE are the most conserved sequences in cold-inducible promoters of rice and soybean, respectively. In cold-inducible promoters, the conserved hexamer sequences were diversified among these three species, suggesting the existence of diverse transcriptional regulatory pathways for cold-inducible genes among the species.

(K. Maruyama, K. Yamaguchi-Shinozaki)

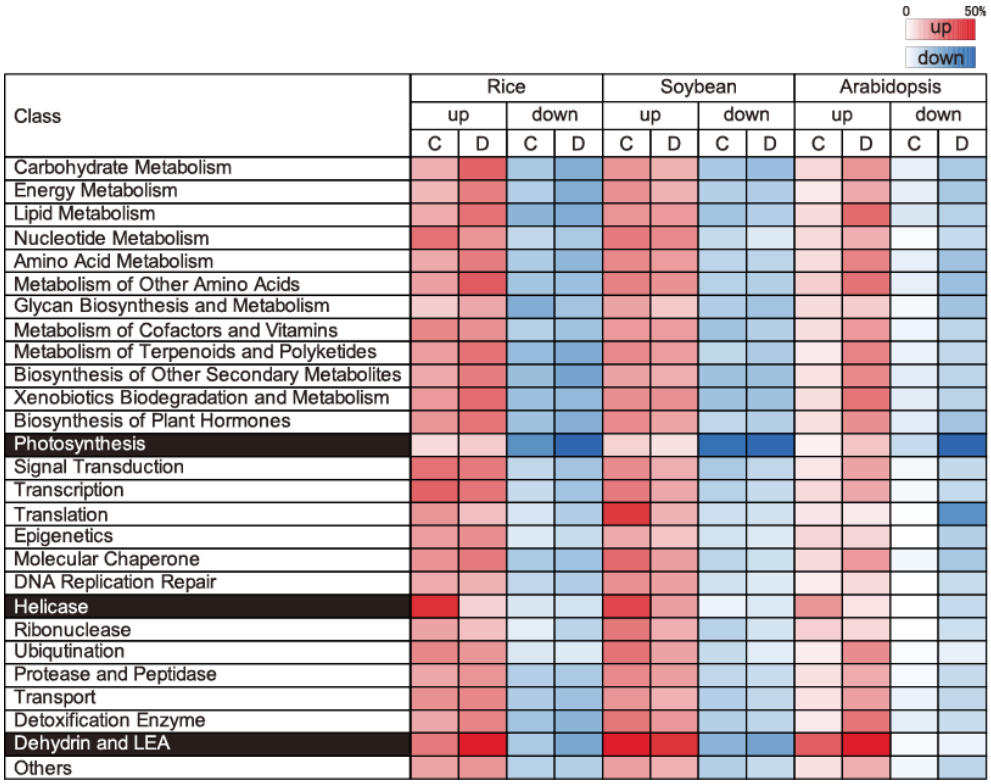

Figure 1. Molecular function of cold- and dehydration-responsive genes in rice, soybean and Arabidopsis. Heat maps of 27 up-regulated (red) or down-regulated (blue) molecular function classes indicating the frequency (%) in cold-treated (C) and dehydration-treated (D) plants.

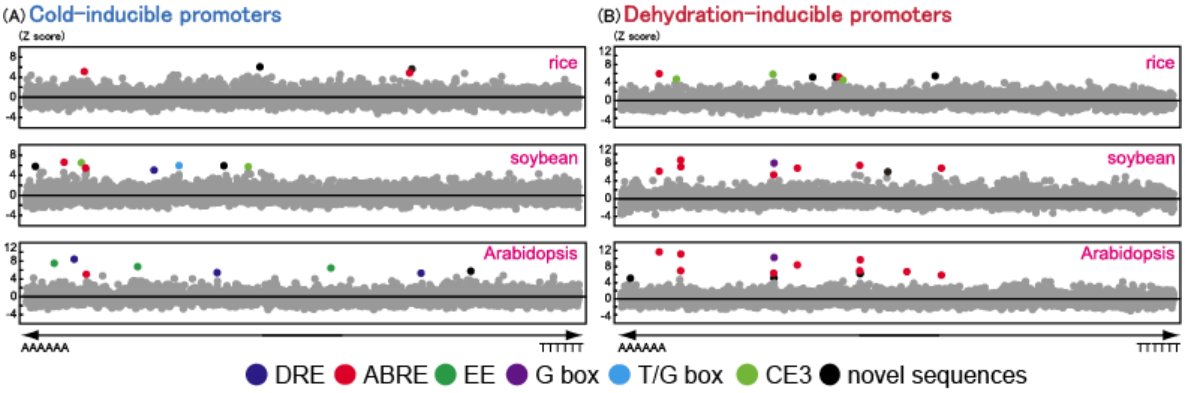

Figure 2. Scatter plots showing Z-scores (y axes) for the observed frequencies of all hexamer sequences (x axes) in cold-inducible (A) and dehydration-inducible (B) promoters in rice, soybean and Arabidopsis compared to standardized promoters. The highly conserved sequences of DRE (blue), ABRE (red), EE (green), G box (purple), T/G box (light blue), CE3 (light green), and novel sequences (black) are shown.

AZF1 and AZF2 proteins regulate plant growth under drought and salt stresses

Plants are exposed to various environmental stress conditions, such as drought, high salt, and low temperature. In response to these stresses, plants regulate growth and development by altering gene expression. Previous reports suggest that overexpression of some stress-inducible transcription factors can increase stress tolerance, resulting in growth inhibition. However, little is known about how such stresses cause plant growth inhibition. This study is aimed to elucidate the mechanism involved in plant growth regulation under environmental stresses by analyzing the functions of two Arabidopsis C2H2-type zinc-finger transcription factors (AZF1 and AZF2) that are induced by abiotic stresses.

AZF1 and *AZF2* genes encode C2H2-type zinc-finger proteins that are thought to function as transcriptional repressors. Expression of these genes are induced by osmotic stresses such as drought and high salt, and a phytohormone abscisic acid. Subcellular localization studies using GFP (green fluorescent protein) fusion proteins showed that the AZF1 and AZF2 proteins are localized to the nuclei in roots under control conditions, whereas the AZF2 protein accumulates in the nuclei of leaf cells under high-salinity stress. To analyze the functions of the AZF1 and AZF2 in plants, we generated transgenic Arabidopsis plants overexpressing *AZF1* and *AZF2* using stress-responsive promoters or a glucocorticoid-inducible promoter. These transgenic plants displayed dwarfed growth with smaller curled leaves (Fig. 1). It was also shown that overexpression of *AZF1* and *AZF2* enhances salt sensitivity in plants. Transcriptome analyses of the transgenic plants demonstrated that AZF1 and AZF2 repress the expression of various genes that are down-regulated by osmotic stresses and abscisic acid treatment. It is noteworthy that many "small auxin-up RNA (*SAUR*)" genes that may be involved in auxin-mediated cell elongation are down-regulated in the plants overexpressing *AZF1* and *AZF2* (Fig. 2). Moreover, gel mobility shift assays revealed that the AZF1 and AZF2 proteins directly interact with the *SAUR* promoter regions to repress the expression of these genes. Collectively, the results indicate that AZF1 and AZF2 function as transcriptional repressors which regulate plant growth under abiotic stress conditions.

The study's findings suggest the possibility that the growth of plants can be artificially controlled under environmental stresses by adjusting the expression levels of some transcription factors including AZF1 and AZF2. Further studies are required to confirm the functions of proteins similar to AZF1 and AZF2 in crops, such as rice and soybean, to develop a technique promoting optimum plant growth under severe environmental conditions, thereby improving abiotic stress tolerance in crops and increasing crop yield.

(K. Kodaira, K. Maruyama, Y. Fujita, K. Yamaguchi-Shinozaki)

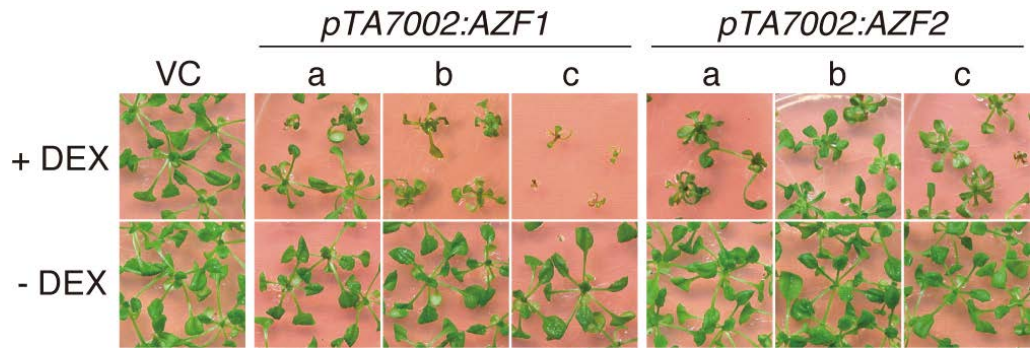

Fig. 1. Growth phenotypes of transgenic Arabidopsis plants overexpressing *AZF1* and *AZF2*. *pTA7002:AZF1*, *pTA7002:AZF2*, and vector control plants were grown for two weeks on GM agar plates supplemented with or without 1 μM DEX.

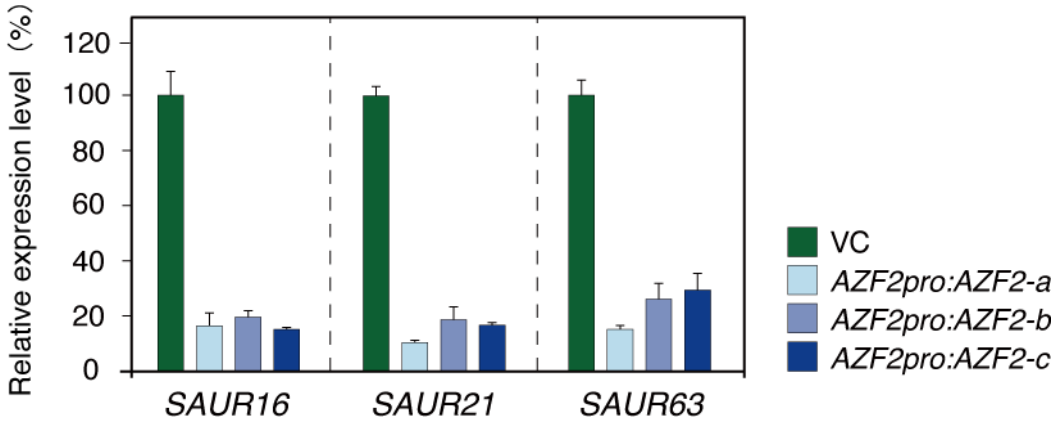

Fig. 2. Expression levels of some *SAUR* genes in transgenic plants overexpressing *AZF2*.

The relative expression levels of the down-regulated genes were determined in the vector control and in three independent transgenic lines that were treated with 200 mM NaCl using qRT-PCR. The highest expression level of each gene was designated as 100.

Soybean line carrying multiple resistance genes against soybean rust

Sustainable soybean production in South American countries such as Brazil, Argentina, and Paraguay is very important because more than half of soybean in the world market is produced in this region and because about 95% of soybean consumption in Japan is dependent on import. In the last 10 years, however, soybean rust has become one of the most serious threats to soybean production in this region causing large yield reduction. Thus far, five resistance loci to this disease have been identified and used for soybean breeding. However, some resistance alleles of these genes were reported to have broken down. The aim of this study was to clarify soybean-rust resistance by pyramiding these resistance genes and to develop soybean line carrying multiple resistance genes for marker-assisted breeding program in South American countries.

An F_2 population segregating Rpp2, Rpp4, and Rpp5, was infected with highly virulent Brazilian rust population-2 (BRP-2). Quantitative trait locus (QTL) analysis revealed that all three genes genetically contributed to the phenotypes of five resistance characters: lesion color; frequency of lesions with uredinia; number of uredinia per lesion; frequency of open uredinia; and sporulation level in a different manner (Fig. 1). Digenic and trigenic interactions were also detected among these thee genes. A soybean line, No6-12F3-1, was screened and identified to carry these three resistant alleles as homozygous by means of marker-assisted selection. It was also identified to have higher resistance than its ancestors, PI230970 (No.3, Rpp2), PI459025 (Bing Nan, Rpp4), PI200487 (Kinoshita, Rpp5), and An76-1 (Rpp2 and Rpp4) by preventing uredinia and spore production in the infections of BRP-2 and its single-uredinia isolates, BRP-2.1, BRP-2.5, BRP-2.6, and BRP-2.49 (Fig. 2).

By using polymorphic DNA markers sandwiching each resistance locus, the presences of three kinds of resistance alleles were successfully identified in the line, No6-12F3-1. In addition, high resistance derived from candidate genetic interactions were observed in this line. Therefore, three resistance genes in this line can be introduced into susceptible soybean varieties in the marker-assisted backcross breeding to confer rust-resistance to susceptible varieties. When this line is used for breeding, however, we must consider that 1) polymorphic DNA markers will be chosen depending on susceptible recurrent parents, 2) high resistance is not always guaranteed because the degree of resistance could be changed by the pathogenicity of rust races and the genetic background of susceptible recurrent parents, and 3) the frequency of backcrossed progenies expected to carry three resistant alleles may be low because of the recombination between resistant loci and DNA markers.

(N. Yamanaka, N. G. Lemos)

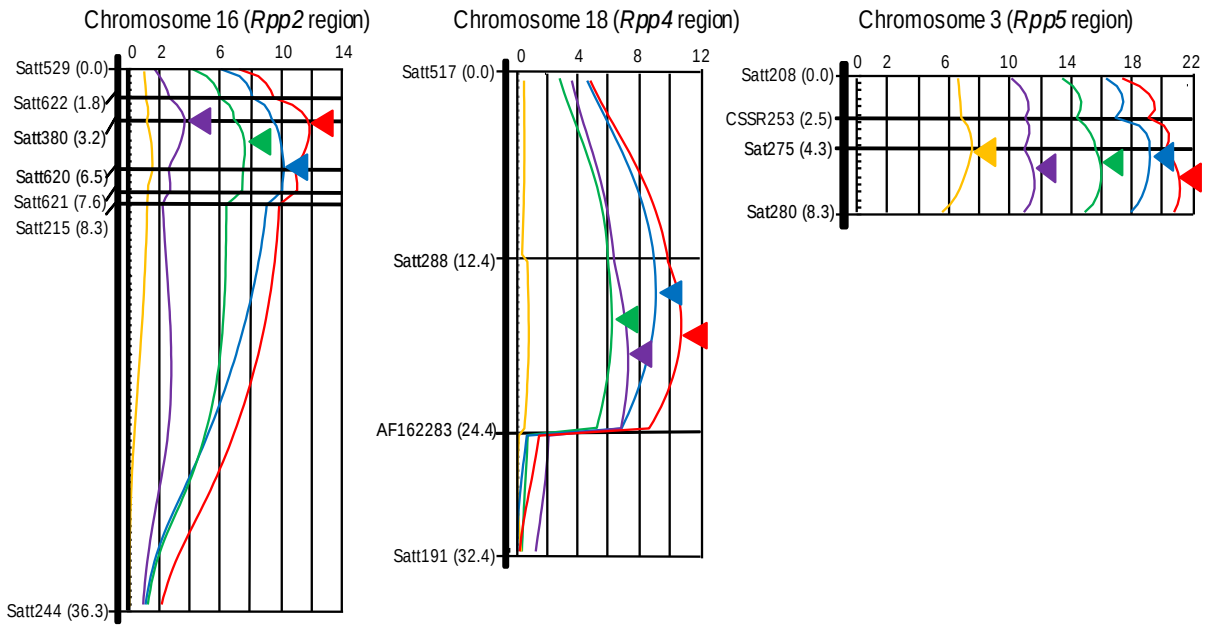

Fig. 1. Logarithm of odds (LOD) curves of the QTLs for five characters related to soybean rust resistance. LOD values and the peaks of graphs of the characters: lesion color (yellow); frequency of lesions with uredinia (green); number of uredinia per lesion (blue); frequency of open uredinia (purple); and sporulation level (red).

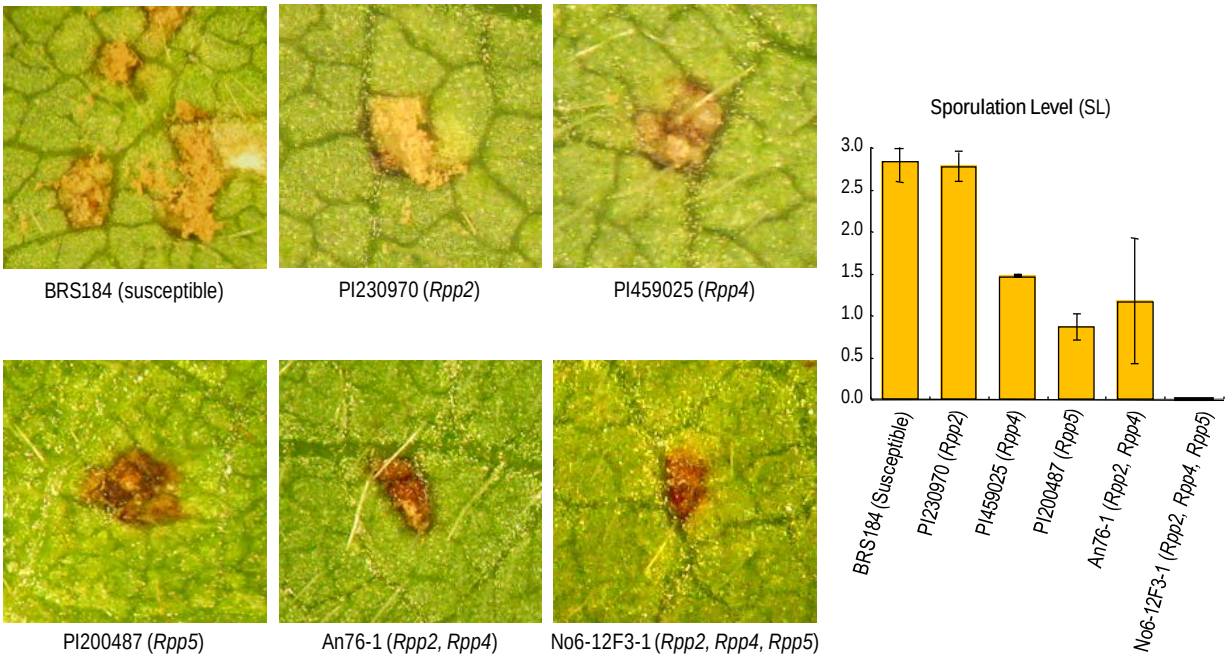

Fig. 2. Lesions and the frequency distribution of sporulation level in the soybean genotypes carrying Rpp2, Rpp4, and / or Rpp5. Pictures and phenotypic data were obtained by BRP-2 infection.

Multiline variety for rainfed lowland rice variety

Rice blast, caused by *Pyricularia grisea* (Cooke) Sacc., is one of the most serious diseases affecting rice (*Oryza sativa* L.). It inflicts severe damage to rice production in temperate and tropical regions. The use of resistant varieties is the most economical and effective method to control blast disease (Yu et al., 1991). However, the true resistance of varieties governed by a major gene is often broken down due to the emergence of virulent races after only several years of use. Increasing host genetic diversity through the use of a multiline variety consisting of several isogenic lines (ILs) or varietal mixtures with different major genes is one of several useful strategies to avoid easy breakdown of resistance. The effectiveness of multi lines or mixed varietal plantings in reducing blast disease has been demonstrated in Japan. However, multiline varieties for blast resistance have yet to be developed and released for use in the tropics.

Rice blast is particularly severe in rainfed lowlands that are prone to droughts. To produce the durable protection system against blast disease in the rainfed lowlands under tropical conditions using genetic diversity of rice variety, the multiline variety consisting of several isogenetic lines with elite Indica-type genetic background was developed.

A set of near-isogenic lines (NILs) for blast resistance genes was developed by using an Indica-type elite rice variety, IR49830-7-1-2-2, as a genetic background suitable for rainfed lowland conditions in the tropics. Initially, it was revealed that IR49830-7-1-2-2 harbors five blast resistance genes – *Pia*, *Pib*, *Pik-s*, *Pita*, and *Pi11*(t) – by using a differential system involving 19 selected standard blast isolates from the Philippines. Based on this result, nine near-isogenic lines were developed targeting eight resistance genes – *Pik*, *Pi7*(t), *Pi3*, *Pi5*, *Pita-2*, *Piz-5*, *Pish*, and *Pi9* – by recurrent backcrossing. The introgression of each resistance gene in the NILs was confirmed by reaction patterns to the blast isolates, allelism tests, and DNA marker analysis. In addition, a genome-wide DNA marker survey revealed that most of the chromosome regions in each NIL were of the IR49830-7-1-2-2 type. The agricultural characteristics of most of the developed NILs were almost the same as those of IR49830-7-1-2-2. Moreover, with one exception, they showed submergence tolerance similar to IR49830-7-1-2-2. The developed NILs could be used as a multiline variety suitable for rainfed lowlands in the tropics.

(Y. Fukuta, Y. Koidea, S. Yanagihara , N. Kobayashia [NICS], H. Kato [NICS], T. Imbe [Kyushu-Okinawa ARC], H. Tsunematsu [NICS], Leodegario A. Ebronb [IRRI], Mary Jeanie Telebanco-Yanoria [IRRI], M. Yokoo [University of Tsukuba], S. Maruyama [University of Tsukuba])

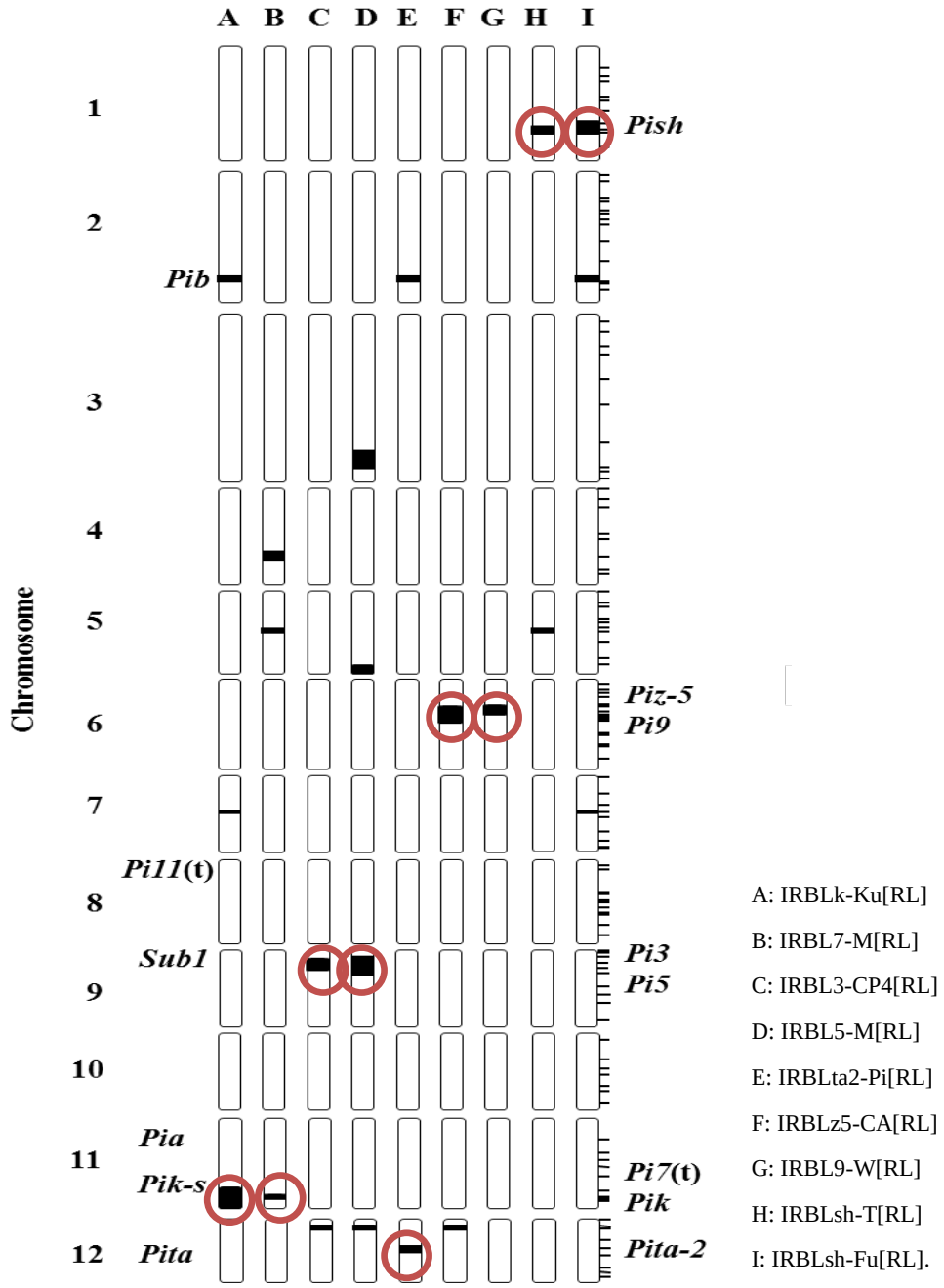

Fig.1. Graphical genotypes of nine near isogenetic lines (NIL) with IR49830-7-1-2-2 genetic background.

Blank regions on each chromosome indicate introgression from donor varieties and red circles show the locations of target resistance genes.

The gene symbols on the left side indicate the resistance and tolerant genes harboring in the genetic background of IR49830-7-1-2-2. The right side gene symbols indicate the introduced resistance genes into IR49830-7-1-2-2.

1-12: Chromosome number

Stock management of the fluvial shrimp *Macrobrachium yui* indigenous to northern Laos based on life-history characteristics

Many *Macrobrachium* species are found in Lao People's Democratic Republic (Lao PDR). In particular, *Macrobrachium yui* indigenous to the northern mountainous areas is well known as local delicacy and is traded at a high price, making shrimp fishing an important source of income for the rural people. However, shrimp catch has decreased year by year due to overfishing and deterioration of aquatic environments. This has become a big problem in the villages surrounding the shrimp's habitat. In this study, we examined species identification, migration pattern, breeding habitat and season, and early life-history of the shrimp in order to develop effective technique of the shrimp stock management . The results are expected to contribute to the recovery and sustainable use of shrimp stock in the future.

Based on morphological characteristics, the species with the highest economic value was identified as *Macrobrachium yui*. Monthly sampling at fixed points showed that larger adults occur mostly in the cave stream; small-sized juveniles in the main river; and various-sized classes from the juveniles to the adults in the forest stream (Fig. 1). This suggests that the shrimp migrates among habitats of the three water bodies during its lifespan. Gonadsomatic index (GSI) of females greatly decreased from July through August and ovigerous females were found only from the inner part of the cave stream, indicating that females spawn in the inner part of the cave stream during the period (Fig. 2). The drifting larvae of the shrimp which had already settled to the bottom emerged from the cave stream from October through May. Based on rearing experiment in the laboratory, it was determined that the hatched larvae remained in the inner part of the cave stream for approximately one month (Fig. 3). In order to achieve recovery and sustain availability of the shrimp stock, prohibiting shrimp fishing at the cave stream during August was considered the centerpiece of fishery regulation. Fishery models were then created based on biological characteristics (i.e. female fecundity, seasonal change in the number of brooding females and growth patterns, etc.) of the shrimp to evaluate the effects of fishery regulation. The fishery model predicted that future catch of the shrimp increased by approximately 30% compared to current catch when the shrimp fishing at the cave stream during August was prohibited under the condition that actual fishing mortality is three times higher than the optimum fishing mortality (Fig. 4).

Backed by the prediction results of the fishery model, the fishery regulation was suggested to the local government. Based on Article 53 of the fishing regulations by the Ministry of Agriculture and Forestry in Lao PDR and at the initiative of the local government and villagers of Pak-Xuang District, Luang-Prabang Province, prohibition of shrimp fishery at the cave stream every August was adopted and enacted starting from year 2011.

(S. Ito,H. Yukio[FRA], K. Iguch[FRA], K. Omori[CMES Ehime University], KounThongBang[LARReC], O. Lasasimma[LARReC], P. Soliyamath[PAFO,Luang-Prabang])

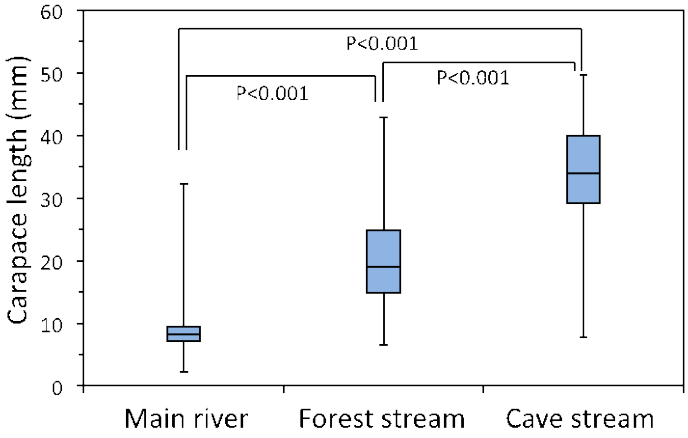

Fig. 1. Comparison of carapace length of the fluvial shrimp *Macrobrachium yui* (except the larvae), among habitats of the three water bodies

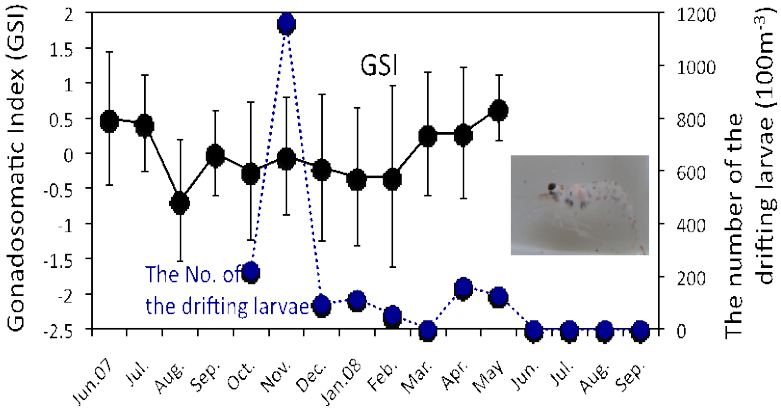

Fig.2 Monthly changes of gonadosomatic index (GSI) of females and the number of the drifting larvae in *M.yui*. The photo in the figure shows the drifting larvae which emerged from the cave stream after settling to the bottom.

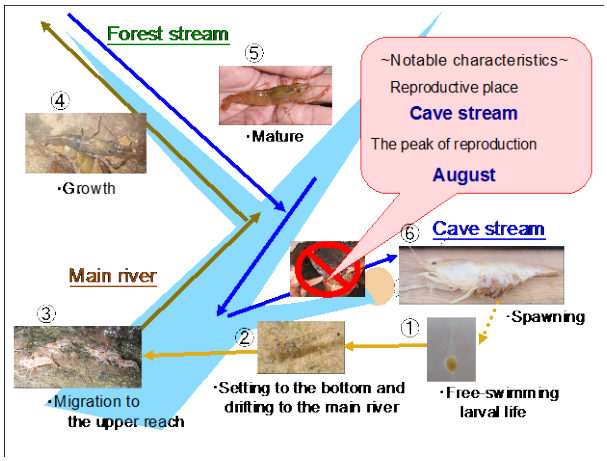

Fig.3 Life-cycle of *M.yui* and the fishery regulations established on the basis of its life-history characteristic.

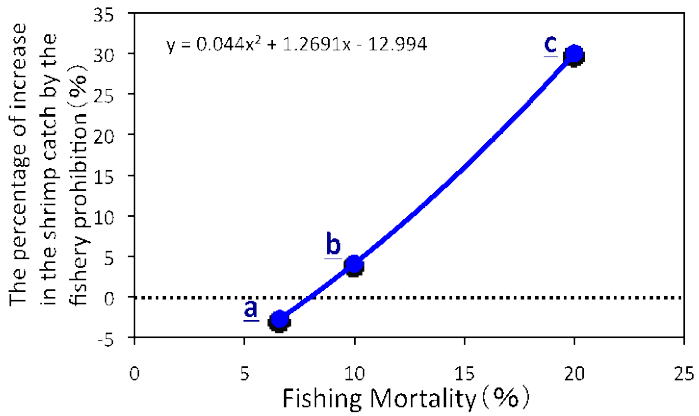

Fig. 4. The percentage of increase in the shrimp catch to fishing mortality resulting from the prohibition of the shrimp fishery in the cave stream in August
a: optimal fishing mortality rate at present (6.6%)
b: fishing mortality rate of approximately 1.5 times a (10%)
c: fishing mortality rate of approximately 3 times a (20%).

Effect of natural fermentation on the textural improvement of traditional fermented rice noodles

Natural fermentation of whole polished rice grains (*Indica*) is a traditional processing method widely applied in southern China and Southeast Asia to improve the texture of rice noodles. Though it is common knowledge that rice starch is degraded during fermentation resulting in poor gel strength, little is known about the effects of fermentation on texture characteristics of starch-based foods; hence, its effect on rice noodles remains unclear. Therefore, exploring the merits of fermentation is crucial in enhancing the quality of fermented rice noodles. The objectives of the present study are: (1) to isolate, identify and characterize the micro-organisms present during the natural fermentation process, (2) to determine the metabolites produced during fermentation and chemical component changes of raw milled rice grains, (3) to investigate their effects on rheological and sensory properties of rice noodles, and (4) to clarify the mechanism for improving the texture of fermented rice noodles.

A total of 170 lactic acid bacteria (LAB) and 96 yeasts were isolated from three local factories in Changde City, Hunan Province, China. *Lactobacillus plantarum* (32 strains) was more frequently isolated than other LAB species during the fermentation process. The number of *Lactobacillus* strains (83.5%) suggested that *lactobacilli* predominated in the fermented rice noodle processing. The predominant yeast species was *Saccharomyces cerevisiae* (57 strains). Table 1 shows the chemical components of raw rice material, fermented rice grains and fermented supernatant at 72 h in three factories. After fermentation, the contents of total starch and amylose, and reducing sugar increased, whereas the contents of starch damage, protein and lipid decreased. However, the changes on total starch and lipid were not significant ($P > 0.05$). Lactic acid was the dominant organic acid produced by fermentation.

Rice starch underwent slight hydrolysis during natural fermentation while its purity increased significantly. Treating the raw milled rice grains with trypsin, lipase or lactic acid (pH 4.0) could modify the rheological characteristics (Fig. 1) and improve the sensory properties of rice noodles. Removal of protein and lipid by a physical extraction method confirmed the result of protease and lipase treatments, indicating that the purity of starch is an important factor in determining noodle texture. Low molecular weight sugars should be rinsed completely from rice grains immediately after fermentation to minimize their negative effects on the tensile and sensory properties of rice noodles.

In conclusion, fermentation of raw milled rice by LAB and yeasts decreased protein and lipid content and increased the purity of rice starch, thereby improving the texture of fermented rice noodles. However, the low molecular weight sugars produced during fermentation should be removed for their negative effect on texture.

(E. Tatsumi, M. Saito, K. Kohyama [National Food Research Institute], Z. Lu [China Agricultural University], L. Li [China Agricultural University])

Table 1. Chemical components of rice grains and fermented supernatants

	Raw rice material	Fermented rice grains		
		Factory A	Factory B	Factory C
Rice grains				
Total starch (%)	89.1 ± 0.9[a]	90.3 ± 1.1[a]	90.7 ± 0.2[a]	89.9 ± 0.7[a]
Amylose (%)	20.6 ± 0.7[a]	21.6 ± 0.3[b]	21.1 ± 0.6[b]	21.9 ± 0.5[b]
Starch damage (%)	2.84 ± 0.13[b]	0.43 ± 0.31[a]	0.57 ± 0.22[a]	0.73 ± 0.17[a]
Reducing sugar (%)	0.35 ± 0.01[a]	3.11 ± 0.11[b]	3.64 ± 0.2[b]	3.77 ± 0.13[b]
Protein (%)	4.5 ± 0.3[b]	3.6 ± 0.1[a]	3.9 ± 0.2[a]	3.2 ± 0.6[a]
Lipid (%)	0.9 ± 0.1[a]	0.7 ± 0.1[a]	0.6 ± 0.3[a]	0.7 ± 0.2[a]
Reducing sugar of fermented supernatant (g/l)	0.12 ± 0.01[a]	3.50 ± 0.03[b]	3.47 ± 0.11[b]	4.09 ± 0.07[b]

Values within the same horizontal row followed by the same alphabetical letter are not significantly different (P > 0.05). Fermented rice grains were obtained from three local factories in Changde City, Hunan Province, China. Raw rice material and tap water in all the local factories were from the same supplier.

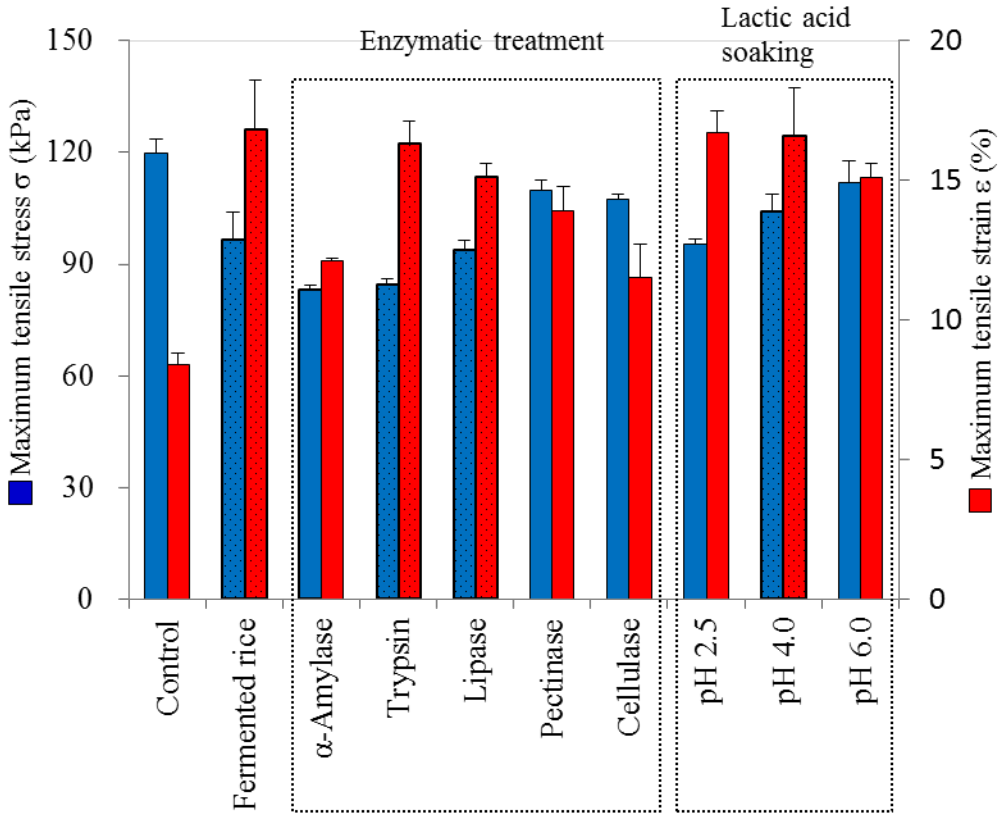

Fig. 1. Tensile properties of rice noodles derived from treated rice grains

Development of cellulose-degrading enzyme recycle system for reducing enzyme cost of saccharification

Lignocellulosic plant biomass is difficult to hydrolyze because cellulose is surrounded by a lignin that has covalent associations with hemicellulose, and cellulose has a tightly packed crystalline structure. Thus, the rate-limiting step in biomass degradation is the conversion of cellulose and hemicellulose polymers to sugars. Among cellulolytic microorganisms, *Clostridium thermocellum*, an anaerobic thermophilic bacterium, is the most potent cellulose degrading bacterium known to produce the cellulosome (2-3.5 MDa). The cellulosome structure of *C. thermocellum* consists of a large non-catalytic scaffolding protein (scaffoldin) named CipA of 197 kDa that is multi-modular and includes nine cohesins, and a family III cellulose binding module (CBM). The catalytic units are non-covalently attached to scaffoldin via the type I interaction between dockerin domains borne by the catalytic units with the cohesins on the scaffoldin.

Recently, to isolate microorganisms that possess effective cellulose-degrading ability, new thermophilic cellulolytic strains were screened from agriculture residues in Thailand using microcrystalline cellulose as a carbon source. We isolated a new strain, *C. thermocellum S14*, which has higher cellulose-degrading ability than several type strains (1).

When rice straw treated by soaking in aqueous ammonia was hydrolyzed by the combination of ß-glucosidase from *Thermoanaerobacter brockii* with the cellulosome from *C. thermocellum* S14, approximately 91% of glucan existing in the rice straw was hydrolyzed (2). On the other hand, enzyme recycling is desired to reduce costs of saccharification process (Fig. 1). In order to recycle the combination, CBM from CipA was fused to the ß-glucosidase. When recycling tests were carried out against crystalline cellulose and ammonia-treated rice straw, combination of cellulosome and ß-glucosidase-fused CBM could recycle at least 5 and 4 rounds, respectively, consistent with high saccharification rates. Based on these results, a recycle saccharification reactor system that can recover saccharified solution through an ultrafiltration membrane using a combination of the cellulosome and ß-glucosidase-fused CBM was developed (Fig. 2). These results indicated that the combination of cellulosomes and β-glucosidase from thermophilic anaerobic microorganisms has great potential as an effective lignocellulose degradation system.

(*Kosugi A, Waeonukul R [KMUTT], Tachaapaikoon C [KMUTT], Mori Y*)

(1) Tachaapaikoon C, Kosugi A, Pason P, Waeonukul R, Ratanakhanokchai K, Kyu KL, Arai T, Murata Y, Mori Y. (2012) Isolation and characterization of a new cellulosome-producing *Clostridium thermocellum* strain. Biodegradation. 23(1):57-68.
(2) Waeonukul R, Kosugi A, Tachaapaikoon C, Pason P, Ratanakhanokchai K, Prawitwong P, Deng L, Saito M, Mori Y. (2012) Efficient saccharification of ammonia soaked rice straw by

combination of Clostridium thermocellum cellulosome and Thermoanaerobacter brockii β-glucosidase. Bioresource Technology doi:10.1016/j.biortech.2011.12.126

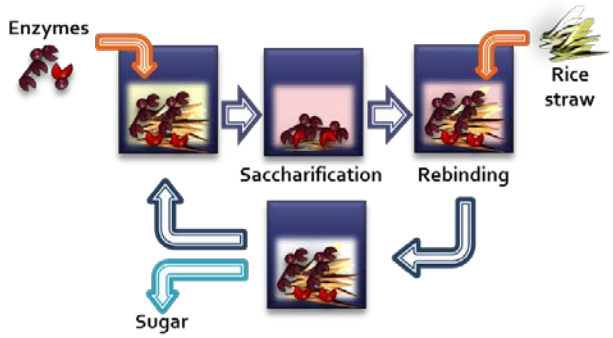

Fig.1. Design of recycle system using a combination of cellulosome and ß-glucosidase-fused CBM

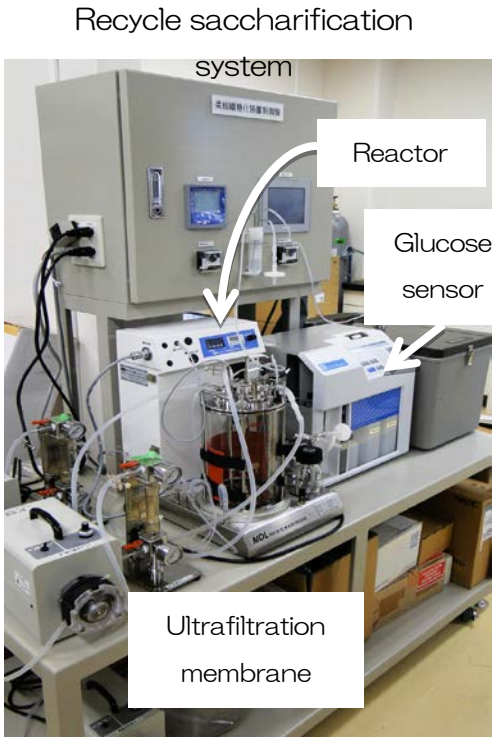

Fig 2. Recycle saccharification system using a combination of cellulosome and CBM-fused ß-glucosidase

Production of biodegradable plastic from oil palm sap

Biodegradable plastic (or bioplastic) are commonly produced from corn starch and edible oil. However, the aforementioned raw materials are also important food components, which could lead to competition between the bioplastic and food industries for the same resources.

Oil palm is a common agricultural product in Southeast Asia. Its source--the oil palm trees--have an economic life span of approximately 20-25 years, after which old trees are felled and the farms, replanted. Meanwhile, the discarded oil palm trunks are suddenly available in large quantities, providing readily-available alternative sources for new products such as bioplastic. These renewable resources derived from agricultural wastes are also called “waste agricultural biomass” or “biomass resources.”

From previous studies, it was shown clearly that ethanol and lactic acid were efficiently producible from oil palm sap using yeast and lactic acid bacteria. It was also found that the concentration of fermentable sugars in the trunk can be drastically increased within a suitable storage period. The polymer that was produced, called Poly-3-hydroxbutyrate (PHB), shows excellent heat resistance and biodegradability, and the expanding demand is expected along with polylactic acid. The objective of this study, therefore, is the development of cheap and efficient PHB-producing techniques from oil palm sap.

Poly-3-hydroxbutyrate (PHB) was produced from sugar in oil palm sap using *B. megaterium* MC1 for the first time (Fig. 1). Different sap sugar concentrations were added to the production medium to analyze its effect on PHB biosynthesis. Sap sugar concentration ranging from 1.0 to 2.5% w/v was tested. With 2.5% w/v sap sugar concentration, a maximal PHB concentration of 1.91 g/L was recorded at 12h of growth (Fig. 2). Nitrogen plays an important role in influencing the process of PHB accumulation in bacterial cells. Different nitrogen compounds were used in this experiment to study the effect of these nitrogen sources on *B. megaterium* MC1. Urea is efficient and economical to produce PHB as a source of nitrogen. Ratio of carbon and nitrogen (C/N molar ratio) in medium has been seen as one of the important factors influencing the biosynthesis of PHB in cell. With a fixed amount of carbon, different C/N ratios were tested by varying the urea concentration. A maximal PHB concentration was obtained with a C/N ratio of 50. To enhance PHA production, *B. megaterium* MC1 was grown in optimum condition. PHB of 3.28 g/L was produced from the juice liquid containing sugar of 2.5 % after 16 hours (Fig. 3).

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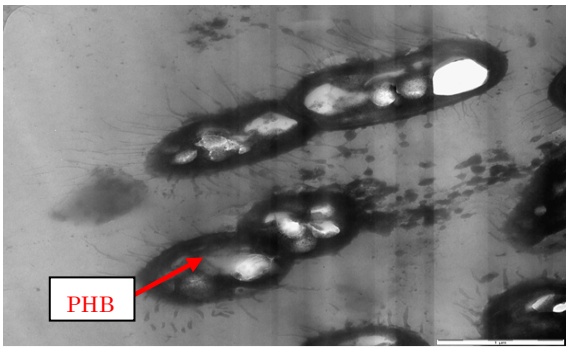

Fig. 1. Accumulation of PHB in B. megaterium MC1

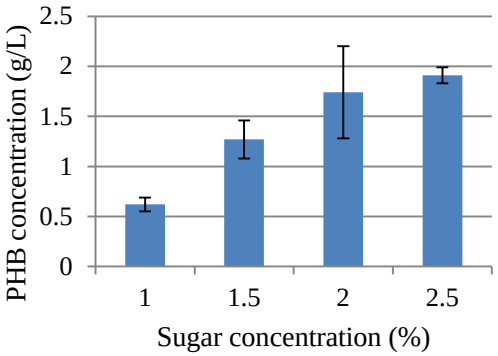

Fig. 2. Effect of sap sugar concentrations on growth and P(3HB) biosynthesis using B. *megaterium* MC1

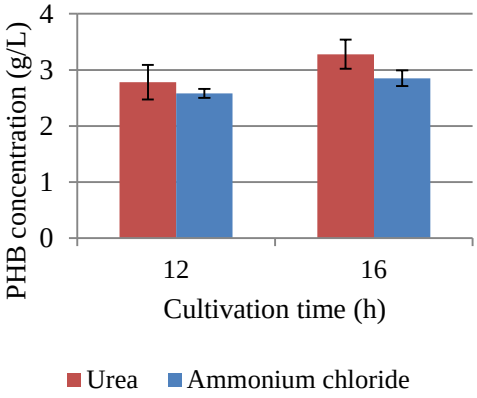

Fig. 3. Optimization of PHB production by B. *megaterium* MC1 using oil palm tree sap as sole carbon source

Analyzing pollen dispersal of seraya (*Shorea curtisii*) to promote healthy seed production to sustain forest regeneration

Trees of timber species larger than 50 cm in trunk diameter at breast height (dbh) are being harvested in ongoing selective logging operations in Malaysia. This is a critical issue as these activities often result to a reduced number of healthy seedlings due to poor seed dispersal across logged-over areas and unsuccessful cross-mating among remaining adult trees.

This research addresses the problem by applying genetic paternity analysis of seeds to identify pollen dispersal patterns and by studying the relationship between tree size (dbh) and male fecundity (i.e. pollen production). It was done in order to come up with a proposal for forest management agencies in Malaysia to revise the selective logging criterion. The results of the study are expected to promote healthy seed production and consequently effect the development of a sustainable forest regeneration program.

Microsatellite genotypes were determined for all adult individuals in an undisturbed forest plot and seeds were collected in three synchronized flowering events for seraya (*Shorea curtisii*). Seraya is one of the major timber species in hill dipterocarp forests of Peninsular Malaysia where selective logging has been undertaken. Paternal donor of each seed was identified by comparing the genotypes of the seed, the mother tree and the paternal donor (paternity analysis). A model based on paternity analysis estimated pollen dispersal patterns. The average pollen dispersal distance was measured to be short at 60 m (Fig. 1).

The current harvesting criterion (dipterocarp trees larger than 50 cm dbh are felled) lowers the density and increases the distance between remaining adult trees, thereby decreasing the chances of pollen reaching other trees. Increasing remaining tree density helps promote cross-mating and improve the production of healthy seeds. Male fecundity of each adult tree estimated from the model based on paternity analysis showed that small-sized trees less than 50 cm in dbh left after selective logging seldom produced pollen (Fig. 2), resulting to fewer seeds produced from cross-mating. Selective logging simulation revealed that conserving the middle-sized trees(70-90cm dbh) ensured about 50% of outcrossing pollen produced to be retained, which was higher than the current logging protocol (5-15%, logging larger than 50 cm trees). This research established pollen dispersal patterns and studied the relationship between tree size (in dbh) and pollen production. The results will be compiled in support of the proposal to the Selangor Forest Department for a revised selective logging criterion to rejuvenate healthy cross-mating of seraya and consequently, sustain forest regeneration.

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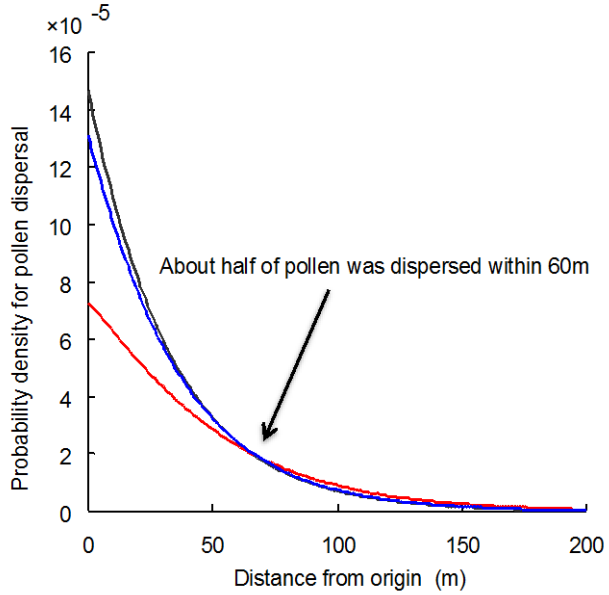

Fig. 1. Pollen dispersal probability curves deduced from the mating process model. Model was based on paternity assignment of seeds conducted on a 6-hectare undisturbed forest plot in Semangkok Forest Reserve, Malaysia. Blue, red and black lines indicate pollen dispersal probabilities for the 1998, 2002 and 2005 flowering events, respectively.

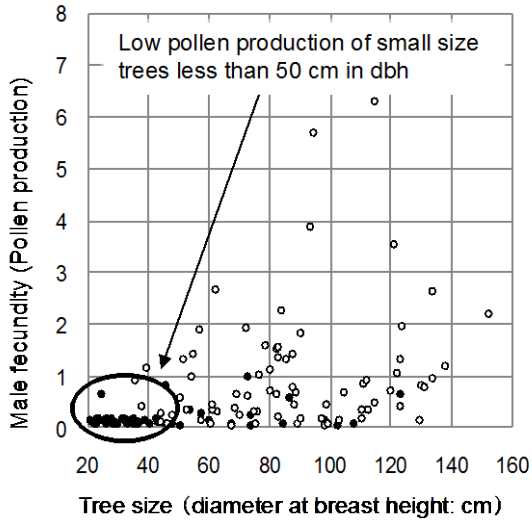

Fig. 2. Relationship between tree sizes in dbh and male fecundity of each adult tree in the plot (larger than 20 cm in dbh). Open and solid circles indicate flowering trees and non-flowering trees based on field observation, respectively.

The importance of large scale mangrove estuaries as feeding grounds for commercially important juvenile fishes

Mangroves contribute substantially to coastal fisheries in terms of providing trophic and refuge support. Typically, the adults of many tropical fish species spawn in the offshore area, producing eggs that develop into planktonic larvae which move or are carried by currents into the inshore and mangroves. However, the mangrove areas surveyed in almost all studies are on the small scale mangroves of normally less than 1 km width, and there are few studies focusing on the large mangrove estuaries. The Matang Mangrove Forest Reserve (MMFR), situated on the northwestern coast of Peninsular Malaysia, is reputed to be the world's best managed mangrove forest. The reserve is the largest single tract of mangrove forest in Peninsular Malaysia (40,151 ha), measuring 52 km between the extreme ends and 13 km wide in the middle, and has been managed as a sustainable production forest since 1905. In this study, stable carbon and nitrogen isotope ratios of juvenile John's snapper *Lutjanus johni*i and Caroun croaker *Johnius carouna* collected from the estuaries of MMFR were measured to investigate 1) the ontogenetic migration of the juveniles into and within the large and complex mangrove estuaries, and 2) the fish's dependence on the food sources provided in the mangrove nursery area.

Prey animals in MMFR showed distinctive $\delta^{13}C$ signatures between habitats and can be divided into two groups: one group with generally enriched $\delta^{13}C$ values closely associated with coastal and lower estuarine areas; the other group with more depleted $\delta^{13}C$ values captured in the upper mangrove areas (Fig. 1). Corresponding with the isotope values of these prey animals, juveniles of John's snappers and Caroun croaker also had more enriched $\delta^{13}C$ values in the lower estuarine areas than in the upper mangrove areas. The remarkable differences observed in the isotope ratios between these sampling sites reflect the difference in food sources between the two habitat groups (Fig. 2, left).

Small individuals of John's snappers caught from the lower mangrove station (S1) had higher $\delta^{13}C$ values that reflect the relatively higher $\delta^{13}C$ values of the prey animals collected in the coastal stations (Fig. 2, left). This suggests that the small fish had recently migrated into the lower mangrove from the coastal area. The $\delta^{13}C$ signature of larger juvenile John's snappers collected from the upper mangrove areas showed more depleted values with their growth (Fig. 2, left). These results indicate the ontogenetic migration of John's snappers spreading into the upper mangrove areas as far as 13 km from the river mouth after entering from the coastal areas (Fig. 2, right). On the other hand, Caroun croaker is a permanent resident in mangrove estuary both as adults and juveniles. The $\delta^{13}C$ signature of Caroun croaker collected from the lower estuary showed more enriched values with their growth, suggesting that the contribution of mangrove-associated prey animals is higher in the smaller juveniles.

These findings of the present study have important implications for the fishery management of juvenile fish in the mangrove estuary. This study shows the importance of

conserving sufficiently large single areas of mangrove with their complex interconnected waterways (including estuary, river, rivulet and creek) as exemplified by the large MMFR, as habitat and feeding grounds for John's snapper and Caroun croaker.

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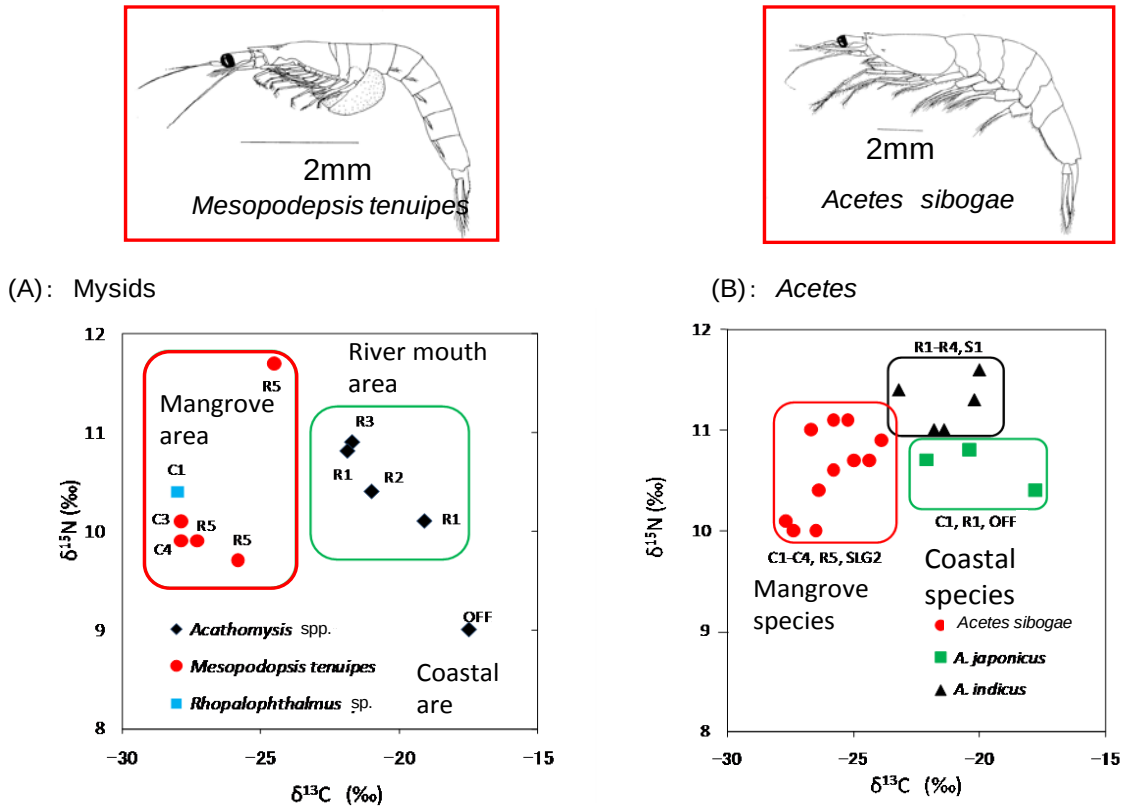

Fig. 1. Plot of $\delta^{15}N$ and $\delta^{13}C$ of prey crustacean animals in the Matang Mangrove Estuary

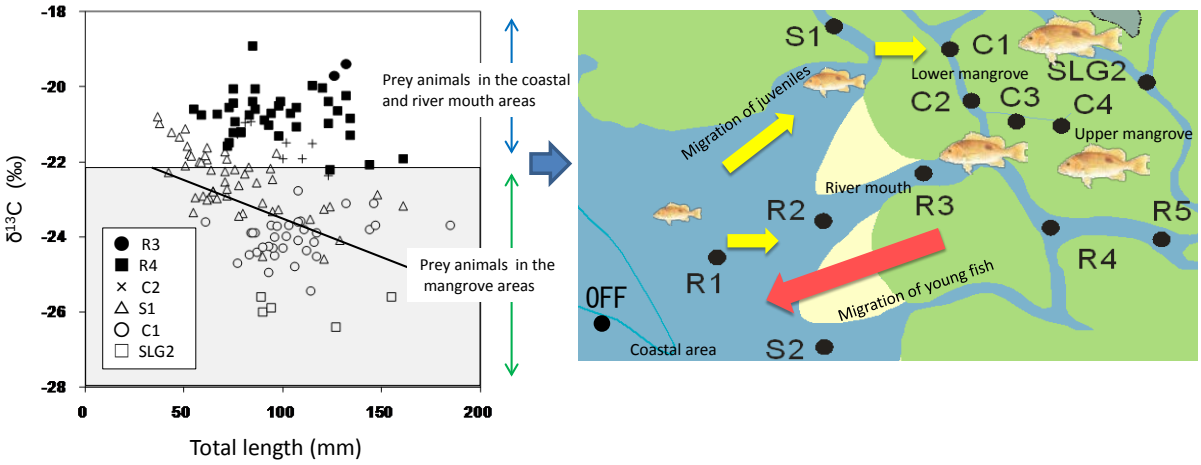

Fig. 2. Relationship between $\delta^{13}C$ values and total length of *Lutjanus johnii* in the Matang Mangrove Estuary (left) and the migration of juvenile and young *Lutjanus johnii* (right)

Size measurement and nutritional condition evaluation methods in sandfish (*Holothuria scabra* Jaeger)

Over 60 species of sea cucumbers are fished commercially and traded in Southeast Asian and Pacific countries, and great demand for these sea cucumbers has caused severe overfishing in these countries. Depletion of natural stocks with high commercial value has encouraged hatchery production, stock enhancement and aquaculture programs of sea cucumbers, especially sandfish, *Holothuria scabra* (Fig. 1), the most valued of tropical sea cucumbers.

High mortality and slow growth are problems in *H. scabra* hatcheries, and rearing techniques must be improved for stable mass production of the seeds. However, there is one fundamental methodological problem: there are no standard methods for size measurement and for evaluating nutritional condition of sea cucumbers including *H. scabra*. Without correct determination of size and nutritional condition, evaluation and improvement of technologies are difficult. The aims of this study, therefore, were to establish accurate size measurement and nutritional condition evaluation methods for *H. scabra*.

Anaesthetization is reported to improve size measurement accuracy in Japanese common sea cucumber, *Apostichopus japonicas*. Although 0.5% KCl and 0.05% $MgSO_4$ did not induce anesthesia, 2% - 4% menthol-ethanol in filtered seawater for 20 - 30 min was found effective and harmless. The anesthetization significantly reduced the coefficient of variation of the mean body length and weight in repeated measurements by 68% and 43%, respectively (Fig. 2).

During starvation, body size and weight decreased concomitantly, resulting in unchanged condition factor (body weight / volume), suggesting that condition factor cannot be used as an index of nutritional condition unlike in animals with axial skeletons or exoskeletons. Protein, cholesterol and carbohydrate concentrations in the body fluid (i.e. coelomic fluid and vascular fluid) were also analyzed to study the relationship with starvation. Since the protein and cholesterol concentrations initially increased and then decreased during starvation period, it is difficult to use them as indices of nutritional condition. The carbohydrate concentration showed a gradual two-fold increase during 10-day starvation (Fig. 3, left). Body fluid density (Fig. 3, right) and volume relative to body size gradually increased and decreased, respectively during 20-day starvation. High correlation coefficients indicate that these factors may be used as proxies for nutritional condition.

The aforementioned methods may be used to correctly monitor the conditions of *H. scabra* in studies for improving aquaculture and stock enhancement techniques. However, further physiological studies to elucidate the mechanisms behind the observed tendencies must be conducted.

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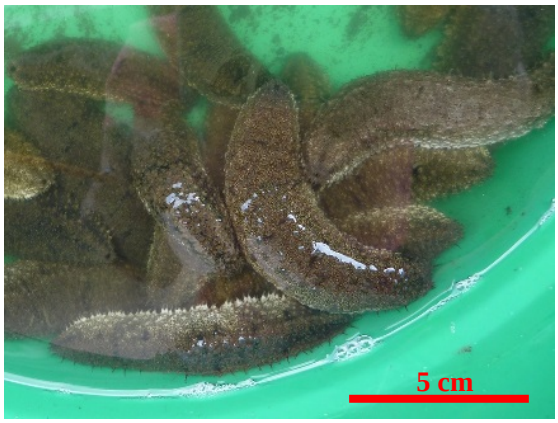

Fig. 1. Sandfish (*Holothuria scabra*)

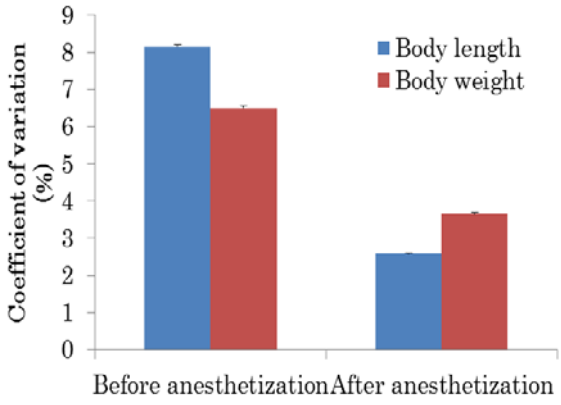

Fig. 2. Coefficient of variation in body length and weight of juvenile *H. scabra* subjected to repeated measurements

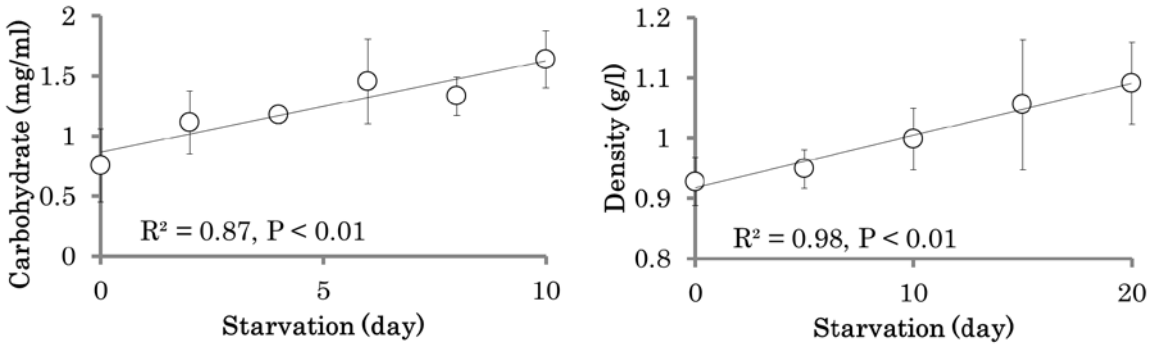

Fig. 3. Relationship between starvation day and carbohydrate concentration (left) and density (right) in body fluid of juvenile *H. scabra*

Stock assessment and management of juvenile orange-spotted grouper (*Epinephelus coioides*) at a tropical mangrove brackish area in Peninsular Malaysia

Juvenile orange-spotted groupers (Fig. 1), the most commercially important fish in the tropics, are usually harvested from brackish waters for use as aquaculture seed, but the actual fishing situation is unclear. In this research, the actual fishing situation of juvenile grouper-species fish was clarified by collecting fishing statistics from the Merbok Mangrove brackish waters in the northwestern coast of Peninsular Malaysia. Also, stock assessment of the juvenile orange-spotted grouper was conducted using fishing statistics and biological analysis. Furthermore, a stock management model for juvenile orange-spotted groupers was constructed and an effective strategy for a sustainable stock management system was proposed to relevant agencies of the Malaysian government.

An investigation on the actual fishing situation of juvenile orange-spotted groupers was conducted in the Merbok Mangrove brackish waters of Malaysia from January 2007 to March 2010. The results showed that they were dominant through all those years (Fig. 2). Spawning behaviour of juvenile orange-spotted groupers was determined based on otolith age-in-days analysis. It was observed that juvenile fish born in the sea migrated into the mangrove brackish waters at the age of two months, stayed there for three to five months, and then moved out to coastal regions by the age of seven months. The relationship between the age in days and total length of the juvenile orange-spotted groupers in the mangrove brackish waters is expressed in the following single regression formula, called the growth formula, where total length=0.91 x age in days + 15.0 (R^2=0.77) (Fig. 3).

The results of the stock assessment conducted on the juvenile orange-spotted groupers in the Merbok Mangrove brackish waters showed that the fishing mortality coefficient (***F***) (coefficient expressing the size of the rate of decline of the number of stock with catching as the cause of death) of fish over four months old increased drastically from September 2008. It was affirmed that juvenile orange-spotted groupers in the same brackish waters were overexploited (Fig. 4). By contrast, stock recovery rate simulation results indicated that a reduction in fishing effort could help expectations for stock recovery. Additionally, the stock recovery rate increased quadratically in association with the reduction in the fishing mortality coefficient (Fig. 5).

With this research, it was established that the mangrove brackish waters are habitats critical to the growth of juvenile orange-spotted groupers. Based on recovery rate model simulation, fishing prohibition would be the most effective method of stock management. If fishing prohibition measures are implemented, up to four times the current amount of juvenile fish could be supplied to the coastal regions. It was estimated that the number of out-migrating stock would be approximately 20,000 fishes per month. These results is expected to be used as fundamental data for stock management in Malaysia.

(*T. Yamamoto, Y. Hanamura, Man Alias, P. E. Chee, S. Ryon[Fisheries Research Institute, Malaysia]*)

Fig. 1. Orange-spotted grouper (*Epinephelus coioides)*

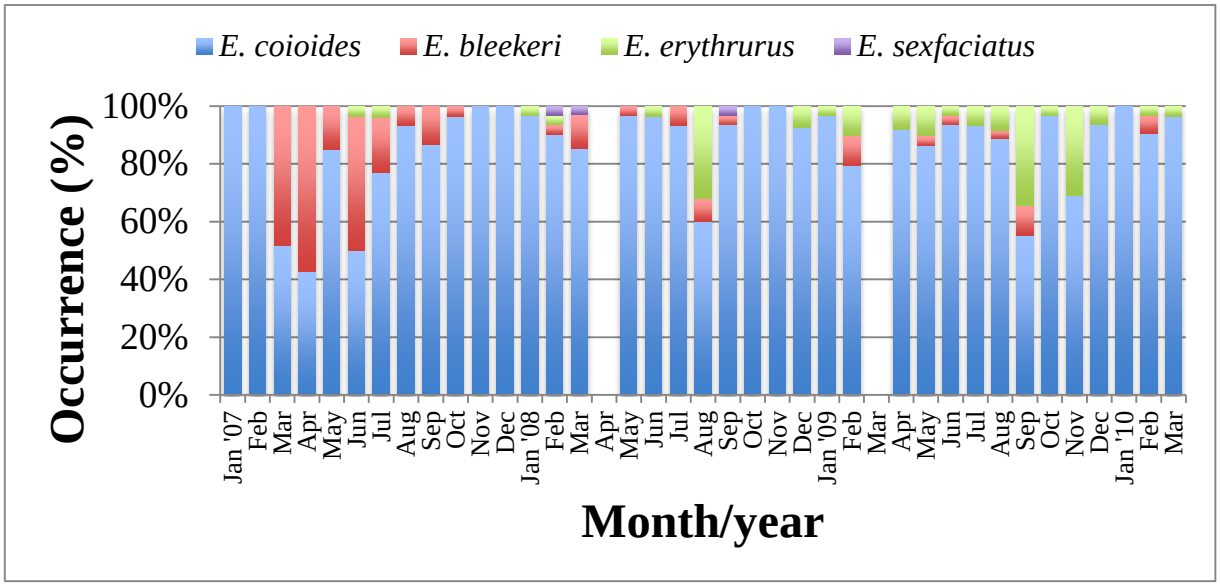

Fig. 2. Species composition of juvenile groupers caught by sampling boat from January 2007 to March 2010

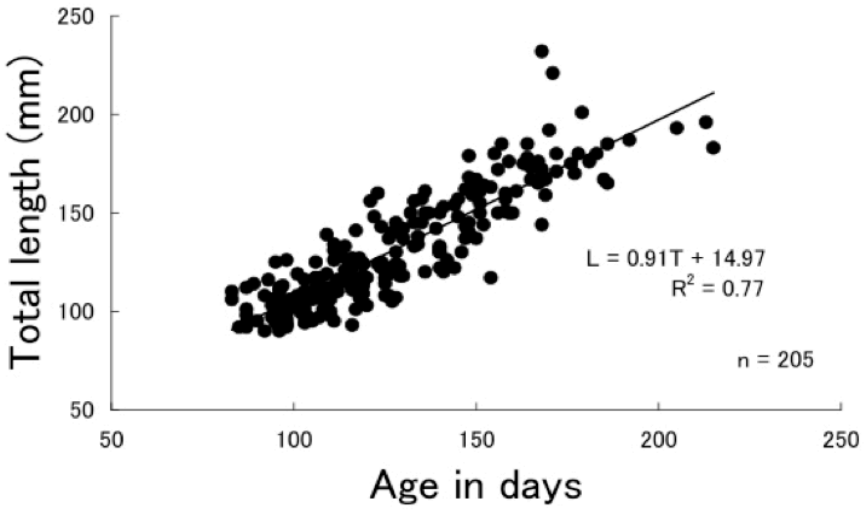

Fig. 3. Scatter plot showing the growth of the orange-spotted grouper (*Epinephelus coioides*) juveniles caught in the Merbok Mangrove area

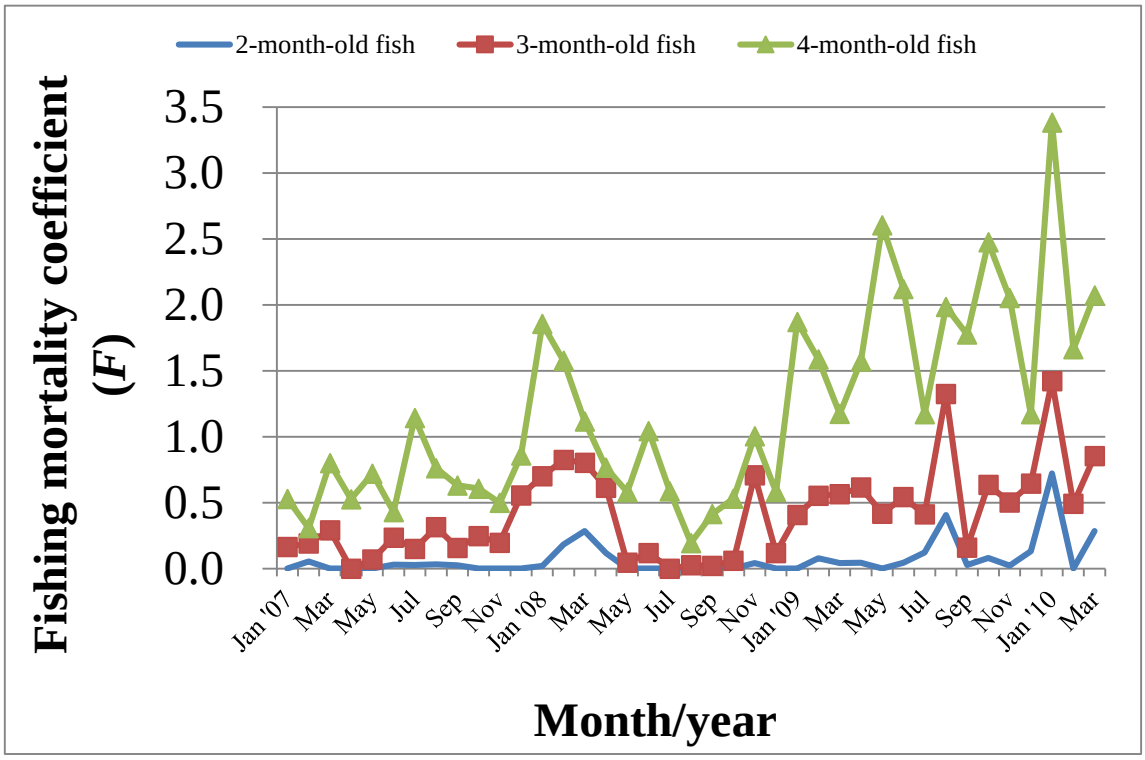

Fig.4. Fishing mortality coefficient (***F***) by age (in months) of juvenile orange-spotted groupers caught in the Merbok mangrove area from January 2007 to March 2010

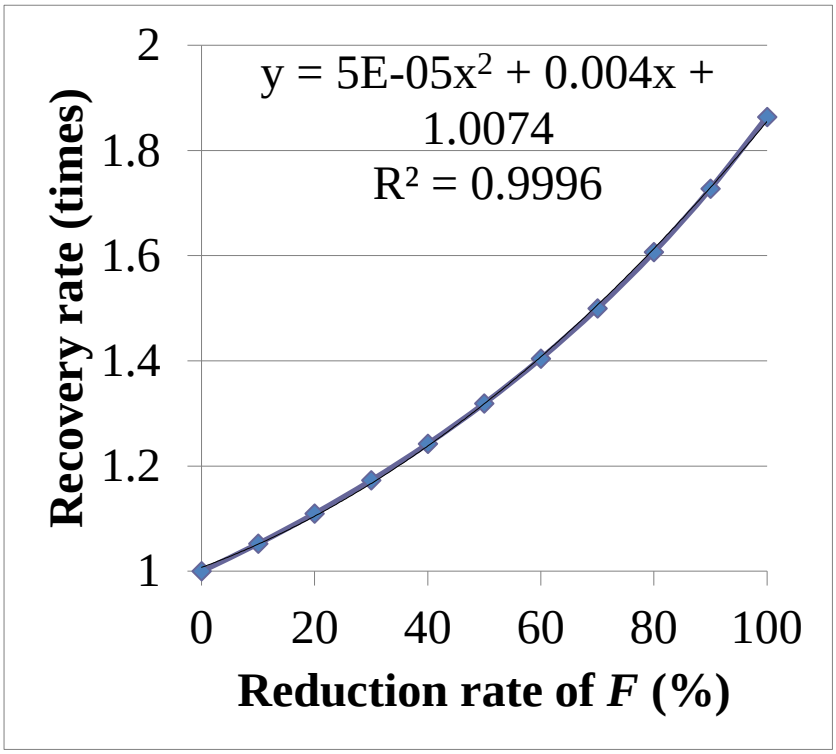

Fig. 5. Recovery rate of stock abundance in number of juvenile orange-spotted groupers in the Merbok mangrove area through arbitrary reductions of ***F*** percentage. The recovery curve of the stock number is expressed as a quadratic equation.