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Research Highlights 2001

Major Research Results from April 2001 to March 2002

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Conformity of agricultural land use and physical stability in Khon Kaen, Northeast Thailand

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Key words : GIS, land evaluation, land suitability, land use, Northeast Thailand

Objectives

In order to consider sustainable land use in Northeast Thailand, the land suitability for cultivation of major crops such as rice, sugarcane and cassava was evaluated based on physical factors, and the conformity of suitability with current land use was revealed using Geographic Information System (GIS) technology and a multi-temporal satellite data set acquired by the LANDSAT-5/Thematic Mapper (TM). The study site was the area within 102 °30' E-103 °00' E and 16 °00' N-16 °30' N located in Khon Kaen Province.

Results

Land suitability for rice, sugarcane and cassava was determined by the assessment of soil properties and water resource availability (Fig. 1). The location of suitable areas for sugarcane and cassava overlapped in the hilly southeastern section of the site. Suitable land for rice was found in the lowland areas near the Chi River, which was unsuitable for upland crops.

Land use map was produced using three TM data sets acquired in July 1989, October 1998 and March 1999 corresponding to the middle and the end of rainy season and the middle of dry season (Fig. 2). More than 60% of the study site was used for agriculture; 120,000 ha or 36% was upland crops and 90,000 ha or 28% was paddy.

The conformity of land use and land suitability were shown by crossing operation in GIS (Table 1). The results were;

1) The suitable areas for sugarcane and cassava were more than 130,000 ha, but that for rice was only 30,000 ha.
2) Fields located in suitable areas were 55% upland crops and 12% rice in current land use area.
3) 40% of the suitable area was used for suitable crops but a large portion of the paddy fields were irrigated. Rainfed paddy located in suitable areas was very scarce.

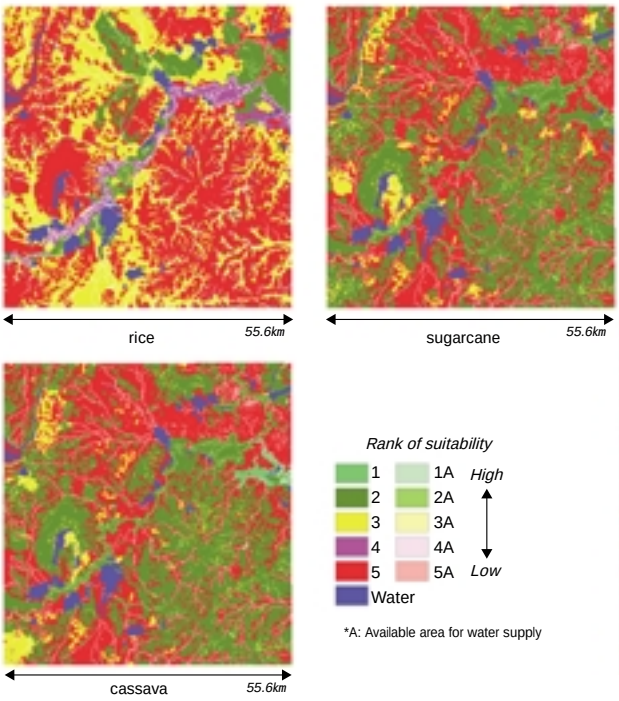

Fig. 1. Land suitability for rice, sugarcane and cassava, evaluated by soil properties and water resource availability.

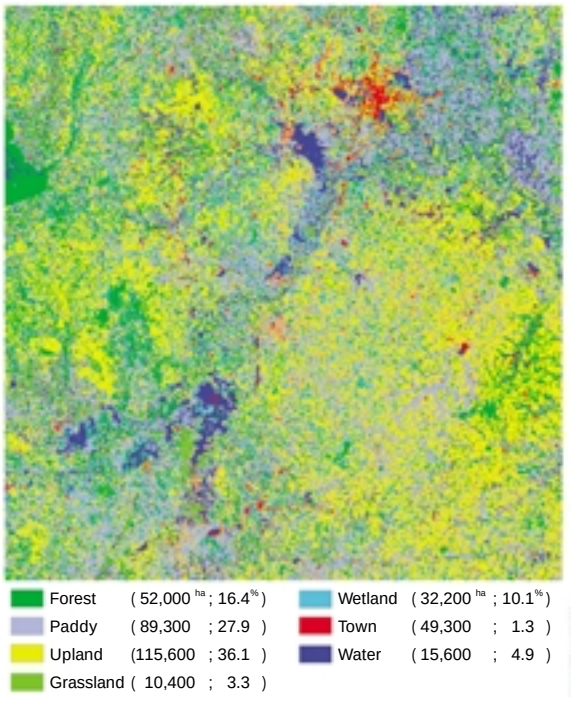

Fig. 2. Land cover / land use map classified by PCA1&2 of TM8907, TM9810 and TM9903.

Table 1. Conformity of land use and land suitability.

		Rice	Sugarcane	
Rank 1	A. area (1000 ha)	0.0	0.0	3.2
	B. conformed land use (1000 ha)	0.0	0.0	1.2
	C. comformity (B/A)	*0.00*	*0.00*	*0.39*
	D. share of current use (B/E)	*0.00*	*0.00*	*0.01*
Rank 2	A. area (1000 ha)	29.0	132.3	131.2
	B. conformed land use (1000 ha)	12.1	55.7	55.2
	C. comformity (B/A)	*0.42*	*0.42*	*0.42*
	D. share of current use (B/E)	*0.14*	*0.48*	*0.48*
Rank 3	A. area (1000 ha)	93.2	18.2	16.1
	B. conformed land use (1000 ha)	33.2	6.6	5.9
	C. comformity (B/A)	*0.36*	*0.36*	*0.36*
	D. share of current use (B/E)	*0.37*	*0.05*	*0.05*
Rank 4	A. area (1000 ha)	23.5	0.3	0.3
	B. conformed land use (1000 ha)	6.7	0.1	0.1
	C. comformity (B/A)	*0.29*	*0.25*	*0.25*
	D. share of current use (B/E)	*0.08*	*0.00*	*0.00*
Rank 5	A. area (1000 ha)	138.7	133.5	133.5
	B. conformed land use (1000 ha)	28.6	34.3	34.3
	C. comformity (B/A)	*0.21*	*0.26*	*0.26*
	D. share of current use (B/E)	*0.32*	*0.30*	*0.30*
others (city, awamp, etc.)		35.5	35.5	35.5
Total (1000 ha)		319.8	319.8	319.8
E. Current land use (1000 ha)		89.3	115.6	115.6

Reference

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Identification of target genes of the DREB1A transcription factor controlling abiotic-stress-responsive gene expression using a full-length cDNA microarray

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Key words : abiotic stress, transcription factor, transgenic plant, stress tolerance, microarray

Objectives

Crop productivity is greatly affected by abiotic stresses such as drought, high salinity, and low temperature. Genetic engineering possesses high potential to improve the stress tolerance of crops through the use of gene transfer technology. A *cis*-acting promoter element DRE plays an important role in regulating gene expression in response to these stresses. We have reported that the *Arabidopsis* transcription factor DREB1A binds to DRE and controls expression of many stress tolerance genes. Overexpression of DREB1A in transgenic *Arabidopsis* activates the expression of target stress tolerance genes and results in improved stress tolerance. To understand how overexpression of DREB1A in transgenic plants increases stress tolerance, cDNA microarray analysis was employed to identify the DREB1A target genes.

Results

First, a cDNA microarray using 1,300 full-length *Arabidopsis* cDNAs was prepared. mRNAs prepared from transgenic *Arabidopsis* plants that overexpress DREB1A under the control of the CaMV 35S promoter (35S:DREB1A), and wild-type control plants were used for the preparation of Cy3-labeled and Cy5-labeled cDNA probes, respectively. These cDNA probes were then hybridized with the cDNA microarray (Fig. 1). Twelve genes were identified as target genes of DREB1A. On the basis of RNA gel blot and microarray analyses, six of them were identified as novel drought- and cold-inducible genes that are controlled by DREB1A (Fig. 2). These target genes contained DRE in their promoter regions.

We comprehensively analyzed further novel target genes using a 7,000 full-length cDNA microarray. More than 40 genes were identified as target genes of DREB1A and confirmed by RNA gel blot and promoter analyses. These target genes encoded enzymes required for the biosynthesis of osmoprotectants such as proline and sugar, membrane proteins, LEA proteins, detoxification enzymes, chaperones and transcription factors. These results indicated that overexpression of the DREB1A proteins in transgenic plants activated more than 40 stress tolerance genes and resulted in improved stress tolerance.

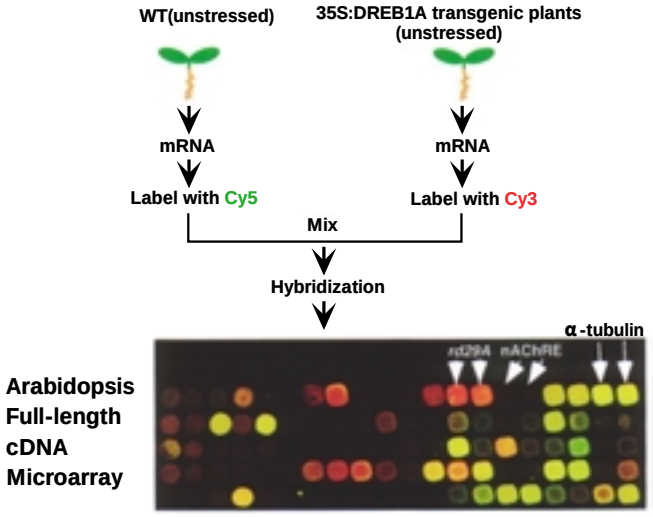

Fig. 1. Strategy for identification of DREB1A target genes. mRNAs from 35S:DREB1A transgenic plants and wild-type(WT) unstressed plants were used for the preparation of Cy3-labeled and Cy5-labeled cDNA probes, respectively. These cDNA probes were mixed and hybridized with the cDNA microarray. In this study, we used the α-tubulin gene as an internal control.

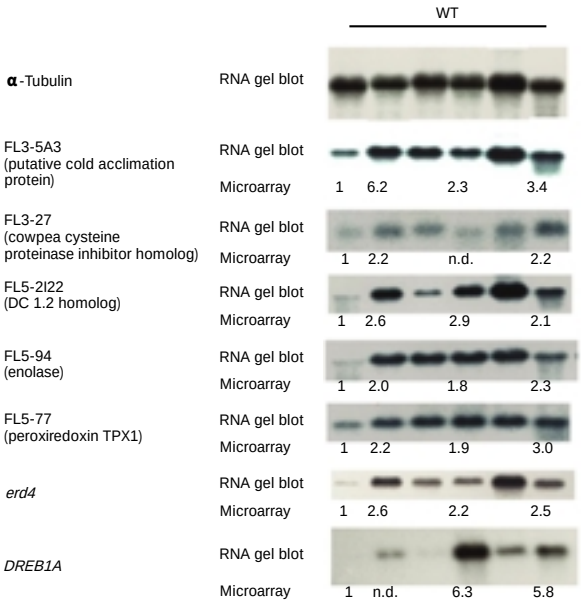

Fig. 2. Comparison of cDNA microarray and RNA gel blot analyses for the identification of new DREB1A target genes controlled by the DREB1A gene.

References

M. Seki, M. Narusaka, H. Abe, M. Kasuga, K. Yamaguchi-Shinozaki, P. Carninci, Y. Hayashizaki and K. Shinozaki (2001) : Monitoring the expression pattern of 1300 *Arabidopsis* genes under drought and cold stresses by using a full-length cDNA microarray. The Plant Cell, 13(1), 61-72.

M. Seki, M. Narusaka, K. Yamaguchi-Shinozaki, P. Carninci, J. Kawai, Y. Hayashizaki and K. Shinozaki (2001): *Arabidopsis* encyclopedia using full-length cDNAs and its application. Plant Physiology and Biochemistry, 39, 211-220.

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Simple and rapid method for the detection of lipoxygenase isozymes in soybean seeds

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Key words : soybean, lipoxygenase isozymes, bleaching abilities, screening method

Objectives

In South American countries, the soybean has been used mainly as a source of edible oil and in livestock feed, while direct food consumption, as is common in Asia, is very limited. Soybeans used for the purposes of food consumption, however, possess great potential for improving the nutrition and health of the local population and for generating income. The limitation on direct consumption in this region is associated with undesirable flavors in soybean food products. Since lipoxygenases have been found to be responsible for grassy-beany flavors, it would therefore be highly desirable to remove these enzymes in locally-adapted soybean cultivars. Through the use of mutants lacking lipoxygenases, L-1, L-2, and L-3, the genetic approach towards breeding triple mutant soybean lacking all isozymes has been employed, aiming to reduce undesirable flavors. In addition, a lipoxygenase isozyme detection method suitable for routine screening is becoming an increasingly utilized approach. The presence or absence of the three lipoxygenase isozymes can be readily and accurately determined by spectrophotometric methods, based on the different bleaching abilities of L-1, L-2 and L-3 isozymes in contact with methylene blue and ß-carotene. However, this method is time-consuming, as different seed samples are necessary for the analysis of each isozyme. The Division's attempt was based upon this above method and was carried out to develop a simpler and more rapid visual method for the detection of individual lipoxygenase isozymes in soybean seeds.

Results

After several attempts, we succeeded in finding a method by which identification of the lipoxygenase isozymes was possible using only a small amount of milled soybean seed samples (Table 1). The new method also enabled the identification of two isozymes, L-1 and L-3, by using the same seed sample and adding the reaction reagent for the L-1 isozyme (Fig. 1). Within five minutes, it was possible to visually determine the individual lipoxygenase phenotypes as follows: colorless – normal soybean having all isozymes; yellow– single mutant lacking L-3; blue – double mutant lacking L-1 and L-2; green – triple mutant lacking all isozymes (Figs. 1, 2). In crosses between triple mutant soybeans and normal soybeans, the other test which is used to identify the L-2 isozyme may be omitted, since the *Lx1* locus is closely linked to the *Lx2* locus. By using this simplified new method, the genetic variation of these isozymes contained in South American soybean cultivars and genetic lines can be efficiently researched.

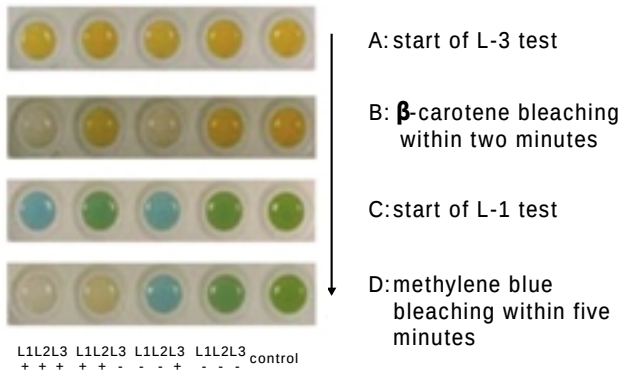

Fig. 1. Visual detection of individual lipoxygenase phenotypes of soybean seeds derived from crosses between triple mutant lacking all isozymes and normal type.

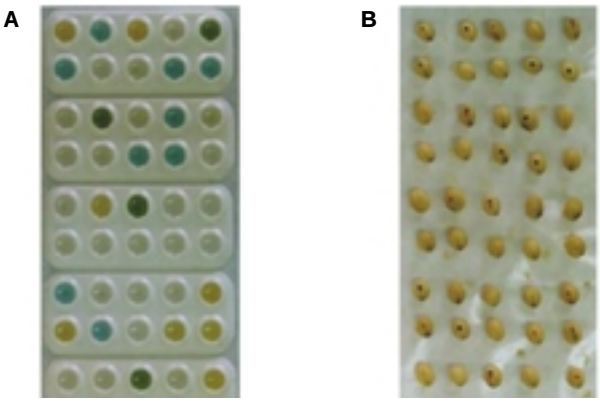

Fig. 2. Analyses of F_2 seeds derived from the cross between triple mutant lacking all isozymes and normal type.
A: result of analyses, B: seeds corresponding to analyses

Table 1. Comparison of original (Suda *et al.*, 1995) and modified methods to identify lipoxygenase isozymes in each soybean seed.

	original	modified
For L-3 test:		
seed material	2.5mg	2.5mg
cludely extracted solution of L-2	0.5ml	10µl
reaction reagent for L-3	2.0ml	250µl
(composition) 0.2M sodium phosphate buffer (pH6.6)	25.0ml	12.5ml
10mM sodium linoleate substrate	5.0ml	5.0ml
distilled water	5.0ml	17.5ml
saturated solution of β-carotene	5.0ml	5.0ml
For L-1 test:		
seed material	2.5mg	not nec.
distilled water	0.5ml	not nec.
reaction reagent for L-1	2.0ml	250µl
(composition) 0.2M sodium borate buffer (pH9.0)	25.0ml	25.0ml
10mM sodium linoleate substrate	5.0ml	5.0ml
distilled water	5.0ml	5.0ml
100mM methylene blue	5.0ml	5.0ml

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Tagging of slow rusting genes for leaf rust, *Lr34* and *Lr46*, using microsatellite markers in wheat

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Key words：durable resistance, QTL analysis, rust, slow rusting, wheat

Objectives

Leaf rust is the most serious disease in wheat. The use of resistant cultivars is very important in achieving sustainable wheat production in developing countries for economic and environmental reasons. Since race-specific genes may be overcome through genetic shifts or new form of virulence in the pathogen population, durable resistance genes are of great interest to wheat breeders. Slow-rusting genes such as *Lr34* and *Lr46*, which are widely used in the CIMMYT breeding program, have been identified to be race-nonspecific and durable. However, the contribution of each gene is small and easily affected by environmental factors, and therefore it is difficult to identify the genes due to their race non-specificity. Tagging these genes with molecular markers increases the efficiency of selecting the resistance genes and facilitates marker-assisted selection for leaf rust.

Results

A doubled haploid (DH) population was produced from cv. Fukuho-komugi x cv. Oligoculm by means of wheat x maize crosses. One hundred and seven DH lines were genotyped for 595 markers, such as microsatellites, RFLP and RAPD. Of the 443 markers mapped, 343 markers were used to construct a framework map to perform QTL analysis for leaf rust resistance in this population. Leaf rust severity was recorded in the field in 2000 and 2001, in Sonora State, Mexico (Fig. 1).

Two QTLs for leaf rust severity were detected on 7DS and 1BL by means of composite interval mapping (CIM, Fig. 2). These QTLs were considered to be due to the effects of the known slow-rusting genes, *Lr34* and *Lr46*. The QTL analysis also indicated that *Lr34* was derived from Fukuho-komugi, while *Lr46* was derived from Oligoculm. These QTLs accounted for about 40% and 26% of the total variation, respectively.

Table 1 shows genetic effects of the two marker loci, *Xgwm295.1* on 7DS and *Xwmc44* on 1BL, which were linked to these QTLs. The mean differences in leaf rust severity were 40.1% for *Xgwm295.1* and 11.3% for *Xwmc44*. Resistant genotype (F/O) was 61.6% more resistant than the susceptible genotype (O/F), and 19.9% more resistant than the overall population mean. Moreover, genotyping of the DH lines using these molecular markers aided in distinguishing the lines with *Lr34* and *Lr46* from those with only *Lr34*. These results indicate that the molecular marker loci *Xgwm295.1* and *Xwmc44* facilitate the identification of *Lr34* and *Lr46* in breeding materials and contribute to the pyramiding of leaf rust resistant genes.

This study was conducted as part of a collaborative program between the Biological Resources Division, JIRCAS, and the Applied Biotechnology Center, CIMMYT, from January 1998 to January 2002.

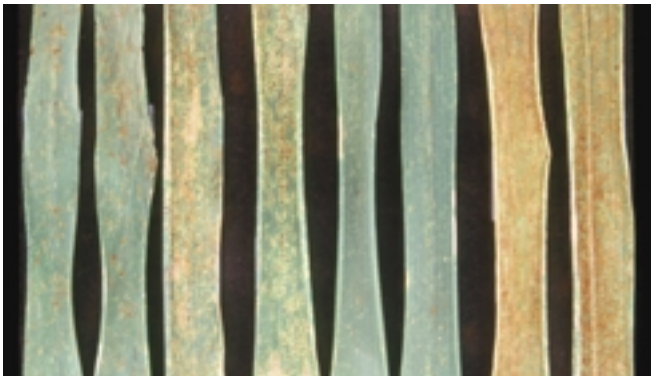

Fig. 1. Symptoms of leaf rust-infected leaves. From left to right (two leaves each): Fukuho-komugi (*Lr34*), Oligoculm (*Lr46*), resistant and susceptible DH lines.

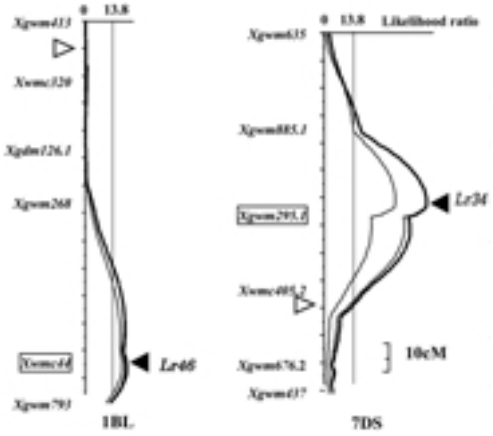

Fig. 2. Likelihood ratio (LR) contour by composite interval mapping for QTL detection of leaf rust severity on chromosome 1B long arm and 7D short arm. Bold contour indicates LR by joint analysis of data for the years 2000 and 2001. LR thresholds, equivalent to LOD=2.5, are 11.5 for a single year and 13.8 for joint analysis. Short arms are toward the top. ▷ indicates centromere.

Table 1. Genotypic effects of flanking loci on leaf rust severity (%).

Locus	Genotype[a]	2000	2001	Mean	
Xgwm295.1	**F**	**12.6**	**10.9**	**11.9**	
(Lr34, 7DS)	**O**	**57.1**	**46.6**	**52.1**	
	dif.	**-44.5**	**-35.7**	**-40.1**	
Xwmc44	**F**	**47.6**	**41.4**	**44.9**	
(Lr46, IBL)	**O**	**23.2**	**16.8**	**19.5**	
	dif.	**24.4**	**24.6**	**25.5**	
Xgwm295.1	**F/O**	**12.6**	**10.1**	**11.3**	**a**[b]
/Xwmc44	**F/F**	**11.6**	**12.5**	**12.5**	**a**
	O/O	**32.8**	**25.6**	**30.2**	**b**
	O/F	**80.4**	**66.4**	**73.0**	**c**
	dif.(Fo vs. OF)	**-67.8**	**-56.3**	**-61.6**	

a F, O and dif. indicate Fukuho-komugi, Oligoculm and genotypic difference between F and O genotypes. F and O are resistant and susceptible for *Lr34*, and susceptible and resistant for *Lr46*, respectively.

b Mean values with different letters are significantly different ($P<0.05$).

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Alternative tillage system for upland cropping in Northeast Thailand

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Key words：sugarcane, sandy soil, hardpan, subsoiling, reduced tillage

Objectives

In recent decades, the development of sugarcane cropping in Northeast Thailand has been accompanied by the utilization of heavy machines rather than draft animals. The operation of heavy machinery for land preparation and product transportation damages the physical condition of the sandy soil, which is extremely vulnerable to mechanical and climatic impacts. In this type of soil, it is not unusual to find hardpan soil having bulk density as high as 1.7 in layers 30-40 cm deep; hardpan soil not only aggravates problems of water runoff and erosion through poor water percolation but, through the limitation of root development, also causes crop growth to be more sensitive to environmental stresses.

Results

In order to find solutions to this problem, the effects of subsoiling treatment were studied in comparison to the conventional tillage system prevalent in sugarcane cropping in Northeast Thailand. With regard to rainy season cropping, the effects of subsoiling on maize yield were not readily apparent during years of abundant precipitation but were quite profound during years of less precipitation. Furthermore, deeper root development of sugarcane was induced and the survival rate of seedlings during the dry season was found to have increased with subsoiling treatment. Soil erosion was also ameliorated through the high levels of water percolation resulting from subsoiling.

Based on the results above, a new working attachment simultaneously incorporating subsoiling, fertilizing and planting functions was developed by modifying a common sugarcane planter (Fig. 1). A chisel with this attachment was useful in breaking up hardpan up to 60 cm in depth and reducing the hardness of the soil from 23 kg·f/cm^2 to less than 10 kg·f/cm^2; this enabled sugarcane to be planted without ridging. It was suggested that this attachment could also work well under no-tillage conditions. Under the conventional system for land preparation and planting, fuel consumption and working time amounted to 50 liters and 15 hours per ha. By using the newly developed attachment, the necessary amount of fuel and time could each be reduced by one-third (Fig. 2). Moreover, the number of required tractor operations could be reduced from five times under the conventional system to just once under the new alternative system, which would also contribute towards reducing the formation of hardpan. The rooting of sugarcane was improved through the use of the new attachment under no-tillage conditions and the growth of sugarcane was superior to that under the conventional planting system (Table 1).

In order to make this system practical and applicable to upland cropping in Northeast Thailand, it is necessary to conduct further studies on the management of sugarcane residue and the control of weeds during the fallow period after the end of ratooning.

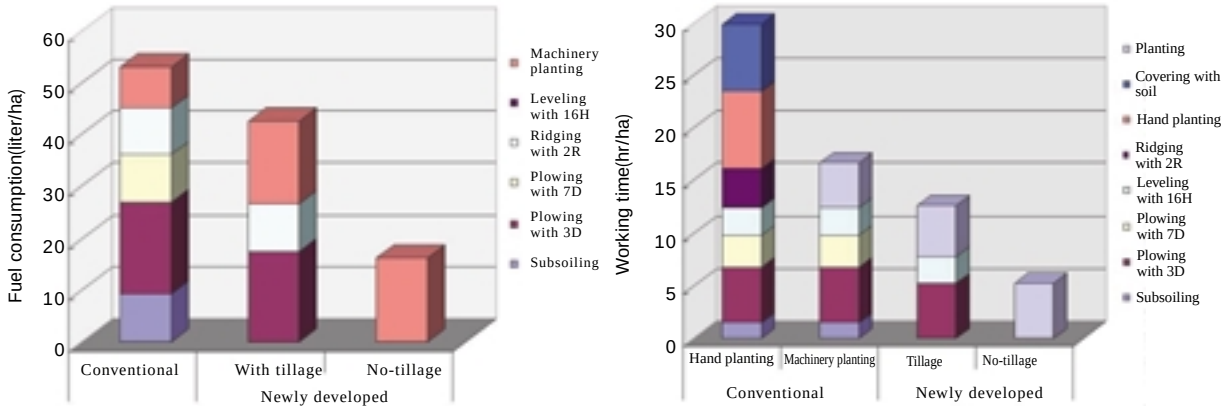

Fig. 1. Comparison of fuel consumption and working times among several planting systems.

Fig. 2. Effects of subsoiling treatment on the growth of sugarcane planted under no-tillage conditions.

Table 1. Effects of planting methods with/without tillage on the growth of sugarcane.

		F.W. of stalk (t/ha)	No. of stalks (/m^2)	Stalk length (cm)	Stalk diameter (mm)
Tillage	**Hand planting**	63.7 ± 12.6	5.0 ± 0.7	206 ± 17	27.5 ± 1.3
	Commercial planter	47.0 ± 10.2	4.9 ± 0.6	178 ± 20	28.3 ± 0.7
	Developed planter	89.3 ± 13.8	6.0 ± 0.3	234 ± 21	29.9 ± 0.3
No-tillage	**Commercial planter**	65.1 ± 5.2	5.9 ± 0.3	186 ± 0	26.9 ± 0.7
	Developed planter	99.6 ± 7.0	5.8 ± 0.2	248 ± 6	29.9 ± 0.9

Note: Sampling was carried out on Sept. 10th 2002. Stalk diameter was determined at the middle of stalk. The values in the table indicate average ± S.E. (n=4). F.W.:Fresh weight

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Eco-physiological characteristics and yielding ability of *Erianthus* spp.

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Key words : *Erianthus* spp., forage production, root development, tolerance to environmental stresses

Objectives

Erianthus spp. is a sugarcane relative that is found throughout the year at various sites ranging from mountainous areas to swamps and riversides in Thailand. Because of its ability to flourish in such diverse environments, its importance as a genetic resource in sugarcane breeding and as a source of roughage for livestock has achieved widespread recognition. With regard to these functions, the eco-physiological characteristics of *Erianthus* were studied in relation to soil moisture, and its yielding abilities were evaluated under the application of animal feces in comparison with napiergrass and maize.

Results

Starting in October 1999, at the beginning of the dry season, root development and the physical conditions of soil were investigated through analysis of soil profiles of neighboring *Erianthus* and napiergrass canopies. Irrespective of plural hardpans and waterlogged conditions, root development of *Erianthus* was observed in layers as deep as 250 cm, while roots of napiergrass were rarely found deeper than 135 cm in soil which was almost completely saturated with water (Fig. 1). The results of soil pF monitoring in the field showed that water loss from soil surface was minimal and considerable amounts of available water existed in the deeper soil layers even in March when there was no vegetation. Meanwhile, the results from pot experiments with adjusted ground water levels suggested that waterlogged conditions were more favorable for the biomass production of *Erianthus* than dry conditions, and well-developed aerenchyma were observed on the roots of plants grown under waterlogged conditions. Therefore, the drought tolerance of *Erianthus* can be attributed to its ability to extend its root system during the rainy season and to take up water from deeper soil layers during the dry season.

The growth and yielding abilities of three forage species including *Erianthus* were evaluated for two years in the field after several fertilizer treatments. The rooting and growth of *Erianthus* after planting were rather slow and its dry matter yield (DMY) was considerably lower than napiergrass, although higher than maize in the first year of planting. However, in the second year, *Erianthus* tended to show higher DMY than napiergrass in all plots excluding those which had received the highest rate of dried feces application, and the maximum DMY of *Erianthus* amounted to 30 t/ha after combining the yields of four harvests. In addition, *Erianthus* showed better yields than did napiergrass under conditions without nitrogen fertilization. There was significant reduction in the DMY of maize and napiergrass associated with the decline of soil pH caused by the annual application of ammonium sulfate, while there was no reduction in the DMY of *Erianthus* (Fig. 2). These results indicate the high yielding ability of *Erianthus*, especially under the acidic and infertile soil conditions common to Northeast Thailand.

The results detailed above suggest the high adaptability of *Erianthus* to the conditions prevalent in Northeast Thailand and its usefulness in sugarcane breeding and biomass production; however, it is necessary to conduct further research focusing on its practical uses as roughage for livestock.

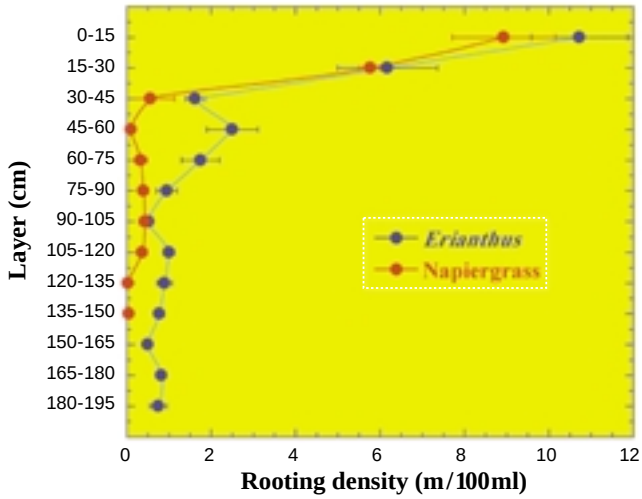

Fig. 1. Root distribution of *Erianthus* spp and napiergrass.

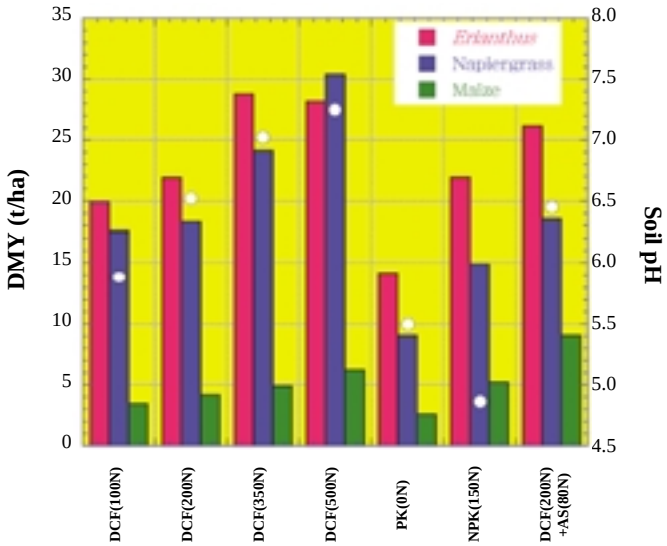

Fig. 2. Dry matter yields of three forages in second year of planting.

Notes: simbol (○) soil pH; DCF dried cattle feces; AS ammonium sulfate; values in parentheses indicate the amount of material in terms of nitrogen.

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Sustainability of sugarcane production evaluated based on N_2 fixation and organic matter cycle

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Key words : N_2 fixation, natural ^{15}N abundance method, organic matter cycle, sugarcane

Objectives

In Northeast Thailand, infertile sandy soil with limited ability to supply and retain nutrients is distributed over large areas. Crops with the ability to fix atmospheric nitrogen endophytically could contribute toward the establishment of sustainable agriculture in these areas, and taking this into consideration, analyses on N_2 fixation in sugarcane and the organic matter cycle of sugarcane production were conducted to evaluate their sustainability.

Results

Possible N input by N_2 fixation in sugarcane planted in the research fileds was estimated by the natural ^{15}N abundance ($\delta^{15}N$) method using cassava as a presumed non-N_2-fixing reference plant. All seven varieties of sugarcane planted in the fields of the research centers showed clearly lower $\delta^{15}N$ values than those of cassava. Average of N input by N_2 fixation in the seven sugarcane varieties was 29% (Fig. 1).

Possible N input by N_2 fixation in field-grown sugarcane was also estimated by the natural ^{15}N abundance ($\delta^{15}N$) method using neighboring weeds growing inside the sugarcane fields as presumed non-N_2-fixing reference plants. Of the total of 54 sugarcane samples, $\delta^{15}N$ values of 19 samples were markedly lower than those of all neighboring plants. Average contribution of N_2 fixation to total plant N of these 19 sugarcane samples was estimated to be 32% (Table 1).

In order to determine the organic matter cycle of sugarcane production, only the stems were removed from the field and most of the other parts remained as residues. Thus, most of the nutrients contained in the residues were returned to the field. In the process of sugar refining at sugar mills, bagasse, filter cake and molasses are discharged as by-products. The main element of the sugar, bagasse and molasses taken out of this organic matter cycle is carbon, on the other hand, filter cake contains large amounts of nutrients. In order to maintain a sustainable cycle of nutrients, the leaves of sugarcane should not be burned during harvesting and filter cake should be properly returned to the field (Fig. 2).

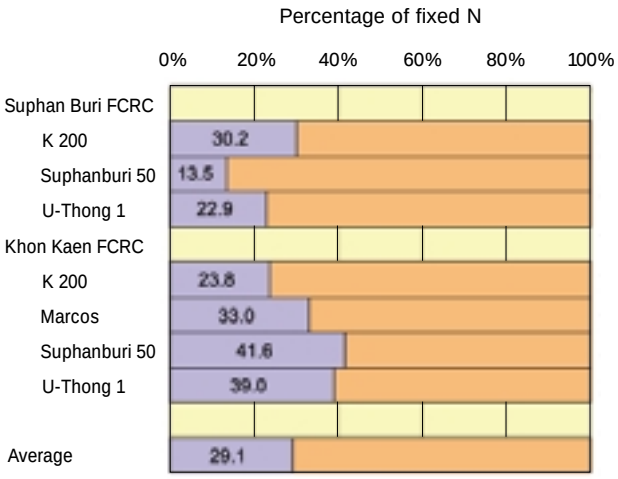

Fig. 1. Estimation of N_2 fixation in different varieties of sugarcane planted in research fields based on the natural ^{15}N abundance method.

Table 1. Estimation of N_2 fixation in field-grown sugarcane in Thailand.

Regions	No. of samples	No. of positive samples[a]	%Ndfa[b] of positive
Norheast	21	7	28
Central	33	11	35
Toral	54	19	32

[a]Number of samples with $\delta^{15}N$ values lower than those of all neighboring plants.
[b]Estimated % nitrogen derived from N_2 fixation.

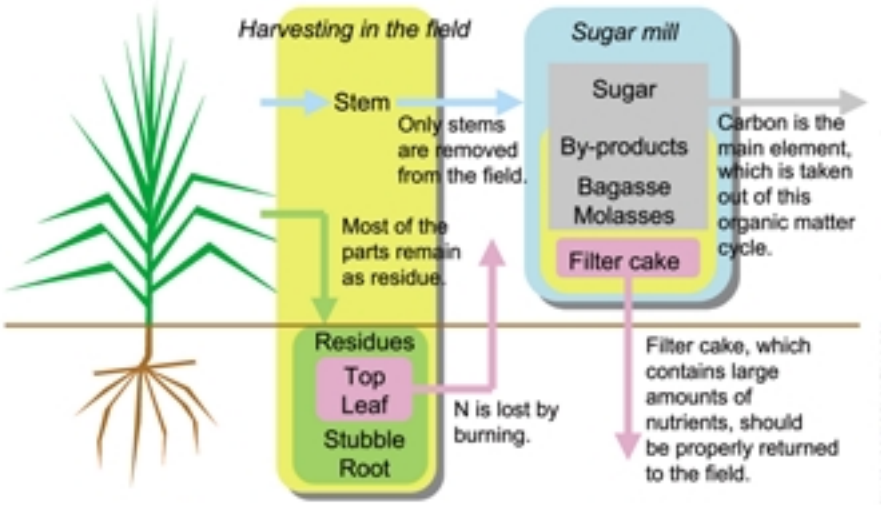

Fig. 2. Organic matter cycle of sugarcane production.

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Technologies for rainfed rice production in Northeast Thailand

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Key words：direct seeding, weed control, land reform

Objectives

The paddy fields of Northeast Thailand account for 55% of paddy area in the country and play a critical role in national food production. However, yield of rice is low and fluctuates due to the rainfed condition without irrigation. Shortage and rising cost of labor threaten the sustainability of production with conventional hand transplanting. To overcome these constraints, a set of technologies was developed for efficient water utilization.

Results

Introduction of dry direct seeding of rice was compatible to irregular rainfall and labor shortage. Growth and yield of direct seeded rice matched those of transplanted rice; and no-tillage was superior to tillage when the amount of rainfall was scarce as a result of higher soil moisture under undisturbed soil conditions. A no-tillage seeder with rotary disk and drill seeder was manufactured and applied in large-scale field trials, which resulted in equal or higher rice yield compared with vicinal transplanted fields (Fig. 1).

Weed control based on soil moisture regime was developed to suppress the dominant paddy weed species, *Cyperaceae*. At the soil moisture rates of 20%, emergence of *Cyperacea* was markedly inhibited (Fig. 2), while the direct seeded rice variety "KDML105" emerged vigorously. The optimum sowing time should be adjusted in order to facilitate adequate germination of rice while suppressing the emergence of weeds.

Paddy fields with dikes surrounded by thick plastic sheets or solidified by soil conditioner (magnesium compounds) were constructed to prevent water leakage. Changes in the depth of standing water before and after construction proved the enhanced water holding capacity of paddy fields with the implementation of both treatments (Fig. 3).

Fig. 1. Large-scale field trial for no-tillage direct seeding cultivation of rice.

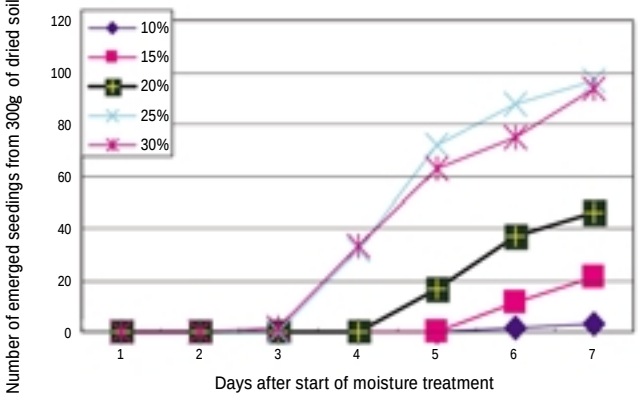

Fig. 2. Emergence of *Cyperaceae* weeds at different soil moisture rates in the soil of rainfed paddy fields.

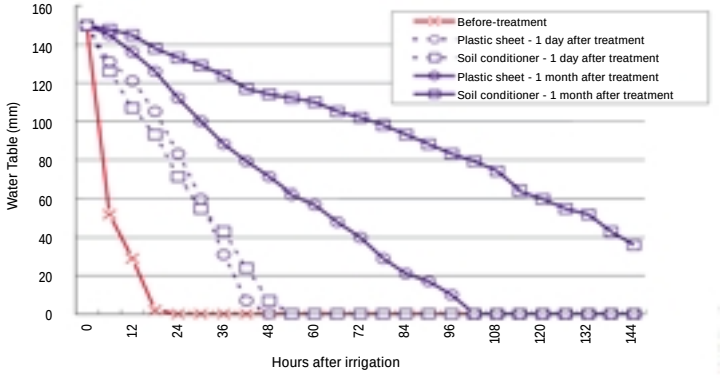

Fig. 3. Changes in the depth of standing water after irrigation.

Reference

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Evaluation of rainfall station networks in tropical monsoon areas

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Key words：tropical monsoon area, rainfall station network

Objectives

Large-scale irrigation projects consisting of reservoirs contribute towards stabilizing rainy season rice cropping in Southeast Asia, but the water storage capacity of some reservoirs is not sufficient for double cropping. Therefore it is necessary to promote appropriate release reduction by monitoring the average amaunt of rainfall in irrigation systems. This study aims to propose a method for evaluating rainfall station density.

Results

The study was carried out in the Muda Irrigation Scheme located in the northwestern region of Peninsular Malaysia (Photo 1). It covers an area of 126,000 ha, of which 96,000 ha consists of rice fields.

First, the spatial variability of rainfall was investigated in the target area. In this study, the simplified Horton method was used. Although the equation is very simple, it accurately portrays the spatial variability of rainfall because rainfall caused by convective lifting is predominant in the tropical monsoon area.

The next step was the simulation of rainfall events with specified rainfall over an area in which rainfall stations were evenly scattered. Accuracy of observation for simulated rainfall events under the given station density was calculated, and the relationship between the accuracy of observed rainfall and rainfall station density was obtained after appropriate simulations.

Standard error of rainfall as a function of station density in the Muda Irrigation Scheme is shown in Fig. 1; the evaluation of the rainfall station network implemented in Isohara by the Meteorological Agency of Japan is shown for comparison.

The rainfall station network began with 20 stations and has expanded to 61 stations in the Muda Irrigation Scheme (Photo 2). The effects of this further investment are not yet readily apparent, but Fig. 1 shows that the expansion of the network has improved the accuracy of rainfall observations.

Photo 1. Aerial view of the Muda Irrigation Scheme.

Photo 2. Rainfall station equipped with a VHF radio.

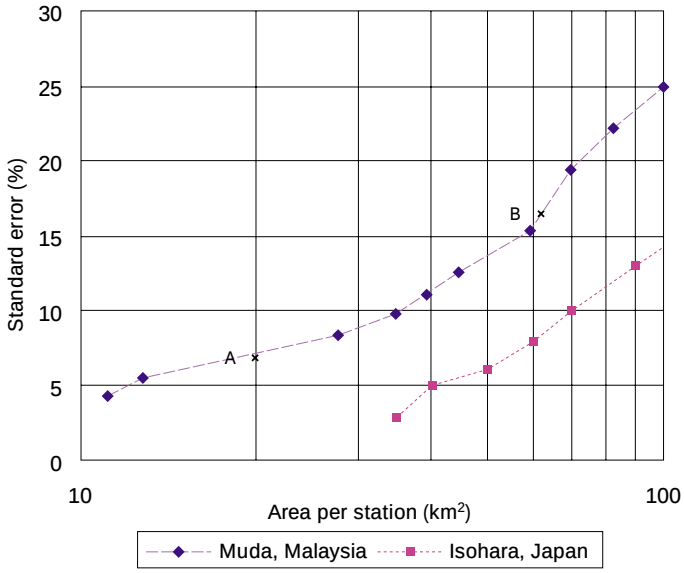

Fig. 1. Standard error of rainfall events as a function of station density.
A: Present station density; B: Initial station density

Reference

N. Horikawa (2002)：Evaluation of rainfall station network for irrigation projects in tropical monsoon area (in Japanese). Applied Hydrology, 15, 54-58.

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Significance of indigenous arbuscular mycorrhizal fungi in forage production in the Brazilian savannas

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Key words : *Brachiaria* grasses, Cerrados, *Panicum maximum*, soil pH

Objectives

Several tropical grasses of *Brachiaria* species or *Panicum* species cover 95% of the improved pastures in the Brazilian savannas. Although it is generally known that arbuscular mycorrhiza (AM) fungi enhances adaptability of the grass species to infertile acid soils in the savanna regions, there is little information regarding the effects of indigenous AM fungi on forage production. In this study, the relationship between the grass species, indigenous AM fungi, and soil pH was examined for 4 tropical grass species.

Results

Seedlings of *Brachiaria decumbens*, *B. brizantha*, *B. humidicola*, and *Panicum maximum* were planted in pots filled with an Oxisol collected from a native savanna area in Campo Grande at Mato Grosso do Sul State, Brazil. They were grown in a greenhouse for 70 days with two inoculating treatments (with/without inoculation of the indigenous AM fungi) in combination with three levels of soil pH (4.3, 5.1 and 6.4).

Inoculation of AM fungi increased the shoot and root dry weights in all species (Photo 1). Phosphorus(P) concentration in the shoots and roots as well as total P accumulation in the examined grass species were improved by AM inoculation (Fig. 1). The mycorrhizal effect on P acquisition was larger in *B. decumbens* and *B. brizantha* than in *P. maximum* and *B. humidicola*. An increase in soil pH had a positive effect on the plant dry weight and P acquisition of the 4 grass species. All grass species showed the highest AM dependency on dry matter production and P accumulation at the lowest pH levels.

In this study, it was discovered that the existence of indigenous AM fungi was necessary for the 4 grass species growing on the Brazilian Oxisol, and that the importance of AM fungi was most significant at the lowest soil pH condition.

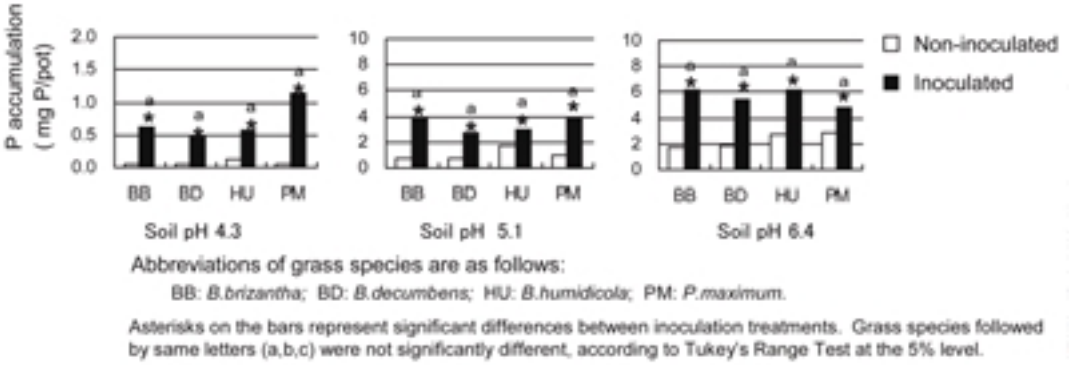

Fig. 1. P accumulation of four grass species affected by arbuscular mycorrhiza inoculation and soil pH.

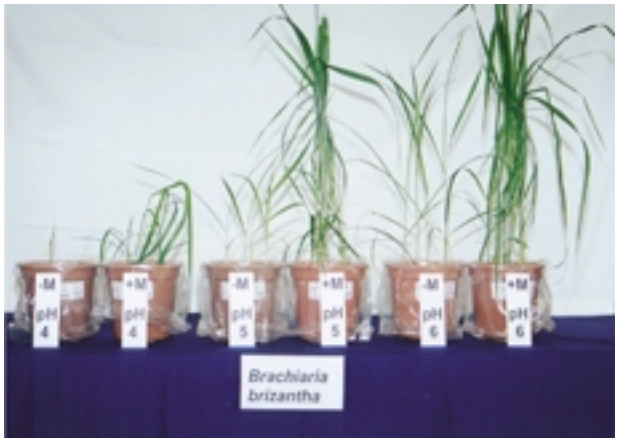

Photo 1. Mycorrhizal (+M) and non-mycorrhizal (-M) *Brachiaria brizantha* at soil pH levels of 4.3, 5.1 and 6.4.

Reference

T. Kanno, M. J. Borges, M. Saito, M. C. M. Macedo, T. Nakamura and C. B. H. Miranda (2001) : Importance of indigenous arbuscular mycorrhiza for dry matter production and phosphorus acquisition in tropical forage grasses. Proceedings of the 28th annual conference of the Brazilian Society of Soil Science, Londrina, Brazil, 80.

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On-farm trial for pasture establishment on wetland in the Brazilian savannas

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Key words : *Brachiaria humidicola*, Cerrados, lowland, *Paspalum atratum*

Objectives

The Brazilian savannas, which cover approximately 200 million hectares (ha) in central Brazil, are among the most important areas for livestock production in the world. It is also estimated that the savannas contain 12 million hectares of wetlands having abundant water resources which remain under-utilized. Since 1998, JIRCAS has been implementing an on-farm trial in Sao Paulo State to improve animal productivity of one of the private farms, which has 450 ha of wetland (Photo 1). In the trial, several experiments have been conducted on the wetland to select available grass species with high flooding tolerance and to pinpoint suitable seeding times for pasture establishment.

Results

In January 1998 (the middle of the rainy season), and November 1998 (the beginning of the rainy season), *Brachiaria decumbens, B. brizantha, B. dictyoneura, B. humidicola, Andropogon gayanus, Setaria anceps* and *Paspalum atratum* seeds were sown in wetland field plots. However, in both experiments, there was no particular species which could survive the wetland rainy season. When the grass seeds were sown in the early half of the rainy season, the small seedlings were flooded at the end of the season and could not survive. These results indicate that the suitable seeding period in the wetlands is the beginning of the dry season, when it becomes possible to use a tractor.

In related work, adult plants of *B. decumbens, B. brizantha, B humidicola, A. gayanus, P. atratum,* and *Cyndon dactylon* were transplanted into the wetland plots in July 1999 (the beginning of the dry season) and their flooding tolerance was compared. After the following rainy season (in June 2000), it was discovered that only *B. humidicola* (cv. Humidicola) and *P. atratum* (cv. BRA-9610) had survived.

Based on those results, seeds of *B. humidicola* were sown on an area of 1.5 ha of the wetland in May of 2001 (beginning of the dry season), and excellent pasture establishment was observed (Photo 2). Results of this study on the private farm indicate that feeding resources can be developed by planting *B. humidicola* or *P. atratum* on the wetland pastures.

Photo 1. Grazing cattle on a wetland pasture during the dry season.

Photo 2. Seeding establishment of *Brachiaria Humidicola* on a wetland pasture at the beginning of the dry season.

Reference

T. Kanno, T. Kawakami, Y. Yoshimura, S. Uozumi and M. C. M . Macedo (2002)：On-farm trial for pasture establishment on wetlands in the Brazilian savannas. Grassland Science (in press).

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Energy requirements for maintenance of Holstein crossbred dry cows in Northeast Thailand

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Key words : tropical zone, energy metabolism, Holstein, dry cow, energy requirement

Objectives

Dairy farming in Northeast Thailand has been growing as an important agriculture sector. But it still faces numerous difficulties in the area of feeding management. Feeding strategies for dairy cattle are clearly described in the feeding standards for cattle in temperate countries, but they cannot be applied directly to the dairy cattle in Thailand because the specific breed of cattle, available feed and surrounding environment in the region are quite different from conditions elsewhere. The nutritional physiology of dairy cattle raised in Northeast Thailand has not yet been well characterized, so the present study aims to examine the effect of protein levels on energy and nitrogen balances in dairy dry cows.

Metabolism trials were conducted with four Holstein crossbred dry cows in order to examine the requirements for maintenance in dairy cows; each cow was fed Ruzi grass hay mixed with different levels of soybean meal. Crude protein (CP) contents in the four dietary treatments were 3.3, 6.4, 9.7 and 13.1%. The dietary treatments consisted of a nine-day preliminary period and a five-day collection period. After the last dietary treatment, the cows were fasted for 4 days. Feces and urine were collected from each animal during the collection period, and only urine was collected over the last two days of the fasting period (Photo 1). Oxygen consumption and the production of carbon dioxide and methane were measured with the ventilated flow-through method during the last 4 days of the feeding period and during the last 2 days of the fasting period.

Results

The metabolizable energy (ME) requirements for maintenance were calculated through a regression analysis of energy retention against ME intake on the basis of metabolic body size. The regression equation was estimated as $Y=0.7851X-320.86$, and the ME requirement for maintenance obtained was 409 $KJ/BWkg^{0.75}$ (Fig. 1). This value for ME requirement was the highest compared to animals examined in previous studies, followed by Brahman cattle (377$KJ/BWKg^{0.75}$), swamp buffalo (334$KJ/BWkg^{0.75}$), and Thai native cattle (245$KJ/BWkg^{0.75}$). It was 17 % lower than the value in the Holstein cattle (487$KJ/BWkg^{0.75}$) as suggested in the feeding standards (Agriculture, Forestry and Fisheries Research Council Secretariat, 1999). Further studies are required to conclude values of ME requirement for the maintenance of Holstein crossbred cattle in Thailand; however, at present it is clear that heat and methane production of the Holstein crossbred cattle in the present studies were higher than those in animals from previous studies.

These result will serve as basic information for the establishment of feeding standards in Thailand.

Photo 1. Cows installed with harnesses to attach feces bags and urine tubes in order to separately collect feces and urine during the collection period.

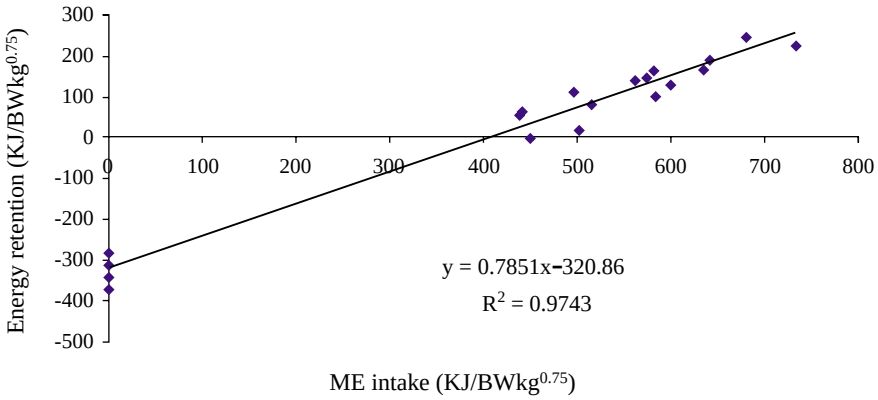

Fig. 1. Relationship between ME intake and energy retention in dairy dry cow.

Reference

T. Kawashima, W. Sumamal, P. Pholsen, R. Chaithiang, W. Boonpakdee and F. Terada (2000)：Comparative study on energy and protein metabolism of Brahman cattle and sheep given Ruzy grass hay with different levels of soybean meal. *In*: T. Kawashima (ed.) Improvement of cattle production with locally available feed resources in Northeast Thailand, JIRCAS and DLD, 123-136.

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Microorganism control in packed tofu manufacture using electrolyzed water

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Key words : anti-microbial activity, heat-resistant bacteria, hypochlorous acid, mixed electrolyzed water, sterilization

Objectives

As a traditional food, tofu is an important part of Asian diets and has now achieved popularity worldwide due to its nutritional value, as it is high in protein, with essential amino acids and isoflavone. However, tofu products decay easily and have a very limited shelf life. To control microorganisms in tofu manufacture, electrolyzed water (EW) was applied at the soybean soaking phase of packed tofu processing. The available chlorine in EW, which is a mixture of hypochlorous ions, hypochlorous acid and chlorine, acts as a sterilizer. The Division focused on the anti-microbial activity of available chlorine and on the distribution pattern in response to pH levels. Under weak acidic conditions around pH 6.5, concentrations of chlorine and hypochlorous ions become minimal, making EW more stable and active as a bactericide.

Results

Acidic EW (pH 2.1; oxidation reduction potential, 1185mv; available chlorine, 100 ppm) and alkaline EW (pH 11.7; oxidation reduction potential, -120mv) were prepared by electrolysis of 0.075% sodium chloride solution. Mixed EW (pH 6.5; oxidation reduction potential, 891mv; available chlorine, 50 ppm) was prepared by mixing acidic and alkaline EW to adjust pH levels to 6.5. Sterilization effects during soybean soaking and the quality of soymilk and tofu produced from soybeans soaked in three types of EW were analyzed. Acidic EW and mixed EW were very effective in killing all microorganisms in soybeans and kept the soaking water aseptic (Fig. 1).

Tofu consistencies produced from acidic EW and alkaline EW soaking were lower than the those obtained from mixed EW and sterilized water soaking (Table 1). We conclude that mixed EW is the most effective disinfectant among the three types of electrolyzed water due to weak acidic pH values, stability, and the lack of damage to soymilk and tofu.

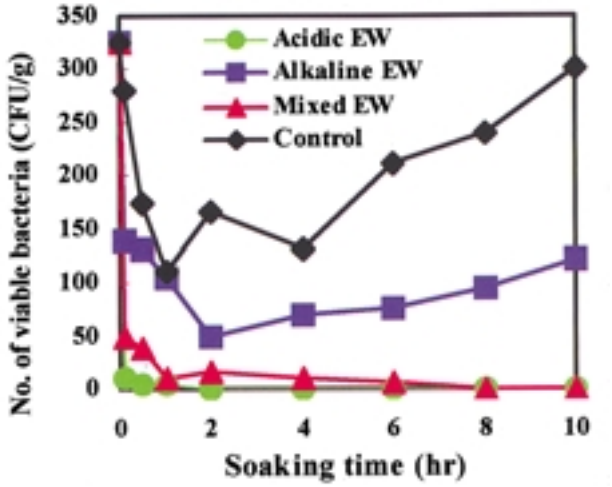

Fig. 1. Changes over time of viable bacteria counts in soybeans after soaking in EW.

Table 1. Effects of soaking soybeans in four types of solutions on soybean, soymilk and tofu quality

	A lkaline EW	A cidic EW	Mixed EW	Sterilized water
olids content in wastewater (%)	0.51	0.47	0.37	0.32
Soymilk (ml)	232.9	230.6	229.1	227.4
Solids content in soymilk (%)	10.85	11.04	10.6	10.64
Tofu gel strength (kPa)	15.14	15.9	17.68	17.78

Reference

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Processing of high-quality rice noodles in China

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Key words： amylose, rice flour, rice noodle, rice starch, rice variety

Objectives

Rice noodles are a traditional food, widely consumed in southern China and Southeast Asia. Various types of rice noodles have been produced during the long history of food processing; in China, most manufacturing has traditionally been carried out by small shops or producers (Photo 1). To industrialize the rice noodle industry, it is necessary to gather basic knowledge about the production process. Towards this objective, a study on the effects of rice varieties, soaking time and milling methods was conducted by the Food Science and Technology Division, JIRCAS.

Results

Generally, Indica rice varieties are used in rice noodle processing. Rice noodle structure depends on the gelatinization and retrogradation properties of starch, and noodle quality is believed to be highly dependent upon amylase content. To determine the influence of rice varieties, the correlation between amylase content and rice noodle quality was analyzed for two Japonica varieties, five Indica varieties and three hybrid Indica varieties. The rice noodles made from the high amylose (greater than 20%) Indica varieties achieved high scores in sensory evaluation (Fig. 1).

Grinding of rice granules is the first step in rice noodle processing, affecting rice flour in terms of particle size distribution, degree of gelatinization and damaged starch content. The effects of dry and wet milling of rice granules were determined, with results showing that the damaged starch content of rice flour after dry milling was greater than that after wet milling. Other results showed that 1) rice granules reached plateaus in water absorbency after soaking for two hours, 2) cooking loss of rice noodles was remarkably decreased through soaking treatment (Fig. 2), and 3) rice noodles made from wet milled flour had higher values in gel strength (Fig. 3).

Photo 1. Rice noodle processing in China.

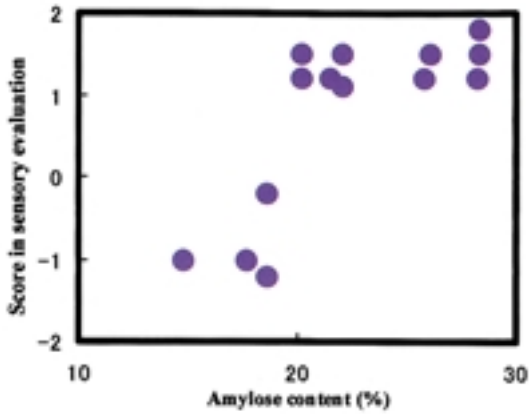

Fig. 1. Correlation between amylose content of rice materials and rice noodle quality.

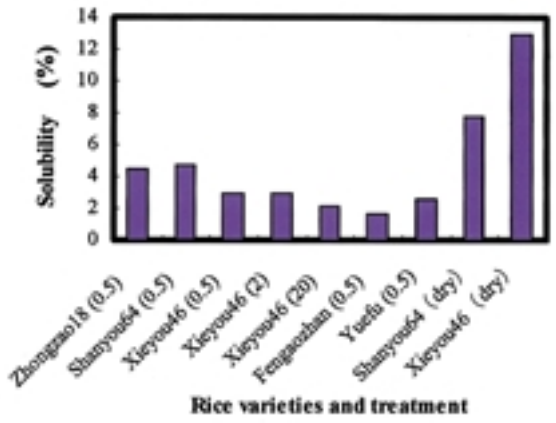

Fig. 2. Cooking loss of rice noodles under different soaking times (hr).

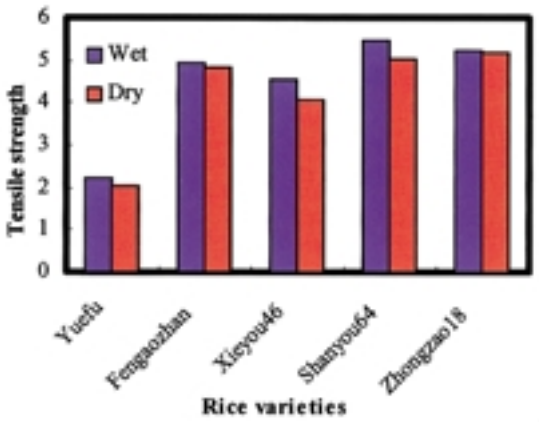

Fig. 3. Effects of milling methods on rice noodle gel strength.

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Preparation of cellulose pulp from oil palm empty fruit bunches

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Key words : oil palm, pulp, cellulose, dissolving pulp, ozone

Objectives

As a source of palm oil, oil palm is one of the most important tree species in Southeast Asian countries such as Malaysia. However, fibrous wood-like residues that remain after oil production have not been effectively utilized so far. These are called empty fruit bunches (EFB, Photo 1) and alternative forms of utilization of EFB include the production of cellulose pulp, or dissolving pulp (DP). DP is used as a raw material of cellulose derivatives or regenerated cellulose. In this study, the preparation of DP from EFB using environment-friendly chemical technologies and methods was conducted.

Results

The pulping process employed in this study used soda– anthraquinone (AQ), which does not include a sulfur element that causes water pollution. EFB fibers were first hydrolyzed with dilute sulfuric acid (pre-hydrolysis), followed by the soda-AQ pulping. Ozone (O_3) bleaching was then carried out on the pulp at room temperature with or without alkali extraction afterward. O_3 is a powerful and less pollutant reagent in pulp bleaching, which has the potential to replace bleaching processes using chlorine-containing reagents. Chemical properties of the pulps are shown in Table 1; a pulp prepared without pre-hydrolysis (Pulp C) and a commercial softwood DP are shown for references. Contents of α-cellulose (an indicator of cellulose purity) approached 90% and above for the pre-hydrolyzed pulps (A and B), which is an acceptable level in comparison with the commercial DP. Ash and pentosan contents of the pulps, which are indicators of cellulose impurity, were also present in comparable levels. Comparing A and B, the higher α-cellulose content of A is indicative of the effectiveness of alkali extraction after O_3 treatment for increasing cellulose purity. Above all, it is concluded that EFB has significant potential as a raw material to be utilized for dissolving pulp in an environment-friendly manner.

Photo 1. An empty fruit bunch (EFB, top) and its fibrous form (bottom).

Table 1. Chemical properties of EFB pulps.

	Preparation condition	α-cellulose content (%)	Ash content (%)	Pentosan content (%)
A	Pre-hydrolysis → pulping → ozone → alkali extraction	95.1	0.09	1.8
B	Pre-hydrolysis → pulping → ozone	88.6	0.06	1.8
C	Pulping → ozone	77.9	0.12	24.2
	Commercial softwood DP	92.3	0.14	2.5

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Characteristics of Chinese domestic freshwater fish: Postmortem changes in the muscle quality of silver carp and grass carp

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Key words : freshwater fish, post-mortem changes, muscle quality

Objectives

A study on the post-mortem changes of fish muscle quality during storage was conducted focusing on the silver carp (*Hypophthalmichthys molitrix*) and grass carp (*Ctenopharyngodon idellus*), which are the most popular Chinese domestic freshwater fish (Photo 1). The fish were kept at temperatures of 5, 10, and 20℃, after death by sacrifice. Changes in muscle quality during storage were then monitored by sensory evaluation of the skin surface, gills and eyes, as well as the smell and hardness of the muscles. The concentration of ATP and its related compounds in the muscles were measured in order to calculate the K-value as a freshness index for fish meat.

Results

Fig. 1. shows deterioration in fish muscle quality as a function of time. If the score was higher than 8, the fish was considered palatable to Chinese consumers. When the fish were kept at 20℃ , scores on the sensory test decreased immediately after death, and within half a day, the scores fell below 8 in both species. At 5℃, the period that the fish was considered palatable extended to about three days. As shown in Fig. 2, the K-value increased rapidly when both silver carp and grass carp were kept at 20℃. Storage at low temperatures such as 5 and 10℃ delayed the increase of the K-value. However, there were significant differences between the two species of fish; K-values in grass carp rose faster than in silver carp, and the K-value of silver carp remained at low levels throughout five days of storage at 5℃. This finding may indicate that silver carp meat itself is stable, although rapid deterioration was evaluated through the sensory test. Poor marks on the sensory test of fish quality is usually indicative of the growth of microorganisms; if the presence of these microorganisms is well-controlled, the shelf life of fish meat may be extended.

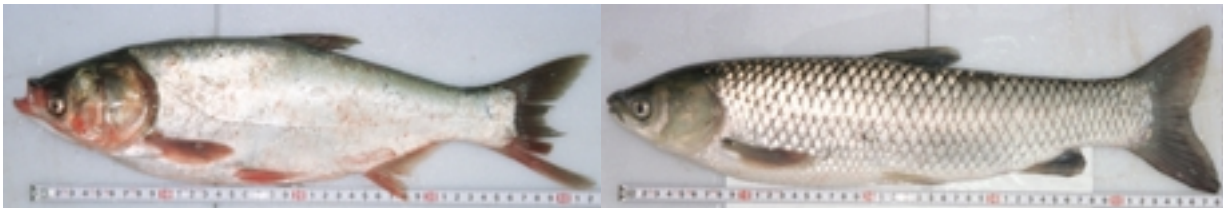

Photo. 1. Chinese domestic freshwater fish: silver carp *Hypophthalmichthys molitrix* (left) and grass carp *Ctenopharyngodon idellus* (right).

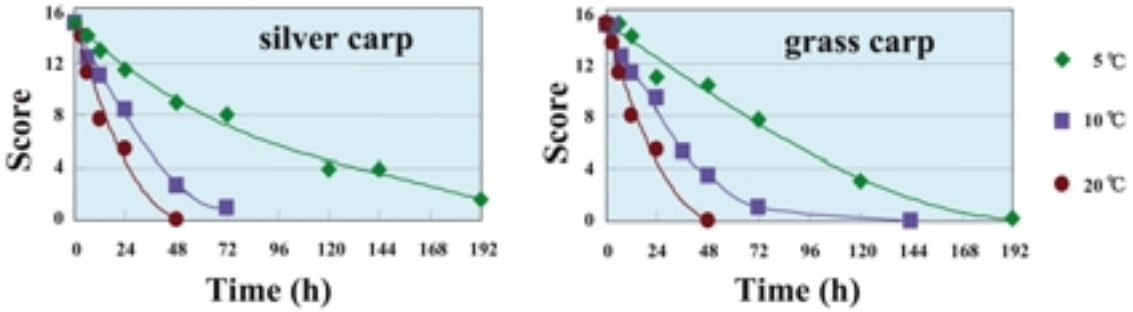

Fig. 1. Changes in sensory evaluation scores of silver carp (left) and grass carp (right) during storage under different temperatures.

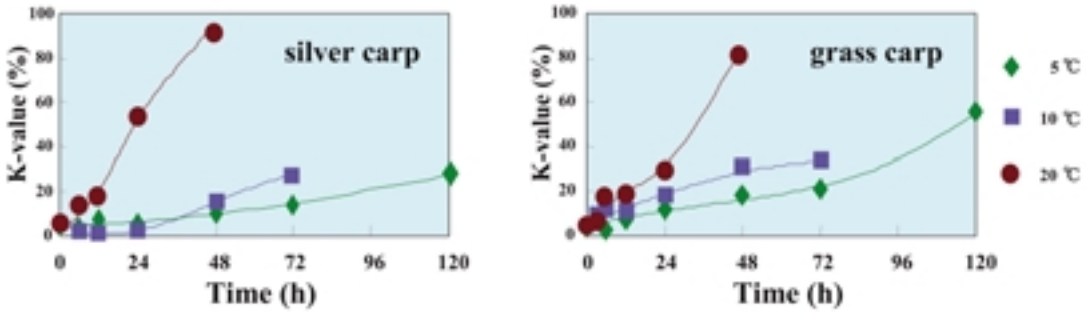

Fig. 2. Changes in K-values of silver carp (left) and grass carp (right) muscle during storage under different temperatures.

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Diagnosis and prevention of viral diseases occurring in cultured Shrimp

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Key words : prawn, viral disease, WSSV, diagnosis, monoclonal antibody

Objectives

In recent years, prawn culture has developed rapidly as an industry in Southeast Asia. However, the production of cultured prawns has decreased markedly as a result of serious viral disease outbreaks such as White Spot Syndrome Virus (WSSV) disease. Therefore, in order to ensure stable production of cultured products, it is essential that preventive countermeasures against viral diseases be adopted. The aim of this study is the development of serological diagnosis and disinfection methods for the prevention of prawn viral diseases.

Results

Serological diagnosis using monoclonal antibodies is one of the most rapid and accurate methods. The production of monoclonal antibodies involves four steps (Fig. 1) ; the preparation of virus antigen, mouse immunization, cell fusion of spleen cells with myeloma cells, and then screening and culture of the hybridoma cells for producing the monoclonal antibodies. As a result, a few strains producing virus-specific antibodies are obtained from more than 100 strains of hybridomas. These monoclonal antibodies are thereafter used in the diagnosis of WSSV.

Viral inactivation against WSSV was tested using chemicals such as formalin and halogenous disinfectants, including sodium hypochlorite and Isodine^{R}. The virus was inactivated with concentrations above 0.25% for formalin, 0.5 ppm for chloride, and 1.25 ppm for povidone-iodone. The data from these experiments indicated that halogenous disinfectants induced an effective inactivation even at lower concentrations.

From these studies, the diagnosis and disinfection techniques for WSSV should enable the prevention of pathogen intrusion into aquaculture farms in Southeast Asia.

Photo 1. Aquaculture pond for prawn farming in Malaysia.

Photo 2. Mass mortality of prawns due to viral disease in Malaysia.

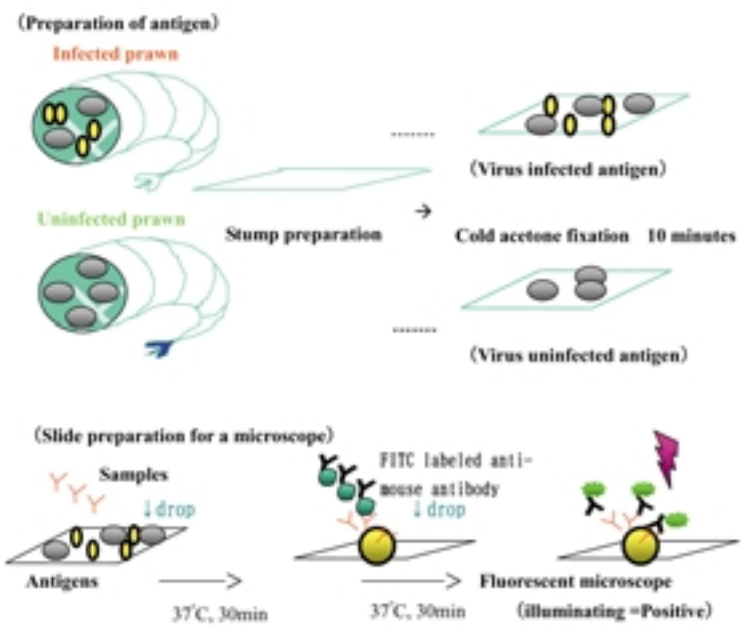

Fig. 1. Screening methods for antibody using indirect immunofluorescence technique.

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Development of male-sterile lines of snap bean (*Phaseolus vulgaris*) varieties using male-sterile cytoplasm detected from a cultivar Kurodane Kinugasa'

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Key words : *Phaseolus vulgaris*, snap bean, cytoplasmic male sterility, recurrent backcrossing, hybrids

Objectives

To increase the diversity of snap bean varieties cultivated in tropical and subtropical areas, we are attempting to incorporate high yielding ability under high temperatures exhibited by 'Haibushi' into other cultivars through conventional crossbreeding methods. During the course of crossing procedures, we found that a cultivar Kurodane Kinugasa' produced semi-sterile hybrids when crossed as a female parent with 'Haibushi'. Since the reciprocal cross, Haibushi' × Kurodane Kinugasa' exhibited a high pollen stainability, the occurrence of male sterility in this cross combination is concluded to be cytoplasmic. We attempted to develop male sterile lines of snap bean varieties using this cytoplasm.

Results

By backcrossing in which Kurodane Kinugasa' was involved as a non-recurrent female parent and other varieties as recurrent male parents, we developed cytoplasmic male sterile (CMS) lines of the following varieties to date; Haibushi(B_{11}), Kentucky Wonder(B_8), Sabel(B_8), Ishigaki No. 2(B_8), Golden Wax(B_8), Kurosando(B_8), Kamogawa Green(B_8), Nerina(B_8), Ichizu(B_6), Sayakazari(B_6), Supander(B_6). The number of the backcrossing generation is shown in the form of B_n in parentheses. By using these CMS lines as female parents for the crossing, we can easily produce hybrids without castration as illustrated in Fig. 1.

The color of hypocotyls of some CMS lines is green, while that of others is purple. Purple coloration is a dominant character as compared to green color. Based on the coloration of the hypocotyl, hybridity of the seeds obtained by pollinating the CMS lines with green hypocotyl (e.g. Kentucky Wonder) with purple varieties (e.g. Haibushi) were examined. The plants obtained from such cross-combinations had purple hypocotyl and were judged to be hybrids (Fig. 2). These CMS lines are thus very useful for producing hybrids efficiently and successfully, and are considered to be useful for studying genetic segregation patterns of morphological and physiological characteristics of snap bean.

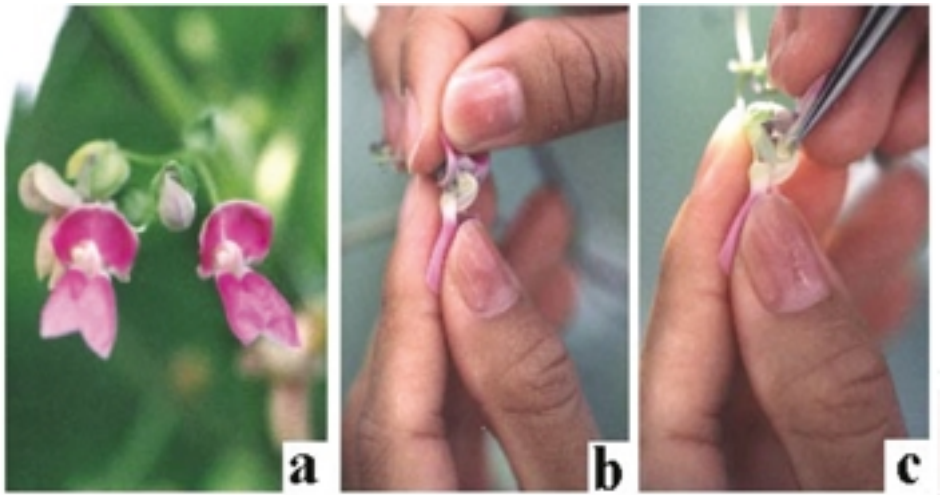

Fig. 1. Cross-pollination using cytoplasmic male sterile (CMS) lines. (a) Flowers that opened on the day of flowering. (b) Stigma can be easily extended from the open mouth of keel petal by pressing down the wing petals with fingers. (c) Then, the stigma was pollinated with fertile pollen.

Fig. 2. Hypocotyls of the plants derived from CMS Kentucky Wonder'(green) × Haibushi'(purple) showed purple color, verifying the hybrid nature of the plants.

Reference

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The function of tomato mitochondrial small heat shock protein under heat stress conditions

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Key words : MT-sHSP, tomato (*Lycopersicon esculentum* Mill.), molecular chaperone

Objectives

Heat stress is one of the most significant constraints on crop production. Under heat stress, synthesis of most proteins is repressed and some proteins, which are called heat shock proteins (HSPs), begin to be synthesized. Accumulation of sHSP in mitochondria (MT-sHSP) under heat stress has recently been reported in a number of plant species, but little is known about the cellular functions of MT-sHSP in the heat tolerance of plants.

The aim of this study was to clone MT-sHSP cDNA from tomato (*Lycopersicon esculentum* Mill.) leaves, evaluate the transcription of the MT-sHSP gene at various temperatures, and assay the molecular chaperone activity of MT-sHSP *in vitro*.

Results

A full-length cDNA (LeHSP23.8: accession number AB017134) encoding the precursor of the MT-sHSP in tomato was successfully cloned. The deduced protein precursor, had a calculated molecular weight of 23.8 kDa.

A single copy of LeHSP23.8 was found in tomato genomic DNA through Southern-blot analysis.

Northern blot analysis revealed the heat-inducible character of LeHSP23.8 mRNA. The threshold temperature was approximately 36℃, and it was accumulated abundantly at 40℃ in tomato leaves (Fig. 1). Among the MT-, ER-, Class I and Class II-sHSP genes, MT-sHSP mRNA responded most quickly at 40℃ in tomato flowers (Fig. 2).

The molecular chaperone function of LeHSP23.8 was demonstrated *in vitro*. When the recombinant LeHSP23.8 was mixed with CS denatured by guanidine hydrochloride, 40% of the native CS activity was recovered after one hour of incubation (Fig. 3A). In another experiment, recombinant LeHSP23.8 protected CS from thermal inactivation and also promoted the renaturation of thermally inactivated CS. The loss of CS activity was relatively slow when CS was incubated with recombinant LeHSP23.8 at 38℃. Furthermore, when the incubation temperature was shifted to 22℃, rapid renaturation of thermal-denatured CS was observed within 10 minutes, and 90% of CS activity was recovered (Fig. 3B).

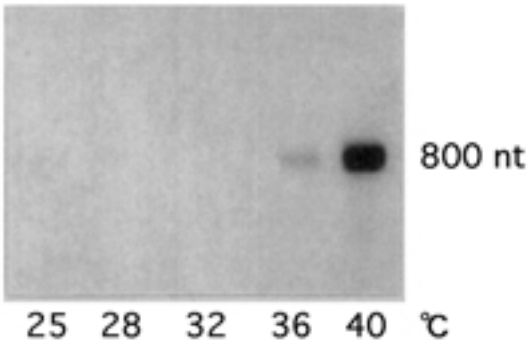

Fig. 1. Northern-blot analysis of temperature-dependent LeHSP23.8 mRNA accumulation in tomato leaves. Tomato plants were grown in greenhouse conditions at 25℃ and treated at 28℃, 32℃, 36℃ or 40℃ for two hours.

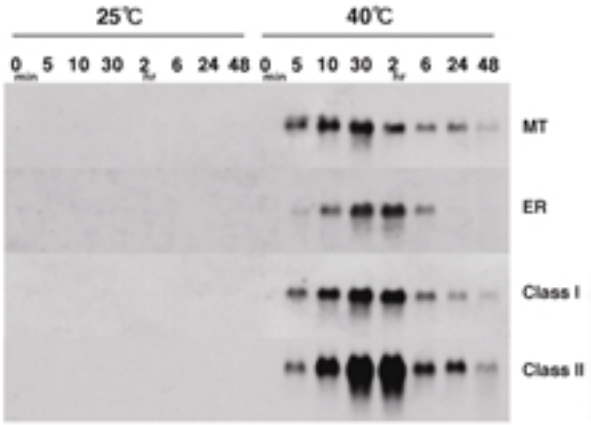

Fig. 2. Northern-blot analysis of heat-induction time course for sHSP genes in tomato flowers. Tomato plants grown under greenhouse conditions were transferred to the growth chamber on the day of flowering, and then incubated at 25℃ or 40℃. The flowers were collected 0 min to 48 hours after the incubation. Panels MT, ER, Class I and Class II show the expression of the genes for MT-, ER-, Class I- and Class II-sHSP, respectively.

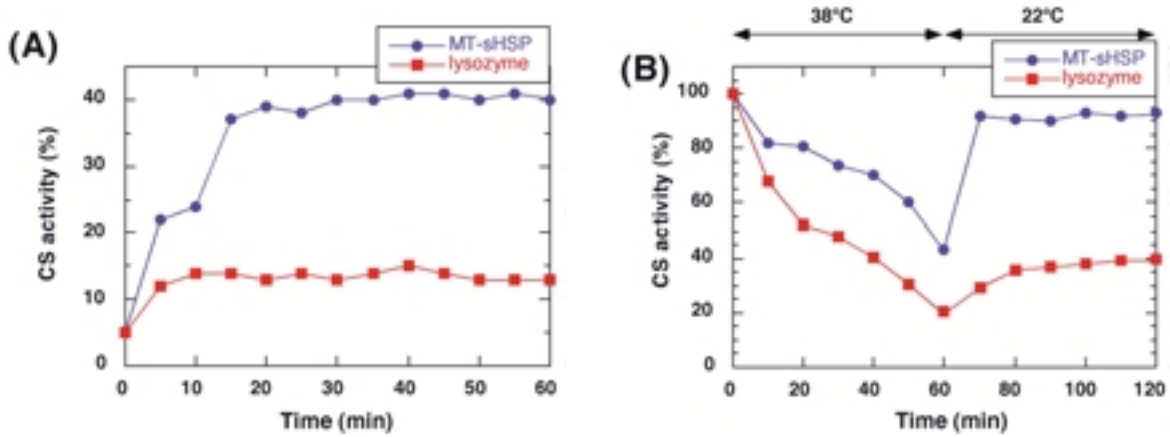

Fig. 3. (A) Effects of LeHSP23.8 protein on the renaturation of chemically denatured citrate synthase (CS). CS (15μM) was denatured in 6 M guanidine hydrochloride for 120 minutes and then diluted 100-fold into a solution supplemented with 150 nM lysozyme (■) or with 1.8 μm recombinant LeHSP23.8 (●).
(B) Effects of recombinant LeHSP23.8 on the thermal inactivation of CS. CS (150 nM) was incubated in the presence of 150 nM lysozyme (■) or 1.8 μm recombinant LeHSP23.8 (●) at 38℃ for 60 minutes and then at 22℃.

Reference

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A breeding index for improving the early growth of sugarcane

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Key words： dry matter, early growth, net assimilation rate, specific leaf area, sugarcane

Objectives

Sugarcane (*Saccharum* sp.) yield remains low compared to its photosynthetic ability. The slow growth of sugarcane at the early stages is one of the primary reasons for this low productivity. A high percentage of sunlight is lost to the soil at this stage. In order to increase sunlight absorption, the rapid expansion of leaf area in sugarcane is considered highly necessary. The delayed expansion of leaf area is attributed to the slow growth of individual plants as well as low density planting in sugarcane crops. Analysis of sugarcane growth at the early stage should be conducted with regard to plant growth as well as crop growth.

Results

The growth of sorghum (*Sorghum bicolor*) was compared to sugarcane during the early growth stages. The dry weight of the sorghum was five times greater 48 days after emergence (Fig. 1), and it was concluded that a higher increasing rate of leaf area enabled rapid growth in the sorghum. The net assimilation rate (NAR) implies the same photosynthetic ability in both species. The higher dry matter percentage of leaves suggests that dry matter partitioning to leaves is higher in sugarcane. Specific leaf area (SLA) is approximately double in sorghum. As relative growth rate correlates to SLA (Fig. 2), the smaller SLA is one of the main reasons for the slow growth of sugarcane at the early stage. Genetic diversity of SLA is observed as an aspect of sugarcane genetic resources (Fig. 3). Some native clones including *S. sinense* are considered suitable for the breeding of varieties having greater SLA values. Even when compared to one of the rapid-growing commercial varieties, a native variety 'Oshima' with greater SLA shows more rapid leaf area expansion and growth (Fig. 4). SLA can be employed as an index for improving the early growth of sugarcane.

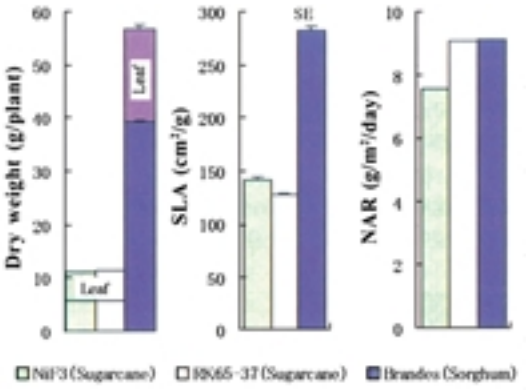

Fig. 1. Comparison of dry weight, specific leaf area (SLA) and net assimilation rate (NAR) between sugarcane and sorghum 48 days after emergence.

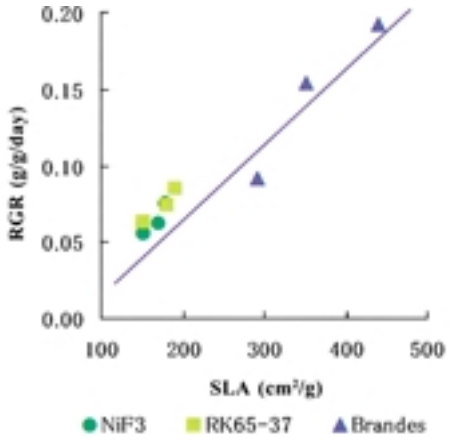

Fig. 2. Relationship between SLA and relative growth rate (RGR).

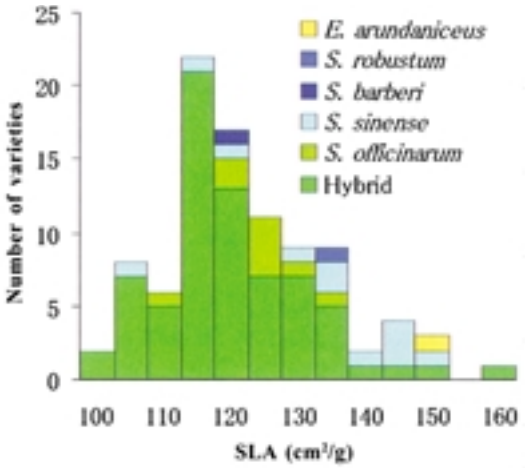

Fig. 3. SLA variation among sugarcane genetic resources.

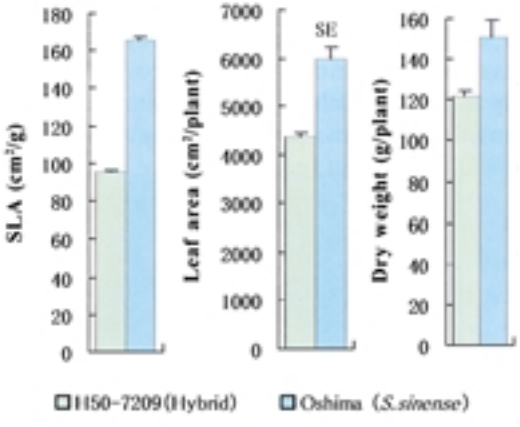

Fig. 4. Comparison of dry weight, leaf area and SLA among sugarcane varieties 84 days after emergence.

Reference

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The relationship between the distribution of citrus psylla, the vector insect of citrus greening disease, and the distribution of jasmine orange

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Key words : *Diaphorina citri*, citrus greening disease (huang long bing), *Murraya paniculata, Citrus* spp., distribution

Objectives

Asian citrus greening disease, or "huang long bing," is the most significant obstacle to sustainable citrus production in tropical and subtropical Asian countries. The first detection of this disease in Japan was in 1988 on Iriomote Island, Okinawa. Subsequently, the disease spread throughout Okinawa prefecture.

The disease is transmitted by grafting or vector insects, like the Asian citrus psylla, *Diaphorina citri* (Fig. 1); therefore, controlling *D. citri* is an important step towards controlling the disease itself. *D. citri* is distributed throughout tropical and subtropical Asia and has also been recorded on major islands among the southwest islands of Japan. The host plants of *D. citri* are restricted to species belonging to the family Rutaceae. Among these species, *Murraya paniculata*, or "orange jasmine"(Fig. 2), and cultivated *Citrus* spp., are the most favored host plants.

In this survey, we investigated the distribution of cultivated *Citrus* spp., *M. paniculata*, *D. citri*, and its parasitic natural enemies on the islands within the southwest islands of Japan displayed in Fig. 3.

Results

Citrus spp. were found on all the islands investigated; however, *M. paniculata* and *D citri* were found only on the islands located south of Amami-Oshima. On the islands where the distribution of *D. citri* was confirmed, at least one of the two species of parasitic natural enemies (an encyrtid wasp *Diaphorencyrtus* sp and an eulophid wasp *Tamarixia radiata*) was found. Distribution of parasitic natural enemies represents the continuous occurrence of the host in the area. Therefore, it is concluded that *D. citri* maintains a continuous presence on the islands south of Amami-Oshima.

In conclusion, the most intensive efforts to prevent invasion by Asian citrus greening disease should be implemented in areas where *D. citri* is distributed, even if the disease has not yet been detected at the present time.

Fig. 1. Infecton of adult *Diaphorina citri* on *Murraya paniculata.*

Fig. 2. *Murraya paniculata* "orange jasmine" planted as a hedge around a house.

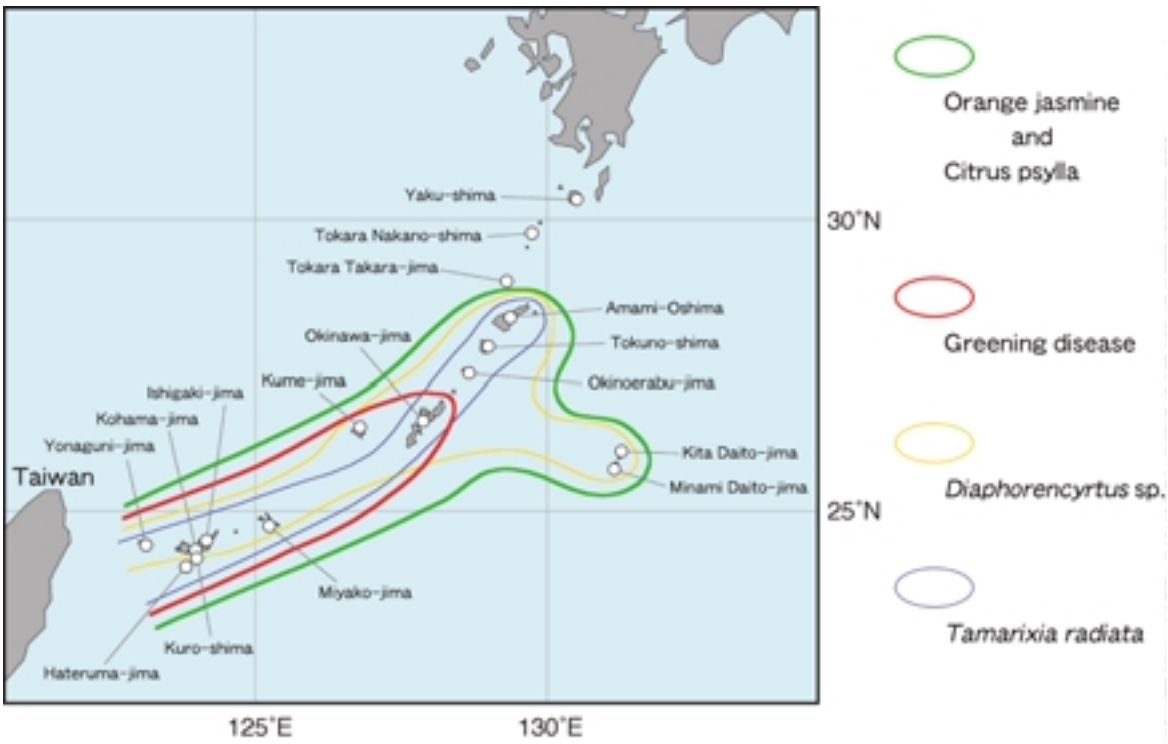

Fig. 3. Distribution of *Murraya paniculata* "orange jasmine," *Diaphorina citri* "citrus psylla," citrus greening disease "huang long bing," and two wasps parasitizing *D. citri* (*Diaphorencyrtus* sp. and *Tamarixia radiata*) on the Southwest Islands of Japan.

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Ecological characteristics of *Antilochus coqueberti*, a specialist natural predator of cotton stainers

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Key words : cotton stainer, predator, *Antilochus coqueberti*, prey preference, development

Objectives

Cotton stainers (*Dysdercus* spp.; Heteroptera: Pyrrhocoridae) are one of the major insect pests of cotton plants. They are difficult to control by insecticide application in cotton fields because they are highly mobile and have many alternative host plants. A pyrrhocoid bug *Antilochus coquebert* (Heteroptera: Pyrrhocoridae) was presupposed to prey upon cotton stainers, but there had been no precise study on its ecological characteristics. In this study, the food preferences and ecological characteristics of *A. coqueberti* were investigated in order to elucidate its validity as a biological control agent against cotton stainers.

Results

Antilochus coqueberti adults and nymphs prey upon two species of cotton stainers (*D. cingulatus* and *D. decussatus*) in the fields of Ishigaki-jima Island (Fig. 1). Laboratory experiments showed that *A. coqueberti* also preys upon all the pyrrhocorids and alydids; however, lygaeids, largids, coreids, and rhoparids are not preyed upon by *A. coqueberti*, although all of these species bear considerable visual resemblance to cotton stainers (Table 1).

Developmental periods from oviposition to adult emergence of *A. coqueberti* fed with *D cingulatus* under each environmental condition are shown in Table 2. Based on these values, developmental zero and the effective cumulative temperature from oviposition to adult emergence were estimated to be 12.8℃ and 606.1 day degrees, respectively.

Reproductive traits of female *A. coqueberti* are shown in Table 3. These values clearly reflect the high reproductive potential of this species.

Female *A. coqueberti* did not exhibit reproductive diapause even if they were reared under short day lengths (10L-14D). The nymphal developmental period and preovipositional period were shorter when compared to those under long day lengths (Table 2).

The results detailed above show that, as a specialist natural predator, *A. coqueberti* is a promising biological control agent against cotton stainers, and its utilization should be highly recommended when this species is artificially reared.

Fig. 1. *Antilochus coqueberti* in copulation preying on *Dysdercus cingulatus*.

Table 1. Suitability of bug species as prey of *Antilochus coqueberti.*

Bug superfamily Bug family Bug species	Suitable?
Lygaeoidea	
Lygaeidae	
Oncopeltus nigriceps	no
Spilostethus hospes	no
Graptostethus servus	no
Thunbergia sanguinaria	no
Pyrrhocoroidea	
Largidae	
Physopelta cincticollis	no
Physopelta gutta	no
Pyrrhocoridae	
Dysdercus cingulatus	yes
Dysdercus poecilus	yes
Dysdercus decussatus	yes
Dysdercus philippinus	yes
Dysdercus sp. probably *D. mesiostigma*	yes
Dysdercus sp. probably *D. solenis*	yes
Armatullus sp.	yes
Coreoidea	
Coreidae	
Dasynus coccocinctus	no
Alydidae	
Riptortus clavatus	yes
Daclera levana	yes
Rhopalidae	
Leptocoris augur	no
Leptocoris rufomarginatus	no

Table 2. Developmental periods from oviposition to adult emergence of *Antilochus coqueberti* fed with *Dysdercus cingulatus.*

Temp (℃)	Day Length	Number of insects	Duration (Days; Mean ± SE)
20.0	14L-10D	21	87.1±1.4
22.5		22	62.3±0.4
25.0		36	49.3±0.5
27.5		8	41.9±0.5
30.0		19	35.5±0.7
22.5	10L-14D	32	56.7±0.3
25.0		23	42.8±0.2

Table 3. Reproductive traits of *Antilochus coqueberti* fed with *Dysdercus cingulatus* under 14L-10D at 25℃.

Properties	value(Mean ± SE)	[min.-max.]
Preoviposition period (days)	10.7±0.7	[10-12]
Adult longevity (days)	97.2±56.9	[34-186]
No. of oviposition	10.9±5.9	[4-20]
Egg batch size	55.2 ±15.5	[20-91]
Total fecundity	601.7±294.5	[222-990]

Reference

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Differences among rice cultivars in CH_4 emission and populations of rhizospheric methanotrophic bacteria at the rice ripening stage

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Key words : methane emission, *methanotrophic bacterium*, rice cultivar, subtropical paddy field

Objectives

In lowland rice cultivation, rice plants grow under flooded conditions and CH_4 is emitted from the fields. Controlling CH_4 emissions from paddy fields is expected to contribute to the mitigation of global warming. Methanotrophic bacteria can utilize CH_4 as their sole carbon and energy source and hence, they are considered to be important regulators of atmospheric CH_4 fluxes. The objectives of this study were 1) to enumerate the populations of methanotrophic bacteria in rice rhizosphere and soil in a subtropical paddy field, and 2) to elucidate the differences among three rice cultivars (Chiyonishiki, Japonica rice; IR72, Indica rice; and IR65598, tropical Japonica rice) in terms of CH_4 emission, CH_4 oxidative activity in roots, and rhizospheric methanotrophic population at rice ripening stage.

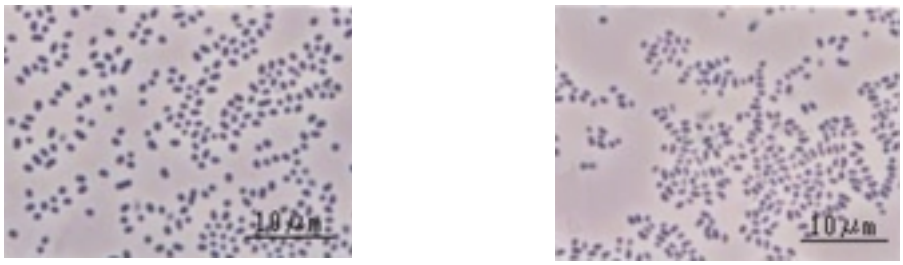
Results

Methanotrophic bacteria in the genus *Methylosinus* (Fig. 1) were isolated from rice rhizospheres in a subtropical paddy field on Ishigaki Island in Okinawa Prefecture. (light clay soil; alluvial origin).

Fluctuations in population levels of methanotrophic bacteria in rice rhizospheres and soil with and without rice straw application at 4 and 10 t/ha (Fig. 2) shows that at the rice heading to ripening stages, the methanotrophic populations in rice rhizospheres increased to about 10^5/g dry matter, whereas the populations in soil concurrently declined to $3x10^3$/g dry soil.

Table 1 shows that CH_4 emission rate for IR65598 was significantly lower than in the other two cultivars at the ripening stage, while its CH_4 oxidative activity in roots was the highest among the three cultivars. Furthermore, the methanotrophic population in roots in IR65598 was significantly higher than in the other two cultivars at the ripening stage.

From this study it was concluded that methanotrophic bacteria *Methylosinus* spp. inhabited rice rhizospheres in the subtropical fields used in this study, and it was also found that CH_4 emission rates and methanotrophic populations in roots differed significantly among rice cultivars at the rice ripening stage.



a b

Fig. 1. Photomicrographs of methanotrophic bacteria *Methylosinus* spp. isolated from rice rhizospheres in a subtropical paddy field. a: Strain R16; b: strain R18. The bar indicates 10 μm.

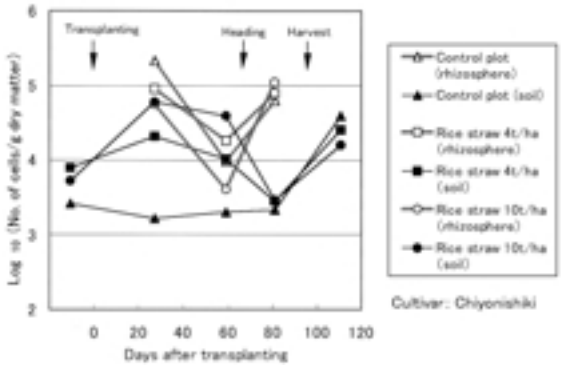


Fig. 2. Fluctuations in population levels of methanotrophic bacteria in rice rhizospheres and soil with and without rice straw application at 4 and10t/ha in a subtropical paddy field.

Table 1. CH_4 emission rate, CH_4 oxidative activity and methanotrophic population level in roots, and root dry weight of three rice cultivars at rice ripening stage (pot experiment, 1 plant pot^{-1}).

Cultivar	CH_4 emission rate (mg pot^{-1} h^{-1})	CH_4 oxidative activity (CH_4 oxidized μg g^{-1} root d^{-1})	Methanotrophic population level in roots (No.g^{-1} dry roots)	Root dry weight (g $plant^{-1}$)
Chiyonishiki	1.78 ± 0.52 a	13.9 ± 2.5 a	4.2 × 10^6 b	2.23 ± 0.43 b
IR72	2.25 ± 0.46 a	11.1 ± 3.4 b	4.5 × 10^6 b	3.63 ± 0.54 a
IR65598	0.66 ± 0.25 b	16.0 ± 3.7 a	6.5 × 10^7 a	3.06 ± 0.84 ab

Values are means of 3 replicates } SD (standard deviation). Data in column followed by the same letters are not significantly different.

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