Continuous application of rice straw compost increases rice yield and contributes to carbon sequestration in paddy fields in the Mekong Delta, Vietnam

The Mekong Delta in Vietnam is the largest rice-producing region in Southeast Asia, contributing more than 20 million tons of rice annually. At present, many farmers in the Mekong Delta simply burn the rice straw in the field, incorporate it into the field after harvest, or ship a part of rice straw for mushroom cultivation. Continuous burning or removing of rice straw from the field may decrease soil organic matter content. Worse, the intensification of land use, combined with a reduced supply of nutrient-laden sediments caused by improved control of floodwater from the Mekong River, may exacerbate the decrease in soil fertility. We cultivated rice 31 times at twice a year (i.e., the dry season crop and the wet season crop) from 2000 to 2015, and evaluated the effects of continuous application of rice straw compost (RSC) on rice yield and total carbon in soil.

We applied RSC (6 Mg ha^{-1} as fresh weight for each crop) and several doses of chemical fertilizer to clarify their effects on rice yields and soil properties. We calculated the yields of treatments where RSC and moderate doses of chemical fertilizer (40% and 60% to a conventional dose) relative to those of the conventionally fertilized plots (i.e., with no added RSC) and analyzed their annual trends. The relative yields gradually increased from 2000 to 2010 (Fig. 1). The rice yields of the treatments where we applied RSC and moderate doses of chemical fertilizer (40% and 60% to a conventional dose) were 0.87 and 0.75 Mg ha^{-1} higher than that of the conventionally fertilized plots for the dry season, respectively, and 0.91 and 0.96 Mg ha^{-1} higher for the wet season in average from 2011 to 2015 (Fig. 2). Available silicon in soil samples from the surface layer (0-10 cm) in treatments with RSC was 10.4 mg kg^{-1} higher on average than that in treatments without RSC (Fig. 3). It seems that the increase of available silicon caused by RSC application contributed to the higher yields. Moreover, RSC application used in combination with moderate doses of chemical fertilizer increased total carbon in the soil at a rate of 356 to 401 kg C ha^{-1} $year^{-1}$ (Fig. 4), indicating that tropical paddy fields can contribute to carbon sequestration.

The results indicate that continuous application of RSC increases rice yield in paddy fields in the Mekong Delta in Vietnam and that tropical paddy soil can contribute to the mitigation of global climate change by carbon sequestration. It is expected that our data will be harnessed by agricultural sectors and/or environmental sectors of the governments of Vietnam and other tropical Asian countries. Regarding the carbon sequestration function of tropical paddy soil, data from other tropical paddy fields needs to be incorporated. Although our RSC is fully fermented with low C/N ratio (less than 17.2), enhanced methane emission accompanied by organic matter input in paddy fields should be considered. Siliceous fertilizer can be an alternative to RSC because it also increases available silicon. However, we need to conduct a cost-benefit analysis to examine its feasibility.

(T. Watanabe, H. M. Luu [Cuu Long Delta Rice Research Institute])

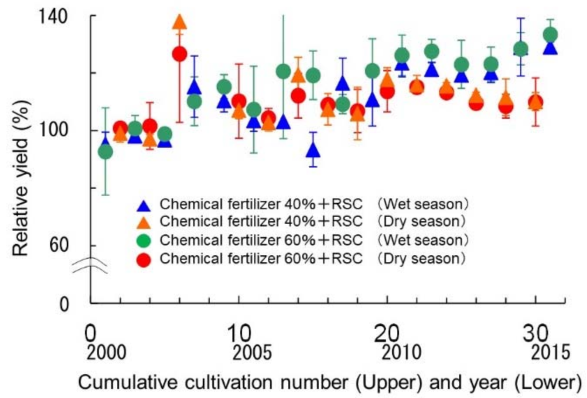


Fig. 1. Annual changes in relative yields.

Bars mean standard deviation (n=3), Relative yield (%) = Yield of treatment / Yield of conventional treatment x 100, For the conventional treatment, chemical fertilizer was applied (as N-P_2O_5-K_2O) without RSC at 80-30-30 (kg ha^{-1}) in the wet season and 100-30-30 (kg ha^{-1}) in the dry season.

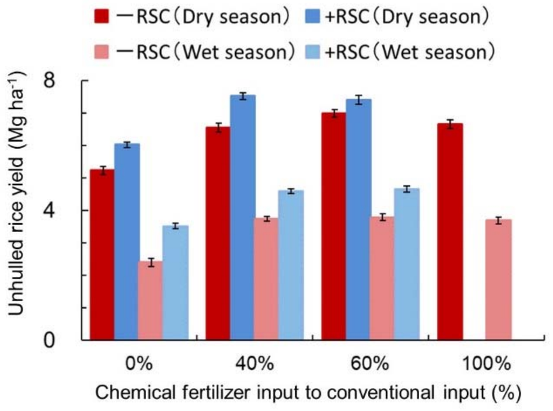
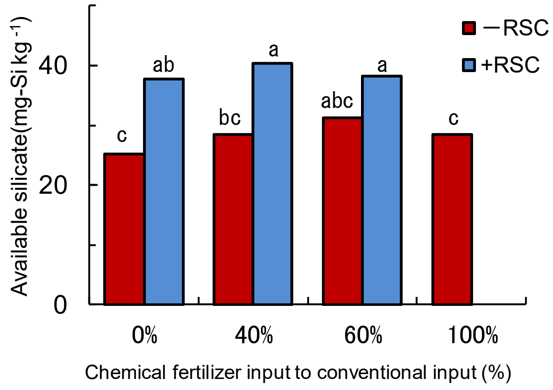


Fig. 2. Average yields for 5 crops (2011 to 2015).

Bars mean standard error (n=3), For the conventional treatment, chemical fertilizer was applied (as N-P_2O_5-K_2O) without RSC at 80-30-30 (kg ha^{-1}) in the wet season and 100-30-30 (kg ha^{-1}) in the dry season.



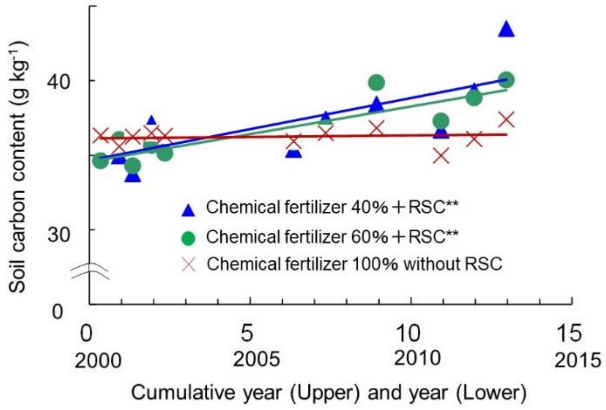


Fig. 3. Available silicon in the soil (0–10 cm) in the experimental paddy field following 25 seasons of rice cropping.

Data with the same letter does not differ significantly (Tukey $p<0.05$). The difference between the average of the +RSC (3 treatments) and average of the −RSC (4 treatments) was 10.4 mg kg^{-1}.

Fig. 4. Change in total carbon (g kg^{-1}) in the soil (0–10 cm) in the experimental paddy.

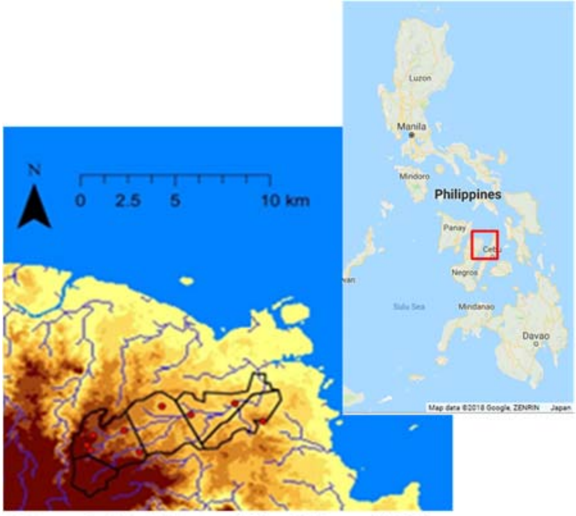
** indicates that the slope was significantly different from 0 ($p<0.01$). The carbon sequestration ratios (kg C ha^{-1} $year^{-1}$) in the text were calculated from the slopes and soil bulk density.

Estimation of nitrogen load to groundwater at a sugarcane monocropping area in the Philippines

The Philippines is one of the major sugarcane-producing countries in Southeast Asia. In particular, a typical sugarcane monocropping area in Negros Island accounts for about 60% of cultivated land throughout the country. The soil in the area consists of a limestone layer with high water permeability, and although groundwater is an important water resource, the nitrogen that is added to the ground surface promptly permeates underground and contaminates groundwater. Therefore, we investigated the actual condition of nitrate nitrogen (NO_3-N) concentration in groundwater. Also, using the annual nitrogen emission data, we calculated the total input amount from various nitrogen inputs in the northern part of the island, which is a typical sugarcane-growing area, and estimated the contribution rate of each input. Lastly, we estimated the nitrogen balance and the potential nitrogen load to the ground due to nitrogen input to the ground surface.

A comparatively high concentration of NO_3-N (i.e., 5.6 ppm average, 8.7 ppm maximum) was detected from shallow groundwater (about 5 to 6 m from ground surface) at nine points in the research area (Fig. 1), indicating that the risk of nitrogen contamination is high (Fig. 2). The sugarcane cultivation area constitutes 77% of land use. The fertilization area, number of livestock, population, and sugarcane cultivation area (Table 1) were assigned to each of the basic units (amount of nitrogen contained per unit amount) of fertilizer, livestock excreta, human excreta, and rainfall (which becomes nitrogen input to the ground surface) to obtain the total nitrogen input amount to the ground surface. The estimated total nitrogen input amount was 192 kgN ha^{-1} (155, 15, 12, 10 kgN ha^{-1}, respectively), of which 81% is nitrogen fertilizer applied in sugarcane cultivation (Fig. 3). Assuming that nitrogen loss through denitrification was 32% among nitrogen inputs to the ground surface (Yoshimoto et al., 2007), its amount was calculated to be 61 kgN ha^{-1}. In addition, from the measurement result of nitrogen absorption amount of stem and leaf, the nitrogen absorbed by sugarcane, and eventually taken out of the system as stem or leaf as harvest product, was 30 kgN ha^{-1} in the case of stem alone and 49 kgN ha^{-1} including leaf, which is equivalent to only 25% of the total nitrogen input amount. Based on these results and according to nitrogen balance estimation in this area, the potential nitrogen load to the underground including surface runoff is lower than that of the nitrogen input to the ground surface when only the sugarcane stem is taken out, and 43% when taking out both stems and leaves of sugarcane (Fig. 3). In the sugarcane cultivation area, the contribution to the underground nitrogen load by fertilizer-derived nitrogen contributes greatly to the NO_3-N contamination of the groundwater. The safe upper limit set by the World Health Organization (WHO) for NO_3-N concentration in drinking water is 10 ppm. A maximum of 8.7 ppm of NO_3-N concentration in the groundwater in the area was detected, thus the amount of nitrogen load to underground should be reduced. It will serve as basic data that can be applied when considering steps to reduce the nitrogen load on the ground surface and underground in this area.

(S. Goto, S. Ando, T. Anzai)



Reference : Jarvis A., H.I. Reuter, A. Nelson, E. Guevara, 2008, Hole-filled seamless SRTM data V4, International Centre for Tropical Agriculture (CIAT), available from http://srtm.csi.cgiar.org.

Fig. 1. Research area (Sagay City, Northern Negros Island)

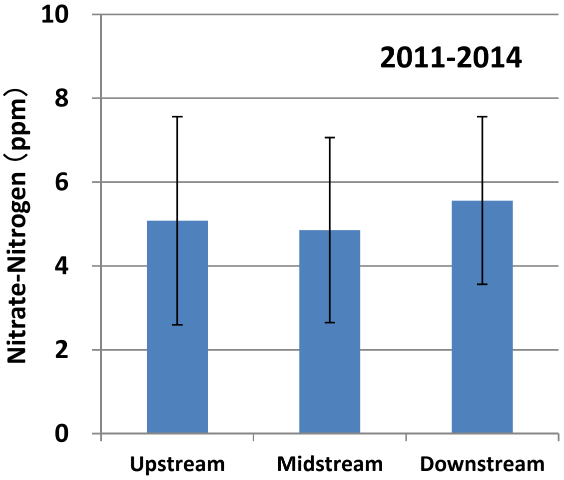


Fig. 2. Nitrate-nitrogen content in shallow groundwater (Error bars indicate standard deviation)

Table 1. Overview of the research area

Area of watershed (ha)	Area of sugarcane cultivation (ha)	Human Population	Animal Population	Amount of rainfall (mm/year)
3,422	2,629	6,577	6,607	1,985

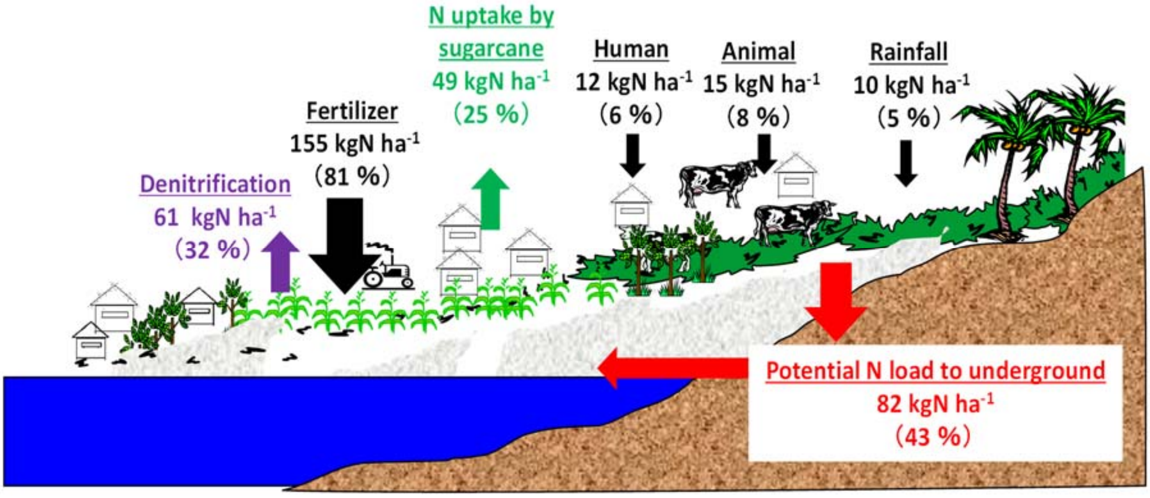


Fig. 3. Estimated nitrogen balance using nitrogen load to ground surface in the research area

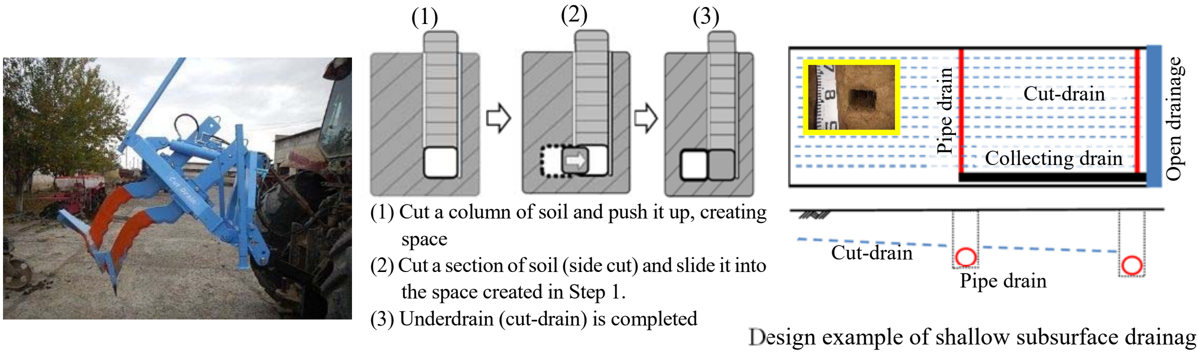
Technical manual on building a low-cost, shallow subsurface drainage system for mitigating salinization

Agricultural productivity in the arid and semi-arid zones of Central Asia increased dramatically during the 1950s owing to large-scale development of irrigated land using the water resources of Amu-darya and Syr-darya Rivers. Simultaneously, however, inadequate water management and poor drainage have led to widespread salinization, especially over a large area in Uzbekistan causing serious damage to agricultural production. Several measures, such as constructing a drainage system, dredging of drainage ditches, and leaching operations to remove salt from farm fields, have been taken to mitigate salinization. Still, salinity levels have remained high in many farm fields as incomplete dredging operations, decreased discharge of deep subsurface drainage, and ineffective vertical drainage systems only had a minimal leaching effect. Thus, we proposed a shallow subsurface drainage technology to ensure effective salt removal (by leaching) from the surface soil layer. The technology was investigated in combination with a new drain drilling machine developed in Japan. The machine can construct relatively stable, low-cost mole holes (hereinafter referred to as “cut-drain”) to depths between 60 to 90 cm from the surface. The cut-drain was initially applied in semi-arid areas for reducing salt loads from shallow subsurface drainage. The information gathered about the technology was later described and compiled into a technical manual after field experiments.

The manual is composed of the following sections, namely, “The mechanism of salt accumulation and countermeasures,” “The factors of salinization in the experimental site,” and “Shallow subsurface drainage technology.” The contents are described in a simple manner and conveyed intelligibly using photos or illustrations (Fig. 1). The shallow subsurface drainage is explained in the manual according to the planning and construction method, the appropriate soil conditions (i.e., suitable soil texture and soil moisture), and problems (e.g., collapse of cut-drain) and its countermeasures. In the farm field where the shallow subsurface drainage technology was applied, high salinity water was discharged from the field to the open drainage (Fig. 2), decreasing soil salinity and subsequently resulting to an approximately 20% increase in cotton yield (Fig. 3).

The manual is expected to be applied in arid or semi-arid zones. Cut-drains should be constructed in areas where the soils meet certain texture and moisture conditions (i.e., the texture should have a low proportion of sand or silt, and it should not be too hard and dry). The manual was translated into Japanese, English, and Russian in agreement with the Farmers’ Council (FC) and concerned research institutes in Uzbekistan. In addition, an abridged version was also translated into English, Russian, and Uzbek for field use. The drilling machine is relatively expensive for farmers. Therefore, FC has an important role to play in supporting farmers, such as providing easy access to use the machine.

(Y. Okuda, K. Omori, and J. Onishi)



(1) Cut a column of soil and push it up, creating space
(2) Cut a section of soil (side cut) and slide it into the space created in Step 1.
(3) Underdrain (cut-drain) is completed

Tractor-mounted drilling machine

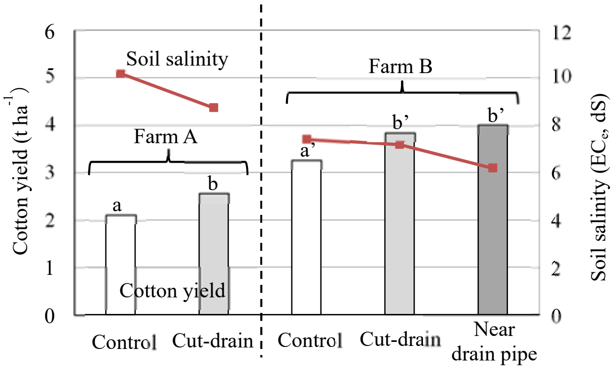
Mole hole formation process

Design example of shallow subsurface drainage in combination with cut-drain
（Left photo shows cut-drain’s hollow core.）

Fig. 1. Examples of photos and illustrations in the technical manual

High-salinity leach water is discharging into an open ditch.

Fig. 2. Outlet of collecting drain



Note) ・Yield survey (Cotton) and Soil sampling (0-100cm) in September 2017
・Different alphabets of cotton yield show significant difference. (Farm A: $p<0.05$, Farm B: $p<0.01$)
・Although soil salinity of cut-drain was lower than control, there was no significant difference.

Fig. 3. Crop yield and soil salinity in the field with shallow subsurface drainage

Further insights into underlying mechanisms for the release of biological nitrification inhibitors from sorghum roots

Sorghum roots release two categories of biological nitrification inhibitors (BNIs) – hydrophilic-BNIs and hydrophobic-BNIs. Earlier research indicated that rhizosphere pH and plasma membrane (PM) H^+-ATPase are functionally linked with the release of hydrophilic BNIs but the underlying mechanisms are not fully elucidated. It was earlier hypothesized that BNIs are transported through anion channels but has not been confirmed. This study is designed to reveal further insights into the regulatory mechanisms of BNI release in root systems using three sorghum genetic stocks. The findings are as follows:

- The levels of sorgoleone (a hydrophobic inhibitor) in root dichloromethane (DCM) wash are positively correlated (Fig. 1).
- A decrease in rhizosphere pH improved the release of hydrophilic-BNIs from the roots of all three sorghum genotypes but had no effect on the release of hydrophobic-BNIs (Fig. 2).
- Fusicoccin, a stimulator of PM H^+ ATPase phosphorylation, promoted H^+ extrusion and stimulated the release of hydrophilic-BNIs. Vanadate, an inhibitor of PM H^+ ATPase phosphorylation, in contrast, suppressed H^+ extrusion and lowered the release of hydrophilic-BNIs.
- The release of hydrophilic-BNIs is positively linked with PM H^+-ATPase activity levels in sorghum roots (Fig. 3a). However, statistical analysis indicates no relationship between PM H^+ ATPase and hydrophobic-BNI release in sorghum root systems (Fig. 3b).
- Anion-channel blockers did not inhibit the release of hydrophilic BNIs, but enhanced H^+-extrusion and hydrophilic-BNIs release. These results indicate that some unknown membrane transporters are operating the release of protonated BNIs, which may compensate for charge balance when transport of other anions is suppressed.

Sorgoleone release from root systems contributes to about 50% of the BNI potential in sorghum roots. The pH insensitivity for sorgoleone release will have implications for BNI-trait expression in soil types with low buffering capacity. Genetic improvements in sorgoleone release can thus result in future sorghum varieties with high-BNI capacity in root systems where the trait is expressed in a range of soil types with varying pH, thus can have wider adaptation to low-N production environments with improvements in agronomic nitrogen use efficiency (NUE).

(G. V. Subbarao, T. Di, T. Yoshihashi, M. R. [Nanjing Agricultural University], Y. Zhu [Nanjing Agricultural University], S. Deshpande [International Crops Research Institute for Semi-Arid Tropics])

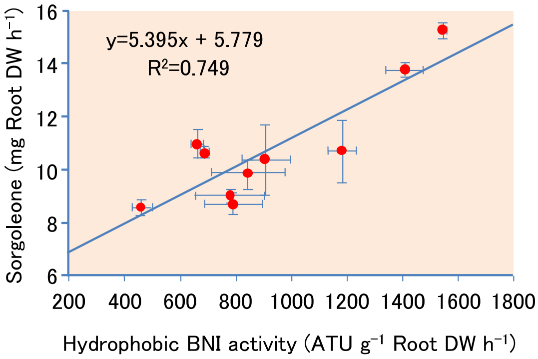


Fig. 1. Relationship between hydrophobic-BNI activity and sorgoleone levels

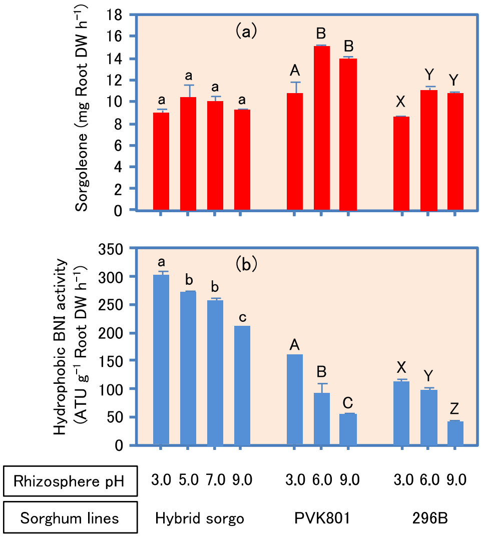


Fig. 2. Rhizosphere pH influence on the release of sorgoleone (a) and hydrophilic nitrification inhibitors (b) from sorghum roots, respectively

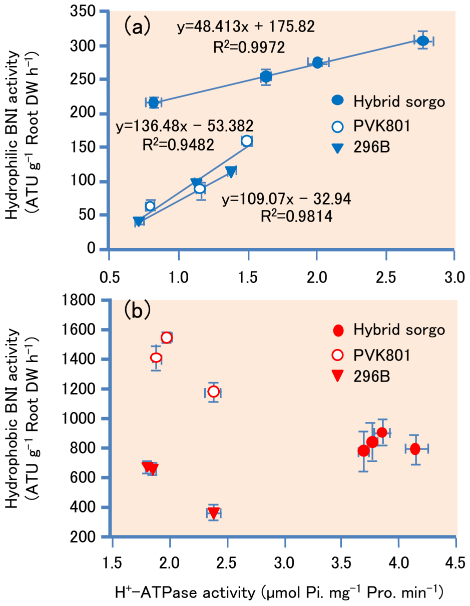


Fig. 3. Relationship between PM H^+-ATPase activity and hydrophilic (a) or hydrophobic (b) BNI activity, respectively, in root systems of sorghum

Genetic variation of blast resistance in rice germplasm from West Africa

Blast disease caused by the fungal pathogen *Pyricularia oryzae* Cavara is one of the most serious rice diseases worldwide, significantly damaging rice production. Development of cultivars resistant to blast is considered the most effective strategy for protecting the crop, and in West Africa, it is the most economical and effective way of controlling rice blast in the fields of resource-poor farmers. Unfortunately, effective and durable use of blast resistance is limited because the genetic information for resistance to blast disease in rice germplasm from West Africa is quite poor.

To understand the genetic variation and enhance the genetic improvement of rice cultivars in West Africa, we used Simple Sequence Repeat (SSR) markers to investigate the genetic diversity in rice accessions, such as *O. sativa* including interspecific hybrids (NERICA varieties *O. glaberrima* and *O. barthii*), and then evaluated the blast resistance of these accessions. Moreover, we elucidated the relationships between blast resistance and the genome chromosome components of the rice accessions, and discussed the diversity of rice germplasm.

We used 195 rice accessions (Table 1), including *O. sativa* L. (114 accessions), *O. glaberrima* (45), *O. barthii* (5), and differential varieties (DVs) and controls for blast resistance (31), which included two susceptible controls (Lijiangxintuanheigu: LTH and US-2), a Japonica Group cultivar (Nipponbare), and an Indica Group cultivar (Kasalath).

These accessions were classified into three clusters -- the Japonica Group (A), Indica Group (B), and *O. glaberrima* and *O. barthii* (C) -- based on the polymorphism data of 61 SSR markers. Moreover, these were classified again into 3 clusters for resistance – low (Ia), middle (Ib) and high (II) -- based on its reactions to 32 international standard differential blast isolates. The resistant group Ia included Nipponbare, LTH, US-2, and five DVs that were lowly resistant. Group Ib had many DVs and accessions from cluster C, and group II were accessions of *O. sativa* including mainly lowland and upland NERICA cultivars. Many accessions of *O. glaberrima* were categorized into resistant group Ib, and their resistance were lower than those of lowland and upland cultivars of *O. sativa* and *O. barthii* (Fig.1).

These results clearly demonstrated the genetic variation of rice accessions from West Africa, and the relationships among cultivars of *O. sativa* and *O. glaberrima* and of wild rice *O. barthii*. The information will be useful for the genetic improvement of rice cultivars in West Africa.

(Y. Fukuta, S. Yanagihara, T. Odjo [University of Abomey-Calavi], Y. Koide [Hokkaido University], D. Silue [AfricaRice], T. Kumashiro[AfricaRice]

Table 1. Classification of rice accessions from West Africa based on the polymorphism data of DNA markers and the genetic variation in resistance to blast disease

Cluster group based on polymorphism data of SSR markers and species		No. of accessions (%)						
		Blast-resistant group						
		Ia		Ib		II		Total
Germplasm form West Africa	A			Upland	4	Upland	15	37
				Upland NERICA	3	Upland NERICA	15	
				O. barthii (Upland)	1	*O. barthii* (Upland)	1	2
	Sum		0		8		31	39
	B			Lowland	4	Lowland	30	77
				Upland	1	Lowland	2	
						Lowland NERICA	40	
				O glaberrima (lowland)	3	*O. glaberrima* (Lowland)	1	4
				O. barthii (upland)	1	*O. barthii* (Upland)	1	2
	Sum		0		9		74	83
	C			*O. glaberrima* (upland)	8			41
				O. glaberrima (lowland)	33			
				O. barthii (upland)	1			1
	Sum		0		42		0	42
Sum in each species	*O. sativa*	0		12(10.5)		102(89.5)		114
	O. glaberrima	0		44(97.8)		1(2.2)		45
	O. barthii	0		3(60.0)		2(40.0)		5
	Total	0(0.0)		59(36.0)		105(64.0)		164(100.0)
Differential varieties and controls	A	Japonica Group differential varieties, *Pik-s, Pish, Pi19*(t))	4	Japonica Group differential varieties, *Pik-h, Pib, Pit, Pii, Pi3, Pi5*(t), *Piz, Piz-5, Pik-m, Pik-p, Pi1, Pi7, Pi12*(t), *Pi20*(t), *Pita*(2), *Pita-2*(2))	19	Japonica Group differential varieties (*Piz-t, Pi9)*	2	
		Nipponbare (Japonica Group)	1					
		LTH (Japonica Group susceptible)	1					
	Sum		6		19		2	27
	B	US-2 (Indica Group susceptible)	1	Kasalath (Indica Group)	1			
		Indica Group differential variety (*Pi12*(t))	1	Indica Group differential variety (*Pi5*(t))	1			
	Sum		2		2			4
	Total	8(25.8)		19(61.3)		2(6.5)		31(100.0)

Clusters A and B are corresponded to Japonica Group and Indica Group, respectively, in *O. sativa*, and cluster C includes *O. glaberrima* and its wild relative *O. barthii.*

Group II is highly resistant, and Ib and Ia follow as middle and lowly resistant group, respectively.

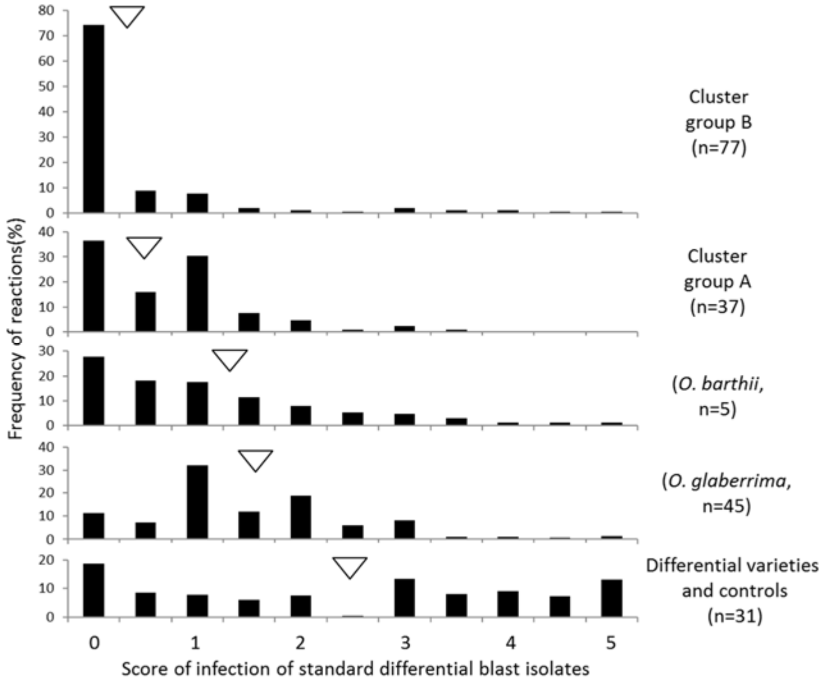


Fig. 1. Resistance of rice accessions from West Africa in each variety group.

The reaction data of rice accessions to 32 standard differential blast isolates are shown in each cultivar group (The data was modified from Odjo et al. 2017).

Whole genome sequence of Guinea yam (*Dioscorea rotundata*) and development of a DNA marker for sex determination

Yam (*Dioscorea* spp.), a widely cultivated tuber crop in Africa, Asia, and South America, serves as a key food source for people in those regions. However, yam breeding has remained ineffective, constrained by the crop's unique inherent attributes including a long growth cycle, dioecy (i.e., having separate male and female plants among varieties), polyploidy, high heterozygosity, and inconsistent year-to-year fluctuations of various traits. Regarding crop characteristics, the use of advanced genetic tools and resources retains enormous potential to boost the breeding efficiency of yam.

As an initial attempt, we focused on Guinea yam (*D. rotundata*), which is one of the most important species for regional food security and farmer income generation especially in West Africa (Fig. 1). Based on our genome analysis of *D. rotundata*, a 594 Mb of genome sequence was successfully assembled. The results of gene prediction using the genome sequence showed a total of 26,198 genes in *D. rotundata* (Fig. 2). By comparison with other known model plant species, 5,557 *D. rotundata* genes were identified with orthologous relationship to the *B. distachyon*, *O. sativa*, and *A. thaliana* gene models. On the other hand, 12,625 *D. rotundata* genes were uniquely found in *D. rotundata* (Fig. 2). The *Dioscorea* genus is characterized by the occurrence of separate male and female plants (dioecy), a feature that has limited efficient yam breeding. To infer the genetics of sex determination, we performed whole-genome resequencing of bulked segregants (quantitative trait locus sequencing [QTL-seq]) in F1 progeny segregating for male and female plants, and identified a genomic region associated with sex determination. Based on the result, a molecular marker for sex identification of *D. rotundata* was developed, enabling us to discriminate male and female plants at the seedling stage (Fig. 3).

The genome sequence information obtained in this work is expected to greatly accelerate the genetic analysis of *D. rotundata* and relative yam species. While the use of a DNA marker for sex identification should immediately contribute to yam breeding for selection of suitable parental materials at the seedling stage, the effectiveness of the QTL-seq approach demonstrated in this study in outcrossing crops and organisms with highly heterozygous genomes should likewise boost the application of genetic analysis in orphan crops such as yam. This in turn could help contribute to food security and improve the sustainability of tropical agriculture.

(S. Yamanaka, S. Muranaka, H. Takagi, M. Tamiru [Iwate Biotechnology Research Center], R. Terauchi [Iwate Biotechnology Research Center], R. Asiedu [International Institute of Tropical Agriculture])

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

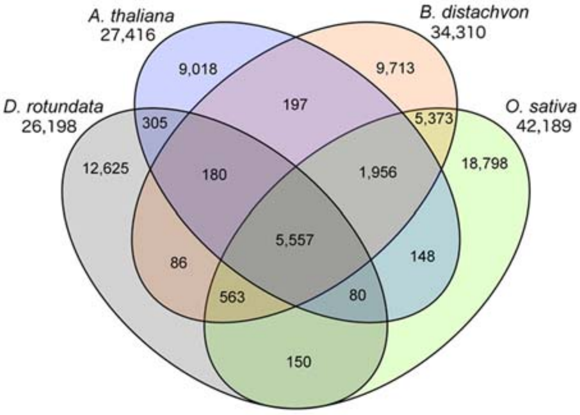


Fig. 2. Venn diagram showing conserved and unique genes at 1:1 correspondence among *D. rotundata*, *Arabidopsis thaliana*, *Brachypodium distachyon*, and *Oryza sativa*. Total gene counts in each genome are given below the species name.

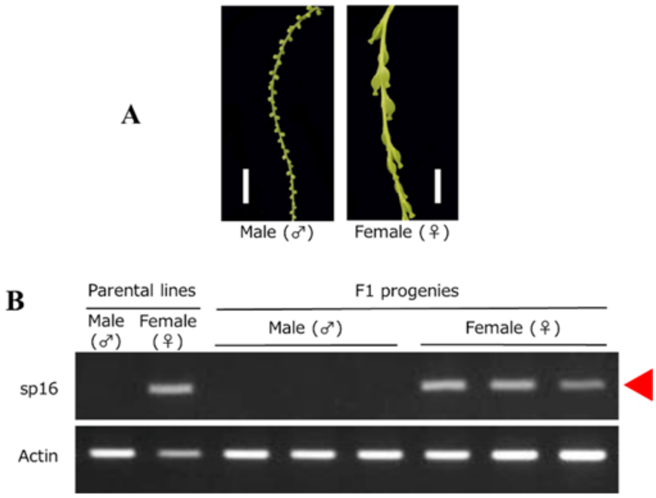


Fig. 3. Sex discrimination by the DNA marker developed in this study. **A**: Male and female inflorescence of *D. rotundata*. Bars = 10 mm. **B**: Results of agarose gel electrophoresis of PCR products amplified by DNA marker sp16 (sp16). Actin from *D. rotundata* (Dr-Actin) served as a control to show that template DNA was present for all samples.

Topographical selection and sulfur application enable high grain yields in rice in currently unutilized floodplain ecosystems of northern Ghana

River floodplains in West Africa, most of which are not currently used for farming, are potential land resources for extending rice cultivation areas. The major constraints that limit expansion of crop production in floodplain areas include difficulties in water management and lack of access to tractor services for land preparation at the appropriate timing for planting. In addition, our previous soil survey and pot experiments indicated severe sulfur (S) deficiency for rice production in these land resources (JIRCAS Research Highlights 2012; available at https://www.jircas.go.jp/en/publication/research_results/2012_14). However, there have been no empirical data to demonstrate the potential of rice cultivation in floodplain ecosystems in West Africa. Therefore, three years of field experiments were conducted in no-till and no-bund conditions to determine rice productivity and the responses to different fertilizer applications on various topographical positions in the floodplain ecosystem.

The experimental results are summarized as follows:

- The target floodplain ecosystem is seasonally flooded in nature, without bunding nor an irrigation system (Fig. 1).
- Unutilized land areas with carbon-rich soils are found within 400 m radius of water reservoirs, i.e., back swamps and main river (Fig. 2).
- Rainfall varied widely, with heavy rainfall during the initial growth stage in 2012, a long dry spell during the middle stage in 2013, and consistent rainfall in 2014.
- Grain yields are consistently high in the currently unutilized lowlands close to water reservoirs (L1-L4) under various rainfall conditions and achieve up to 5.4 t ha^{-1} with N and S application (Fig. 3).
- Sulfur application enhances agronomic N use efficiency (AE$_N$) from 13.4 to 22.8 kg grain per kg N applied, and its effect is particularly large in the currently unutilized lowlands (L1-L4) with AE$_N$ values ranging from 28.4 to 32.6 (Table1).
- The grain yields and agronomic N use efficiencies of L1 to L4 are as high as those in irrigated paddy fields in West Africa.

Based on these results, it is concluded that cultivation of rice in the currently unutilized floodplain areas offers good opportunities to increase rice production in West Africa. It should be noted that more quantitative risk analysis for the yield reduction by complete submergence and infrastructural enhancement for the tractor access will be required to facilitate the use of these land resources.

(Y. Tsujimoto, M. Oda, K. Katsura (Tokyo University of Agriculture and Technology), Y. Fujihara (Ishikawa Prefectural University), J. Sakagami (Kagoshima University), B. Inusah (SARI-CSIR), A. Fuseini (SARI-CSIR), W. Dogbe (SARI-CSIR), A. I. Zakaria (SARI-CSIR)

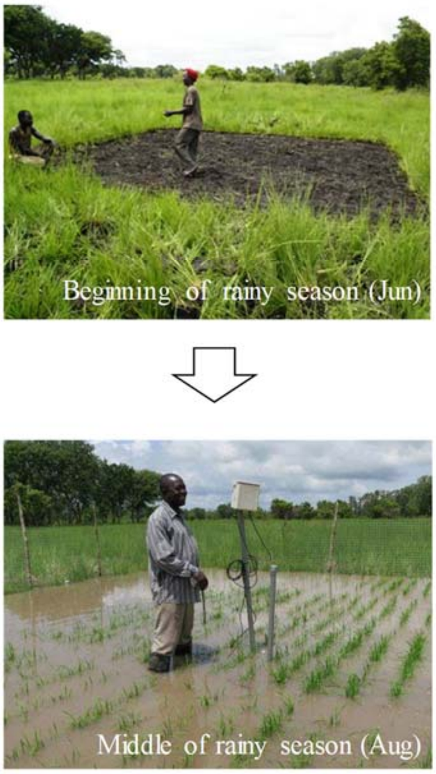


Fig.1. Natural flooding during rainy season in the floodplain ecosystem of Volta river (near L1)

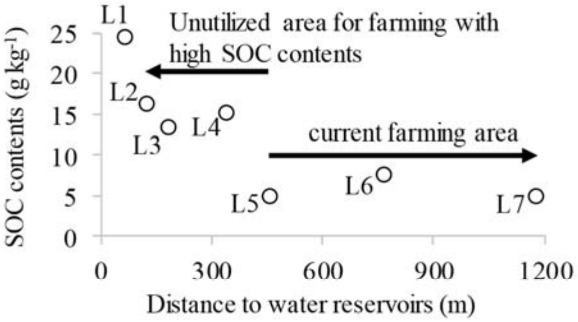


Fig. 2. Distance to water reservoirs and soil organic carbon (SOC) contents of seven experimental fields

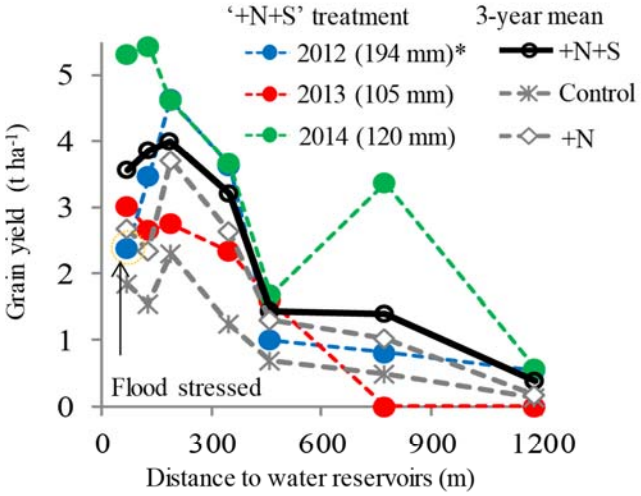


Fig. 3. Effect of topographical condition and fertilizer management on rice yield

*Cumulative rainfall for a month during the initial growth stage from June 15 to July 15. The value was highest in 2012 in our observation from 2010 to 2015. In 2012, the L1 field was completely flooded for 6 days just after emergence.

Table 1. Effect of topographical positions and S application on the agronomic N use efficiency

Treatment*	L1	L2	L3	L4	L5	L6	L7	mean
+N	14.0^{b}	11.0^{b}	23.6^{a}	23.0^{b}	8.7^{a}	8.8^{b}	0.6^{b}	13.4^{b}
+N+S	28.8^{a}	32.1^{a}	28.4^{a}	32.6^{a}	10.2^{a}	15.0^{a}	4.0^{a}	22.8^{a}

Values with the same alphabets indicate no significant differences by Tukey HSD (5%). Values are shown as the mean of three experimental years.

*N was applied at the rate of 60 kg ha^{-1} either as urea or ammonium sulfate. S was applied at the rate of 10~68 kg ha^{-1} as ammonium sulfate or sodium sulfate of which any types or amounts had equivalent effect on rice production.

Reinforcement technology that can be applied by farmers themselves using ground-cover plants for irrigation facilities in paddy fields

In Ghana, locally produced rice comprises only 34% of consumption, resulting in the importation of the remaining 66%. It is thus necessary to improve paddy rice productivity, and irrigation facilities in paddy fields play an important role in achieving sustainable irrigated rice cultivation. However, irrigation facilities in inland valleys in Ghana have not performed efficiently due to high intensity rainfall and poor maintenance. According to the 1995 FAO Report, 40% soil surface coverage reduced raindrop erosion by 90%. Thus, a reinforcement technology using ground-cover plants to prevent raindrop erosion is being developed in accordance with research policy on preventive maintenance.

Local species are recommended as ground-cover plants as they minimize the impacts on ecosystems and overcome the psychological obstacles for the farmers. Vegetation around paddy field irrigation facilities are relatively resistant to changes in soil hardness and soil moisture, and therefore, has advantages over other plants. The targets of this technology are the levees and irrigation canals at paddy fields and, more specifically, the slopes and crowns of canals except for the wetted perimeter. Ground covers are planted by cuttage (i.e., propagation by plant parts) in a zigzag pattern at 15cm intervals. Local farmers are already familiar with this through rice transplanting, hence they can develop and manage it themselves (Fig. 1). Figure 2 shows that cover plants, especially "*Chrysopogon aciculatus,*" strengthen the soil around the irrigation facilities. Employing this technology, therefore, will eventually establish a dense plant community, which in turn will improve the facilities' functionalities and durability (Fig. 3). The planting will be scheduled based on farmers' viewpoint with due consideration to the following: 1) the dry season from Dec. to Feb. must be avoided as root extension growth tends to be difficult under the hardened topsoil, 2) the agricultural off-season is from Nov. to Mar. when the farmers themselves undertake planting work and implement plans to reduce construction costs, 3) the end of the longer rainy season from Jul. to Nov. promises moderate water supply to rice without significant raindrop impact (Fig. 4). The total cost of implementing this technology, including maintenance costs per 100m of irrigation canal in 10 years, is equal to the current cost of an unlined canal or 50% cost of a concrete-lined canal. Moreover, farmers do not need to spend cash if they install the reinforcement technology by themselves.

This technology is expected to be applied in West Africa where high intensity rainfall is common and irrigation facilities are poorly maintained. Prior to implementation, native plant characteristics will be elucidated and plants preferred by farmers would be selected. Control strategies will be identified and established before introducing new plants so they cannot invade the paddy fields. Thorough maintenance during the first year is key to ensuring the durability of the irrigation facilities because planting work will continually improve the cover plants' erosion prevention function against raindrop impact in the succeeding years.

(H. Dan, S. Hirouchi, C. Hirose, O. Emmanuel [Kwame Nkrumah University of Science and Technology], H. Adzraku [KNUST], S. Agodzo [KNUST])

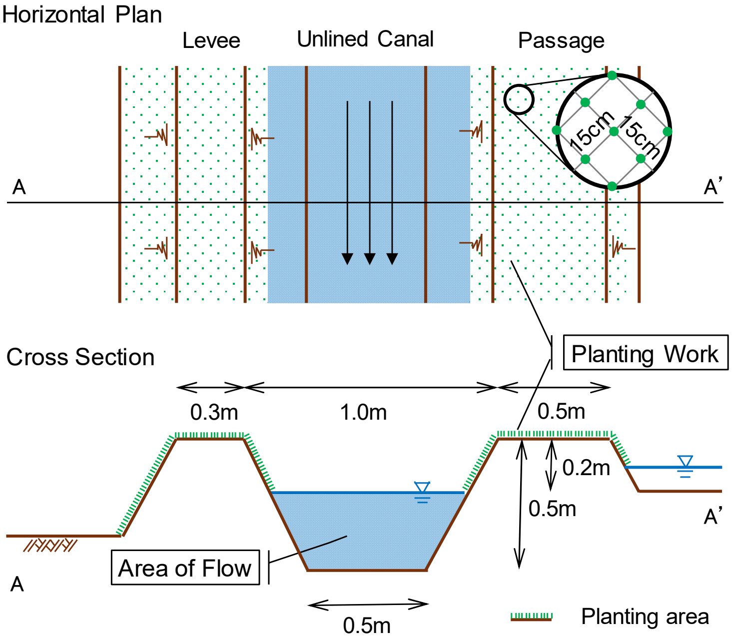


Fig. 1. Standard design of the reinforcement technology

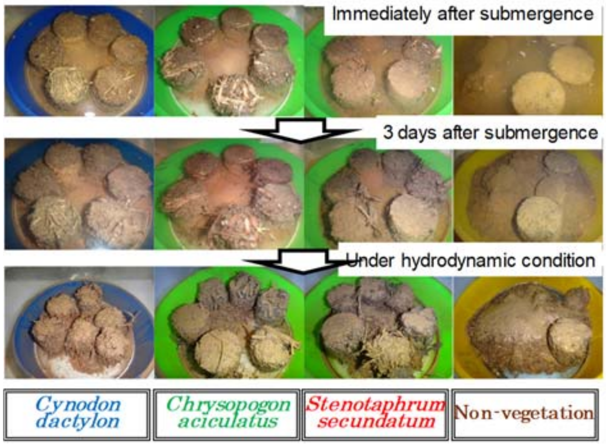


Fig. 3. Test canal 6 months after installing
Left: Canal without vegetation
Right: Canal planted with ground covers

Fig. 2. Grade and process of collapse between three test plants and under non-vegetation condition

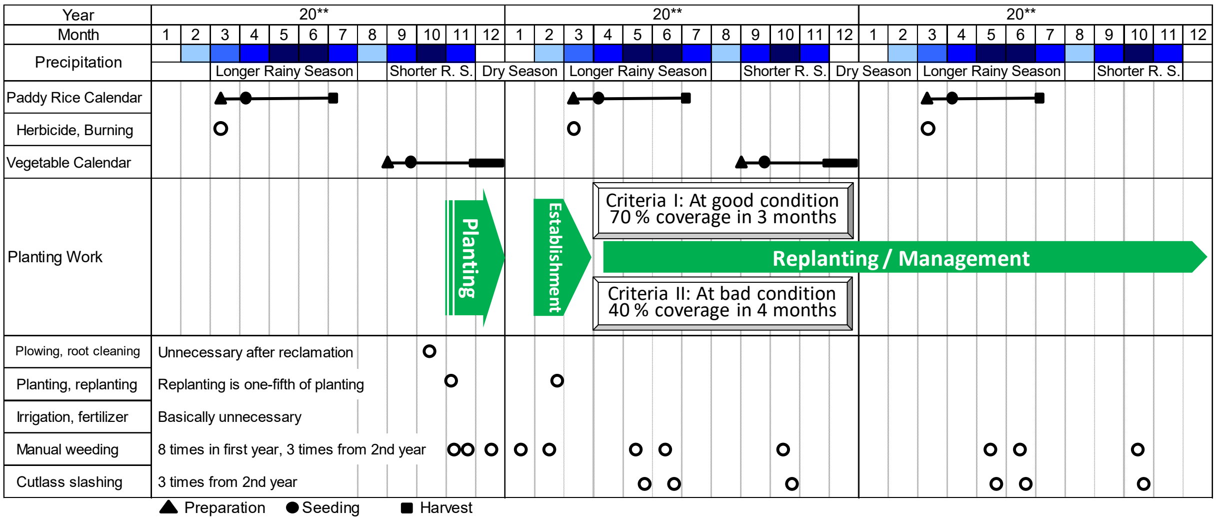


Fig. 4. An example of a construction schedule and maintenance plan for this technology

Identification of a gene that promotes ammonium nitrogen (NH_4^+-N) uptake in rice

Nitrogen is the most essential nutrient to plant growth and grain yield. There are two forms of inorganic nitrogen available to plants: one is NO_3^--form and the other is NH_4^+-form. NH_4^+-form is the major form in paddy fields, thus rice plants grown in the fields uptake mainly NH_4^+-form nitrogen as nitrogen source in roots. Therefore, promoting NH_4^+-form uptake is one of the major targets to increase grain yield in rice. It is known that NH_4^+ influx into roots by a high-affinity transport system (HAT) is down-modulated in response to elevated NH_4^+ concentration around soil surface. We hypothesized that canceling the down-modulation of NH_4^+ influx by HAT would be beneficial on the uptake of NH_4^+-form nitrogen in rice. However, no gene that modulates NH_4^+ influx by HAT has been identified in rice. In this study, we aimed to identify the gene that modulates HAT of rice roots with gene expression analyses. Furthermore, we tried to isolate a gain-of-function gene to promote uptake of NH_4^+-form nitrogen with rice *Tos17* insertion mutants.

To isolate candidate genes concerned in down-modulation of HAT in roots of rice, we performed four-biological-repeat transcriptome analyses. A total of 28,381 out of 36,444 filtered probes were selected as differentially expressed genes based on a false discovery rate of ≤ 0.05. A strong candidate gene for down-modulation of HAT, the coding protein kinase gene *OsACTPK1*, showed 1,071 times higher expression in roots under NH_4^+-rich condition as compared with NH_4^+-deficient condition (Table 1). We then analyzed the detail of *Tos17*-inserted mutant of *OsACTPK1* (*actpk1* mutant) to elucidate *OsACTPK1* as down-modulator of HAT. Kinetic analyses of NH_4^+ influx by HAT revealed that *V*max value of *actpk1* mutant was 2 times higher than that of wild type, whereas there was no significant difference of *K*m value between these two lines (Fig. 1). These results indicated that down-modulation of HAT was canceled in *actpk1* mutant due to the loss-of-function *OsACTPK1*. Total nitrogen content of the *actpk1* mutant was significantly higher (+32%) than that of wild type in 1,000 μM NH_4Cl condition (NH_4^+-rich condition), while the significant difference was not observed in 5 μM NH_4Cl condition (NH_4^+-deficient condition) (Fig. 2A). Furthermore, the longest-root length of the *actpk1* mutant was significantly lower (-22%) than that of wild type in 1,000 μM NH_4Cl condition, while the significant difference was observed in 5 μM NH_4Cl condition (Fig. 2B).

We concluded that *OsACTPK1* was a down-modulator of HAT and that loss-of-function *ACTPK1* (*actpk1* gene) could maintain activity of NH_4^+ influx even in elevated NH_4^+ concentration. The *actpk1* gene could be effective in improving nitrogen use efficiency in a rice molecular breeding program. Also, reduction of root elongation in *actpk1* mutant would be used as phenotypic marker in the program. Further analyses to characterize nitrogen use and grain yield of *actpk1* are required for the program since *OsACTPK1* would function to avoid NH_4^+ toxicity.

(M. Obara, M.P. Beier [Tohoku University], T. Hayakawa [Tohoku University])

Table 1. Details for the *OsACTPK1* gene, a strong candidate for down-modulation of HAT

Item	Description
Increase of gene expression in response to elevated external NH_4^+ concentration	1,071-fold
RAP ID	Os02g0120100
Protein function	protein kinase

Rice Oligo DNA Microarray (4X44K RAP-DB) was used in this research. Total RNA was extracted from roots of rice plants grown for 10 days in 5 and 1,000 µM NH_4Cl.

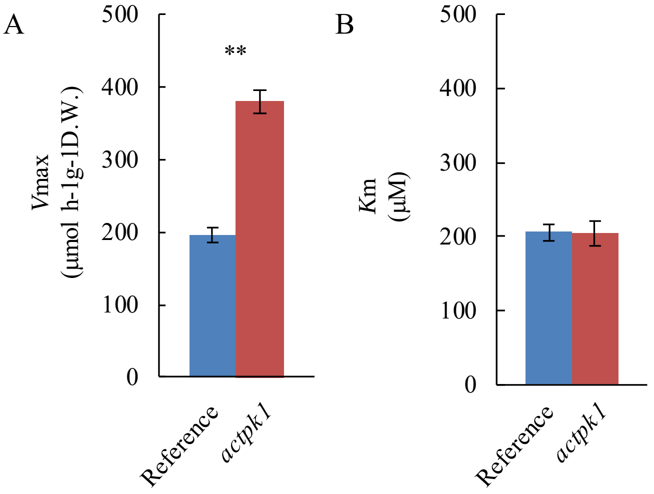


Fig. 1. Kinetic properties of HAT in *actpk1* mutant.
*V*max value (A) and *K*m (B) to NH_4^+ were expressed in blue column for reference, Nipponbare and red column for *actpk1*. Plants were hydroponically grown for 10 days in 1,000 µM NH_4Cl. Mean value with standard error (n=3-6) was plotted. Asterisks indicate probability of less than 0.01 between reference and *actpk1* (ANOVA).

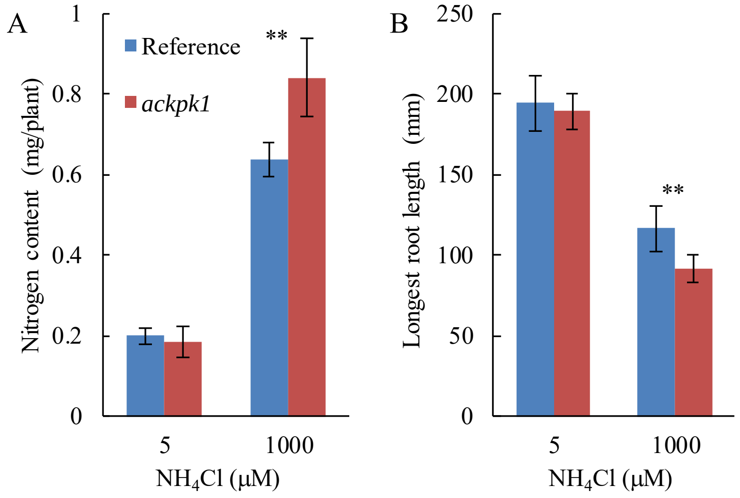


Fig. 2. Total nitrogen content (A) and longest root length (B).
Plants were hydroponically grown for 10 days in two NH_4^+ conditions, 5 µM as deficient and 1,000 µM as rich condition of NH_4^+. Mean value with standard deviation (n=6 for total nitrogen content, n=14 for longest root length) was expressed in blue column for Nipponbare as reference and red column for *actpk1*, respectively. Asterisks indicate probability of less than 0.01 between reference and *actpk1* (ANOVA).

Evaluation method of root angle distribution at seedling stage in rice (*Oryza sativa* L.)

Root type has been one of the breeding targets after the Green Revolution in rice, but research efforts related to genetic improvement of root type was not focused and knowledge accumulation was slow. Evaluation of rice root type is a difficult research subject. Hanzawa et al. (2013) investigated root architecture using a basket as an evaluation method. Unfortunately, the basket method cannot handle a large number of plants and is unsuitable for variation or genetic analyses using many germplasms and big population sizes in hybrid populations. A more efficient evaluation method for root architecture of rice is therefore necessary to clarify the genetic variation of rice germplasms and hybrid populations.

A new method using seedling trays was developed, with the angles (as well as the distribution) and numbers of crown roots of seedlings at 14 days after sowing investigated using a nine-score scale (10: 0-10°, 20: 10-20°, 30: 20-30°, 40: 30-40°, 50: 40-50°, 60: 50-60°, 70: 60-70°, 80: 70-80°, 90: 80-90°) (Fig. 1B). The experiments revealed that introgression lines and isogenic lines with the common genetic background of an Indica Group rice cultivar, IR 64, showed a wide variation from shallow to deep root. Details on root angle distribution in each line were also clarified (Fig. 2).

The results of investigation using the seedling tray method agreed with those of the basket method (Fig. 3), and it confirmed the effect for the evaluation of root angle distribution in rice.

The advantages of the seedling tray method are as follows: shorter duration of rice seed cultivation, bigger population size of rice samples per square meter, and more detailed score scaling compared with that of the basket method for evaluating root angles (Table 1).

The seedling tray method will be useful in evaluating root types or architectures in rice cultivars, conducting genetic analysis of hybrid populations with big population sizes, and surveying genetic variation in germplasm, which means that this method will be a key technology for root type breeding in the future.

(Y. Fukuta, M. Obara, A. Tomita [University of Tsukuba],
T. Sato [Tohoku University], Y. Uga [National Agriculture and Food Research Organization])

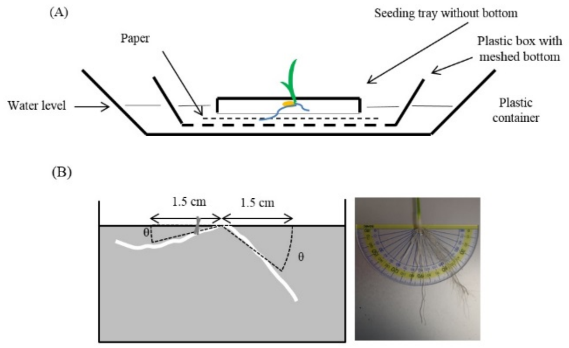


Fig. 1. Seedling tray method for evaluation of crown root angle distribution in rice seedling stage. (A) Set up the seedling tray without bottom in the container. Seedling tray is divided into 17 rows and 2 steps, and 34 rice seedlings are cultivated at the same time. (B) Investigation of growth angle of crown roots from horizontal line, at 14 days after sowing (Modified from Tomita et al. 2017).

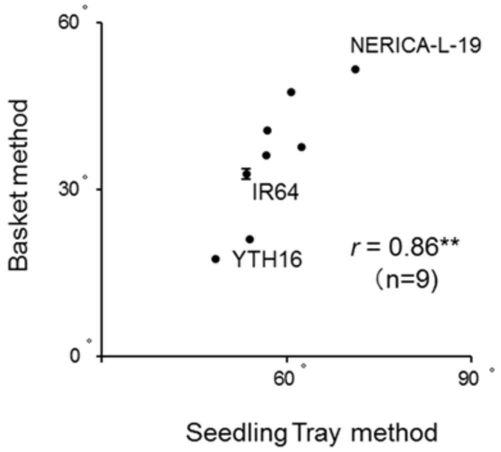


Fig. 3. Root vertical angle (RVA) relationship between the basket and seedling tray methods in IR 64 and eight accessions with the IR 64 genetic background. Average values of RVA for each accession were used as representative data in the seedling tray method and in the basket method of Hanzawa et al. (2013). Error bars indicate SD for each accession. **: significant at P = 0.01 (Modified from Tomita et al. 2017).

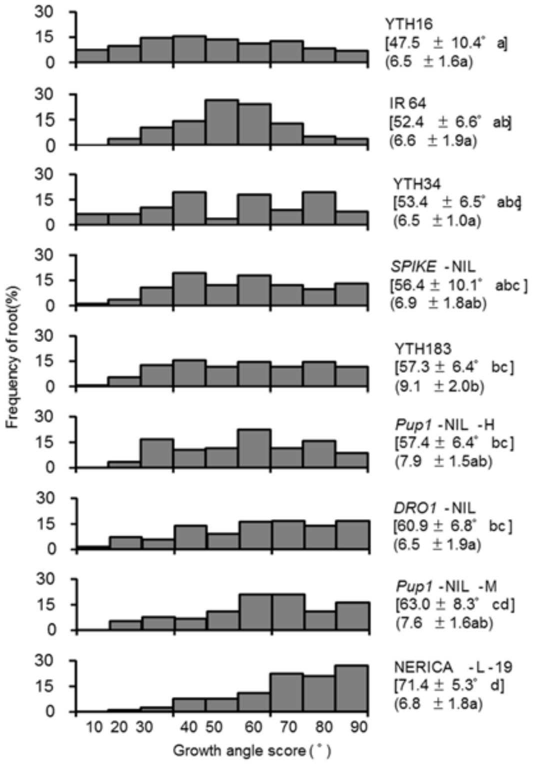


Fig. 2. Root angle distributions in IR 64 and eight accessions with the IR 64 genetic background were investigated using the seedling tray method. The averages of YTH16 and Dro1-NIL were calculated using 22 plants, and the others were calculated using 12 plants. [ ]: Average ± SD of RVA, ( ): Average ± SD of TRN, TRN: total root number, RVA: root vertical angle. Values denoted by different letters are significantly different at P = 0.05 by the Tukey-Kramer test. (Modified from Tomita et al. 2017).

Table 1. Differences of effects between seedling tray method and basket method

Item	Seedling tray method	Basket method
Duration of cultivation (days)	14	21
No. of plants per 1 m^2	137	44
Scales for root angles	9 (0-90)	4 (0, 15, 45, 75)

Overexpression of *AtGolS2*, an Arabidopsis galactinol synthase gene, increases grain yield in rice under drought stress in the field

Drought is a major abiotic stress, critically limiting the yield of food crops including rice. Many reports have demonstrated that overexpression of stress-related genes could improve drought tolerance in rice. However, very few reports have shown improved grain yields in transgenic rice under drought conditions in field environments. Many genes that may play an important role under drought have been mostly introduced in a single model variety of rice, such as Nipponbare, to understand the functions of the genes. To improve rice varieties by transgenic approaches, it is necessary to consider both adaptation to the target environments and fulfillment of local grain quality and of taste preferences. Here, we describe the generation of transgenic rice lines that overexpress *AtGolS2*, which is a candidate gene for drought tolerance encoding a galactinol synthase identified in Arabidopsis, in the background of two commercial varieties, and present the increased grain yield of transgenic rice under drought in the field.

We generated transgenic rice lines that express *AtGolS2* in two varieties, Curinga and NERICA4. Curinga is a Brazilian local upland rice variety, and NERICA4 is a popular upland rice variety in African countries. Each transgenic line accumulated significantly higher amounts of galactinol as compared with non-transgenic rice plant (Fig. 1). The transgenic lines grown under drought had higher relative water content in leaves and higher photosynthetic activity than non-transgenic plants, leading to lesser reduction in plant growth. To test the performance of the transgenic lines under drought in the field, three consecutive field trials were carried out from 2012 to 2015. The extent of drought varied among trial years. For instance, trial years 2012-13 and 2013-14 were very dry with continuous rain-free days of 31 and 39, respectively, including flowering periods. Rain-free days in trial year 2014-15, however, were only 19 after flowering. A transgenic Curinga line numbered 2580 and a transgenic NERICA4 line numbered 1577 consistently had higher grain yield than each non-transgenic variety (Fig. 2). Our results provide strong evidence that *AtGolS2* is a useful biotechnological tool to reduce grain yield losses in rice under drought in the field.

For commercialization of the transgenic lines developed in this study, multi-location trials in South America and Africa are required, and the laws and ordinances of each country related to the treatment of genetically modified organisms have to be followed. For dissemination, collaboration with international and local institutes and with private sectors is required.

(T. Ishizaki, T. Ogata, K. Maruyama, K. Nakashima, M. Ishitani [International Center for Tropical Agriculture (CIAT)], M. Selvaraj [CIAT], M. Kusano [University of Tsukuba], F. Takahashi [Riken], K. Shinozaki [Riken])

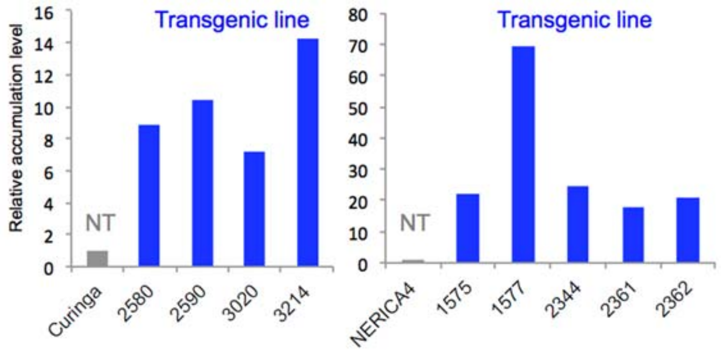


Fig. 1. Accumulation of galactinol in transgenic lines for *AtGolS2*. Numbers indicate ID for each transgenic line. NT, non-transgenic plants.

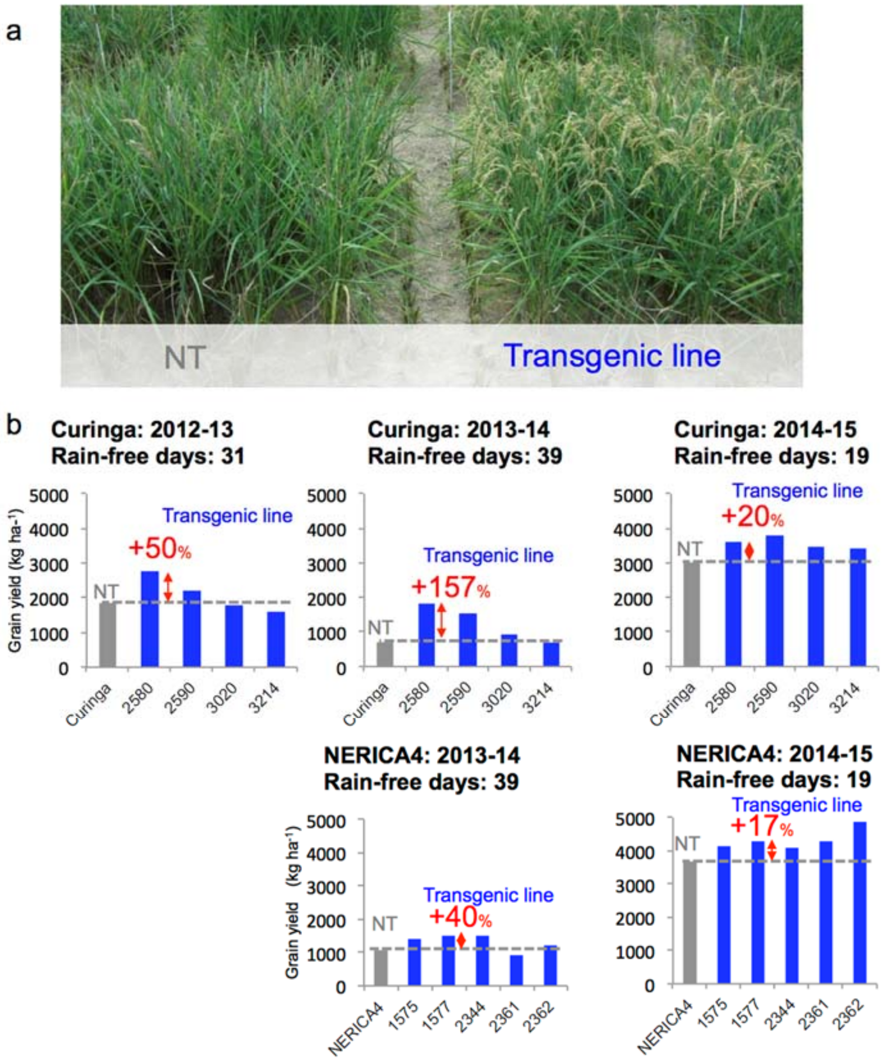


Fig. 2. Improved grain yields of transgenic lines for *AtGolS2* under drought in the field. (a) Evaluation of transgenic rice in a confined field of International Center for Tropical Agriculture located in Colombia. Left, non-transgenic Curinga; right, transgenic lines of Curinga numbered 2580. (b) Grain yield of transgenic lines for *AtGolS2* in the three consecutive field trials. Numbers indicate ID for each transgenic line. NT, non-transgenic plants.

Enhancement of drought tolerance in soybean plants by down-regulation of *GmERA1* genes

Drought is one of the biggest issues affecting global soybean production. A lot of drought tolerance-related genes have been identified in model plants such as *Arabidopsis thaliana*. Functional validation of candidate genes could provide gene resources for breeding varieties of non-model staple crops, such as soybean, that can withstand drought conditions. Previous research has implicated that farnesyltransferase gene *ERA1* in *A. thaliana* is one of promising candidates for genetic manipulation of drought stress tolerance. Although *ERA1* homologs in soybean plants (*GmERA1*) could be a candidate for improving drought tolerance, the function of *GmERA1* had been unrevealed. As soybean is recalcitrant to transformation and gene expression analysis, we employed a virus vector system to validate the potential candidate gene *GmERA1* for drought resistance in soybean.

A plant virus vector derived from *Apple latent spherical virus* (ALSV) was used for the functional analysis of *GmERA1* in soybean plants. Soybean leaves subjected to ALSV-mediated *GmERA1*-down-regulation showed increased drought stress responses with reduced water loss, gas exchanges and higher leaf surface temperature (Fig. 1). *GmERA1*-down-regulated soybean plants also showed reduced wilting and higher survival rate under water-limiting conditions compared to control plants (Fig. 2).

Our data support the proposal that *GmERA1* can be downregulated to increase drought tolerance in soybean. We also demonstrate that the virus vector system, which bypasses the need to generate transgenic plants, is a useful tool for evaluating candidate drought-resistance genes in soybean in the short term.

(T. Ogata, Y. Nagatoshi, Y. Fujita, N. Yamagishi, N. Yoshikawa [Iwate University])

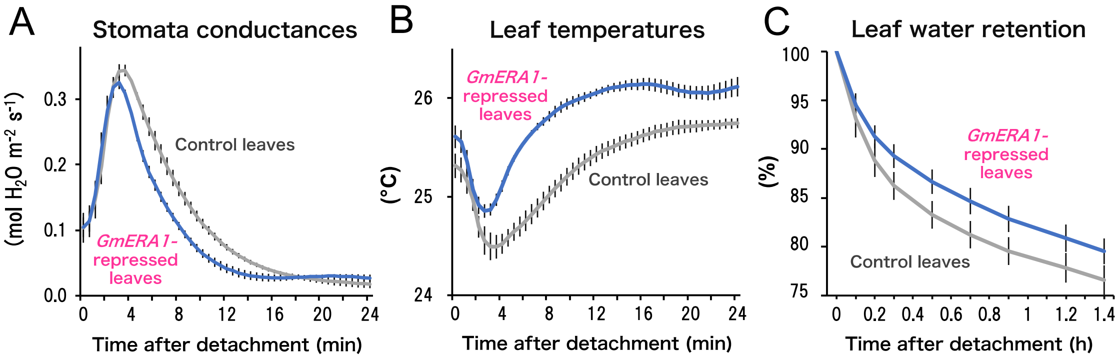


Fig. 1. Improved drought stress responses in *GmERA1*-repressed soybean leaves
Leaf detachment-induced response of stomatal conductance (A), leaf temperature (B), and water retention (C) in soybean leaves infected with *GmERA1*-recombinant virus were measured. Data are means ± SE (*n* = 3 to 6). Figures are modified from Ogata et al. (2017).

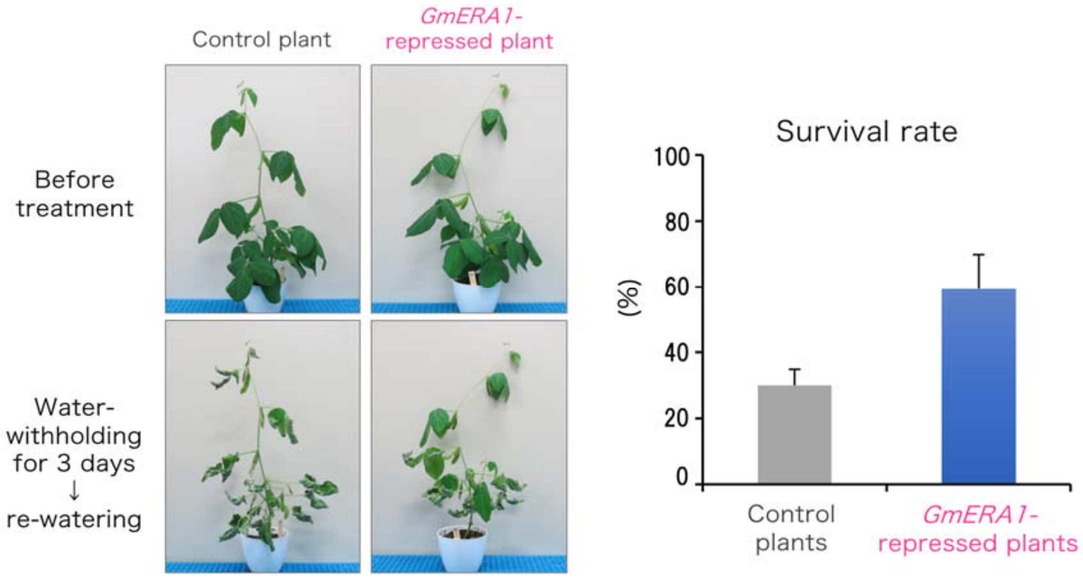


Fig. 2. Improved drought tolerance in *GmERA1*-repressed soybean plants
The soybean plants infected with *GmERA1*-recombinant virus were withheld water for three days before re-watering (left panels). Survival rate of the plants were recorded after re-watering (right panel). Data are means ± SE (*n* = 3 to 6). Figures are modified from Ogata et al. (2017).

Delayed heading technique for early heading *Erianthus arundinaceus* collected in Japan

Erianthus arundinaceus (Retz.) Jeswiet, a wild relative of sugarcane, has received attention as a sugarcane breeding material because of its high biomass productivity in ratoon crops and excellent tolerance to environmental stresses such as drought. Therefore, it has been increasingly utilized to improve sugarcane in several sugarcane breeding countries. However, many *E. arundinaceus* germplasm show earlier heading than that of sugarcane and this mismatch of heading periods between them is one of the biggest limiting factors to achieving intergeneric crossings. The aim of this study is to develop an effective delayed heading technique for early heading *E. arundinaceus* to achieve diverse intergeneric crossings with sugarcane.

Using the early heading Japanese *E. arundinaceus* accessions (JW630 and JW4) as experimental materials, we evaluated the effects on their delayed heading by photoperiodic treatment (PT) with different ratooning times (April, June, and July) (Fig. 1). In the PT plot, long-day treatment (i.e., the day length was extended for 14 h) was applied from 22 June to 23 August, and short-day treatment (i.e., the day length was gradually reduced by 30 min every 2 weeks) was applied from 24 August to 8 November. The illuminance below 1 m from the lamps was about 500 lux.

The heading periods in natural day length of JW630 (mid-September) and JW4 (late October) were earlier than the heading period of sugarcane breeding materials (early November to late December). The effects on their delayed heading were enhanced when we applied PT to the later-ratooning materials (Figs. 2, 3). The difference of average heading date between PT and natural day length plots was 2 days in JW630 and 8 days in JW4 with April-ratooning, 8 days in JW630 and 13 days in JW4 with June-ratooning, and 20 days in JW630 and 18 days in JW4 with July-ratooning. As a result, headings could be delayed in JW630 and JW4 until mid-November and late November, respectively, making it possible to cross them with sugarcane heading during these periods. Since pollen-germination rates in PT plots exceeded 25%, they can be used as a male parent for crossing with sugarcane.

This delayed heading technique combining PT and late-ratooning time will expand the possibility to utilize diverse early heading *E. arundinaceus* accessions for sugarcane improvement.

(Y. Terajima, A. Sugimoto, H. Takagi, S. Ando, S. Irei [Okinawa Prefectural Agricultural Research Center], S. Tagane [Kyushu University], H. Hayashi [University of Tsukuba])

Fig. 1. Photoperiodic treatment of *E. arundinaceus*.

Photo: Aug. 2011 at JIRCAS-TARF

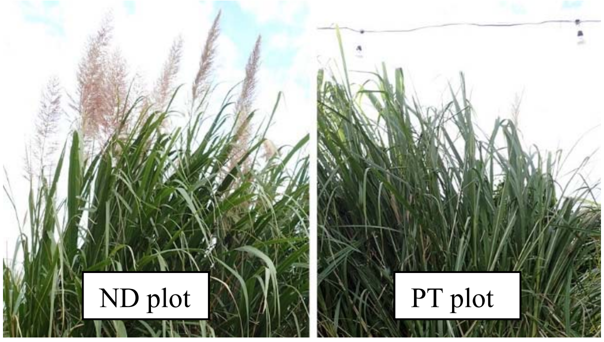


Fig. 2. Heading of JW4 in ND and PT plot.

ND and PT indicate natural day length and photoperiodic treatment, respectively.

Photo: 20 Oct. 2011 at JIRCAS-TARF

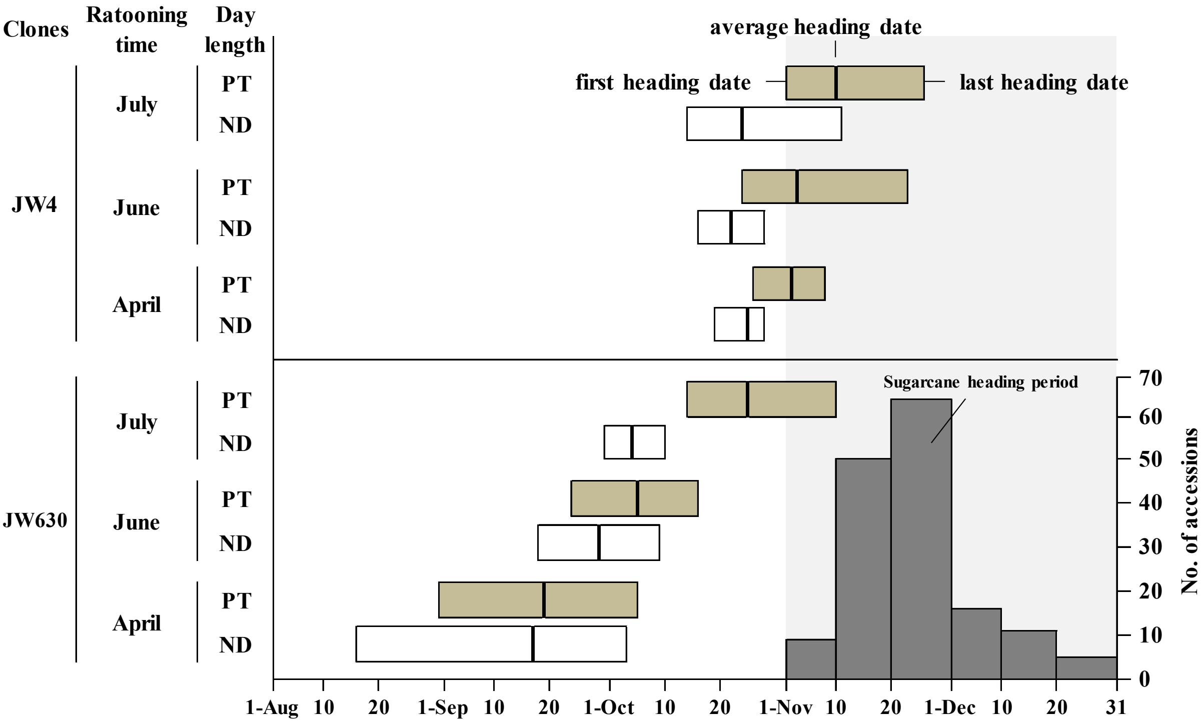


Fig. 3. Effects of ratooning time and photoperiodic treatment on heading dates of JW4 and JW630.

The data in the figure shows the average values of two-year experiments (2010, 2011).

PT and ND indicate photoperiodic treatment and natural day length, respectively.

The black bar means the distribution of the heading period in sugarcane varieties and clones (n=155) in 2009.

PT was more effective on delayed heading in later-ratooning plots, and a significant interaction was observed between ratooning time and day length in ANOVA ($P < 0.01$).

Complete chloroplast genomes of *Erianthus* and *Miscanthus*, and phylogenetic relationships within the *Saccharum* complex

The genera *Erianthus* and *Miscanthus*, both members of the *Saccharum* complex, are of interest as potential resources for sugarcane improvement and as bioenergy crops. Ongoing studies focus on the conservation and use of wild *Erianthus* and *Miscanthus* accessions as breeding materials. However, despite current interest, the taxonomy and phylogenetic relatedness of *Saccharum* and these related genera have been controversial. Because the chloroplast (cp) genome has conserved gene content and uniparental inheritance, polymorphism within the chloroplast genome is a valuable tool for phylogenetic and evolutionary studies. In this study, we determined the complete cp genome sequence of *Erianthus arundinaceus* and *Miscanthus sinensis*. Our analysis of these cp genomes provides insight into the phylogeny and the evolution of the *Saccharum* complex based on the sequence variations of these cp genomes.

The complete cp genomes of *E. arundinaceus* (Accession No.: LC160130) and *M. sinensis* (LC160131) had typical circular structures with 141,210 bp and 141,416 bp in length, respectively (Fig. 1). The number of genes was 143 in *E. arundinaceus* and 141 in *M. sinensis*, including 79 and 78 protein-coding genes, respectively. Alignment of the *E. arundinaceus* and *M. sinensis* chloroplast genome sequences with the known sequence of *S. officinarum* demonstrated a high degree of conservation in gene content and order. Using the data sets of 76 chloroplast protein-coding genes, we performed phylogenetic analysis in the *Saccharum* complex. Our results show that *S. officinarum* is more closely related to *M. sinensis* than to *E. arundinaceus*. We estimated that *E. arundinaceus* diverged from the subtribe Sorghinae before the divergence of *Sorghum bicolor* and the common ancestor of *S. officinarum* and *M. sinensis* (Fig. 2).

This is the first report of the phylogenetic and evolutionary relationships inferred from maternally inherited variation in the *Saccharum* complex. Our study provides an important framework for understanding the phylogenetic relatedness of the economically important genera *Erianthus*, *Miscanthus*, and *Saccharum*.

(S. Tsuruta, M. Ebina [NARO-ILGS], M. Kobayashi [NARO-ILGS], W. Takahashi [NARO-ILGS])

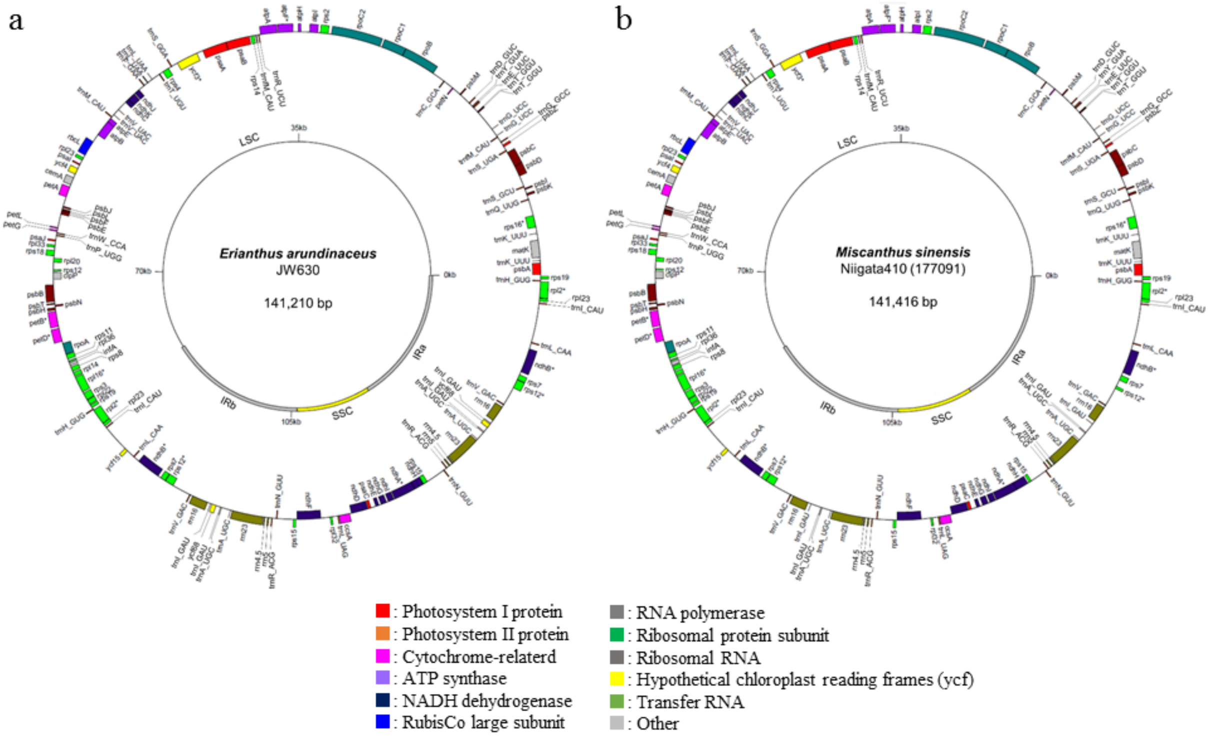


Fig. 1. Chloroplast genome maps of *Erianthus arundinaceus* (a) and *Miscanthus sinensis* (b). LSC: large single-copy, SSC: small single-copy, IRa and IRb: inverted repeat a and b

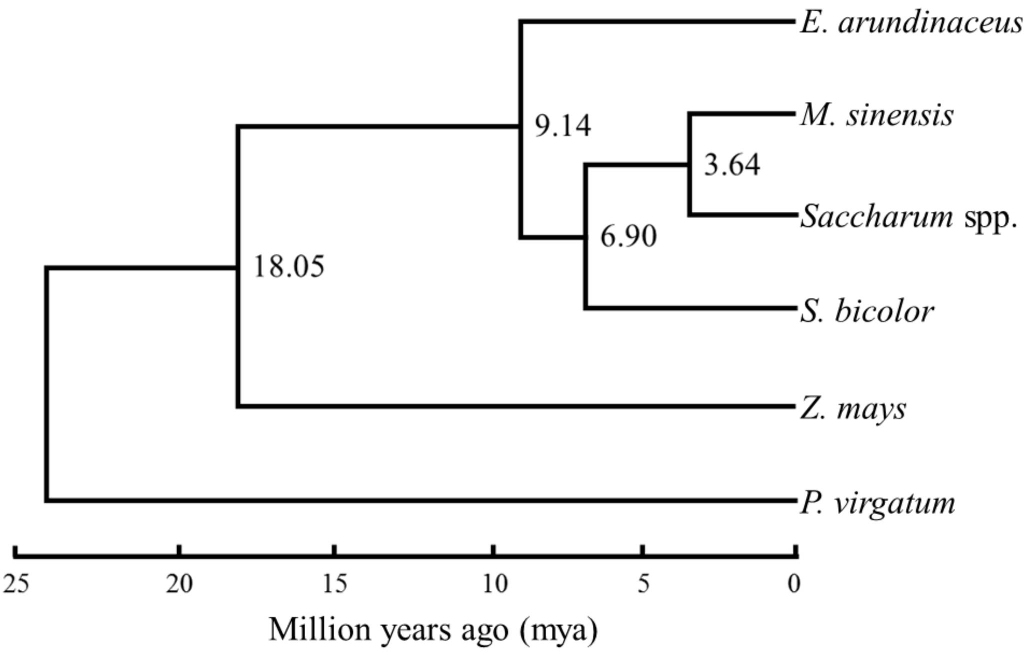


Fig. 2. Divergence times of the *Saccharum* complex estimated based on variation of 76 concatenated protein-coding chloroplast genes

Characterization of *Erianthus arundinaceus* collected from Japan based on nuclear DNA content and simple sequence repeat markers

Erianthus arundinaceus, a member of the *Saccharum* complex, has been receiving increased attention as a bioenergy crop and a potential resource for sugarcane improvement. Although *Erianthus* species are widely distributed in tropical and subtropical climates, some wild accessions are well adapted to temperate Japan. To our knowledge, these are wild *E. arundinaceus* accessions that have adapted to the northernmost regions, and thus are potentially novel genetic resources for the development of cultivars or breeding lines suitable for growth in the temperate zone. However, little is known about the genetic characteristics of the Japanese accessions. To facilitate genetic studies and breeding programs, we developed simple sequence repeat markers from *E. arundinaceus* and obtained basic knowledge about the genetic background of this species collected in Japan.

We investigated the 2C DNA content and SSR polymorphisms in *E. arundinaceus* accessions collected from three climatic zones (temperate, subtropical, and tropical) in Japan and Indonesia with flow cytometric analysis. All examined accessions fell into two groups reflecting collection location: the temperate zone in Japan (mean 8.06 pg) and the subtropical and tropical zones in Japan and Indonesia (mean 7.56 pg). Although DNA content differed significantly between the accessions from temperate and tropical/subtropical zones, chromosome number was inferred to be identical in all accessions. A phylogenetic tree of 29 accessions based on detected fragments from 39 SSR primer pairs classified Japanese (26 accessions) and Indonesian (3 accessions) accessions into well-defined distinct groups. Both 2C DNA content and phylogenetic analysis subdivided Japanese accessions into two groups, suggesting that some of the Japanese accessions had different genetic characteristics from other accessions from Japan and Indonesia. In addition, of the 39 SSR primer pairs, 31 primer pairs were amplified in other genera of the *Saccharum* complex including *Saccharum* spp., *Miscanthus sinensis* and *Narenga porphyrocoma* (Fig. 3).

It is unclear how the hexaploidy of *Erianthus* was established in evolution, but the significant differences in DNA contents between the two *E. arundinaceus* groups might reflect a geographical distribution and ecological adaptation after ancestral hexaploidization. Further comprehensive studies including additional genetic resources from other countries and genomic information are needed to fully understand the basis of DNA content variability in *E. arundinaceus*.

(S. Tsuruta, Y. Terashima, M. Ebina [NARO-ILGS], M. Kobayashi [NARO-ILGS], W. Takahashi [NARO-ILGS])

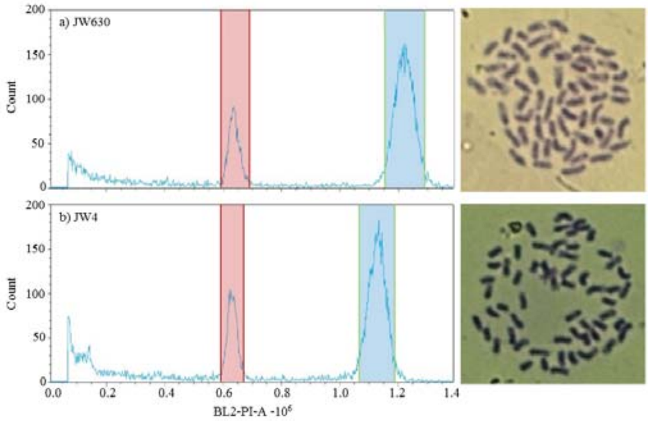


Fig. 1. Histograms of relative DNA content and mitotic metaphase chromosome preparations of *Erianthus arundinaceus* from temperate (a) and subtropical (b) zones.
The peaks marked in red and blue were detected in the internal control and the experimental samples, respectively. The x-axis shows the number of propidium iodide (PI)-labeled cells detected using a BL2A filter (488 nm). The y-axes are on cell counts.

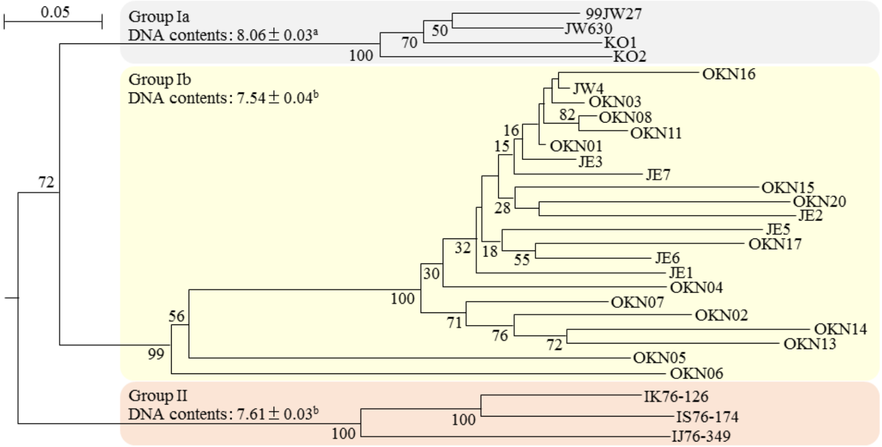


Fig. 2. Phenogram of the 29 accessions based on SSR genotyping data from 39 primer pairs. Numbers beside the branches indicate the values (%) of 1,000 bootstrap replicates. Values of DNA content are mean ± standard deviation within each group. DNA content with different letters indicate statistically significant differences among groups (p<0.001).

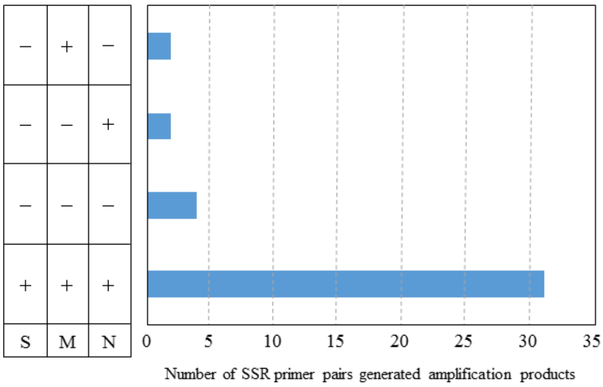


Fig. 3. Application of 39 SSR primer pairs to the *Saccharum* complex.
S: *Saccharum* spp. (7 species), M: *Miscanthus sinensis* (5 accessions), N: *Narenga porphyrocoma* (1 accession). Successful amplification (+), No amplification (–).

An effective pesticide against the dominant insect vector of sugarcane white leaf disease

Sugarcane white leaf (SCWL) disease is an insect-borne disease that severely affects sugarcane production in Southeast Asia, especially in Thailand. The pathogen is phytoplasma. Effective treatments against SCWL disease have not been developed yet, thus an integrated pest management system based on healthy seed cane production by meristem culture is being considered. However, SCWL disease has been spreading to healthy seed cane propagation fields due to transmission by the SCWL disease vector insect, *Matsumuratettix hiroglyphicus,* among others. Therefore, we screened insecticides and determined those that have shown high efficacy against *M. hiroglyphicus*, with the aim of developing an infection risk reduction technique targeting the insect vectors at healthy seed cane propagation fields.

Seven commercial insecticides from among registered insecticides in Thailand were applied on 2-month-old, healthy potted sugarcane plants in a greenhouse (Fig. 1a). Results showed that three of them (i.e., lambda-cyhalothrin 2.5% emulsifiable concentrate, thiamethoxam 25% water dispersible granule, and dinotefuran 1% granule) had long-term residual effects on the mortality of *M. hiroglyphicus* (Table 1). These three selected insecticides from the greenhouse condition test were applied on sugarcane fields (Fig. 1b), and the residual periods were evaluated using a small leaf cage (Fig. 1c). Data revealed that dinotefuran resulted in high mortality and that it had the longest-lasting residual effect among the three pesticides (Table 2). The impacts of the three insecticides on two natural enemies of sugarcane stem borers, *Cotesia flavipes* (Cameron) (larval parasitoid) and *Trichogramma confusum* Viggiani (egg parasitoid), were also evaluated, with the results of the experiments indicating that dinotefuran had no negative effects on the two natural enemies of *M. hiroglyphicus* (Table 3). From these results, we found that dinotefuran showed high efficacy against *M. hiroglyphicus* and that the impact to the natural enemies was not significant. Thus, this pesticide may be used toward developing techniques to reduce the risk of SCWL disease invasion by insect vectors on healthy seed cane at propagation fields.

Planting of healthy seed cane and managing the risks of invasion by insect vectors are essential to producing healthy seed cane at the propagation fields. The findings of this study contribute to the latter factor. To develop an appropriate chemical management technique, we need to understand the residual period of dinotefuran in each growth stage of sugarcane, and analyze the population dynamics and abundance estimation of *M. hiroglyphicus*. Moreover, the registration requirements must first be complied with before using dinotefuran for commercial application and treatment of sugarcane fields.

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

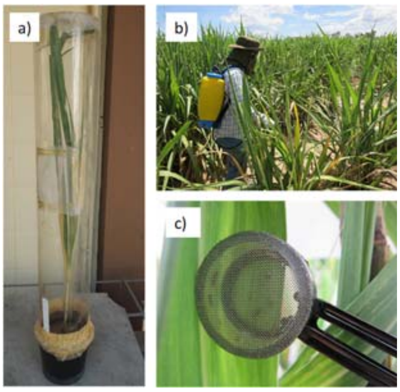


Fig. 1. Greenhouse (indoor) and field (outdoor) testing methods.

a) The pot used for the greenhouse condition test. The insect vectors were kept in the cage.
b) Sugarcane field for the field test. The plant height was about 160cm.
c) The small leaf cage for the field test. The diameter was 20mm.

Table 1. Residual effect of pesticide treatments in *M. hiroglyphicus* mortality under laboratory condition

	Mortality ± S.E. (%)		
Chemical treatment	1 day after	7 days after	30 days after
Carbaryl 85% WP	100 ±0a	100 ±0a	0 ±0b
Carbosulfan 20% W/V EC	100 ±0a	100 ±0a	0 ±0b
Carbofuran 3% GR	0 ±0b	40.6±6.0b	0 ±0b
EPN 45% W/V EC	100 ±0a	100 ±0a	0 ±0b
Lambda-cyhalothrin 2.5% W/V EC	100 ±0a	78.1±6.0a	6.3±6.3b
Thiamethoxam 25% WG	100 ±0a	100 ±0a	87.5±5.1a
Dinotefuran 1% GR	93.8±6.3a	100 ±0a	100 ±0a
Distilled water	3.1±3.1b	0 ±0c	0±0b

The pesticides were applied on two-month-old potted plants and the insect vectors were released 1 day, 7 days and 30 days after the treatment.

The numbers indicate the mortality rates 48 hours after pesticide treatment.

Values with different letters within columns indicate significant difference based on Tukey's honest significant difference test ($P<0.05$).

Table 2. Residual effect of selected pesticide treatments in *M. hiroglyphicus* mortality under field condition

	Mortality ± S.E. (%)			
Chemical treatment	1 day after	7 days after	30 days after	60 days after
Lambda-cyhalothrin 2.5% W/V EC	34.7±18.5b	4.0±2.3b	1.3± 1.3b	2.7± 1.3ab
Thiamethoxam 25% WG	100 ± 0a	98.6±1.3a	30.7±10.4a	9.3± 5.8ab
Dinotefuran 1% GR	98.7±1.3a	100 ±0a	98.7± 1.3a	49.3±13.1a
Distilled water	5.3±2.7b	1.3±1.3b	2.7± 2.7b	1.3± 1.3b

The pesticides were applied on 6- to 7-month-old plants and the insect vectors were released 1 day, 7 days, 30 days and 60 days after the treatment.

The numbers indicate the mortality rates 48 hours after pesticide treatment.

Values with different letters within columns indicate significant difference based on Tukey's honest significant difference test ($P<0.05$).

Table 3. Residual effect of the selected pesticides to *C. flavipes* and *T. confusum*

	C. flavipes			*T. confusum*		
	Mortality ± S.E. (%)			Mortality ± S.E. (%)		
Chemical treatment	1day after	7days after	30days after	1day after	7days after	30days after
Lambda-cyhalothrin 2.5% W/V EC	79.0±6.4a	11.0±8.6a	5.0±1.6a	73.0±3.4b	20.5±3.0b	11.0±3.3a
Thiamethoxam 25% WG	65.0±5.0a	8.0±2.0a	8.0±2.6a	98.0±2.0a	41.5±7.2a	12.0±3.7a
Dinotefuran 1% GR	6.0±1.9b	4.0±1.9a	2.0±1.2a	17.0±3.7c	14.0±2.0b	5.0±2.2a
Distilled water	1.0±1.0b	2.0±1.2a	0 ±0a	10.0±2.2c	9.5±2.2b	3.0±2.0a

The pesticides were applied on five-month-old potted plants and the insects were released 1 day, 7 days and 30 days after the treatment.

The numbers indicate the mortality rates 48 hours after pesticide treatment.

Values with different letters within columns indicate significant difference based on Tukey's honest significant difference test ($P<0.05$).

Genetic variation in resistance in rice germplasm and differentiation of blast races in Bangladesh

Rice cultivations are conducted under three conditions in Bangladesh, namely, Aus (upland in early rainy season), Aman (rainfed lowland in late rainy season), and Boro (lowland in winter/dry season). Rice blast disease has been reported in recent years, the most serious of which occur during Boro season. Unfortunately, local information related to the genetic variation in resistance of rice cultivars and the differentiation of blast races is scarce. To build up a stable protection system against the disease, the accumulation of relevant information in rice cultivars and blast races will be important.

A total of 331 blast isolates from Bangladesh were classified into two cluster groups, I and II, based on the reaction patterns to differential varieties for 23 kinds of resistance genes (Fig. 1). Those in cluster I showed higher frequencies of virulence to differential varieties (DVs) for *Pii*, *Pi3*, *Pi5*(t), *Pik-m*, *Pi1*, *Pik-h*, *Pi-k*, *Pik-p* and *Pi7*(t) than those in cluster II (Fig.1). Clusters I and II were dominant in rainfed lowland and irrigated lowland, respectively, and there were no major differences between I and II in each region (Fig. 2). The results, however, were different in the case of other countries such as Japan, Cambodia, and West Africa. Blast isolates from rice cultivars BRRI dhan34 and Sadamota in rainfed lowland were virulent to DVs in [i] and [k] groups (except for *Pik-s*), while those from BRRI dhan28 and BRRI dhan47 in irrigated lowland were avirulent.

On the other hand, 334 rice accessions conserved in Bangladesh Rice Research Institute (BRRI) showed a wide variation in resistance. Variations among the groups in cultivated conditions (Aus, Amam, and Boro) and cultivated groups (Indica Group and Japonica Group) were changed, and showed complexity. BRRI dhan34 and Sadamota (but not BRRI dhan 28 and BRRI dhan 47) were found to harbor the resistance genes in DV groups [i] and [k], but not BRRI dhan 28 and BRRI dhan47.

These results indicated that wide variations in resistance of rice cultivars and blast races were present, and that the differentiation of blast races corresponded with rice cultivars that are cultivated in each region and cultivated condition. This will be the first step in the development of a durable protection system against blast disease in Bangladesh.

(Y. Fukuta, S. Yanagihara, M. Obara, M. Khan [Bangladesh Rice Research Institute], M. Ali [BRRI], M. Monsur [BRRI], M. Mia [BRRI], M. Latif [BRRI], M. Khalequzzaman [BRRI], A. Kawasaki-Tanaka [Tottori University], N. Hayashi [National Agriculture and Food Research Organization], A. Tomita [University of Tsukuba])

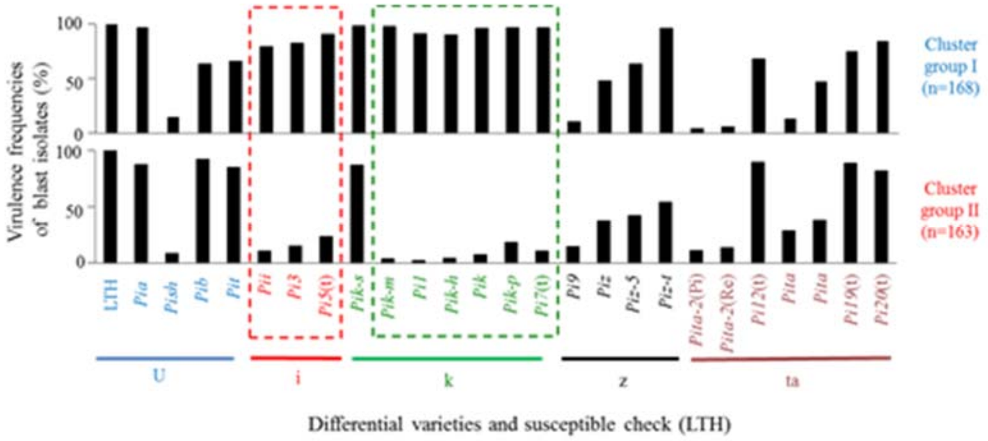


Fig. 1. Virulence frequencies of blast isolates from each cluster group on differential varieties. Cluster analysis using Word's hierarchical clustering method was used to classify a total of 331 blast isolates on the basis of the reaction pattern of 25 differential varieties as well as Lijangxintuanheigu (LTH) for susceptibility checking (Modified from Khan et al. 2017).

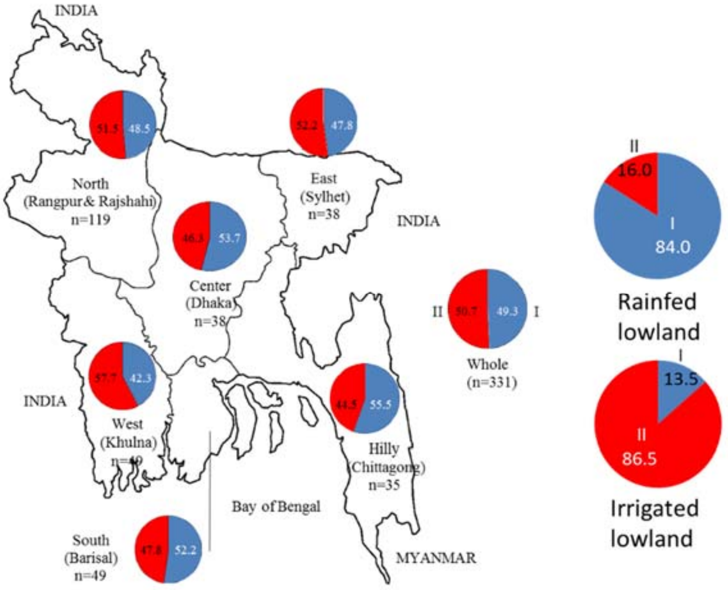


Fig. 2. Geographical distribution of cluster groups for blast isolates in Bangladesh.

There were no big differences in frequencies between clusters I and II in each region, but the frequencies were completely different between rainfed lowland and irrigated lowland.

Table 1. Estimated resistance genes in rice cultivars of Bangladesh

Cultivation season	Variety	Estimated resistance gene in genetic background									
		Differential variety									Unknown
		「U」				「i」	「k」		「z」	「ta」	
		Pia	*Pish*	*Pib*	*Pit*	*Pii* *Pi3* *Pi5* (t)	*Pik-s*	*Pik-m* *Pi1* *Pik-h* *Pik* *Pik-p* *Pi7* (t)	*Pi9* (t) *Piz* *Piz-5* *Piz-t*	*Pita-2,* *Pi12* (t) *Pita* *Pi19*(t) *Pi20* (t)	
Aman (Rainfed)	BRRI dhan34				○	1		1			1
	Sadamota	○			○	1		1	1	1	1
Boro (Irrigated lowland)	BRRI dhan28	○	○	○	○		○		1	1	1
	BRRI dhan47		○	○	○		○			1	1

○ : indicates presence of resistance gene, 1: indicates presence of any of the resistance genes

Improvement of functionality of broccoli sprouts using slightly acidic electrolyzed water

Sprouts are simply germinated seeds of soybean, mung bean, and radish, among others, that are consumed as food. They contain many nutrients and functional components, and provide health benefits. Broccoli sprout, in particular, contains sulforaphane, which has an antioxidant function, and has been attracting attention as a health/functional food. Sulforaphane in broccoli sprout is produced by the myrosinase enzyme from 4-methylsulfinylbutyl glucosinolate (glucoraphanin). Several factors that can promote the accumulation of bioactive compounds have been studied, among them internal factors such as genotypic effect, and external factors including environmental conditions such as light, salinity, sugars, and plant hormones. Slightly acidic electrolyzed water (SAEW) with a near-neutral pH and containing available chlorine concentration (ACC) can be generated by electrolyzing dilute hydrochloric acid using non-membrane electrolytic cell. SAEW has been recognized as having antimicrobial ability and is a promising non-thermal sanitizer for use in the food industry. In Japan, SAEW has been an authorized food additive since 2002 and a specified agricultural chemical since 2014. Previous studies have found that electrolyzed water can enhance the inhibitory activity of angiotensin I-converting enzyme (ACE) in soaked soybeans, influence the germination of mung bean and some grains, elevate the content of γ-aminobutyric acid (GABA) in germinated brown millet, brown rice, and affect antioxidant enzymes in mung bean sprouts. In this study, the effects of SAEW on sulforaphane content and total bacterial count of broccoli sprouts were investigated.

SAEW was prepared with different ACCs (10, 20, 30, 40, 50 ppm) by electrolyzing the tap water with pH 4.35 for a period of time. Broccoli seeds were submerged in different treatment solutions for 3 h. The soaked seeds were then evenly placed on sterile cheesecloth in a polypropylene box consisting of two layers. The upper layer was used for seed germination and the lower layer was filled with the solutions. The seeds were cultivated at 25 °C at a relative humidity of 80% in the dark. The treatment solutions were changed every 24 h until the whole sprouts were harvested after 8 d (Fig. 1). The sulforaphane content increased significantly ($p < 0.05$) by 95% after treatment with SAEW 40 compared with tap water (Fig. 2A). All of the SAEW solutions increased the content of sulforaphane in broccoli sprouts compared with tap water, although the increase between using the SAEW 20 and tap water control treatments was not significant. SAEW 40 significantly enhanced the activity of myrosinase (Fig. 2B). Moreover, the number of microorganisms on the broccoli sprout decreased by 1.71 log CFU/g after using the SAEW with ACC value of 50 mg/L treatment compared with tap water treatment. Figure 3 shows that the bactericidal activity of SAEW on the surface of broccoli sprouts increased as the ACC values increased.

Overall, with a suitable ACC it can be a useful tool for enhancing the amount of sulforaphane and reducing the microbial counts on broccoli sprouts intended for fresh consumption as a functional food.

(S. Nirasawa, H. Liu, L. Li [China Agricultural University])

Fig. 1. Broccoli sprouts cultivated with slightly acidic electrolyzed water

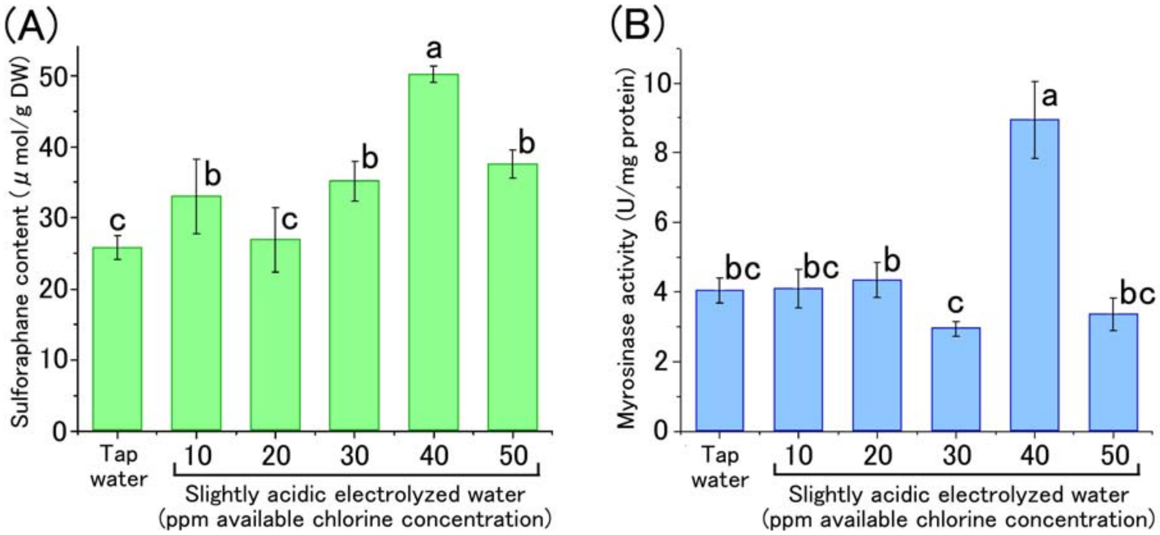


Fig. 2. Sulforaphane content (A) and myrosinase activity (B) of broccoli sprouts treated with different available chlorine concentrations of slightly acidic electrolyzed water.
Different letters mean statistically significant difference ($P<0.05$).

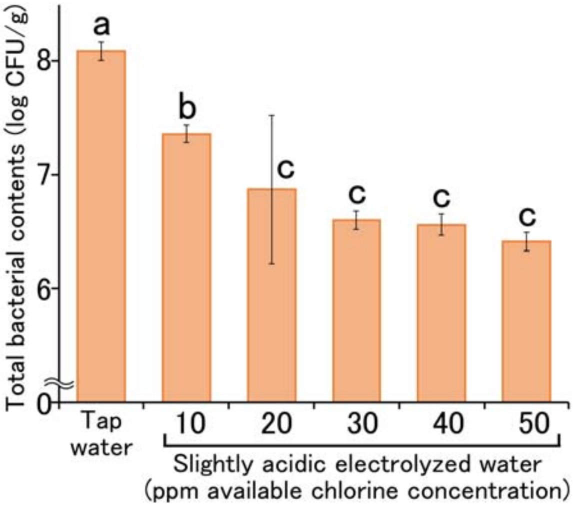


Fig. 3. Total bacterial counts on broccoli sprouts treated with slightly acidic electrolyzed water.
Different letters mean statistically significant difference ($P<0.05$).

pH adjustment of oil palm sap improves lactic acid fermentation

Oil palm sap (OPS) holds great promise as a naturally derived nutrient medium for microbes because it contains large amounts of amino acids, minerals, and free sugars. We have shown the usefulness of OPS as nutrient medium when we investigated ethanol production by fermentation using yeast. However, declining fermentation ability and delayed fermentation were observed during lactic acid fermentation. Lactic acid fermentation is very important in beverage applications and organic acid production, hence it is necessary to develop an improved method (of using OPS) that can also be applied for general purposes. We have developed a simple method to adjust the pH of OPS to remove fermentation inhibitors and revive lactic fermentation ability.

Lactic acid fermentation was obstructed by an ingredient of OPS, and the conversion efficiency of sugar was only about half of the theoretical yield (Table 1). When the pH of OPS was raised progressively, insoluble sediments were formed in weak alkali (Fig. 1). The insoluble sediments were removed and the pH of OPS returned to near neutral. Lactic acid fermentation ability was restored when treated OPS was used for lactic fermentation. In particular, when lactic acid fermentation using the treated OPS (which had been adjusted to alkaline pH 9.0) was performed, the lactic acid amount, the conversion rate, and productivity improved by 1.5-3 times in comparison with lactic acid fermentation that used untreated OPS (Table 1). Fermentation inhibitors such as aromatic compounds contained in plant raw materials have been thought to inhibit the growth of lactic acid bacteria. Identification of aromatic compounds in the treated and untreated OPS was performed by gas chromatography mass spectrometry, and it was found that aromatic compounds such as p-hydroxybenzoate, vanillin acid, syringe acid, p-coumaric acid, and ferulic acid greatly decreased in the treated OPS (Fig. 2).

(T. Arai, A. Kosugi, K. Balakrishnan [Universiti Sains Malaysia], S. Sudesh [Universiti Sains Malaysia])

Table 1. Lactic acid production from oil palm sap pretreated using acidic and alkaline precipitation by *Bacillus coagulans* strain 191

pH adjustment	Sugar concentration (g/L)	Concentration (g/L)	Yield (g/g)	Productivity (g/L/h)
Untreated (pH 5.6)	78.7 ± 2.4	42.7± 1.1	0.54	0.89
pH 6.0	61.4± 1.2	53.9 ± 2.2	0.88	2.25
pH 7.0	66.8 ± 0.7	57.1 ± 2.8	0.85	2.38
pH 8.0	65.9 ± 0.7	60.8 ± 2.5	0.92	2.53
pH 9.0	69.0 ± 2.3	63.3 ± 1.4	0.92	2.64
pH 10.0	66.4 ± 1.2	61.2 ± 2.8	0.92	2.55

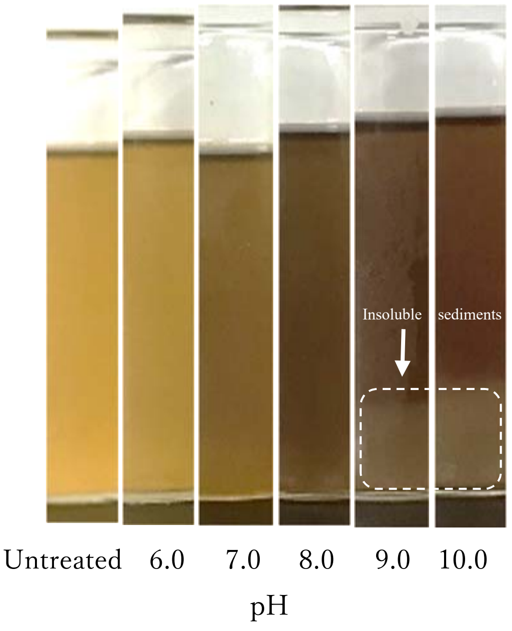


Fig. 1. Treatment of sap at various pH

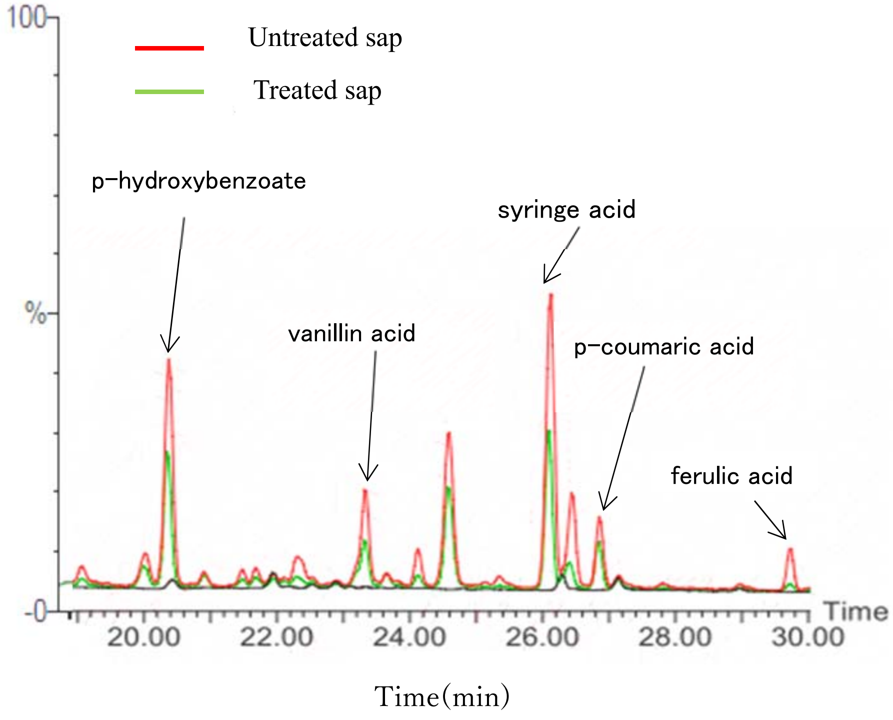


Fig. 2. Identification of aromatic compounds in the treated and untreated sap

Forecasting mass synchronized flowering in Southeast Asian tropical forest by analyzing the dynamics of flowering gene expression

In Southeast Asian tropical forests, irregular and prolonged synchronized flowering is observed not only on dipterocarp species (the major canopy species in these forests) but also on various tree species belonging to different taxa. Synchronized flowering is basically triggered by environmental factors such as low temperature and drought. However, the mechanisms and the relationship between these factors have not been well studied, making synchronized flowering difficult to forecast. Dipterocarp trees, generally known as Lauan and mainly used for timber and plywood, constitute about 80% of canopy species in Southeast Asian tropical forests. Difficulties on its vegetative propagation have forced us to make planting materials throughout seed collection during fruiting season. However, unpredictable flowering and seeding of dipterocarps restrict the planned production of planting materials. Furthermore, climate change could alter environmental factors, modifying the synchronized flowering pattern in the region and possibly affecting not only the regeneration of tropical tree species but also forest ecosystems. Therefore, we monitored the flowering phenology and expression of flowering genes of two dipterocarp species (*Shorea curtisii* and *S. leprosula*) for about four years, and developed a flowering forecasting model from climate data.

High homology of the RNA sequences with the *Arabidopsis* gene database indicates that the *FT*- and *LFY*-like genes of *S. curtisii* also regulate flowering of the species. These genes were expressed in leaf and bud tissues at least one month before synchronized flowering (Fig. 2). We then developed a flowering forecasting model to estimate the parameters of gene expression and degradation, and to determine the environmental factors that cause flowering. The estimation showed that both low temperature and drought were necessary for flowering. The environmental thresholds for daily temperature and precipitation (drought) ranged from 25.5 to 25.7 and from 156 to 182 mm over 9-11 weeks, respectively (Fig. 2), suggesting that lower temperatures and dry conditions (i.e., the values are below threshold over 9-11 weeks) express the flowering genes. Flowering is initiated after more than one month. The estimation can contribute to planned seed collection because it enables stakeholders to prepare seed collections at least 9-11 weeks before flowering.

Our model and estimated parameters can also be utilized for forecasting flowering under climate change conditions. We can input environmental data (temperature and precipitation) and incorporate climate change scenarios into our model to predict gene expression behavior, thus contributing to the discussion on the effects of climate change on forest regeneration and the ecosystem. It should be noted, however, that the estimated parameters and environmental conditions were based only on two flowering events over the four-year observation period, and that more monitoring data are required to develop a more precise model.

(N. Tani, A. Satake [Kyushu University], T. Ichie [Kochi University], S. Numata [Tokyo Metropolitan University], SH. Yeoh [University of Malaya], SL. Lee [Forest Research Institute Malaysia], N. Basherudin [FRIM], N. Muhammad [FRIM])

Fig. 1. Seeding of *S. curtisii* (at Semangkok Forest Reserve in July 2014)

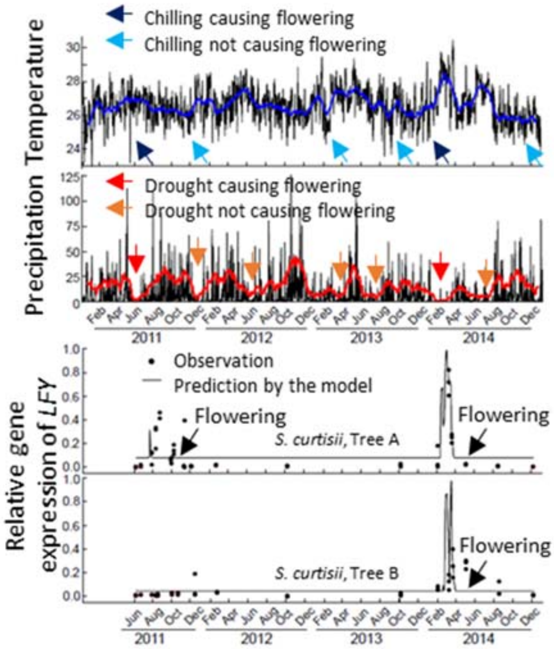
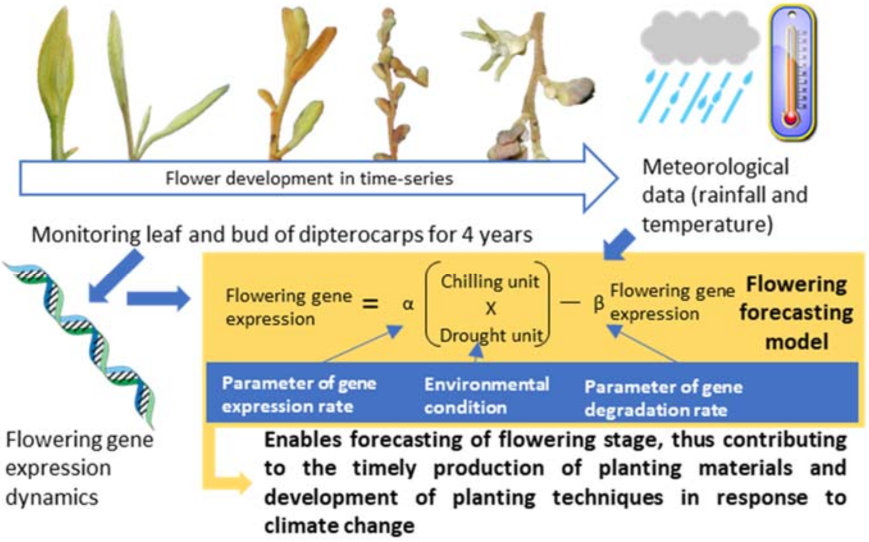


Fig. 2. Daily temperature and rainfall (top), and expression of flowering gene during observation period (bottom)



Enables forecasting of flowering stage, thus contributing to the timely production of planting materials and development of planting techniques in response to climate change

Fig. 3. Method for developing the flowering forecasting model

Development of a rearing technique for the free-swimming zoea larvae of the freshwater shrimp *Macrobrachium yui* from Northern Laos

In Laos, many freshwater prawns (*Macrobrachium* spp.) have been found in a variety of freshwater environments including rivers, ponds, and caves. They have high market value and thus are economically important for the local farmers. *Macrobrachium yui* (Holthuis 1950, Fig. 1), which mainly inhabits the northern part of Laos, fetches the highest price at the local market. The females of *M. yui* have been reported to migrate toward the inner part of the cave stream to spawn and hatch their eggs. After the zoea larvae hatch out (Fig. 1A), they stay inside the cave until development into postlarvae (Fig. 1B). Then, they change their habitat from the cave stream to the aboveground river and develop into juveniles. Thus, this species inhabits two different water environments in its life cycle. In Laos, *M. yui* catch has decreased dramatically, and the genetic diversity in some local populations has already been greatly reduced by environmental deterioration and overfishing. In order to aid in conserving and recovering local populations of *M. yui*, production of *M. yui* larvae may be one promising solution. In this study, we aimed to develop a technique for rearing *M. yui* larvae without using cave stream water.

According to the relationship between egg size and salinity tolerance of larvae of the *Macrobrachium* species, it was hypothesized that (1) the larvae of *M. yui* can develop at a certain water salinity during zoea larvae stage and (2) they can adapt to freshwater after development into postlarvae. We compared the survival rate of the zoea larvae when reared in 4 different salinities (0-10.5 ppt). Among the tested waters, the zoea larvae showed the highest survival rate, at 91.7 % on average, in 3.5 ppt (Fig. 2). Next, we tested acclimation to freshwater during the postlarval stage using three experimental treatments: rearing at 0 ppt salinity (freshwater) for both the first and second weeks (treatment I), rearing at 1.7 ppt for the first week and 0 ppt for the second week (treatment II), and rearing at 3.5 ppt salinity for both the first and second weeks (treatment III). The postlarvae survived better in treatments II (78%) and III (73%) than in treatment I (51%) (Fig. 3). Finally, the water from the Xuang river in Luang Prabang Province, Laos, where *M. yui* inhabits, was analyzed using ion chromatography to know ion concentrations. The concentrations of SO_4^{2-}, Mg^{2+}, and Ca^{2+} of 3.5 ppt artificial seawater were similar to those in cave water (Table 1).

This study succeeded in rearing zoea larvae of *M. yui* using 3.5 ppt artificial seawater instead of cave water. The postlarvae showed high survival rates when rearing salinities were gradually decreased. The technique developed in this study will contribute to artificial seed production of *M. yui* in aquaculture facilities without cave water, and help in recovery efforts for the endangered local populations of *M. yui*.

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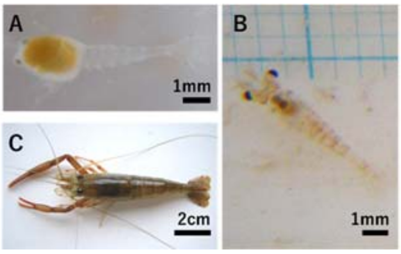

Fig. 1. The developmental stages of *M. yui*.
A: Hatched larva
B: Postlarva
C: Female broodstock

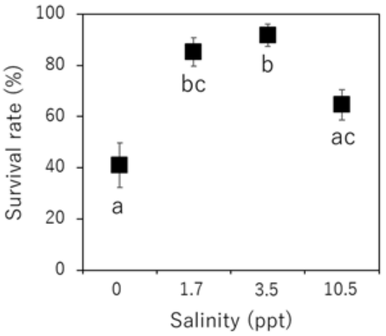

Fig. 2. Survival rates of larvae during the free-swimming zoea larval stages when reared at 4 different (0-10.5 ppt) salinities. The results are expressed as the mean ± SE of 23 replicates. Different letters indicate significant differences ($P < 0.05$).

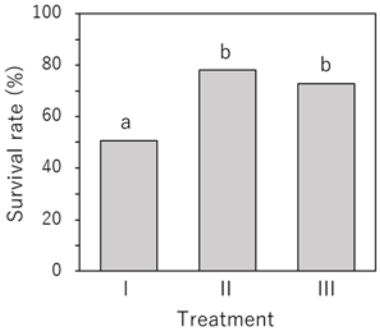

Fig. 3. Survival rates of postlarvae during the 2 weeks after settling to the bottom, in three treatment groups. Treatment I: n = 93, 100, and 107 in Treatment I, II, and III, respectively. Different letters indicate significant differences ($P < 0.01$).

Table 1. The concentration (mg/l) of major ions in 3.5 ppt artificial seawater, cave stream water, and aboveground water. The U and P values indicate statistical results of the comparison of seven ions between cave stream water and aboveground water by the Mann-Whitney U test.

Ions	Artificial seawater at 3.5ppt*	Cave stream water (n=4) (mean (SE))	Aboveground water (n=12) (mean (SE))	*U* value	*P* value
Cl^-	1767.9	1.04 (0.14)	0.34 (0.12)	53	0.025
NO_3^-	N.D.**	2.06 (0.63)	0.19 (0.06)	57	0.006
SO_4^{2-}	270.4	67.48 (46.88)	9.52 (3.96)	52	0.034
Na^+	1053.9	6.34 (0.46)	8.85 (1.33)	28	0.505
K^+	41.5	0.68 (0.06)	1.07 (0.09)	12	0.009
Mg^{2+}	134.9	16.61 (3.60)	8.49 (1.48)	53	0.025
Ca^{2+}	62.5	108.28 (4.80)	41.18 (5.12)	58	0.004

*Kester et al., (1967) Limnol. Oceanogr., 12: 176-179.

**N.D., no data