Estimation of rice production in 1km mesh in Bangladesh using multiple regression analysis and GIS

Impact assessments and countermeasures to global climate change are urgently required in developing countries vulnerable to environmental fluctuations. Agricultural systems in developing countries significantly depend on natural conditions, hence principal production areas are likely to be established in areas where meet adequate land and climate conditions for cultivation. Potential land suitability maps independent from climate condition are necessary in order to assess the impact of climate change to principal production areas, i.e., suitable areas, spatially and quantitatively.

To this end, a method to estimate rainy and dry season rice production in 1km mesh in Bangladesh was developed using multiple regression analysis and GIS with fundamental geospatial dataset. This proposed model (Fig.1) employed several combinations of six land factors representing Slope (3 categories), Land Type (5 categories), Soil Texture (5 categories), Drainage (6 categories), Soil Permeability (3 categories), and Soil Salinity (4 categories) derived from Bangladesh Country Almanac (BCA) Ver.3. The BCA, prepared and provided by CIMMYT-Bangladesh, is a national geospatial dataset containing sub-district level information.

The distributed areas of 26 attributes, representing six land factors in 463 sub-districts (or Thana), were assumed as explanatory variables while the productions of rainy and dry season rice in 2002-2003 were respectively assumed as response variable and then, a multiple regression analysis was executed. Adjusted coefficients of determination (adjusted R2) of the multiple regression equations for rainy and dry season rice were significantly high at 0.903 and 0.823, respectively.

The partial regression coefficients were assigned into attribute tables for six land factor maps, and then rice production per 1 km x 1 km mesh was estimated by map calculation in GIS (Figs. 2 and 3). The total amount of map calculation compiled by new and old provincial boundaries showed high correlation with rice productions in the statistics, and the mean error in each province was within 23-33% (Table 1). This implied that the distribution of production for each mesh was accurate.

The estimated rice production in 1-km mesh is available for “baseline,” i.e., the initial value under current climate condition for climate change impact assessment. When statistics and map data are available, categorical data can be manipulated as quantitative variables in such a manner that the area of each attribute is applied for explanatory variables. It provides scalability and versatility for model development. However, this method is intended only for estimating production by the combination of attributes, not for determining the contribution of each attribute.

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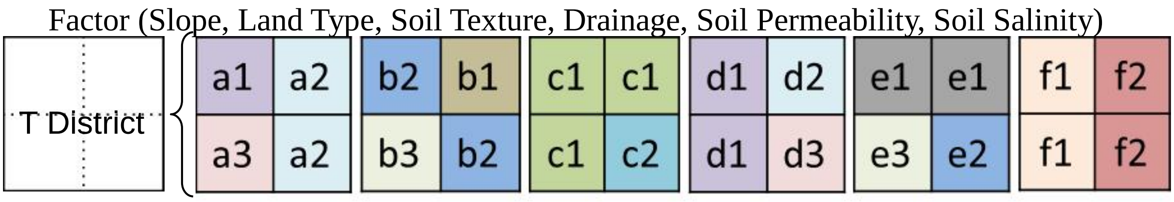

Supposing that:

- Production in T district = Σ (Production in each mesh)
- Production in the mesh which has same combination of attributes are same

Production in T district $=\Sigma(\alpha_i \cdot Ma_i)+\Sigma(\beta_i \cdot Mb_i)+\cdots+\Sigma(\varepsilon_i \cdot Mf_i)$

where

$\alpha_i, \beta_i \cdots \varepsilon_i$ *: coefficients of attribute i in factor α, b, ･･･ f*

Fig. 1. Concept of the model

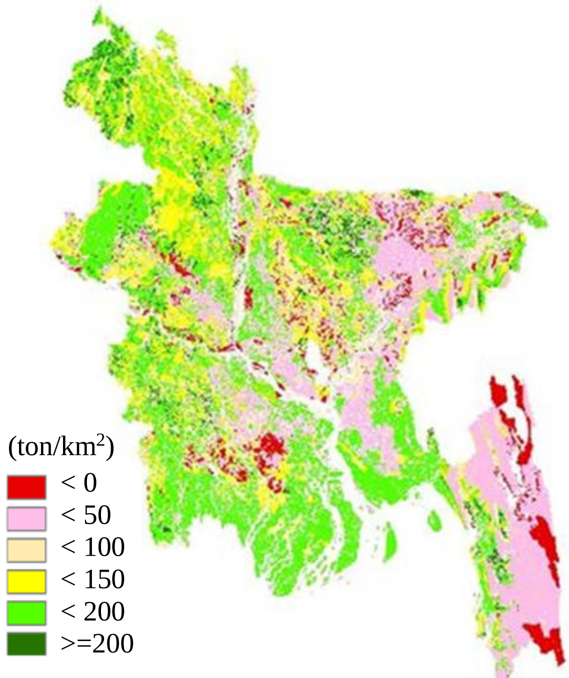

Fig. 2. Estimated production map for rainy season rice

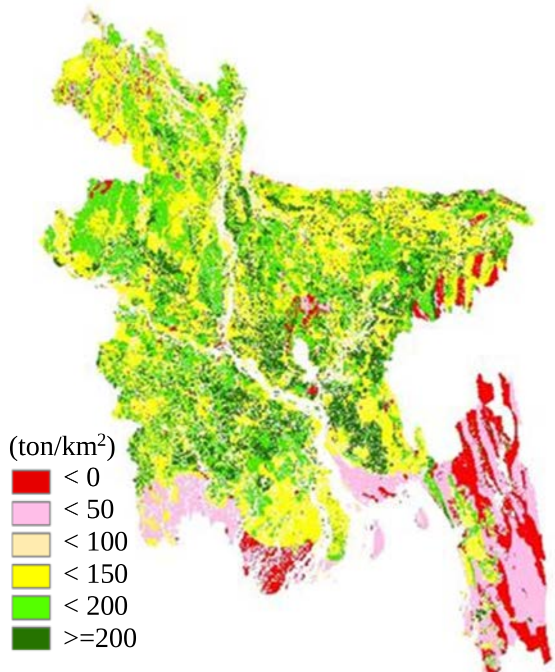

Fig. 3. Estimated production map for dry season rice

Table 1. Accuracy of estimation to statistics of 21(old) / 64 (new) provinces

Target	Accuracy	21 Provinces	64 Provinces
Rainy season rice	Correlation to statistics	0.827	0.925
	Mean error (%)	23.2	31.3
Dry season rice	Correlation to statistics	0.806	0.867
	Mean error (%)	28.6 [a]	33.3 [b]

[a] Excluded one prefecture in which production was under 2,000 tons
[b] Excluded four prefectures in which production was under 20,000 tons

A projection method to estimate global warming impacts on vegetable production

Projecting future impacts of climate change is important in considering appropriate coping and adaptation measures. The importance of projections also applies to agriculture and food supply, and many have already been generated to predict future trends. However, most projections are limited to cereals with only a few studies focusing on vegetables.

One reason why vegetables are less focused on is that the number of items and varieties of vegetables are too many to develop respective crop models used for projections. Another reason is that vegetables are considered less important than cereals in terms of food security. However, there are some regions whose economies depend largely on vegetable production. For such regions, projecting climate change impacts on vegetable production is more important. Therefore, the purpose of this study is to consider a projection method for vegetables as an alternative to crop model.

There are some countries, including Japan, where vegetables are cultivated all year round, using both open fields and horticultural facilities like greenhouses, and following product standards. In such countries, production costs of vegetables grown during unfavorable seasons are likely to be higher than those grown during favorable seasons due to the use of adaptation technologies. Therefore, assuming that average air temperature during cultivation period is a representative index of meteorological condition, we hypothesized that production costs increase when the average temperatures of cultivation environments are higher or lower than the optimal temperature ranges, and that the costs make downward-convex curves (Fig. 1).

To investigate the hypothesis, regression analysis was applied to data on production costs and cultivation environments of 15 vegetable types in Japan. Regression analysis showed that: some items have relatively high determination coefficients and follow the convex curves of the hypothesis (Fig. 2); other items have relatively high determination coefficients but do not include the interior minimum points of the convex curves (Fig. 3); and others have low determination coefficients. These three patterns are summarized in Fig. 4.

Relatively high determination coefficients imply that vegetable cultivation at temperatures below or above optimal range has introduced some adaptation methods, which vary production cost. To ascertain the substance of adaptation methods, regression analysis between temperature and itemized production costs was employed. Results show that the cost of fertilizers, chemicals, energy and power, seedlings, and management are sensitive to temperature, and that they are thought to be closely related to adaptation methods. Of the vegetables whose curves have interior minimum points (Fig. 2), some have minimal cost points lower than optimal temperature ranges for plant growth. Investigation of itemized production cost data of the vegetables reveals that costs for quality preservation after harvesting, e.g., cleaning, shipping preparation, and packing costs, can reach high values even within optimal temperature ranges. This implies that temperature ranges optimal for plant growth are not necessarily optimal in terms of production cost.

The impact of temperature change on vegetable production due to climate change can be projected by measuring the change in production cost along the estimated production cost curve (Fig. 4). The data used for this analysis was derived from main production areas. If data can also be derived from small production areas whose temperatures are warmer, anticipated dot-lines in Fig. 4 can be estimated. In order to deal with many vegetables, this study adopted only average air temperature during cultivation period as the explaining variable, and did not distinguish varieties of a vegetable. Use of variables suitable for each vegetable and varietal analysis are expected to improve the statistical significance of the regression analysis.

(*S. Kobayashi, J. Furuya, and Y. Yamamoto*)

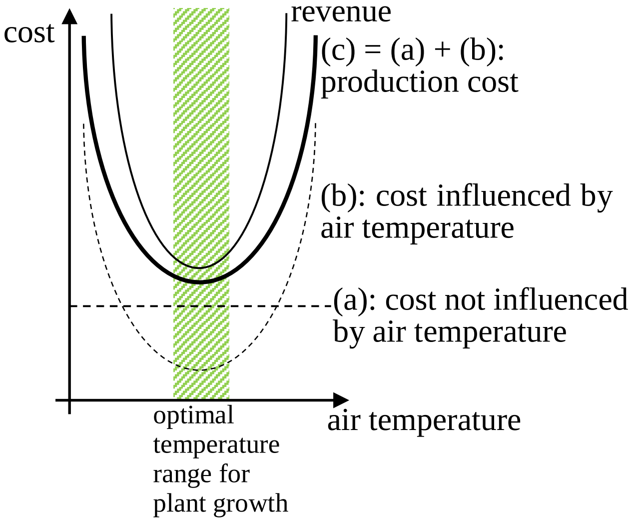

Fig. 1. Hypothesis of vegetable production cost

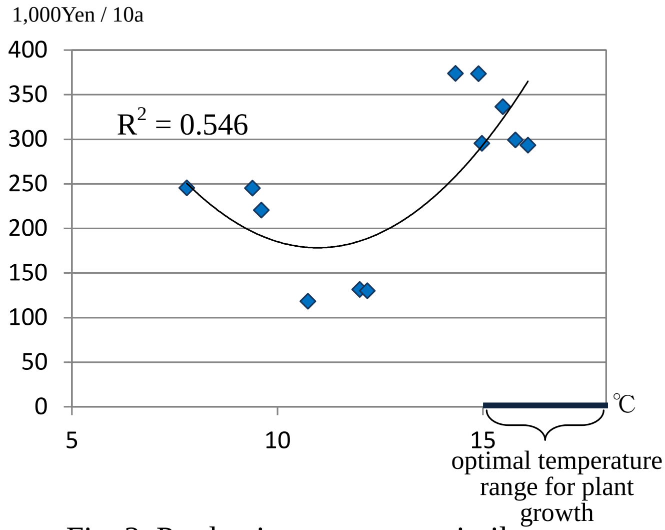

Fig. 2. Production cost curve similar to the hypothesis (spinach).

Shishito pepper and eggplant reveal the same pattern. They are summarized as pattern (i) in Fig. 4.

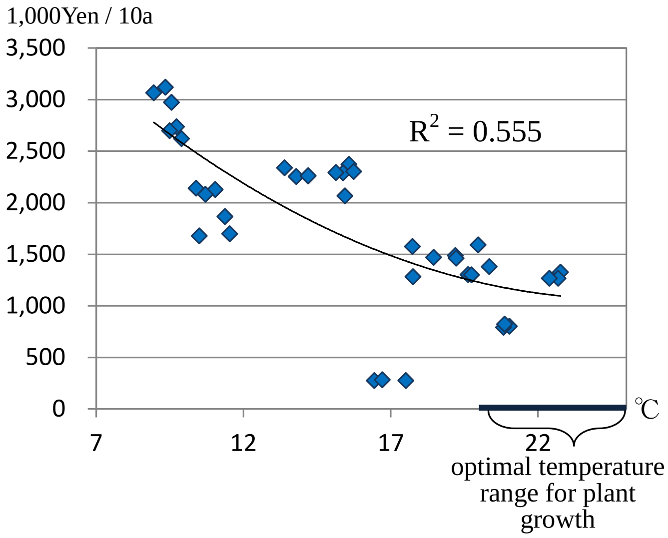

Fig. 3. Production cost curve without interior minimal point (tomato).

Cucumber and watermelon reveal the same pattern. They are summarized as pattern (ii) in Fig. 4.

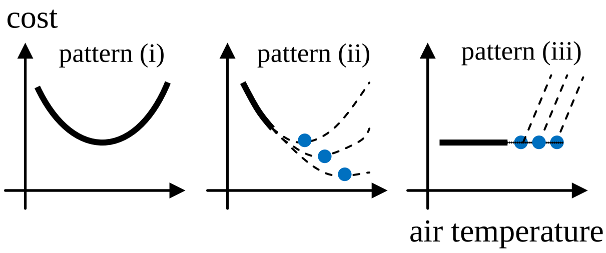

Fig. 4. Auxiliary hypothesis of vegetable production cost.

Dot-lines in patterns (ii) and (iii) cannot be observed from the data used for this analysis, but they are believed to exist. Pattern (iii) includes green onion, carrot, and Chinese cabbage. In Japan, they are usually cultivated not in horticultural facilities but in open fields. Differences in temperature have the possibility of influencing not only production cost but also land productivity. Additional analysis for pattern (iii) may be required.

Near-isogenic lines carrying QTL for high spikelet number with the genetic background of IR64, an Indica-type rice variety

IR64, recognized globally as a high quality Indica-type rice variety, was first released by the International Rice Research Institute (IRRI) in 1985. To improve the yield potential of IR64, a set of near-isogenic lines (NILs) of IR64 with increased total spikelet number per panicle (TSN) was developed using New Plant Type (NPT) varieties as tropical Japonica-type donor parents.

A total of five NILs derived from different donor parents were developed through marker-assisted selection. Regardless of the donor parents, Quantitative Trait Locus (QTL) for high TSN was detected on common region of the long arm of chromosome 4 (Table 1, Fig. 1). We designated this QTL as *qTSN4*. Marker information linked to *qTSN4* is shown in Table 1. NILs have 196-239 spikelets per panicle (i.e., 40-70% greater TSN than IR64), which is attributed to the increase in larger number of spikelets on the secondary and ternary rachis branches. Variation in TSN was observed among NILs; the number of spikelets was greatest in IR64-NIL2 (IR65564-2-2-3 as donor parent) and in IR64-NIL4 (IR66215-44-2-3 as donor parent).

In this study, we succeeded in developing five NILs for high TSN with a rice variety (IR64) genetic background by employing molecular marker-assisted selection. The materials are expected to be useful for enhancing the yield potential of rice varieties. These NILs are also available for understanding the genetic basis of TSN and the effects of single QTL/gene by testing it under different environmental conditions. Further investigation is required to determine if the variation in panicle architecture is simply due to the allelic effects or not.

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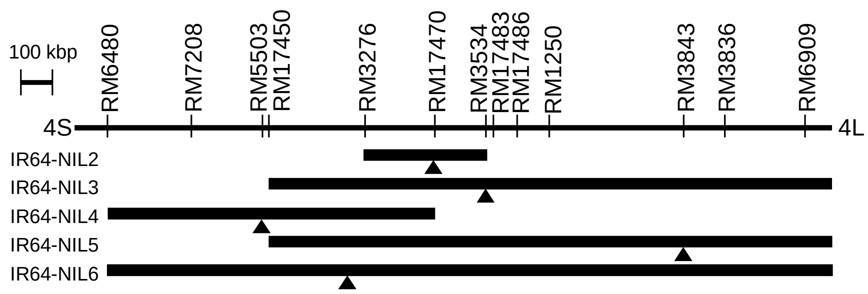

Fig. 1. The location of QTLs for spikelet number per panicle on the long arm of chromosome 4. Arrowheads indicate the location of peak LOD score.

Table 1. Marker information on QTLs and panicle structure of IR64 and its NILs for high TSN

Variety/Line	Donor parent (NPT variety)	Marker[A]	Total spikelet number per panicle[B]	Number of rachis per panicle[B]		
				Primary rachis	Secondary rachis	Ternary rachis
IR64	-		141.2±17.8 c	9.2±0.4 c	28.6±4.0 c	1.3±1.1 c
IR64-NIL2	IR65564-2-2-3	*RM17470* - *RM3534*	233.9±22.6 a	10.6±0.8 ab	46.8±4.7 a	11.2±3.2 a
IR64-NIL3	IR69093-41-2-3-2	*RM3534* - *RM17486*	196.4±19.1 b	11.3±0.7 a	37.2±3.5 b	1.9±2.3 c
IR64-NIL4	IR66215-44-2-3	*RM6480* - *RM5503*	239.4±36.4 a	10.9±0.7 a	46.2±6.4 a	6.8±4.3 b
IR64-NIL5	IR68522-10-2-2	*RM3843* - *RM1113*	197.6±19.6 b	10.8±0.8 ab	39.4±3.8 b	2.3±1.5 c
IR64-NIL6	IR66750-6-2-1	*RM17450* - *RM17470*	213.5±25.3 ab	9.8±0.9 bc	43.1±5.3 ab	6.9±1.8 b

A) Flanking markers for *qTSN4*. Underlines show the nearest marker.

B) Data was obtained in the dry season of 2009 in IRRI (values are indicated as average ± SD). Different letters (a, b and c) indicate significant difference at 5% by Tukey-Kramer's test.

Fig. 2. Panicle architecture of IR64 and its NILs for increased spikelet number

Identification and characterization of biological nitrification inhibition (BNI) substances in sorghum root

Nitrification, one of several pathways in the soil-N cycle, results in the microbiological conversion of relatively immobile NH_4^+ into highly mobile NO_3^- (which is susceptible to losses through leaching [NO_3^- leaching]), and gaseous N emissions (N_2O, NO and N_2) by denitrification. The price of nitrogen fertilizer has been rising in recent years. Controlling nitrification through suppression of nitrifier activity is thus critical to improving nitrogen use efficiency (NUE) of agricultural production systems. Suppressing soil nitrification through the release of nitrification inhibitors from plant roots is termed 'biological nitrification inhibition' (BNI). This present study aims to characterize BNI function in sorghum, in particular the production of inhibitors, their chemical identity, functionality, and factors regulating their release.

Sorghum roots release two types of nitrification inhibitors: hydrophilic-BNIs and hydrophobic-BNIs. The former were those released into water-based collection medium while the latter are those released by washing the roots for 30s with dichloromethane (DCM), which has high affinity for hydrophobic compounds.

Three nitrification inhibitors -- MHPP (methyl 3-(4-hydroxyphenyl) propionate), sakuranetin (5,4'-dihydroxy-7-methoxyflavanone) (isolated from hydrophilic BNI activity), and sorgoleone (2-hydroxy-5-methoxy-3-[(8'Z,11'Z)-8',11',14'-pentadecatriene]-*p*-benzoquinone) (isolated from hydrophobic BNI activity) -- were isolated from the inhibitory activity released from sorghum roots (Fig. 1). The release of nitrification inhibitors required the presence of NH_4^+, whose stimulatory effect lasted 24h, in the root environment (Fig. 2). The release of hydrophilic-BNIs declined at rhizosphere pH > 5.0. Nearly 80% of hydrophilic-BNI released was suppressed at pH ≥7.0 (Fig. 3). A bioluminescence assay using recombinant *Nitrosomonas europaea* was employed to determine BNI activity. The ED_{80} (effective dose for 80% inhibition Nitrosomonas function) for sakuranetin, sorgoleone, and MHPP was 0.6 µM, 12.0 µM, and >120 µM, respectively (Fig. 4).

These results are useful as fundamental knowledge towards utilization research of BNI in sorghum. We should clarify the field conditions in which sorghum BNI is the most efficient and investigate the BNI activity of each substance in the soil. We need to establish reliable screening techniques and selection criteria for breeding.

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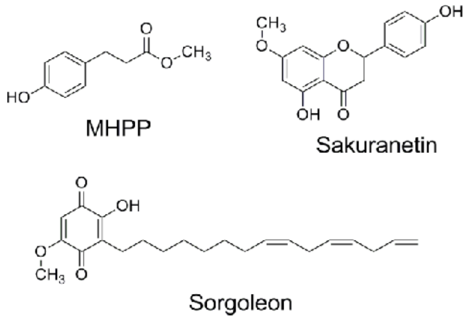


Fig. 1. BNI compounds from sorghum root

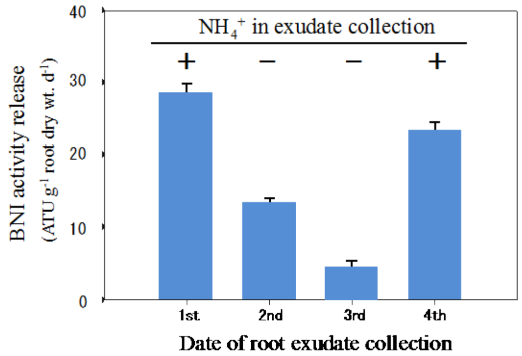


Fig. 2. Influence of NH_4^+ in the root exudate collection solutions on BNI activity release from sorghum roots; root exudate was collected every day by exchanged collection solution using one plant

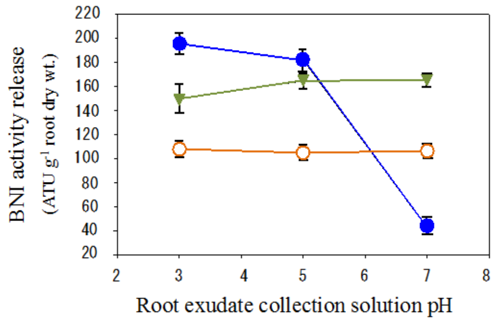
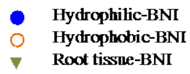


Fig. 3. Influence root exudate collection solution pH on the BNI release in sorghum



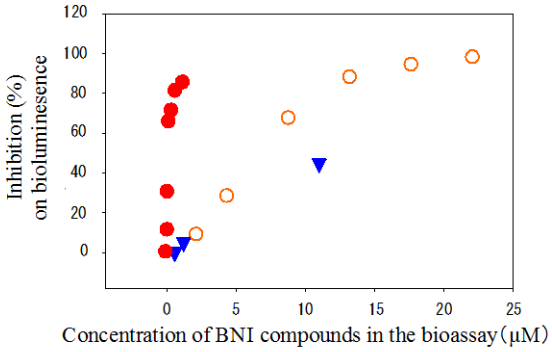


Fig. 4. Relative effectiveness of sakuranetin (●), sorgoleone (○) and MHPP (▼) in inhibiting *Nitrosomonas* activity *in vitro*

Registration of a biogas CDM project in Viet Nam with the UNFCCC CDM Executive Board (CDM-EB)

The Clean Development Mechanism (CDM) is a system which aims to reduce emission of greenhouse gases (GHGs) through the implementation of emission reduction projects in developing countries. The reduced emission is converted to credits (Certified Emission Reduction: CER) and traded in developed countries (Annex I countries in the Kyoto Protocol).

In Viet Nam, a CDM project that reduces GHG emission and contributes to rural development was established, expecting that the methodology developed from the research study would be used as basis for similar future projects. Biogas digesters (BDs) for treating farm wastes, sewage, etc. were introduced to low-income communities to produce biogas, a renewable fuel that can be used as fuel wood and LP gas substitutes for heating and cooking purposes.

In formulating a CDM project in rural areas of developing countries, the most important thing is to establish an organized system, from selecting beneficiary households in small and wide areas, to introducing GHG emission reduction technologies, monitoring, and obtaining carbon credits. In this CDM project, 34 farmer leaders (called “Key Farmer: KF”) from three districts were trained and developed to serve as promoters and provide guidance to beneficiary households in installing, operating and maintaining BDs, as well as in monitoring emission reduction.

After introducing BDs to individual households, the volume of GHG emission reduction was estimated. JIRCAS and Can Tho University (CTU) researchers conducted studies and experiments to quantify the following: 1) woody biomass stock in Can Tho City, 2) fuel demand in rural area, 3) fraction of non-renewable woody biomass, 4) reduction of nonrenewable fuel use which will be substituted by BD and 5) number of participant households. Annual GHG emission reduction by the project was 1,203 t-CO_2, with 961 households participating (Tables 1 and 2).

The above-mentioned CDM project, titled “Farm Household Biogas Project Contributing to Rural Development in Can Tho City,” was registered on 15 August 2012 with the UNFCCC CDM-EB after approval by the governments of Japan and Viet Nam (Fig. 1). This project, introducing an economical plastic type BD (Fig. 2), was the first biogas CDM project formulated by a Japanese entity to directly benefit low-income households (by contributing to livelihood improvement) as well as the environment (by emission reduction of GHG).

In order to use the BDs continuously, appropriate pig-raising techniques and maintenance of BDs should be conducted based on the technical manuals prepared by JIRCAS and CTU.

Small-scale CDM projects like this one has scale demerit (i.e., the cost of project formulation, registration, subsidy of materials, technical support, validation and verification may not be covered by its advantages). The expectation is that private companies will participate in the project and purchase carbon credits, taking into account the expected co-benefits such as compliance to CSR (Corporate Social Responsibility), creation of BOP (Base of the pyramid) business opportunity to low income communities, and provision of additional fund to cover the shortfall.

(E. Matsubara, T. Izumi, A. Taminato, Y. Iizumi)

Table 1. Data for estimating the volume of GHG emission reduction

Item	Value	Remarks	
① Woody biomass in Can Tho City			
Average biomass	18.82 tC/ha	a	Baseline survey
Area of forest and orchard	14,592.82 ha	b	Can Tho City
Total biomass in forest and orchard	274,637 tC	c=a*b	
Annual growth rate of woody biomass	12.38 %	d	IPCC
Annual incremental woody biomass	34,000 tC/yr	e=c*d	
② Fuel demand in rural area			
(Fuel wood)	1.58 t/yr	f	3.8 persons / household
Rural population	563,326 人	g	2008, Can Tho City
Fuel wood use in rural area	233,799 t/yr	h=f/3.8*g	
Carbon volume	116,900 tC/yr	i=h*0.5	Carbon fraction: 0.5 (IPCC)
(LPG)	17.8 kg/yr	j	
③ Fraction of non-renewable woody biomass			
	70 %	k=1-e/i	
④ Substitution of fuel use with renewable biogas			
	Based on monitoring activities, it is confirmed that fuel for cooking was fully substituted with biogas from BD installed at households.		
⑤ Number of participant households			
	961 hh	l	

Table 2. Volume of GHG emission reduction

Present GHG emission		
Fuel wood	Fuel wood demand per household	1.58 t/yr
	Fraction of non-renewable woody biomass	70%
	non-renewable woody biomass demand per household	1.11 t/yr
	GHG emission from fuel wood use per household	1.41 tCO_2/yr
LPG	LPG demand per household	17.80 kg/yr
	GHG emission from LPG use per household	0.05 tCO_2/yr
Total	GHG emission from cooking per household	1.46 tCO_2/yr
	GHG emission from cooking per 961 households	1,403 tCO_2/yr
GHG emission reduction by CDM project		
Year 1	Unit in operation: 241	352 tCO_2
Year 2	Unit in operation: 721	1,053 tCO_2
Year 3	Unit in operation: 961	1,403 tCO_2
Year 4	Unit in operation: 961	1,403 tCO_2
Year 5	Unit in operation: 961	1,403 tCO_2
Year 6	Unit in operation: 961	1,403 tCO_2
Year 7	Unit in operation: 961	1,403 tCO_2
Total		8,420 tCO_2
Average		1,203 tCO_2

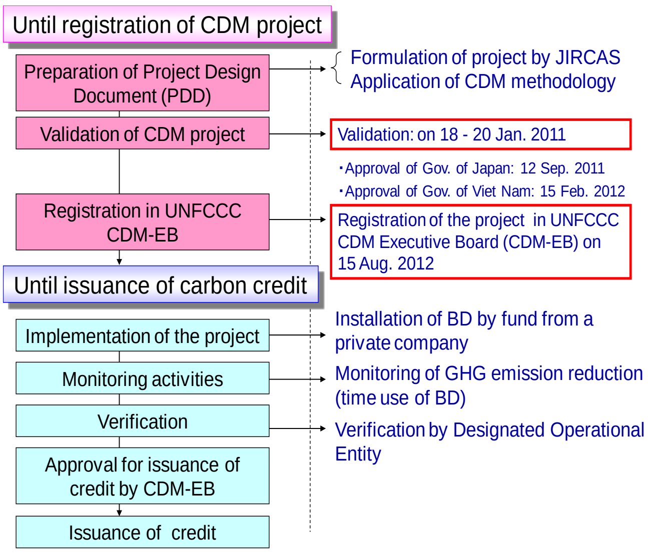


Fig. 1. CDM project implementation procedure

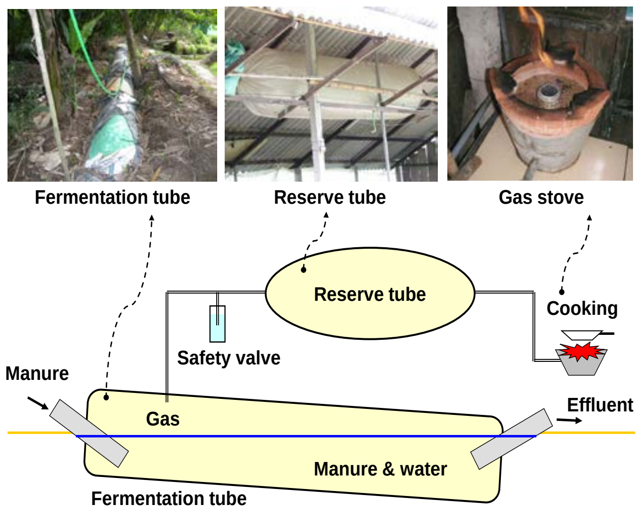


Fig. 2. Plastic type biogas digester system

Development of a guideline for the conservation and management of natural resources in Mali and Niger

Arable lands in the Sahel region in semi-arid West Africa are being degraded through intensive land use activities such as exploitative farming, extensive animal husbandry, and excessive fuelwood collection. Hence, it is necessary not only to introduce conservation techniques against degradation of soil and vegetation, but also to facilitate and get the villagers involved in conservation efforts. In this way, villagers become part of the solution. In addition, it is essential to have a regional or local support system to encourage and guide the villagers in tackling natural resource management issues.

Against this backdrop, JIRCAS implemented the “Study on the Establishment of Methods of Management and Conservation of Resources for Agricultural Production” with the Institute of Rural Economy in the Republic of Mali and the Ministry of Agriculture in the Republic of Niger. This study was subsidized by the Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan from 2008 to 2012.

Four villages in Mali and two villages in Niger were selected as target areas for verification. Conservation activities (e.g. erosion control, reforestation, and soil fertility improvement) were carried out at these villages using approaches and techniques to clarify the constraints and to emphasize and reinforce the role of villages and relevant organizations. All applied approaches were compiled to form the guideline and technical manuals.

In the guideline, problems are resolved by applying the ‘question method’. The question method brings existing problems to light, hastening the problem-solving process with the cooperation of villagers (Fig. 1). The method lets local government officers act as facilitators, thereby allowing wide participation and enabling the villagers to clarify and solve the problems by themselves. In addition, the approach focuses on land management policy (COFO system, Fig. 2), which was developed based on Niger’s Rural Code (Code Rural du Niger, 1993). It is designed to enhance the establishment, management, financing and implementation capabilities of the village organization (COFOB) responsible for the conservation of natural resources, and it strengthens cooperation among relevant organizations. This approach thereby improves the ability of villagers to solve every problem they could face during implementation of conservation activities.

The approach used in the guideline is widely applicable because it contains detailed procedures and examples that local government officers in agriculture, forestry and environment can carry out during conservation activities with villagers. The guideline has already been certified by the Ministry of Agriculture in Niger and the Institute of Rural Economy, Ministry of Agriculture in Mali. Likewise, the technical manuals which were presented in 10 separate volumes including one on forest conservation, have been highly appreciated by officials from relevant agencies in Mali and Niger. Because these technical manuals clearly illustrate the information and procedures needed by local government officers to guide and advice villagers.

(T. Higashimaki, K. Takenaka, M. Yamada, T. Takeuchi, C. Hirose, T. Kobayashi, R. Miyazaki, N. Shimizu, J. Yasuhisa, K. Suzuki, T. Shinohara)

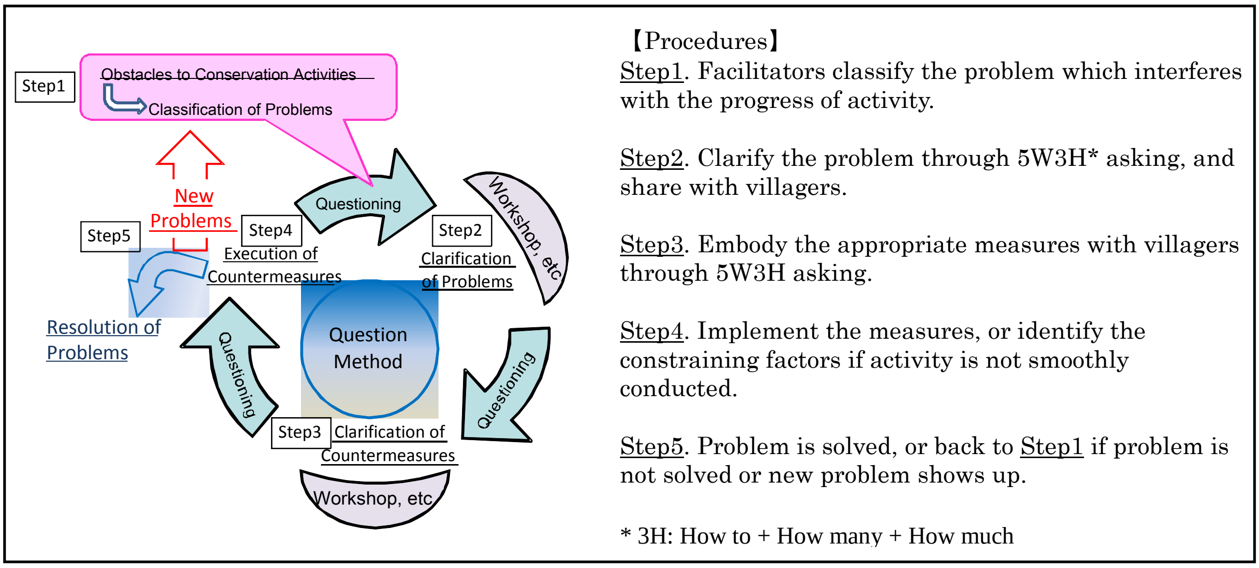


Fig. 1. Problem resolution through questioning

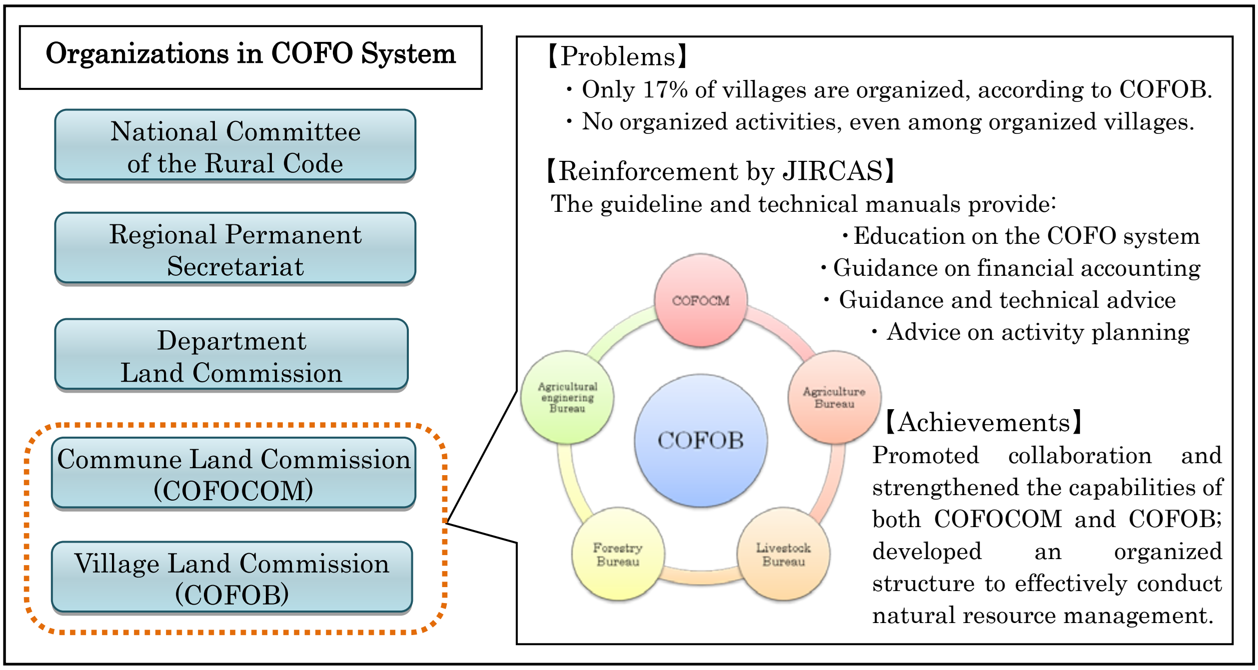


Fig. 2. Reinforcement of COFO system (local policy for land management) in Niger

Local government-led support measures and technical assistance to settled nomads in Xinjiang Uygur Autonomous Region

In order to mitigate the intense degeneration of winter pasture, the local government of Xinjiang Uygur Autonomous Region developed a policy on grazing prohibition and grazing rest by combining stall-feeding during winter with grazing at natural pasture during summer. This policy is aimed at providing the nomads a stable life while protecting and restoring natural grassland. It requires the nomads to form settlements and promotes a new mode of agricultural management.

Thus far, results have been unsatisfactory owing to the settled nomads' lack of experience in planting forage and feeding livestock. In addition, the distribution of technical guidance documents from the county level to the settled nomads had to pass through several local administrative divisions.

Therefore, there is a need to demonstrate a comprehensive and systematic technical assistance program, in accordance with the development policy of the village, to enable the nomads to cope with the transition as soon as possible.

Through the first half of the pilot project, we were able to confirm that the township government played an important role in improving the infrastructure of the villages for settlement and for providing technical guidance to settled nomads. Therefore, enhancing the administrative skills of township government officials is very important towards the stable management of villages for settlement.

At the second half of the pilot project, the technical capabilities of field extension workers were strengthened in order to promote cooperation with local officials. We also gave the nomads directions and on-the-job trainings to get them more actively involved in the settlement project.

The outcome of this research was the publication (in Chinese) of a guidebook and several field-specific manuals, whose contents came from ideas obtained from work and technical problems confronted by the pilot project. All aforesaid books and manuals have graphs or pictures that are easy to understand.

The guidebook, titled "Stable management of the settled nomads,"provides extensive, knowledge-based information to technical personnel tasked to help the nomads. It gives ideas on how to deal with problems over time, through negotiations and dialogues. Manuals on planting and livestock-raising were also published for the Kazakh nomads who cannot read Chinese.

The autonomous government may begin enforcing the nomads' settlement policy, for example, by building new settlement sites around the two demonstration villages, in the hopes of accelerating the dissemination of the new policy and the results of this study. The local (city) and county science bureaus who participated in the management and operation of the pilot project at the two demonstrative villages are currently making plans for its widespread implementation.

This project was carried out with JICA as implementing agency.

(Hirofumi Iga, Keisuke Omori, Katsumi Hasada, Tsutomu Kobayashi, Mitsuru Marumoto, Seiichi Chiba [private individual])

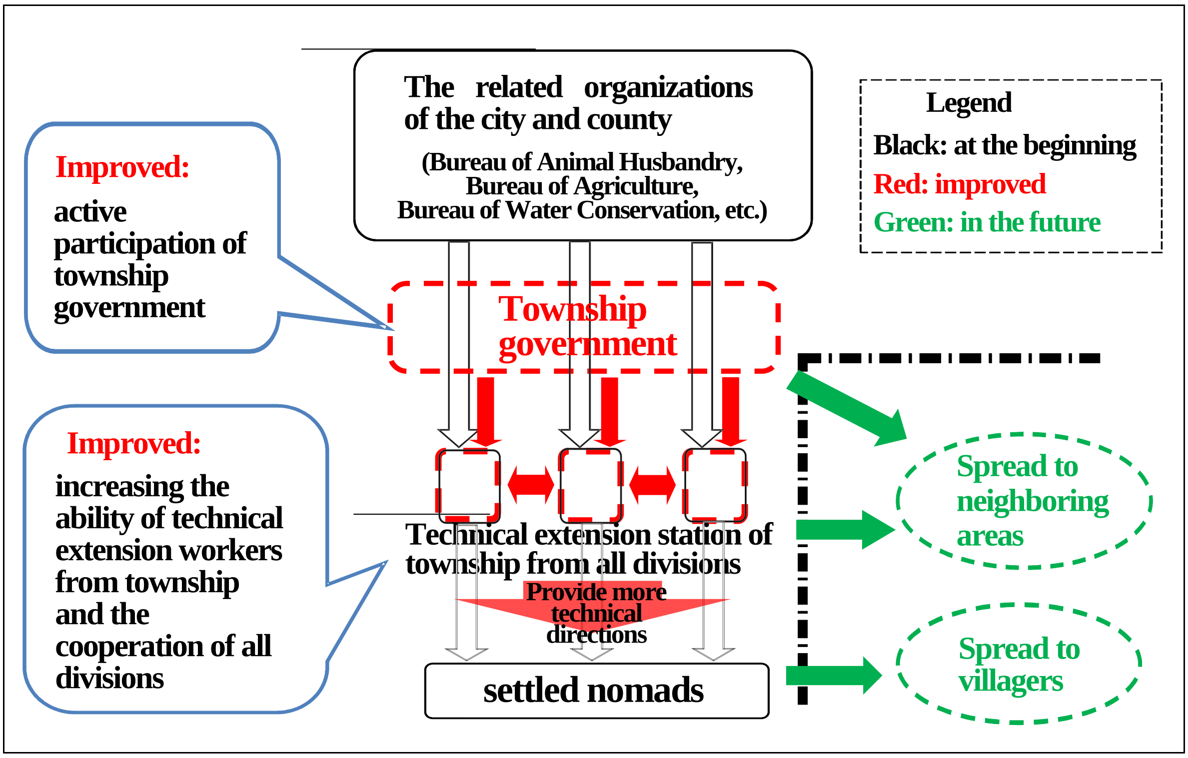


Fig. 1. Strengthening the role of township government and technical extension stations in support of the settled nomads

A: The old teacher (has valuable experiences in nomads' settlement)

Has also experienced many failures in the past;

Strongly believes that "since the conditions at each settlement village is different, it is impossible to get the same results even if the same method is applied. The key to success is to train qualified personnel who are flexible and can handle different villages under different conditions or scenarios."

B: The young official (a young county government personnel in charge of nomads' settlement)

Has gained knowledge on animal husbandry and settlement policy from university, but has little actual experience in the field.

Still trying to find out what and how to give guide and suggestion to the township officials and technical extension workers in his administrative areas.

Fig. 2. The characters in the guidebook, titled "Stable management of the settled nomads"

Contents of the technical manual

1. Grassland management
2. Planting of forage crops
    - 2-1. Guidance for alfalfa planting and use
    - 2-2. Guidance for planting and use of corn as silage
    - 2-3. Guidance for making fully ripe compost
    - 2-4. Guidance for ammonia (urea) treatment
3. Rearing management of dairy cattle
4. Management, market sales, and household
5. Water use and conservation, salt injury, and water management-
6. Brief introduction to making cheese

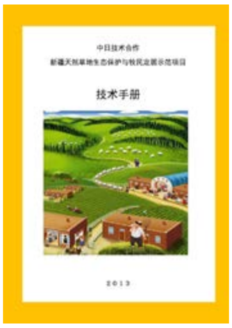


Fig. 3. The contents of the technical manual

Two cryptic species in the coconut hispine beetle, *Brontispa longissima*

The coconut hispine beetle, *Brontispa longissima*, is a serious invasive pest of coconut palm, *Cocos nucifera*. It has recently invaded Southeast Asia and further expansion of risk areas (e.g., into India, Sri Lanka, or Africa) is of great concern. This insect is difficult to control with just chemical pesticides because it stays in young unopened coconut fronds that locate the crown, and coconut trees are too tall for growers to spray pesticides. Moreover, coconut trees are grown near houses and pesticide pollution is a concern; therefore, an alternative to pesticides (i.e., biological control) is necessary.

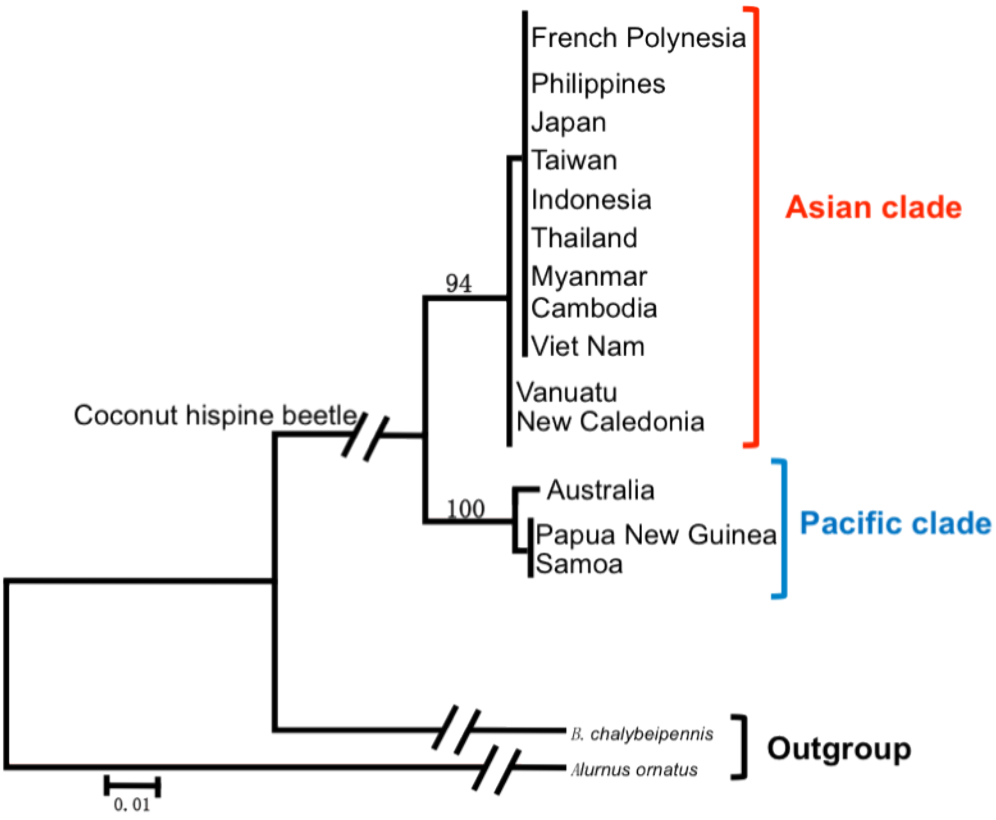
The larval parasitoid, *Asecodes hispinarum* (Hymenoptera: Eulophidae), was introduced to Southeast Asia from Papua New Guinea (via Samoa) through a FAO project. To study genetic variation in this species and to determine the place of origin or distribution pathways of *B. longissima*, we compared partial mitochondrial cytochrome oxidase subunit I gene (*COI*) sequences of specimens obtained from Pacific, Southeast Asian, and East Asian countries. Because our phylogenetic analysis based on the mtDNA sequences showed that *B. longissima* comprises two well-defined monophyletic and allopatric groups, we compared life-history traits between the two groups for effective biological control.

Results of our study show that there are two monophyletic groups: one distributed over a limited area (Australia, Papua New Guinea, Samoa, and Sumba Island; referred to as the Pacific clade) and the other dispersed over a wide area of Asia and the Pacific region (referred to as the Asian clade) (Figs. 1 and 2).

Recent invasions and outbreaks have been reported only in areas where the Asian clade has been found. The Asian clade has a significantly longer life span and produces more eggs than the Pacific clade, suggesting that the former has advantageous life-history traits with the potential to become serious pests. To support further study of geographic distribution and effective control, we developed a PCR–RFLP method for differentiating the two clades. Digestion of the PCR product of a 1,014-bp region within the *COI* with *Bsl*I resulted in clade-specific patterns.

The native habitat of *A. hispinarum* is considered to be Papua New Guinea suggesting that the original host of this parasitoid belongs to the Pacific clade, whereas beetles that invaded Southeast Asia belong to the Asian clade. Another potential natural enemy of the beetle, *Tetrastichus brontispae* (Hymenoptera: Eulophidae), is distributed, originally from Java, Indonesia, *Tetrastichus brontispae* (Hymenoptera: Eulophidae), another potential natural enemy of the beetle, is distributed where the Asian clade exists. Therefore, we believe that we should study *T. brontispae,* which is rather useful as a "real" natural enemy of the beetle in Southeast Asia. We recommend that when *B. longissima* happens to invade new areas, the clade of the beetle should be examined using our technique before introducing a natural enemy.

(S. Nakamura, R. Ichiki, S. Takano, M. Murata, N. T. Huong, A. Mochizuki [NIAES], K. Takasu [Kyushu Univ], K. Konishi [NARO], J. C. Alouw [ICOPRI], D. S. Pandin [ICOPRI])



・Bootstrap values (>50%) (1,000 replications) are indicated near the branches.

Fig. 1. Neighbor-joining phylogenetic tree (modified from Takano et al. (2011)).

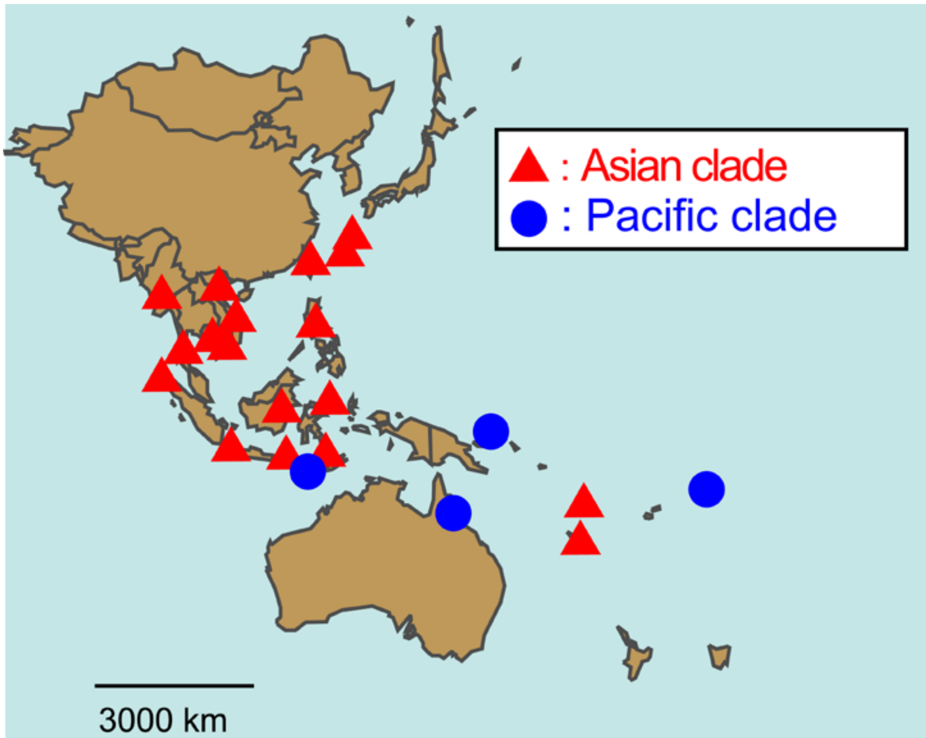


Fig. 2. Geographical distribution of the two cryptic species in *Brontispa longissima* (modified from Takano et al. (2011)).

Pathogenic variation of soybean rust in South America

Soybean [*Glycine max* (L.) Merrill] is an economically important crop and is a valuable source of oil and protein worldwide as well as of food products traditional to the Orient. South American countries are the largest soybean producers in the world, with production centered in Brazil, Argentina, and Paraguay. Soybean rust, caused by *Phakopsora pachyrhizi* Sydow & P. Sydow, is one of the most destructive and economically important diseases of soybean. Understanding the pathogenicity of indigenous fungal populations is useful for identifying resistant plant genotypes and targeting effective cultivars against certain populations. The objective of this study was to investigate pathogenicity of *P. pachyrhizi* infecting soybean in the 3 South American countries in 2007–2010, and to compare the rust pathogenicity geographically and temporally.

Soybean rust samples were collected in Argentina, Brazil, and Paraguay in the 3 cropping seasons of 2007/2008, 2008/2009, and 2009/2010. For comparative analysis of soybean rust pathogenicity between South America and Japan, rust samples were collected in Japan in 2007 and 2008. A total of 16 soybean genotypes including cultivars and lines (Table 1) were selected as a differential set to test soybean rust populations from South American countries. Nine differentials were reported to carry *Rpp* (resistance to *P. pachyrhizi*) genes (Table 1): *Rpp1* in Plant Introduction (PI) 200492, PI 368039, PI 587880A, and PI 587886; *Rpp2* in PI 230970 and PI 417125; *Rpp3* in PI 462312; *Rpp4* in PI 459025; and *Rpp5* in Shiranui. Sixteen soybean differentials were grown at 24°C with a 14 h photoperiod under rust-free conditions in a growth chamber and inoculated with *P. pachyrhizi* urediniospore suspension using a paintbrush or a glass atomizer. Two weeks after inoculation, lesion appearance [presence (+) or absence (−) of lesions] and sporulation level (SL) on the differential set were determined macroscopically. SL was rated using a 0–3 scale: 0, none; 1, little; 2, moderate; 3, abundant (Fig. 1). A few soybean leaves for each differential were detached from the inoculated plants, and the number of uredinia per lesion (NoU) formed on the abaxial side of the leaves were counted under a stereomicroscope. The NoU was calculated from 30 lesions per genotype. Data for the 3 parameters, (i) presence (+) or absence (−) of lesions, (ii) SL, and (iii) NoU, were collected for all rust populations and converted into infection types caused by the rust populations (Table 2). Infection types without lesions (immune) and with lesions showing SL 0 or 1 and NoU <1.5 were classified as resistant (R) indicated in red, whereas those with lesions showing SL 2 or 3 and NoU ≥ 1.5 were classified as susceptible (S) indicated in blue. When lesions with SL 2 or 3 and NoU <1.5 or SL 0 or 1 and NoU ≥ 1.5 were observed, the infection types were classified as intermediate (IM) indicated in yellow.

Fifty-nine rust samples from Argentina, Brazil, and Paraguay in 3 cropping seasons of 2007–2010 were evaluated for pathogenicity using 16 soybean differentials (Table 3). In the South American populations analyzed, only 2 pairs of populations yielded identical pathogenicity profiles in the 16 differentials: BE4-2 and PA5-3 from Brazil and Paraguay, respectively, and PC1-1 and PA9-1 from Paraguay, indicating substantial pathogenic variation in the rust populations. Each of the rust samples with identical pathogenicity profiles was collected from different locations, suggesting no association between pathogenicity and geographical origins of the samples. Comparative analysis of 59 South American and 5 Japanese samples revealed that pathogenic differences were not only detected within South America but also distinct between the *P. pachyrhizi* populations from South America and Japan. In addition, pathogenic differences were observed among South American *P. pachyrhizi* populations with the same geographical origin but different temporal origins. Thus, yearly changes in rust pathogenicity were detected during the sampling period. The differentials containing resistance genes *Rpp1*, *Rpp2*, *Rpp3*, and *Rpp4*, except for PI 587880A, displayed resistant reaction to only 1.8%–14%, 24%–28%, 22%, and 36% of South American *P. pachyrhizi* populations, respectively. In contrast, PI 587880A (*Rpp1*), Shiranui (*Rpp5*), and 3 *Rpp*-unknown differentials, PI 587855, PI 587905, and PI 594767A showed resistant reaction to 78%–96% of all populations. This study demonstrated pathogenic diversity of *P. pachyrhizi* populations in South America and that the known *Rpp* genes other than *Rpp1* in PI 587880A and

Rpp5 have been less effective against recent pathogen populations in the countries studied.

(H. Akamatsu, N. Yamanaka, Y. Yamaoka [University of Tsukuba], A. J. G. Ivancovich [Instituto Nacional de Tecnologia Agropecuaria], R. M. Soares [Empresa Brasileira de Pesquisa Agropecuária], W. Morel[Instituto Paraguayo de Tecnología Agraria], A. N. Bogado[Instituto Paraguayo de Tecnología Agraria])

Table 1. Soybean differential genotypes used for the evaluation

Differential		Resistance gene [a]	Origin
1	PI 200492	*Rpp1*	Japan
2	PI 368039	*Rpp1*	Taiwan
3	PI 230970	*Rpp2*	Japan
4	PI 417125	*Rpp2*	Japan
5	PI 462312	*Rpp3*	India
6	PI 459025	*Rpp4*	China
7	Shiranui	*Rpp5*	Japan
8	PI 416764	ND	Japan
9	PI 587855	ND	China
10	PI 587880A	*Rpp1*	China
11	PI 587886	*Rpp1*	China
12	PI 587905	ND	China
13	PI 594767A	ND	China
14	BRS 154	ND	Brazil
15	TK5	ND	Taiwan
16	Wayne	ND	USA

[a] *Rpp1*–*Rpp5* have been mapped to different loci.
ND: not determined.

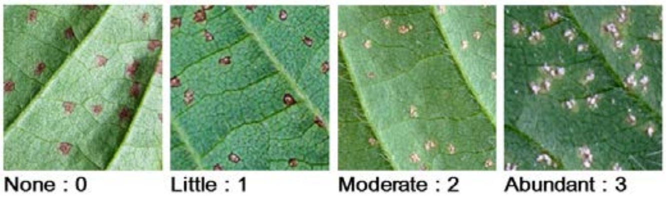


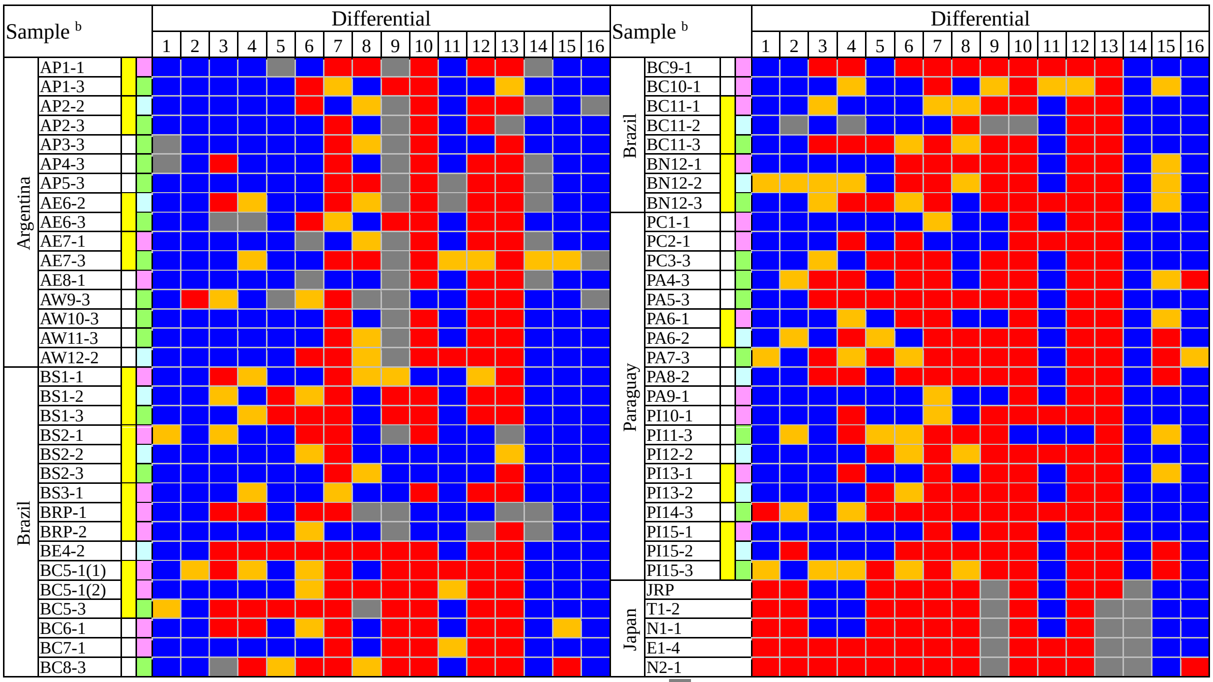
Fig. 1. Assessment of fungal sporulation on *Phakopsora pachyrhizi*-inoculated soybean leaves.

Table 2. Classification of infection types produced by soybean rust

Lesion	Sporulation level	No. of uredinia [a]	Infection type
Absence	-	-	Resistant
Presence	0 or 1	<1.5	Resistant
Presence	2 or 3	<1.5	Intermediate
Presence	0 or 1	1.5 or more	Intermediate
Presence	2 or 3	1.5 or more	Susceptible

[a] No. of uredinia per lesion was calculated from 30 lesions per differential

Table 3. Infection types of *Phakopsora pachyrhizi* samples from South America collected in 2007–2010 on the 16 differentials [a]



[a] Infection types were classified according to Table 2. The column with grey indicates that infection type was not determined because of preparation failure.

[b] AP: Pampa region; AE: Northeast region; AW: Northwest region; BS: South region; BE: Southeast region; BC: Central-West region; BN: North region; PC: Canindeyú prefecture; PA: Alto Paraná prefecture; PI: Itapúa prefecture. The following numbers represent sampling locations in each country (1–12, 1–12, and 1–15) and sampling season (1: 2007/08 ; 2: 2008/09 ; 3: 2009/10). Samples with the same location number were derived from the same location. Samples derived from the same location were indicated in yellow in each location. BRP-1, BRP-2, and BS3-1 were collected from the same location and season.

Molecular markers enable identification of upland NERICA varieties

Upland NERICA（New Rice for Africa）was developed by Africa Rice Center (AfricaRice, former name: West Africa Rice Development Association or WARDA) to meet increasing demand for rice in Africa. It originated from combinations between each of following three Asian rice (*Oryza sativa* L.), WAB 56-50, WAB 56-104, and WAB 181-18, and one African rice (*Oryza glaberrima* Steud), CG 14, comprising 18 varieties.

Although some varieties have identical seed morphological characters, most of them have to be observed in the field (plant morphology) to enable identification of each variety. However, plant morphology is sometimes affected by environmental factors specific to each cultivation area, making identification of varieties difficult. JIRCAS has been characterizing upland NERICA varieties based on various aspects such as agricultural traits, components and distribution of chromosome segments derived from parents or others, tolerance to abiotic stresses, and resistance to biotic stresses. The purpose is to evaluate their potential and develop new breeding materials. A survey of chromosome segments of upland NERICA varieties revealed that 18 microsatellite markers (SSR markers) enable classification of NERICA into varieties or groups.

The original seeds consisting of 18 upland NERICA varieties were provided by AfricaRice and multiplied at JIRCAS field (Tsukuba, Japan). Each variety was tested to confirm the uniformity of the plants. A total of 295 SSR markers distributed in whole genome chromosomes were used to genotype upland NERICA varieties, of which 243 markers showed polymorphism among CG 14, WAB56-104 and 18 upland NERICA varieties. The polymorphisms were derived not only from the difference between CG 14 and WAB56-104, but also from an unknown provenance. After comparing their chromosome components, 18 markers were finally selected based on their capability to differentiate upland NERICA varieties. The NERICA varieties were classified by those markers as follows: upland NERICA1, 5, 6, 7, 10, 14, and 17 were classified by their respective single markers; upland NERICA2, 12, 13, and 18 were classified by combining more than one marker (Table 1). On the other hand, the following varieties did not show any polymorphisms: upland NERICA3 and 4, upland NERICA8, 9 and 11, upland NERICA15 and 16 (Table 1). Thus, they were identified as three separate groups. This information can be used for quality control in seed production of upland NERICA varieties and their further utilization in breeding.

(*Seiji Yanagihara, Yoshimichi Fukuta, Sachiko Namai, Ayumi Fukuo, Kunihiko Konishyo, Hiroshi Tsunematsu, Takashi Kumashiro*)

Table 1. Differential markers for the 18 upland NERICA varieties and their differentiation capabilities

DNA markers (Chromosome)		WAB-56-104	CG 14	NERICA																	
				1	2	3	4	5	6	7	8	9	11	10	12	13	14	15	16	17	18
a	RM7187(4)	A	B	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
b	RM3471(4)	A	B	A	C	A	A	C	A	A	A	A	A	A	A	A	A	A	A	A	A
c	RM7318*(1)	A	A	A	A	A	A	C	A	A	A	A	A	A	A	A	A	A	A	A	A
d	RM7356(8)	A	B	A	A	C	C	A	C	A	A	A	A	A	A	A	A	A	A	A	A
e	RM5704*(11)	A	B	A	A	A	A	A	C	A	A	A	A	A	A	A	A	A	A	A	A
f	RM7318*(1)	A	A	A	A	A	A	C	A	A	A	A	A	A	A	A	A	A	A	A	A
g	RM5704*(11)	A	B	A	A	A	A	A	C	A	A	A	A	A	A	A	A	A	A	A	A
h	RM566(9)	A	A	A	A	A	A	A	A	C	A	A	A	A	A	A	A	A	A	A	A
i	RM406(2)	A	B	A	A	A	A	A	A	A	C	C	C	A	A	A	A	A	A	A	A
j	RM3392(3)	A	B	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A
k	RM1347(2)	A	B	A	A	A	A	A	C	A	A	A	A	A	A	C	A	A	A	C	A
l	RM6948(8)	A	B	A	A	A	A	A	A	A	C	C	C	A	B	B	C	A	A	A	A
m	RM5481(7)	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A
n	RM7383(1)	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	A	B
o	RM5599*(11)	A	B	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	B
p	RM6335(12)	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A
q	RM5599*(11)	A	B	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	B
r	RM5704*(11)	A	B	A	A	A	A	A	C	A	A	A	A	A	A	A	A	A	A	A	A

WAB 56-104：One parent for upland NERICA 1-8. CG14：Common parent among upland NERICA varieties.
A: WAB 56-104 type. B: CG 14 type. C: Other types
Polymorphism patterns enclosed by heavy lines are the unique patterns used to differentiate NERICA varieties.
＊indicate markers which show more than one band.

Improvement of drought resistance of upland New Rice for Africa (NERICA) by expression of *DREB1C*

The demand for rice in Africa is increasing, and the need to improve rice production is urgent. In this context, one group of cultivars, called New Rice for Africa (NERICA), has become the focus of high expectations, and is becoming increasingly popular in Africa. Since the majority of rice production in Africa relies on rainfall, drought resistance is one of the most important traits for further improvement. The dehydration-responsive-element–binding protein 1 (DREB1) gene is one of the best-characterized candidate genes for conferring tolerance to abiotic stresses, including drought. Here, we introduced *Arabidopsis DREB1C* under the control of a stress-inducible rice lip9 promoter (lip9::*DREB1C*) into NERICA1, which is one of the most popular upland NERICA cultivars. We investigated survival under severe drought, and vegetative growth performance and several agronomic traits under moderate drought.

The constructs, lip9::*DREB1C*, was introduced into NERICA1 by *Agrobacterium*-mediated transformation. T_3 plants carrying the transgene as a single homozygous copy were used for analysis. We evaluated the ability of plants to survive under rapid drying. Seeds were sown on soil in 50-mL conical tubes with a hole at the bottom (1 seed/tube), and the tubes were immersed in water. After 3 weeks, tubes with plants were taken out of the water and left unwatered for 10 days. The withering plants were then returned to water, and their recovery was recorded 1 week later. The survivors were considered to be the plants that were expanding new leaves. Transgenic lines had greater survival than the non-transgenic plants (Table 1). We also investigated the growth of plants under long-term moderate drought. Fourteen-day-old seedlings were planted on saturated soil in 4-L pots and then the soil was allowed to dry naturally. When the volumetric content dropped below about 15%, we added enough water to keep it at around 15%. Control plants were grown in permanently flooded soil. Each pot held 2 transgenic plants and 2 non-transgenic plants for comparison. First, we measured dry weight at the late vegetative growth stage (2 months old). The transgenic plants tended to have higher dry weights under moderate drought than non-transgenic plants, and 3 of those differences were significant (Fig. 1). Then we replanted transgenic lines 476, 482, and 749, which had higher shoot dry weights than non-transgenic plants under moderate drought, to investigate heading time, number of spikelets, number of filled grains, and dry weight of straw. All 3 transgenic lines headed significantly earlier than non-transgenic plants (Table 2). The culm length of transgenic plants was significantly shorter than that of non-transgenic plants in most cases. Transgenic plants tended to have more spikelets per plant (an indicator of sink capacity) and more filled grains per plant (an indicator of yield ability) than non-transgenic plants under moderate drought, line 482 significantly so. Under flooding, all 3 transgenic lines had significantly more spikelets than non-transgenic plants, and lines 476 and 749 also had significantly more filled grains than non-transgenic plants. Transgenic plants had significantly less straw than non-transgenic plants under flooding.

We demonstrated that *DREB1C* improves both drought resistance and traits relating to yield of NERICA1. In the case of successful field trials, the selected transgenic lines can be potentially used in a number of arid regions in Africa.

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Table 1. Survival of NERICA1 lines transformed with lip9::*DREB1C* under severe drought

Line [z]	No. of plants tested (A)	No. of plants survived (B)	% survival (B/A) [y]
408	67	52	77.6 [b]
465	61	48	78.7 [b]
466	53	25	47.2 [a]
482	42	35	83.3 [b]
713	58	48	82.8 [b]
724	58	52	89.7 [b]
749	65	56	86.2 [b]
Non-transgenic	58	16	27.6 [a]

[z] 408 - 749 are lines transformed with lip9::*DREB1C*.

[y] Different letters denote significant differences at P < 0.05 by Tukey's test.

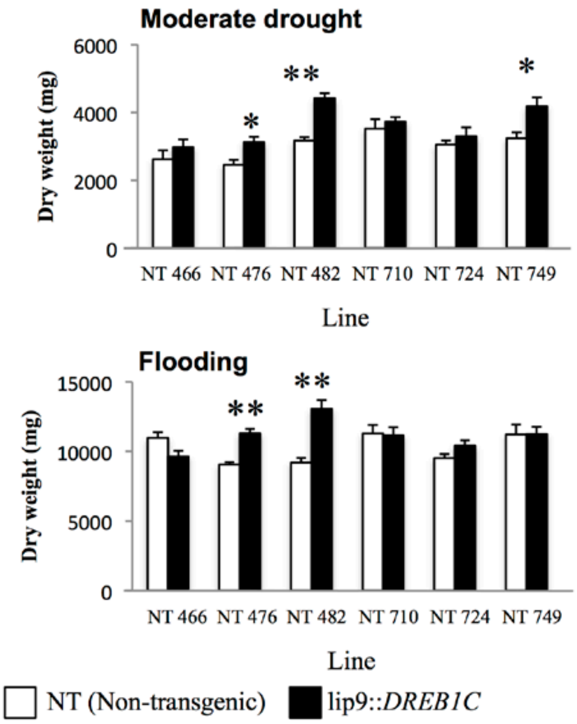

Fig. 1. Growth of lip9::DREB1C transgenic plants in pots. *Asterisks* denote significant differences at * *P* < 0.05 or ** *P* < 0.01 by *t* test. *Bars* represent SEM (n = 5).

Table 2. Agronomic traits of NERICA1 lines with lip9::*DREB1C* grown under flooding or moderate drought [z].

Growth condition	Line	Days to heading	Culm length (cm) [y]	No. of spikelets/ plant [y]	No. of filled grains/ plant [y]	Dry weight of straw (mg) [y]
Moderate drought	476	60.7 ± 0.4	67.7 ± 2.0	112.3 ± 9.0	72.0 ± 4.4	2565 ± 205
	Non-transgenic	65.5 ± 0.7	80.1 ± 2.3	94.0 ± 5.9	58.1 ± 5.4	2446 ± 171
	P value [x]	<0.01	<0.01	0.13	0.08	0.67
	482	59.6 ± 0.6	67.6 ± 3.1	122.4 ± 4.6	71.3 ± 3.9	2734 ± 70
	Non-transgenic	66.9 ± 0.5	77.5 ± 1.1	81.4 ± 6.4	52.2 ± 4.6	2349 ± 151
	P value [x]	<0.01	0.02	<0.01	0.01	0.05
	749	60.9 ± 0.4	71.3 ± 1.9	97.0 ± 5.2	62.5 ± 3.5	2280 ± 214
	Non-transgenic	65.7 ± 0.7	78.9 ± 3.3	88.4 ± 3.7	52.9 ± 4.0	2507 ± 185
	P value [x]	<0.01	0.08	0.21	0.11	0.44
Flooding	476	63.3 ± 1.0	87.3 ± 0.5	308.1 ± 8.2	170.8 ± 4.9	5312 ± 135
	Non-transgenic	71.1 ± 0.2	97.8 ± 0.5	178 ± 9.4	143.7 ± 8.6	6004 ± 230
	P value [x]	<0.01	<0.01	<0.01	0.03	0.03
	482	61.7 ± 1.0	84.1 ± 0.6	298.1 ± 2.1	165.5 ± 3.2	5342 ± 121
	Non-transgenic	69.8 ± 0.5	98.0 ± 0.9	232.6 ± 14.9	170.9 ± 8.1	6901 ± 190
	P value [x]	<0.01	<0.01	<0.01	0.55	<0.01
	749	62.3 ± 0.4	87.5 ± 1.9	312.1 ± 8.6	198.8 ± 7.4	5633 ±115
	Non-transgenic	69.7 ± 0.3	97.7 ± 1.3	239.1 ± 3.4	173.7 ± 4.0	6940 ± 217
	P value [x]	<0.01	<0.01	<0.01	0.02	<0.01

[z] Values are mean ± SEM (*n* = 5).

[y] Measured 1 month after heading.

[x] Determined by *t* test.

Measuring the hydraulic gradient using a laser rangefinder

African paddy fields are lined by earth canals which are often vulnerable to natural disasters; hence, appropriate measures based on knowledge of local weather, landform and soil conditions are necessary. To validate the effectiveness of the measures and to evaluate earth canal functions, hydraulic gradients (surface water gradients) have to be measured. In contrast with concrete canal beds, earth canal beds accumulate sediments (soil and sands), making bed level measurements inaccurate. Traditionally, surface water levels are measured directly using metal tapes (Fig. 1); however, this method is difficult for the following reasons:

i) At least two people are needed in order to confirm by visual inspection whether the edge of the metal tape is in contact with the water surface.

ii) An accurate surface water level cannot be measured if the staff does not have good estimation skills, as surface water levels often fluctuate.

iii) Bench marks set on the shoulder of the canal hinder farming activities.

Therefore, a simpler and easier way to measure the hydraulic gradients of earth canals was developed. For this purpose, the laser rangefinder (LR) was found suitable and convenient. Measurements are carried out as follows:

1. The hydraulic gradient is easily measured by taking the height (h2) from the water surface at two different points on the canal (Fig. 1, right; Fig.2). Measurement accuracy depends on the resolution of the instrument (resolution of LR is around ±2mm).
2. LR is a device that measures distances in a noncontact manner by measuring the phase difference between the laser emitted from the instrument and the laser reflected from the object. Depending on the object, some improvised device may be necessary for the laser to be reflected exactly from the object (surface water in this case) to the LR.
3. For this purpose, a tube is made by cutting the top and bottom of a PET bottle after which it is stuck into the earth canal. A float (e.g., a styrofoam board) is placed inside the tube so that the LR can catch the reflected laser light from the water surface.
4. Holes or slits are made to the tubes in order to synchronize the water levels inside and outside the tubes. There are two ways to make holes: one is to make a circle (punch a hole); the other is to cut a slit. There are no significant differences in measured data using both methods; however, the ‘punched hole’ method is deemed harder, and consequently, better.
5. Measurement accuracy is higher in cases where the cross-section of PET bottles are rectangular.
6. The sides of the PET bottle are set parallel to the stream (Type 2) (Table 1, Fig. 3).

The roughness coefficient (i.e., a representative value indicating resistance to flow) of a canal can be calculated by measuring its hydraulic gradient. Not only does it help in developing irrigation plans, but it can also be utilized as an index in evaluating an earth canal’s deterioration. This method can be applied to earth canals of different types, widths, and sizes.

Also, if combined with a rotating laser (the setup consists of an apparatus for generating a level laser beam and the receiving instrument), the relative height (h1) of LR can be measured by oneself (i.e., unassisted). However, the use of rotating lasers is not yet widespread in developing countries. Thus, if local governments or consultants decide to use this method, they will need two or several people and a leveling device to obtain measurements.

(Shinji Hirouchi, Haruyuki Dan, Chikako Hirose)

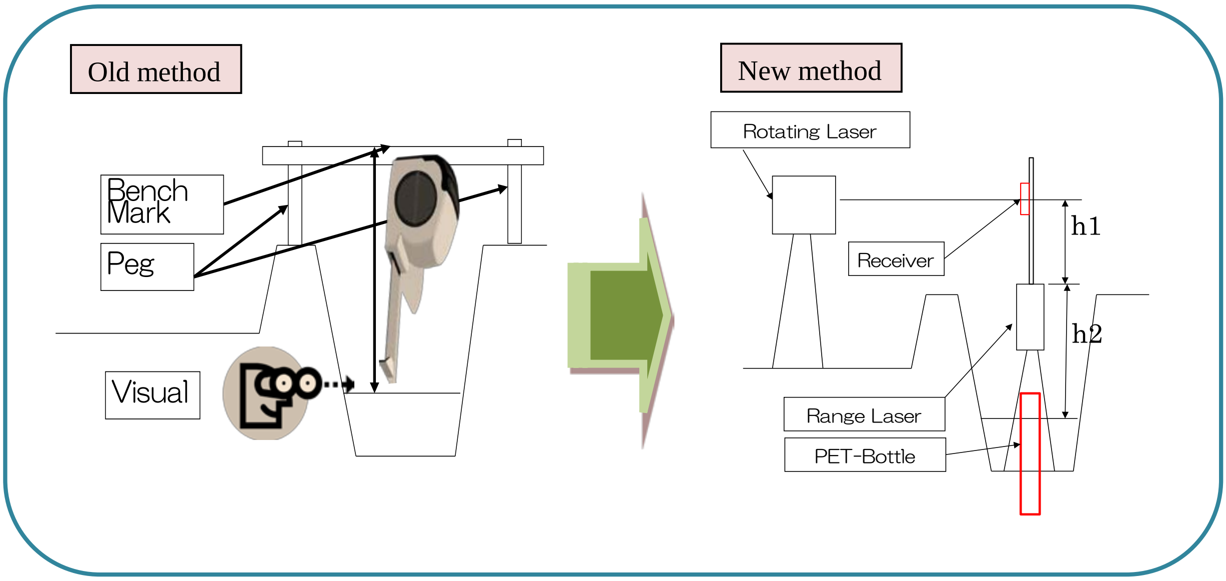

Fig.1. Observation system

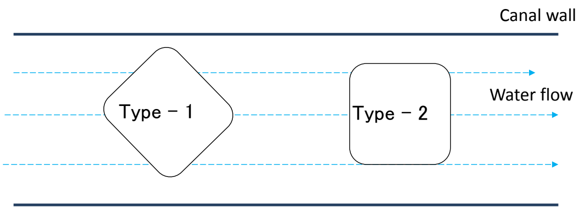

Fig.3. PET bottle installation types. (Type 1: PET bottle sides not parallel to canal wall; Type 2:

Fig.2. Observation setup

Table1. Measured values of h2, by installation type

	A		b	
	Type 1	Type 2	Type 1	Type 2
Maximum	940	934	1, 047	1, 041
Minimum	935	926	1, 042	1, 032
Difference	5	8	5	9
Average (A)	938	931	1, 044	1, 038
SD	1. 4	1. 8	1. 5	2. 0
Observed (O)	929		1, 037	
(O) - (A)	9	2	7	1

Notes: units in mm; a, b: observation points)

Assessing the probability of land submergence for lowland rice cultivation in northern Ghana

Sub-Saharan African countries are being strongly urged to enhance their rice production, because their rice consumption and importation rates have been rapidly increasing in recent years. Areas planted to rice in Africa are classified agro-ecologically into rainfed upland, rainfed lowland, and irrigated. Rainfed lowland includes extensive areas of unexploited land that has great potential for the promotion of rice-growing. To develop low-cost rice-farming systems that require no large-scale irrigation or land reclamation, it is important to select suitable areas where water for rice-farming can be obtained naturally; floodwaters offer promise for this purpose. Here, we propose a method of assessing flood probability from submergence frequency, as estimated from satellite imagery and geospatial data.

ALOS/PALSAR images acquired in May, June, August, and September 2010 were used to classify land and water, and then a submerged-area map was produced. In the study site, submergence frequency (y) was approximated by distance from reservoirs (x):

$y = -1.281\ln(x) + 9.0566$ ($R^2 = 0.9415$) (Fig. 1)

Flood extent derived from reservoirs was simulated using Digital Elevation Model (DEM) and the flood simulation program “SimFlood,” which was developed during the study. The results implied that the submerged areas identified by PALSAR were distributed in areas where flood water inflow exceeded 20 times (Fig. 2).

A flood probability assessment map was produced by integration of the estimated submergence frequency and flood extent simulation (Fig. 3). Validation of results using field monitoring data obtained in September 2011 confirmed the adequacy of the estimation (Table 1). Thus the possibility of floodwater use in the study area could be adequately determined.

The flood simulation program “SimFlood” is available for distribution in executable form. When DEM and water resources map can be prepared, the probability assessment of land submergence in other similar areas would be possible.

All-weather microwave sensors that can penetrate clouds have substantial advantages, particularly in identifying the extent of water resources during rainy season. ALOS/PALSAR has been inactive since April 2011, however, archived data are available for analysis.

(Y. Yamamoto, Y. Tsujimoto, Y. Fujihara [Ishikawa Prefectural University], J. Sakagami, M. Fosu [Savanna Agricultural Research Institute])

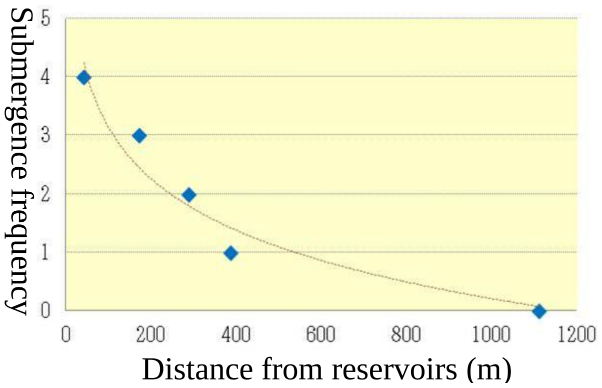

Fig. 1. Relationship between submergence frequency and distance from reservoirs

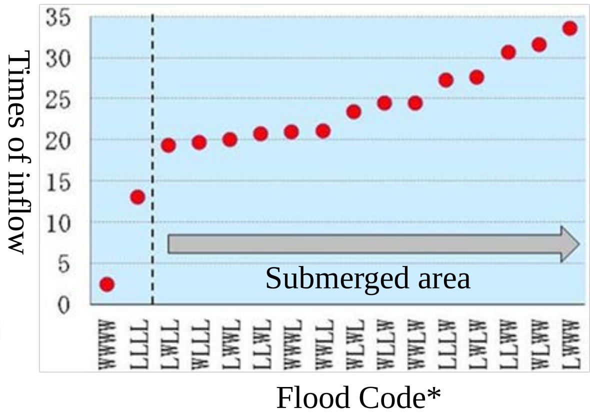

Fig. 2. Times of inflow by flood water in submerged areas

* The results of classification in May, June, August, and September (from bottom to top). W: submerged; L: not submerged.

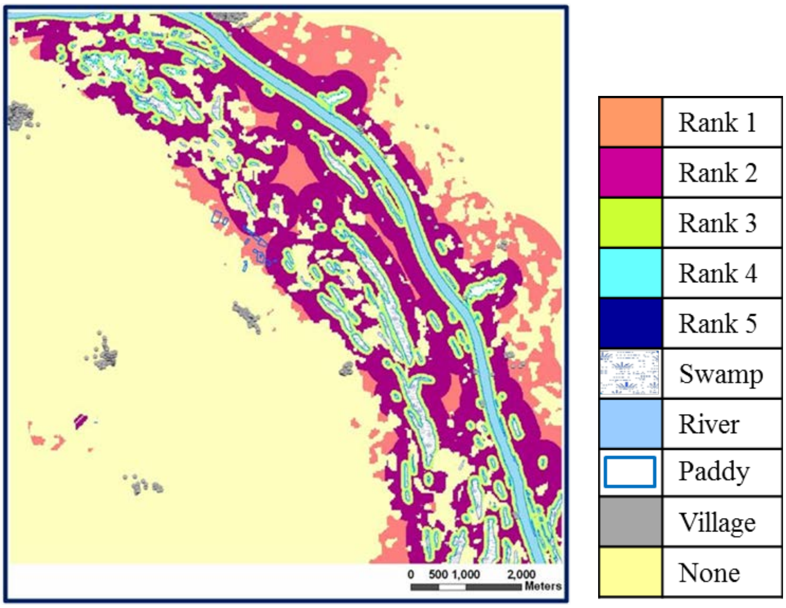

Fig. 3. Flood probability map assessed by geospatial features

Table 1. Soil moisture* by flood probability assessment rank

Flood probability rank	Soil moisture*
None	0.776
Rank 1	0.786
Rank 2	0.819
Rank 3	0.856
Rank 4 & 5 (water boundaries)	0.879

* Average values measured using a portable soil moisture meter equipped with a voltage sensor (DIK-311K, Daiki Rika Kogyo Co.).

Topographic distribution of the soil total carbon content and sulfur deficiency for rice cultivation in a floodplain ecosystem of the Northern region of Ghana

River floodplains, consisting of wide and flat alluvial plains bordering rivers, are expected to support a large expansion in the rice cultivation area and production, the major share of which is currently unexploited in West Africa. An understanding of the spatial variation in soil fertility is fundamental for developing appropriate fertilizer management practices and extending rice cultivation in this floodplain ecosystem. This study focuses on a floodplain along the White Volta River in the Northern region of Ghana, the major rice-producing region of the country. Here, we proposed a topographic distribution model to estimate the total carbon contents of soils, and then identified specific nutrient deficiencies in growing rice by combining satellite imagery analysis, soil chemical analysis, and phytometric pot experiments.

The total carbon (TC) contents of soils (0-15 cm in depth) largely differed from 2.0 to 40.2 g kg^{-1}, among 89 samples in the target floodplain. In the analysis using the four different topographic parameters, i.e., ‘Elevation’, ‘Distance to the White Volta river’, ‘Distance to reservoirs’, and ‘Slope angle’, the single logarithmic model of the ‘Distance to reservoirs’ was selected for estimating the soil TC contents with the greatest determination coefficient at 0.54 (Fig.1). The estimation model depicted the distribution map of soil TC contents as shown in Fig. 2, where we can find non-cultivated areas with high soil TC contents relatively close to the village compound. The soil TC contents showed close correlation with the N uptakes of rice grown under non-fertilized pot experiments (Fig.3). This result indicated that soil TC content can be used as an appropriate parameter of the soil N-supplying capacity for rice in the target ecosystem. Limited sulfur concentrations in plant tissues and a significant response to S application indicated that inherent sulfur deficiency restricted rice growth (Table 1). Therefore, fertilization of N, P, and K without S should increase only the concentration levels of these elements in rice plants but not biomass production. The effect of sulfur application on rice growth was greater with increased proximity to reservoirs.

The distribution of soil TC contents corresponded to the length of waterlogging period, which also logarithmically increased with proximity to reservoirs, as estimated by satellite imagery images. This corresponding pattern indicated that extension of rice cultivation areas near reservoirs can be regarded productive in terms of both water availability and soil fertility. Internal (soil-derived) and external nitrogen can be efficiently utilized for rice production by supplementing S-containing forms of fertilizer (e.g., ammonium sulfate). The potential risks of complete submergence should be further studied for extending rice production in this floodplain ecosystem.

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Institute])

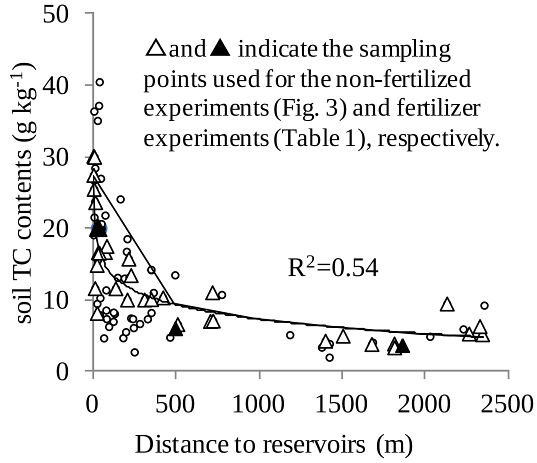


Fig. 1. Relationship between soil total carbon (TC) contents and distance to water resources (river and backswamp)

The approximate equation is 'soil TC = 29.28-3.3 x ln (Distance to reservoirs)'. The main Volta River, backswamps, and ponds were extracted as 'reservoirs' by the visual classification of the Quickbird image captured at the beginning of the dry season in November 2009.

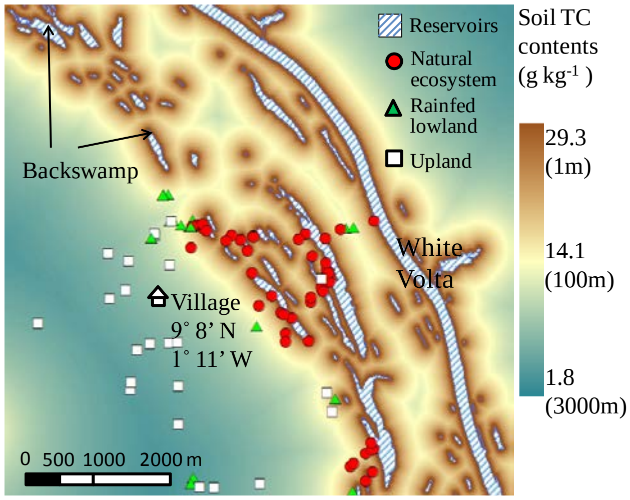


Fig. 2. Spatial distribution in the soil total carbon (TC) contents within the floodplain using the model equation in the Fig. 1

Based on interviews with local farmers, the sampling points are classified into ● natural ecosystem with no cropping history, ▲rainfed lowland, and □upland.

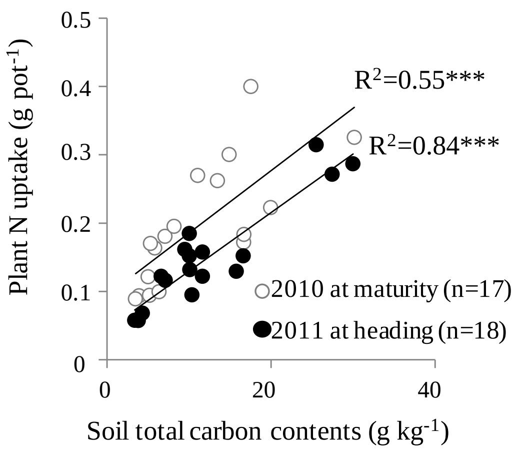


Fig. 3. Relationship between soil total carbon contents and plant N uptakes under non-fertilized treatments.

Table 1. Top dry matter yields and N:S concentration ratios as affected by soils and fertilizer treatments.

	Distances to reservoirs (river and backswamps) of the 3 experimental soils		
	Near(40m)	Middle(501m)	Far(1870m)
Fertilization	Dry matter g pot^{-1} (N:S conc. ratio)		
control	14.7 (29.2)	23.5 (13.0)	12.7 (12.9)
N	12.4 (**51.6**)	27.0 (25.4)	10.1 (**47.9**)
P	14.6 (27.8)	22.2 (11.3)	12.8 (14.7)
K	18.6 (26.9)	17.1 (12.5)	11.5 (14.9)
NP	10.7 (**45.0**)	24.5 (**31.6**)	9.3 (**50.4**)
NK	14.6 (**57.1**)	20.3 (**34.0**)	7.9 (**50.8**)
NPK	16.5 (**51.8**)	16.5 (**43.8**)	5.9 (**47.1**)
NPKSi	18.9 (**49.6**)	25.2 (**33.6**)	12.0 (**48.3**)
NPKZn	19.5 (33.7)	**12.0** (**44.6**)	**3.2** (**56.5**)
NPKS	**95.2** (**13.4**)	**71.2** (6.6)	**42.4** (7.1)

Bold numbers are significantly different from control at 5%. Nutrient rates are N=0.70g, P=0.22g, K=0.36g, Si=1.87g, Zn=0.05g, S=0.23g per pot.

Identification of a gene that regulates growth under drought conditions in rice

Drought causes growth reduction in plants. Although drought changes expression of a variety of genes, the physiological and molecular mechanisms for plant growth restriction during drought conditions remain unclear. In this study, we identified a gene for a phytochrome (phy)-interacting basic helix-loop-helix transcription factor (PIF)-like protein, OsPIL1, which acts as a key regulator of reduced internode elongation in rice under drought conditions.

The level of *OsPIL1* mRNA in rice seedlings grown under non-stressed conditions with light/dark cycles oscillated in a circadian manner with peaks in the middle of the light period (Fig. 1). When drought started in the middle of the dark period, expression of *OsPIL1* was not elevated during the light period. When drought started early in the light period, the *OsPIL1* expression was drastically decreased to a level similar to that observed in the dark period (Fig. 1). We found that *OsPIL1* was highly expressed in the node portions of the stem using promoter-GUS analysis. Transgenic rice plants overexpressing *OsPIL1* (*OsPIL1*-OXs) showed promoted stem elongation, resulting in a strikingly tall plant (Fig. 2). This was mainly due to increased elongation in each internode. In contrast, transgenic rice plants with a chimeric repressor (*OsPIL1*-RDs) displayed short internode sections. The internode cells of *OsPIL1*-OXs were larger than those of non-transgenic control plants. Smaller internode cells were found in *OsPIL1*-RDs. The transcriptome analysis identified 1396 genes up-regulated (FCA > 2.0) and 1358 genes down-regulated (FCA < 2.0) in the 1st node portion of *OsPIL1*-OXs. Expression of more than half of the up-regulated genes was decreased under drought conditions (790/1396 genes), and expression of large numbers of the down-regulated genes was increased by drought (480/1358 genes), suggesting that these were down-stream genes for OsPIL1. The up-regulated gene set in *OsPIL1*-OXs was enriched for cell wall related genes responsible for cell elongation. Using the transient assay system, we verified that OsPIL1 could activate expression of the cell wall related gene via the G-box element. These data suggest that OsPIL1 functions as a key regulatory factor of reduced plant height via cell wall related genes in response to drought (Fig. 3).

The regulatory system by OsPIL1 may be important for morphological stress adaptation in rice under drought conditions. We think that the *OsPIL1* gene has great potential to produce crops with good growth even under drought conditions.

(D. Todaka, K. Nakashima, K. Maruyama, Y. Fujita, K. Yamaguchi-Shinozaki)

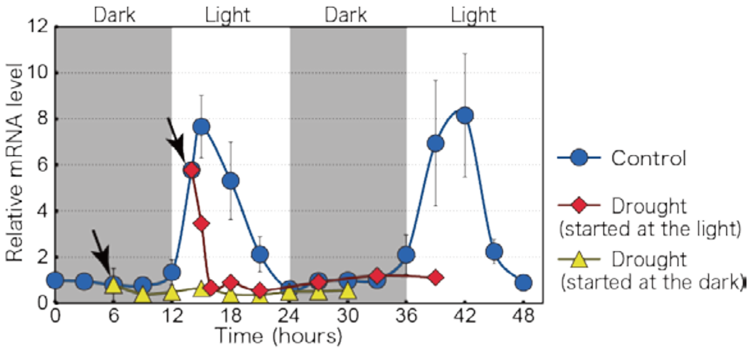


Fig.1. Expression analysis of *OsPIL1* under non-stress (Control) or drought (Drought) conditions by quantitative RT-PCR. Arrows indicate the starting point of the stress treatment.

Fig. 2. Plant heights of transgenic rice plants overexpressing *OsPIL1* (*OsPIL1*-OX) and vector control plants (Control).

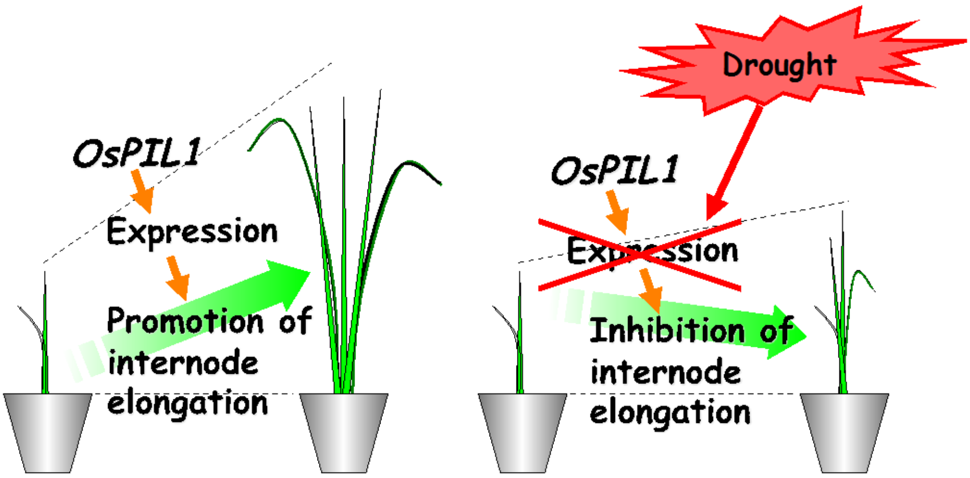


Fig. 3. A model for the plant height regulatory system of OsPIL1. The *OsPIL1* expression promotes internode elongation. Drought decreases the *OsPIL1* expression, resulting in inhibition of internode elongation. Consequently, the plant shows a dwarf phenotype.

The protein kinase *PSTOL1* from traditional rice confers tolerance of phosphorus deficiency

In Asia, around 60% (29 Mha) of the rain-fed lowland rice is produced on poor and problem soils that may be naturally low in P or P fixing (Fig. 1). Resource-poor farmers have limited access to increasingly expensive P fertilizer, and this situation may further aggravate given that phosphate rock, the source of P fertilizer, is a finite and non-renewable resource. The development of rice varieties with high productivity under low P is therefore a valid and necessary approach to improve yield and enhance food security.

The major quantitative trait locus for P deficiency tolerance, *Pup1*, was identified in the traditional *aus*-type rice variety Kasalath. *Pup1*-specific molecular markers revealed that the putative protein kinase gene (*OsPupK46-2*) was closely associated with tolerance to P deficiency in stress-adapted germplasm. This result was confirmed by qRT-PCR and thus the gene was named as Phosphorus starvation tolerance 1 (*OsPSTOL1*). An in-vitro phosphorylation assay confirmed that *OsPSTOL1* is a functional Ser/Thr protein kinase.

Phenotypic analyses of transgenic lines with constitutive overexpression (OX) of the full-length *OsPSTOL1* coding region (35S::*OsPSTOL1*) in varieties IR64 (*indica*) and Nipponbare (*japonica*) showed that high expression of the *OsPSTOL1* transgene (OX high) enhanced grain yield by about 30% under low P conditions (Fig. 2). The superior performance of IR64 OX-high lines was due to higher root dry weight and subsequent experiments in nutrient solution showed that total root length and root surface area were significantly higher in transgenic seedlings. This was confirmed for near isogenic lines (NILs) in an IR64 background: IR64-NILs +*Pup1* showed significantly enhanced root growth (Fig. 3).

Transgenic IR64 plants expressing the GUS-reporter gene under the control of the native *OsPSTOL1* promoter were used to analyze the *OsPSTOL1* expression (Fig. 4). Specific GUS staining was detected in stem nodes (crown roots) of young seedlings, while within nodes, GUS staining was restricted to crown root primordia and parenchymatic cells located outside of the peripheral vascular cylinder. No GUS staining was observed in older, emerging crown roots or in the initial (seminal) seedling root. Taken together our data suggest that *OsPSTOL1* is a regulator of early crown root development and growth in rice.

Pup1-rice varieties developed by marker-assisted breeding will be beneficial not only under low P conditions but in a wider range of rice environments, since plants with vigorous root may show improved crop establishment and weed competitiveness. This may contribute to food security and to establishing more sustainable rice production systems.

(M. Wissuwa, J. Pariasca-Tanaka, R. Gamuyao, J.H. Chin, S. Catausan, C. Dalid, I. Slamet-Loedin, S. Heuer, P. Pesaresi, E.M. Tecson-Mendoza)

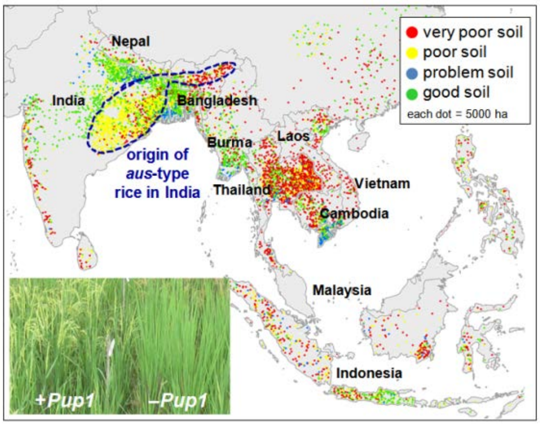


Fig. 1. Problem soils in Asia and origin of stress-tolerant *aus*-type rice varieties

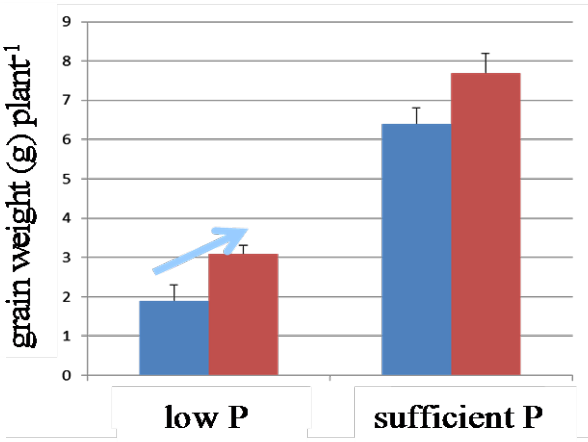


Fig. 2. Grain weight of *PSTOL1*-overexpressing IR64 and corresponding null plants. Error bars indicate SEM. Blue: null; red: OX high

Fig. 3. Representative root of a IR64-NIL +*Pup1* (left) compared to parental IR64 –*Pup1* (right), grown under low-P in nutrient solution

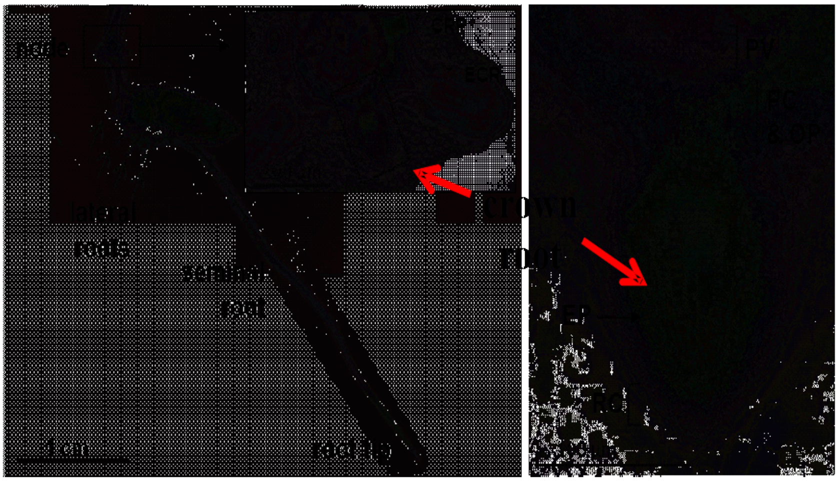


Fig. 4. GUS expression driven by the native *PSTOL1* promoter in young IR64 seedlings is observed in parenchyma (PC) and outer parenchyma (OP) cells adjacent to the peripheral vascular (PV) cylinder of the coleoptilar node and in crown root primordia (CRP; indicated by asterisks), but not in emerging crown roots (ECR; arrows). RC, root cap.

Reference : Gamuyao et al., 2012. Nature **488,** 535–539.

Guideline on “On-farm mitigation measures against salinization under high groundwater level conditions”

Excessive irrigation raises the groundwater table and causes water-logging. In arid or semi-arid zones, salts carried by river water or groundwater accumulate, resulting to decreased crop yields and abandonment in the case of highly salinized agricultural fields.

In order to mitigate rising groundwater levels and solve this issue, governmental organizations in Uzbekistan have improved irrigation facilities, maintained drainage systems and recommended saving water to farmers. However, field conditions have not improved much despite government’s efforts. The salinization issue could not be solved fundamentally until the farmers understand the need to save water and improve drainage.

For this reason, JIRCAS, in cooperation with Uzbekistan’s Ministry of Agriculture and Water Resources (MAWR) and the Farmers’ Council (formerly Farmers’ Association), formulated a guideline for farmers to implement practical on-farm measures in Uzbekistan, which, according to reports, has the largest salinized land area in Central Asia.

Detailed research studies and experiments in farmers’ fields have been conducted to come up with necessary measures. Technical background on mitigating measures as well as cost-benefit analysis through assumed model farms are described intelligibly and simply in the guideline. The contents are as follows:

First, the mechanism of salinization is explained to recognize the relationship among its causes and its influence on farming activities. Monitoring results are displayed visually to help farmers understand the scope of the problem (Fig. 1).

Second, water-saving irrigation methods such as simple surge flow method, alternative dry furrow (ADF) method and land leveling are proposed. The effects of these methods are compared with conventional method (Fig. 2). Also, a low-cost land leveling method is proposed by combining farmers’ prior leveling work with laser land leveling.

Third, a drainage maintenance system is suggested, where the farmers themselves can cooperate with the government to check drainage canals or sub-surface drainage.

Fourth, improved crop rotation is proposed through cultivation of cotton, wheat, summer crop and green manure. The extra income that will be generated will help provide the necessary budget for the measures.

Finally, the costs and benefits of introducing stepwise measures are calculated from the assumed farm models (Fig.3).

The guideline, which has been approved by the MAWR, is available in Russian and Uzbek languages. Furthermore, a popular edition of the guideline is being prepared for greater content accessibility by farmers in the field.

(Y. Okuda, H. Ikeura, J. Onishi, N. Nitta, A. Fukuo, K. Shiga, M. Naruoka, T. Oya, I. Yamanaka, Y. Shirokova[Irrigation and Water Problem Research Institute of Uzbekistan])

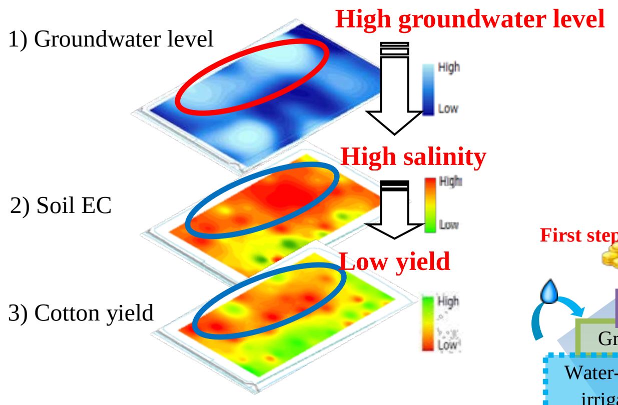


Fig. 1. Field monitoring results

(cause and effect)

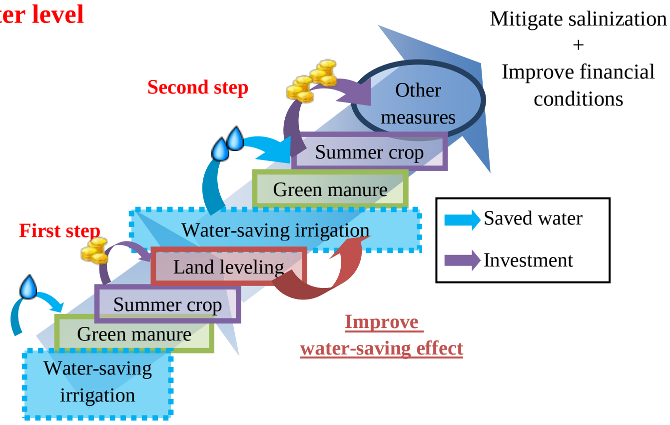


Fig. 3. Proposed stepwise measures

Fig. 2. Excessive irrigation (left photo) and countermeasure technologies against salinization (center and right photos)

（Left） Excessive irrigation：Flooding the furrows results to increased infiltration loss

（Center） ADF irrigation method：Irrigation water is distributed alternately, limiting water losses

（Right） Accurate laser land leveling：Water-saving effect is increased by minimizing undulation

Influence analysis of climatic and input factors on maize yield in Hebei Province

When China became a net maize-importing country in 2010, full attention was given to the possibility of further increasing domestic supply capability. As the expansion of arable land nears its limit, further increase in supply can only be achieved by increasing maize yield. However, increasing maize yields is very difficult due to the deterioration of production environment caused by excessive use of chemical fertilizer, climate change, and shortage of farm labors. As Hebei Province is among the three biggest maize-producing provinces and the fact that maize supply depends mainly on its yield, clarifying the main factors that influence yield is important for predicting maize supply trends. Further, this study may also lead to risk analysis and policy making for stable production. Farmer household survey data of nine counties in Hebei Province were used in the study, taking into account the various regional differences, in order to achieve higher accuracy.

We built several analytical models, including the ordinary least squares (OLS) regression, the maximum likelihood (ML), and the residual maximum likelihood (REML) multilevel models, to analyze the impact of input and climatic factors on maize yield using survey data collected between 2003 to 2010 from 4152 farmer households. Based on the results of panel analysis, the ML model was selected for its good modeling capacity. The model was used to estimate the elasticity and the contribution ratio of input factors (seed, chemical fertilizer, pesticide, fixed asset, labor, machine operation, irrigation) and climatic factors (temperature, precipitation) on maize yield.

The elasticity of inputs affecting maize yield is 0.176. As for the climatic factors (during the growth period from June to September), the elasticity of temperature is -2.159 and the elasticity of precipitation is 0.019. While the influence of precipitation is positive, the influence of temperature is negative and quite big. Specifically, the negative impact on yield due to high temperature in September is significant (Table 1).

Regarding the influence of input factors on maize yield, the positive effect of fixed assets, labor, chemical fertilizer, irrigation, and seed selection has been confirmed. The elasticity of seed selection is 0.076, which is higher than other inputs and reveals the fact that seed selection is important for yield increase (Table 1).

As shown by the contribution ratio of each factor for yield increase during the measurement period, moderate rainfall in June, August, and September, as well as seed selection, produced positive contributions. On the other hand, high temperatures in July and September led to negative contributions. The influence of climatic factors was relatively large.

The analytical method based on panel data analysis should also be applied to similar studies in areas other than Hebei Province. By doing so, it is hoped that the accuracy of supply predictions can be improved. To obtain influence parameters for median and long-term supply predictions, the measurement period should be further increased.

(H. Chien, J. Ma [China Agricultural University], Y. Chen [China Agricultural University], E. Kusano)

Table 1. The elasticity coefficients of impact factors on maize yield

Variables	Elasticity	STD	
Input factor	0.176	0.016	***
Fixed assets	0.014	-0.004	***
Pesticide	-0.003	-0.007	
Fertilizer	0.030	-0.010	***
Seed	0.076	-0.009	***
Labor	0.021	-0.009	**
Irrigation	0.033	-0.003	***
Machine operation	0.003	-0.007	
Climatic factor			
June temp.	-0.157	(-0.129)	**
July temp.	-0.782	-0.273	
August temp.	-0.082	-0.296	***
September temp.	-1.139	-0.297	***
Jun. to Sep. accumulated temp.	-2.159	-0.323	***
June ppt.	-0.147	-0.014	***
July ppt.	0.057	-0.013	***
August ppt.	0.065	-0.014	
September ppt.	0.045	-0.011	***
Jun. to Sep. precititation	0.019	-0.025	***

Note：***, ** and * denote statistical significance at the 1%, 5% and 10% levels.

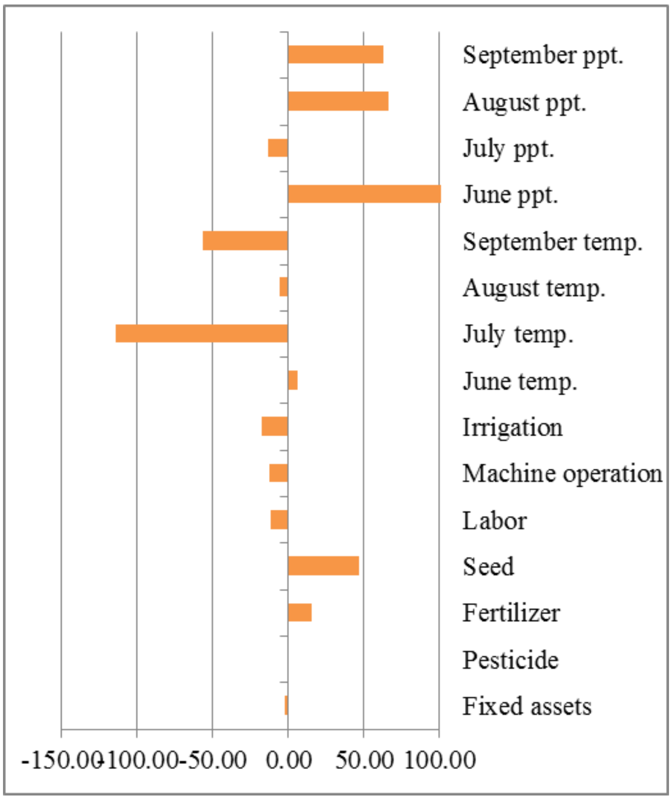


Figure 1. Contribution ratios of input factors on maize yield increase

Notes: 1. Ratio in percentage. 2. Average ratio from 2003 to 2010 (simple average)

Cooking method affects antioxidant capacity and total phenolic content in basils

The effects of thermal treatment on the antioxidant properties of fruits and vegetables are important issues in home scale cooking as well as food manufacturing. In Southeast Asia, a variety of locally distinct conventional vegetables, including basil, are produced. Basil, a leafy herb, has recently been attracting research attention because it contains a variety of phenolic compounds which exhibit desirable physiological characteristics believed to provide health benefits. These native vegetables are mainly consumed as an ingredient in cooked dishes though they may also be eaten raw. This study, therefore, examined the changes in antioxidant capacity and total phenolics under five different heat cooking methods. We measured the antioxidant capacity (using the stable free radical diphenylpicrylhydrazyl / DPPH method) and total phenolic content (by Folin-Ciocalteu method) in methanol extracts of four *Ocimum* species commonly consumed in Thailand (Fig. 1) through blanching, boiling, steaming, sautéing, and autoclaving.

The results indicated that among the five cooking methods, sautéing was the best at increasing both antioxidant capacity and total phenolic content in all types of basil. Steaming also saw increases in all types except for sweet basil (Fig. 2).

Among the four species of basil, sautéed hairy basil showed the largest increases in both antioxidant capacity and total phenolic content, at more than double the amount of the untreated sample. On the other hand, both antioxidant capacity and total phenolic content in sweet basil were significantly reduced by cooking methods other than sautéing (Fig. 2). In particular, antioxidant capacity and total phenolic content of vegetables were reduced through blanching as existing components were released from plant tissues into cooking water. Additionally, the types of phenolics in basil extracts did not change greatly during sautéing, but the contents were observed to have increased. This is probably because the phenolics bound to methanol-insoluble materials tend to be isolated by heat cooking.

These results show that antioxidant capacity and total phenolic content in basils are affected by the method of cooking. It is also believed that heat cooking does not intend to promote phenolic contents, but changes it to a state that can be used. Consequently, it is possible to make processed rice that imparts antioxidant properties, for example, by boiling it in liquid in which antioxidants are eluted (JIRCAS Research Highlights, 2010).

(G. Trakoontivakorn [Kasetsart University], P. Tangkanakul [Kasetsart University], K. Nakahara)

Fig. 1. Basils common in Thailand. From left: *O. americanum* (hairy basil), *O.tenuiflorum* (holy basil; syn. *O. sanctum*), *O. basilicum* (sweet basil) and *O. gratissimum* (wild basil)

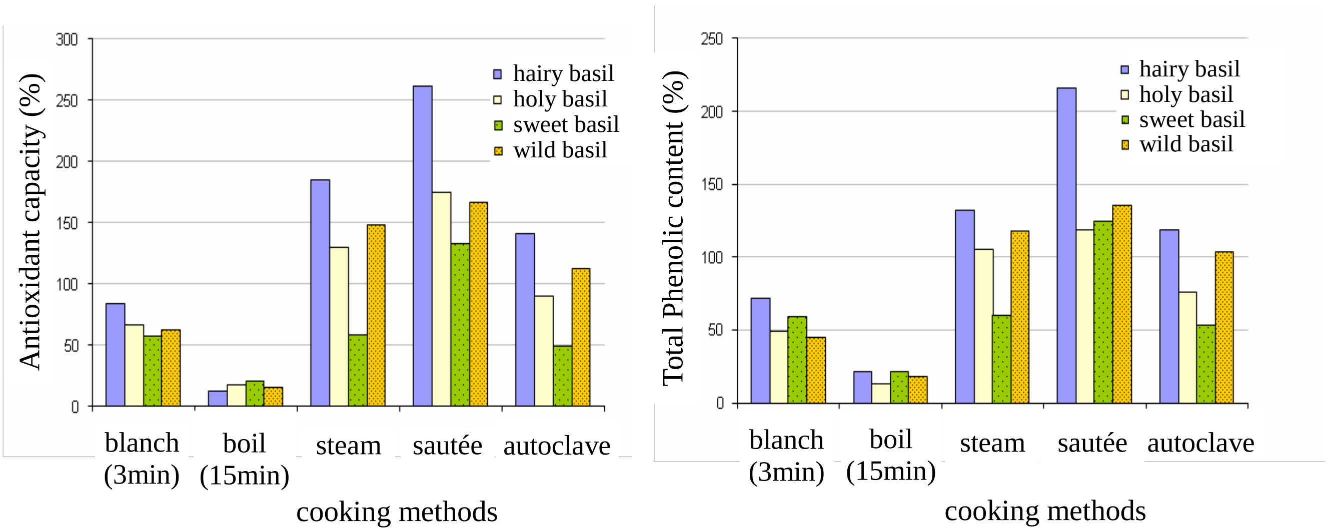


Fig. 2. Antioxidant capacity and total phenolic content remaining in leaves after various cooking methods compared to fresh *Ocimum* herbs

(Data is the average value of the results from multiple experiments; reproducibility has been confirmed.)

Reference: Trakoontivakorn et al. (2012) JARQ 46: 347 353.

Characterization of novel anaerobic alkalithermophilic, cellulolytic-xylanolytic bacteria

Lignocellulosic plant biomass is difficult to hydrolyze because cellulose has a tightly packed crystalline structure and is surrounded by a lignin that has covalent associations with hemicellulose. Thus, the rate-limiting step in biomass degradation is the conversion of cellulose and hemicellulose polymers to sugars.

Pretreatment of lignocellulose can be enhanced to enzymatic digestibility. Alkaline pretreatment is one of several chemical pretreatment technologies that have been intensively investigated. It employs various alkaline reagents including sodium hydroxide, calcium hydroxide, potassium hydroxide, aqueous ammonia, ammonia hydroxide, and sodium hydroxide in combination with hydrogen peroxide. Mechanistically, alkali is believed to cleave hydrolysable linkages in lignin and glycosidic bonds of polysaccharides, which causes a reduction in the degree of polymerization and crystallinity, swelling of the fibers, as well as disruption of the lignin structure.

In general, alkaline pretreatment is more effective on hardwood, herbaceous crops, and agricultural residues, which have lower lignin content, than on substrates such as softwood, which contain high amounts of lignin.

The alkaline-treated lignocelluloses must adjust to neutral pH and/or remove completely alkaline reagents because cellulase and hemicellulases in subsequent saccharification process are unable to act under alkaline condition.To develop enzymes which have high degradation ability under alkaline condition, it is necessary to isolate alkalinephilic microorganisms having high cellulolytic ability.

We were able to successfully isolate an anaerobic alkalithermophilic, cellulolytic-xylanolytic bacterium from coconut garden soil in the Bangkuntien district of Bangkok, Thailand. The bacteria were Gram-stain positive, catalase-negative, endospore-forming, motile and rod-shaped, with a cell size of 0.2-0.3×2.0-3.0 µm. Optimal growth occurred at pH 9.5 and T=55 °C. The bacteria strain fermented various carbohydrates, and the end products from the fermentation of cellobiose were acetate, ethanol, propionate and a small amount of butyrate. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the strain represented a new phyletic sublineage within the family Clostridiaceae, with <93.0% 16S rRNA gene sequence similarity to recognized species of this family. On the basis of phenotypic, genotypic and physiological evidence, the strain represents a novel species of a new genus, for which the name *Cellulosibacter alkalithermophilus* is proposed. *C. alkalithermophilus* showed high ability to degrade not only microcrystalline cellulose, but also hemicellulose such as xylan under alkaline condition at pH 9.5, suggesting that the bacteria might possess unique abilities to degrade polysaccharides .

The type strain of the type species has been deposited in the culture collection of Thailand Institute of Scientific and Technological Research as TISTR 1915(T).

(A. Kosugi, A. Watthanalamloet, C. Tachaapaikoon, K. Ratanakhanokchai [King Mongkut's University of Technology Thonburi])

Table 1. Characteristics of novel bacterial isolate *Cellulosibacter alkalithermophilus*

Properties	*Novel isolated strain Cellulosibacter alkalithermophilus*	*Closest relatives Clostridium thermosuccinogenes DSM 5807*
Cell length (μm)	0.2–0.3×2.0–3.0	0.3–0.4×2.0–4.0
Gram staining	Positive	Positive
Spore forming	Positive	Positive
GC contents (mol%)	30.0	35.9
Optimum growth temperature (ºC)	55	58
Optimum growth pH	9.5	7.0
Cellulose degradation ability	++	-
Xylan degradation ability	++	-

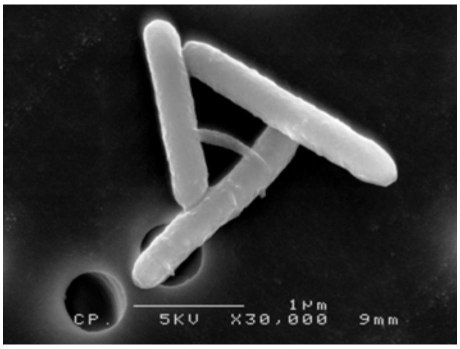


Fig. 1. Scanning Electron Microscope picture of *Cellulosibacter alkalithermophilus*

Isolation of thermotolerant yeasts for a non-cooling fermentation system in tropical areas

Ambient temperatures in tropical areas are high, therefore ethanol fermenters also show a rise in temperature (up to around 40 °C) during fermentation. *Saccharomyces cerevisiae*, the typical fermenting yeast, cannot carry out fermentation under high temperatures because it does not have thermotolerance.

We isolated the thermotolerant yeasts, *Kluyveromyces marxianus* (Y2) and *Issatochenkia orientalis* (C19). These yeasts, which have shown greater thermotolerance than *S. cerevisiae*, can produce ethanol under high temperature conditions (more than 40 °C) and can tolerate the fermentation inhibitors contained in saccharified lignocellulosic biomass. Consequently, ethanol fermentation without cooling can be achieved using these yeasts. It is expected to save energy through reduced cooling costs.

These thermotolerant yeasts can grow at higher temperatures than *S. cerevisiae*. Y2 and C19 strains can grow and ferment at 45 °C and 42 °C, respectively (Figs.1A and B). Y2 strain has shown tolerance to furfural, a fural compound, and is relatively insulated from the inhibition by the furfural induced from biomass hydrolysis (Fig. 2A); C19 strain has displayed tolerance to weak acids, hence it is relatively insulated from the inhibition by acetic acid induced from hydrolysis of lignocellulose biomass (Fig. 2B). The kinds of inhibitors to fermentation depend on the type of lignocellulose, therefore, we can select these yeasts as adequate for fermentation in biomass hydrolysis.

In terms of productivity, it can be pointed out that ethanol yield of C19 (73%) is lower than that of Y2 (90%). Additionally, Y2 often produces glycerol as a byproduct when it is incubated under stress conditions.

(Yoshinori Murata、Takamitsu Arai、Akihiko Kosugi、Yutaka Mori)

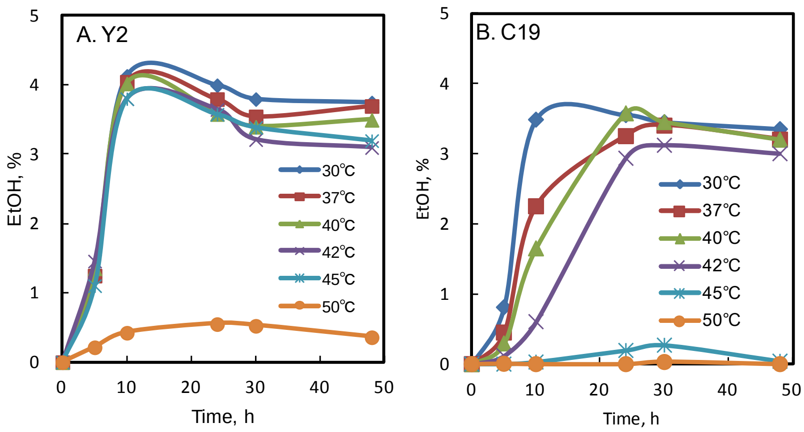


Fig. 1. Ethanol fermentation by thermotolerant yeasts, Y2(A) and C19(B) in 10%YPD（10% glucose, 2% peptone, 1% yeast extract）at different temperature conditions. These yeasts have the thermotolerance to produce ethanol under 42 °C.

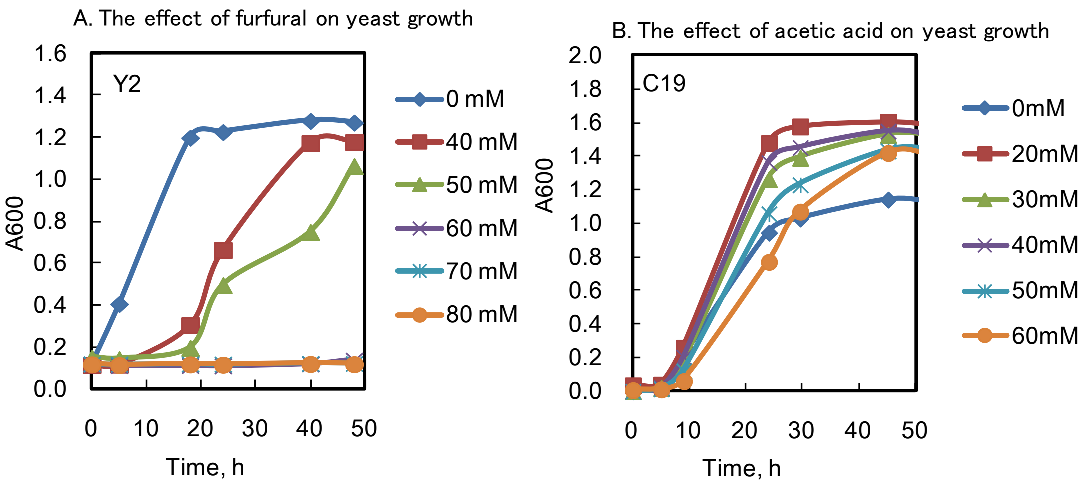


Fig. 2. The influence of inhibitors on yeast growth. (A) Y2 shows tolerance to 40mM furfural at 42 °C. (B) C19 shows tolerance to 60mM acetic acid at 42 °C.

Reference :

Y. Mori, A. Kosugi, Y. Murata, and T. Arai, Ethanol Production from Sap of Old Oil Palm Trunks Felled for Replanting (2010)Journal of the Japan Institute of Energy 89, 1147-1152

Non-sterilized bio-plastic production from oil palm sap using halophilic polyhydroxyalkanoate-producing bacteria

We have previously reported that we can produce polyhydroxyalkanoate (PHA), a type of bio-plastics, from oil palm sap (OPS) using PHA-producing bacteria. OPS is a good natural medium. It contains fermentable sugars such as glucose, various amino acids, vitamins, and minerals; however, it is prone to microbial contamination. To prevent microbial contamination, we developed a new PHA fermentation method in which OPS, containing salt (NaCl), is fermented by halophilic PHA-producing bacteria, *Halomonas* sp. SK5.

Studies on the bacterial strain's ability to synthesize PHA were initiated by screening for the most suitable carbon source. Four carbon sources were evaluated, namely, fructose, glucose, sucrose and sodium acetate.

The results showed better growth of the bacterial strain in a medium supplemented with glucose and sucrose. We examined the optimal NaCl concentration in a medium for bacterial growth. Although the growth patterns were almost similar at 5 and 10% (w/v) salinity (Fig. 1), the best cell growth and PHA production were obtained at 10% (w/v) salinity (Table 1). Therefore, we hypothesized that it is possible to avoid microbial contamination by adding a 10% (w/v) saline solution.

We evaluated the potential of OPS in mixtures of artificial salt water and seawater as a growth and production medium. OPS was diluted (1:1) with sterile distilled water or artificial salt water. Results showed that high cell biomass (2.8 ± 0.2) and PHA content (44 ± 6 wt%) were attained (Table 2).

In addition to the utilization of OPS, the potential of natural seawater as growth medium was also evaluated. It showed no microbial contamination even in the case of using sea water. PHA accumulation utilizing mixtures of OPS and seawater showed steady increase, yielding approximately 2.4±0.2 g/L cell biomass and 24±8wt% PHA content at the end of 48h of cultivation (Fig. 2). OPS and seawater as the carbon source and culture medium, respectively, facilitated significant accumulation of PHA. This study showed the potential of OPS and seawater for use in low-cost culture medium to support bacterial cell growth and PHA production.

(*Takamitsu Arai, Akihiko Kosugi, DN Rathi [Universiti Sains Malaysia], K Sudesh [Universiti Sains Malaysia]*)

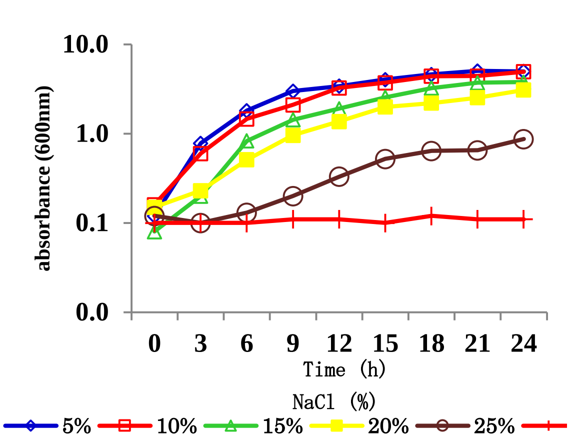


Fig. 1. Growth profile analysis (OD_{600nm}) of *Halomonas* sp. SK5 grown at 30 °C

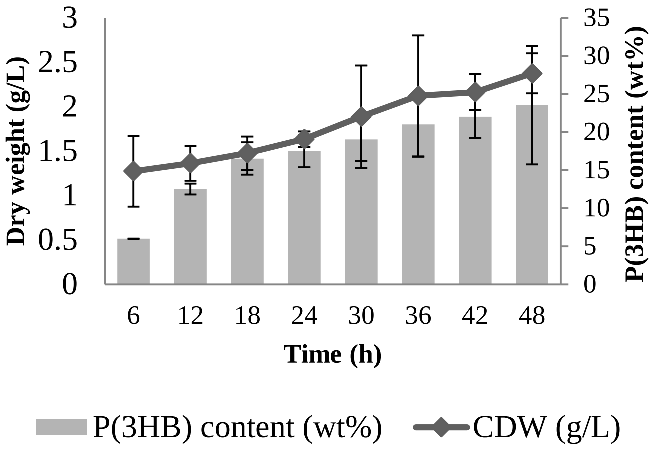


Fig. 2. Time profile analysis of P(3HB) biosynthesis by *Halomonas* sp. SK5 grown in OPS seawater medium at 30 °C and 200 rpm

Table 1. Effects of varying salinity on the cell growth PHA biosynthesis in *Halomonas* sp. SK5

Salinity (w/v %)	**Dry weight (g/L)**	**PHA content (wt%)**
5	**2.4 ± 0.1**	**40 ± 4**
10	**3.5 ± 0.2**	**41 ± 4**
15	**3.6 ± 0.2**	**30 ± 5**
20	**3.4 ± 0.3**	**26 ± 4**
25	**2.6 ± 0.1**	**15 ± 4**

Table 2. Evaluation of the potential of oil palm trunk sap as carbon source as well as sole growth medium in *Halomonas* sp. SK5

	Dry weight (g/L)	**PHA content (wt%)**
Sap	**1.7 ± 0.2**	**24 ± 4**
Sap and salt water	**2.8 ± 0.2**	**44 ± 6**

Soil suitability map for teak plantation in the Northeast of Thailand

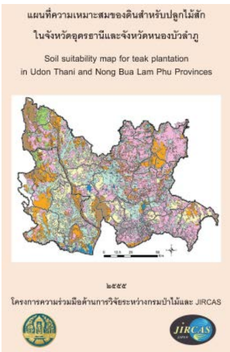
Teak (*Tectona grandis*) is a valuable indigenous tree species in Thailand. Teak wood products are used in making high quality furniture and as building material for houses, etc. It is one of several economic tree species suitable for farm forestry management, and plays a vital role in promoting forest restoration and regional development, thanks to rapid tree growth and high timber prices. Teak has several characteristics, including affinity to specific soil types and conditions, hence future yield depends on site suitability for teak plantation.

Decision support information on determining site suitability is important to teak farmers. There had been no such maps that quantitatively showed soil suitability for teak plantation; thus, not a few farmers came to grief after realizing that they had planted on unsuitable sites.

In general, the northeast region of Thailand is not suitable for teak growing; however, we have observed some promising areas. Thus, we endeavored to produce soil suitability maps for Udon Thani and Nong Bua Lam Phu Provinces in order to promote teak farm forestry for livelihood improvement. Based on field surveys and the soil group map of Thailand created by the Land Development Department, the soil suitability class (SSC) for teak plantation in the two provinces was developed. The SSC was categorized into 5 ranks, ranging from 1 (good) to 5 (poor). Letter suffixes were appended to SSC ranks 2-5 to denote site limitations (i.e., n: nutrition, f: flooding, g: gravel, d: drainage). The areas of slope complex (SC) and water (W) on the soil group map were not classified. The SSC is equivalent to the site quality class shown in 'Yield table for Teak plantation in the Northeast of Thailand' (Fig. 1R). The future yield of a teak forest stand can then be projected by combining data from the soil suitability map and the yield table (Figs. 2 and 3).

Matching accuracy is about 69%; however, actual teak growth might be different from the SSC because of the effects of maintenance (Sukchan and Noda, 2012). The map shows the landmarks (in Thai) and includes photographs of typical soils by SSC to provide guidance to farmers and Royal Forest Department (RFD) extension staffs (Fig. 2). Currently, the soil suitability map covers only Udon Thani and Nong Bua Lam Phu Provinces, but this know-how will be made available so that the map extends to other provinces.

(*I. Noda, T. Vacharangkura, W. Himmapan [Royal Forest Department], S. Sukchan [Land Development Department]*)



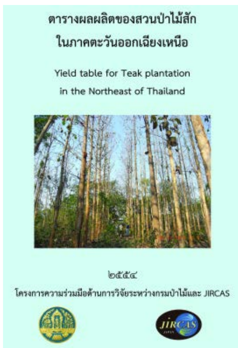


Fig. 1. Left: Soil suitability map for teak plantation in Udon Thani and Nong Bua Lam Phu Provinces (Noda et al., 2012); Right: Yield table for teak plantation in the Northeast of Thailand (Vacharangkura et al., 2011)

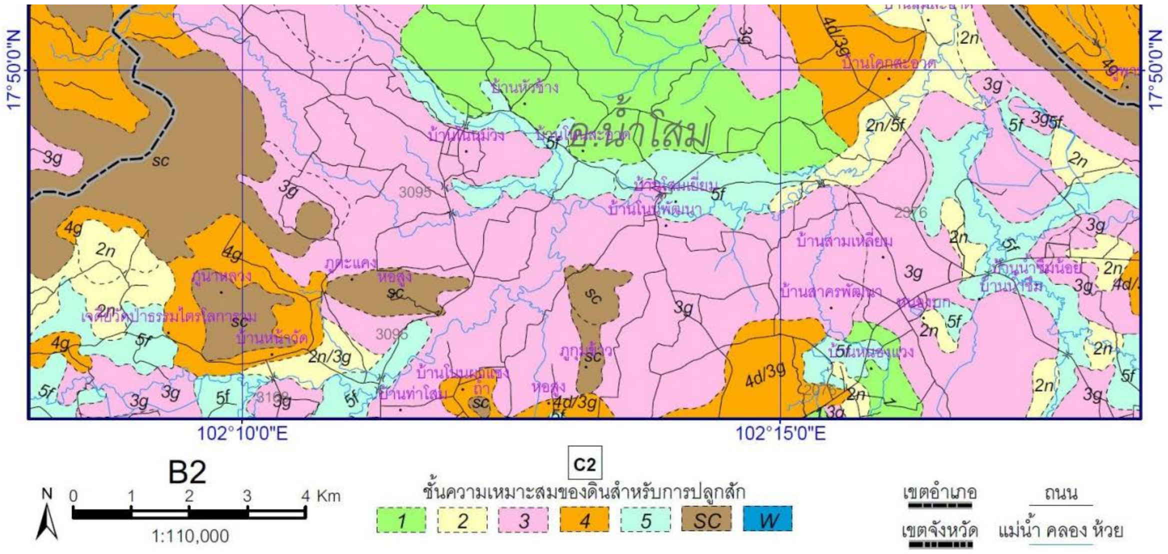


Fig. 2. Soil suitability map (sample page, in Thai). Legend on the map means that SSC 1: green colored area, SSC 2: cream colored area and so on. Also, dashed line: district boundary; dashed-two dotted line: province boundary; solid black line: road; solid blue line: channel or river.

ระยะปลูก 4 ม. X 4 ม. ชั้นคุณภาพพื้นที่ระดับดี (SI = 26)						
อายุ (ปี)	ความสูงไม้เด่น (ม.)	ความสูงเฉลี่ย (ม.)	ความโตเฉลี่ย (ซม)	พื้นที่หน้าตัด (ม.^{2}/ไร่)	ปริมาตรต่อต้น (ม.^{3}/ต้น)	ปริมาตร (ม.^{3}/ไร่)
...	...	...	...	...	...	...
14	18.7	15.7	59.2	2.035	0.253	17.341
15	19.2	16.2	61.6	2.134	0.279	18.522
16	19.8	16.8	64.0	2.230	0.305	19.698
...	...	...	...	...	...	...

Fig. 3. Yield table (sample page, in Thai). Case scenario: A teak plantation spaced at 4m x 4m, with site index (SI) = 26 and SSC rank = 2. The teak plantation is projected at age 15 to have a dominant tree height of 19.2 m, an average tree height of 16.2 m, an average tree girth of 61.6 cm at breast height, a basal area of 2.134 m^2/rai, a stem volume of 0.279 m^3/tree and a stand volume of 18.522 m^3/rai. (1 rai = 0.16 ha)

References

1*) Noda I., et al. (2012) Soil suitability map for teak plantation in Udon Thani and Nong Bua Lam Phu Provinces. 70pp, RFD-JIRCAS Project. (in Thai)

2) Sukchan S. and Noda I. (2012) Improvement of soil suitability mapping for teak plantations in Northeast Thailand. JIRCAS Working Report 74: 27-32.

3*) Vacharangkura T., et al. (2011) Yield table for Teak plantation in the Northeast of Thailand. 54pp, RFD-JIRCAS Project. (in Thai)

(*) http://forprod.forest.go.th/forprod/ebook/e-book.html

Genetic diversity of the shrimp, *Macrobrachium yui*, indigenous to Laos

The indigenous shrimp, *Macrobrachium yui*, is utilized as an important living aquatic resource for cash income in northern Laos. Recently, shrimp catch has declined greatly in many fishing sites due to overfishing and deterioration of the river environment. In order to aid recovery of the shrimp stock, we estimated the genetic diversity within a population, the genetic differentiation among populations, and the dynamics of population size in the past using partial mitochondrial DNA control region sequences. In this study, we will suggest measures for shrimp stock recovery suited to each fishing site.

In total, 570 nucleotide sequences were obtained from 355 specimens at seven sampling sites along the Xuang, Ou, Khan, and Houng Rivers of the Mekong River system and the Et River of the Ma River system, in which 58 variable sites were noted and 74 haplotypes were detected (Fig. 1). A minimum-spanning tree (MST) for the shrimp showed two major clades which are linked to each other by 13 base substitutions (Fig. 1). Coefficient of genetic differentiation significantly differed among the five river groups and among localities (Table 1), but not among localities within the same river (Tables 1 and 2), suggesting that the population is structured by the river almost without gene flow. The population at Et River indicated extremely lower genetic and nucleotide diversities than other populations (Fig. 2). Loss of genetic diversity in the Et River population would decrease population growth rate through inbreeding and miniaturization and increase the risk of extinction due to rapid environmental changes. According to the MST (Fig. 1) and mismatch distribution of haplotypes, the Ou (H・K) and Et River (ET) populations have decreased greatly in the past whereas the Khan (XK) and Houng River (NS) populations have remained stable over a long period of time. Only the Et River (ET) population has not reached recovery yet.

Genetic differentiation among *M. yui* populations along the rivers and stock enhancement without regard to genetic diversity could possibly lead to genetic disturbance of local populations. Therefore, we primarily recommend stock management as a concrete measure for recovery of the shrimp stock.

Shrimp migrating into cave streams for reproduction are easily caught by villagers using bamboo traps. Moreover, some shrimp populations have been greatly been reduced in the past, implying that *M. yui* is vulnerable to dramatic environmental changes. This suggests the importance of stock management measures such as specifically fishing regulation at the cave stream and habitat conservation.

Stock management of the shrimp should then be carried out at the rivers through the initiative of the villagers with support from the local government. Genetic diversity of shrimp population at the Et River (ET) has been severely reduced; hence, artificial shrimp production under government control may be required as one of effective measures for shrimp stock recovery in the future.

(H. Imai [University of Ryukyus], S. Ito, A. KounThongbang, O. Lasasimma [LARReC], P. Soliyamath [Na-Luang Fisheries Station, Luang-Prabang])

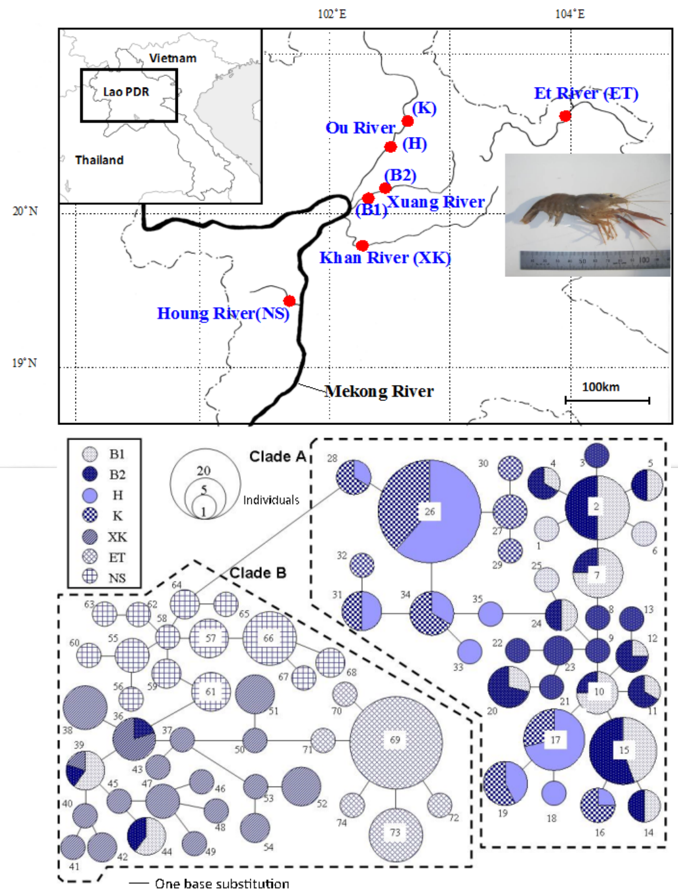


Fig. 1. Sampling sites and minimum spanning tree presenting relationships among 74 haplotypes of the shrimp, *Macrobrachium yui* (Photo inset : the indigenous shrimp, *Macrobrachium yui*) .

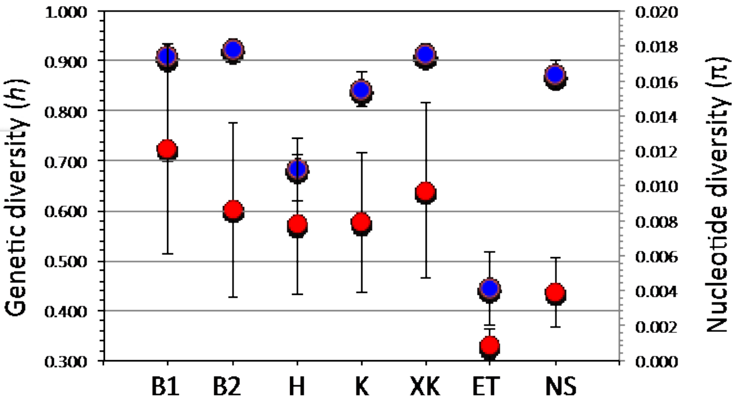


Fig. 2. Genetic diversity (h) (●) and nucleotide diversity (π) (●) at seven localities in five rivers. Error bars show standard deviation.

Table 1. Analysis of molecular variance for pairwise differences (AMOVA) in *Macrobrachium yui*

Comparisions	Percentage of variation	F	P
Among the river groups (F_{CT})	73.34	0.733	<0.01
Among localities within the river group (F_{SC})	0.34	0.013	0.23
Among localities (F_{ST})	26.32	0.737	<0.01

Table 2. Pairwise F_{ST} values (below the diagonal) and pairwise F_{ST} P values (above the diagonal) for the mitochondrial DNA control region at seven localities of *Macrobrachium yui*.

Site	B1	B2	H	K	XK	ET	NS
Xuang R. (B1)	-	0.174	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Xuang R. (B2)	0.011	-	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Ou R. (H)	0.385	0.441	-	0.340	<0.001*	<0.001*	<0.001*
Ou R. (K)	0.423	0.481	0.000	-	<0.001*	<0.001*	<0.001*
Khan R. (XK)	0.669	0.739	0.743	0.741	-	<0.001*	<0.001*
Et R. (ET)	0.820	0.874	0.884	0.883	0.616	-	<0.001*
Houng R. (NS)	0.755	0.816	0.812	0.810	0.544	0.838	-

Bonferroni correction *P<0.001

Occurrence of causative dinoflagellates of paralytic shellfish poisoning in Selangor coast, Peninsular Malaysia

The blood cockle, *Anadara granosa* (Fig. 1a), is an important shellfish aquaculture species in Southeast Asia. In Peninsular Malaysia, the center of cockle culture is in Selangor coast, where huge, suitable mud flat habitats are extensive (Fig. 1b). The spats occur in high density throughout the region, for export to neighboring countries as culture seeds as well as for domestic culture.

In recent years, causative dinoflagellates of paralytic shellfish poisoning have been spreading in Southeast Asia, and have become a serious problem to bivalve culture activities. Toxification of bivalves has resulted to big economic losses due to regulations on aquaculture shipping as well as food safety. The main purpose of this study, therefore, is to collect useful knowledge for the future management of cockle fisheries in Selangor coast, by conducting habitat status and distribution surveys for causative dinoflagellates of paralytic shellfish poisoning.

Between January to May 2012, surveys were carried out in wide areas of Selangor coast using plankton nets (mesh size 20μm). Two causative species of paralytic shellfish poisoning, *Gymnodinium catenatum* (Fig. 2a) and *Alexandrium tamiyavanichii* (Fig. 2c, 2d), were detected in the plankton samples. It was evidently clear that the distribution of *G. catenatum* vegetative cells is wide at a 100-km stretch along Selangor coast, from north to south (Fig. 3). The resting cysts, *G. catenatum*, were observed in surface sediment samples (0-2cm) taken by a sediment corer in Selangor coast (Fig. 2b). Sample density was especially high in the northern part, averaging about 0.5 cells/g dried mud (Fig. 4).

Future collaboration with the Malaysian government will focus on the establishment of a monitoring system to detect paralytic shellfish poisoning including the conduct of toxicity analysis in blood cockle. The presence of causative dinoflagellates in the culture grounds will be examined in order to improve food safety of the cultured shells. This monitoring system should enhance stabilization of the cockle culture industry in the region. Local and foreign shipments of the cockle spats, from Selangor coast into other coastal waters, can be a diffusion factor for the resting cysts, and may result to the spread of paralytic shellfish poisoning in Southeast Asia. As part of its future activities toward effective fisheries management, transportation of spats to other culture grounds must take into consideration the diffusion of the resting cysts.

(T. Yurimoto, A. Mohd Nor Azman [FRI Malaysia], Y. Takata [SK-Laboratory], M. Kodama [Univ. of Tokyo], K. Matsuoka [Nagasaki Univ.])

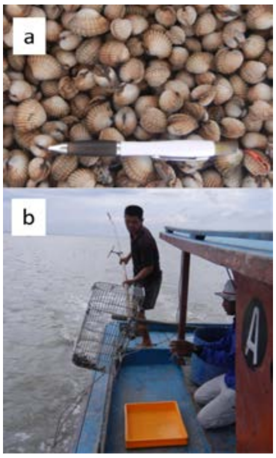


Fig. 1. (a) Blood cockle; (b) A fisherman harvesting cultured cockle

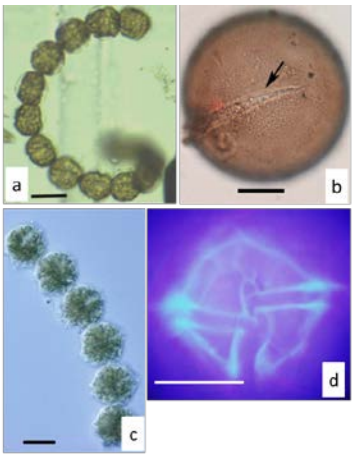


Fig. 2. Vegetative cells of (a) *Gymnodinium catenatum*, (b) the resting cyst and (c) *Alexandrium tamiyavanichii*; (d) fluorescence microscope image of *A. tamiyavanichii* stained with chemical solution. Scale bars correspond to 50 µm (a) and 20 µm (b, c, d)

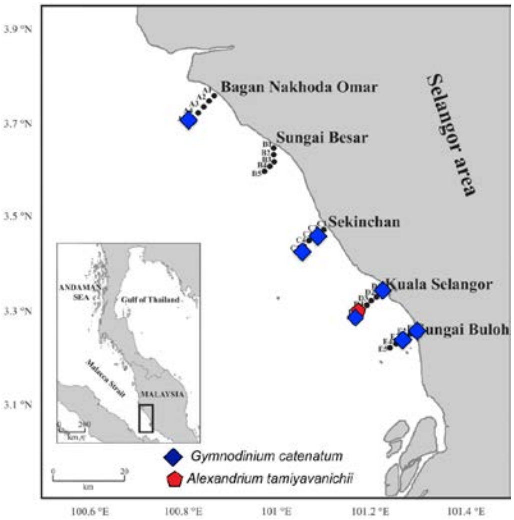


Fig. 3. Occurrence of the vegetative cells, *Gymnodinium catenatum* and *Alexandrium tamiyavanichii*, along Selangor coast

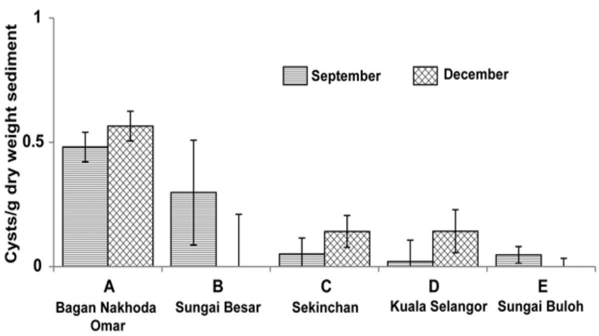


Fig. 4. Comparison of resting cyst (*Gymnodinium catenatum*) cell densities in surface sediments at each site