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Research Highlights 2002

Major Research Results from April 2002 to March 2003

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Production of a digital map of the hazardous conditions of soil erosion for the sloping lands of West Java, Indonesia using geographic information systems (GIS)

S. UCHIDA[1], S. RITUNG[2], D. KUNTJORO[2], W. W. MARTA[2] and WAHYUNTO[2]

[1]Development Research Division, JIRCAS
[2]Center for Soil and Agroclimate Research and Development, Indonesia

Key words: soil erosion, USLE, vegetable fields, GIS, remote sensing

Objectives

Soil erosion caused by precipitation is a kind of land degradation commonly appeared in the sloping lands of humid areas. In this study, we attempted to estimate the hazardous conditions of soil erosion by using geographic information systems (GIS). The study site used for this project was Langensari Village located in the Lembang Sub-District, Bandung District, West Java Province in Indonesia, where temperate vegetable fields were spread widely over the sloping highlands. Fig. 1 shows a typical scene of vegetable field in the site. We employed the Universal Soil Loss Equation (USLE) to estimate soil loss in the site. The objectives were 1) to develop a method to obtain factors used in the USLE from various geographic data including remote sensing data, and 2) to demonstrate the applicability of the system to land use planning.

Results

We developed a method to estimate rain factor (R) from the daily rainfall data. Fig. 2 shows the results of the estimated factor R during the period from 1990 to 2000. Soil factor (K) was calculated by using the results of physical analysis performed on soil samples taken from the site. Slope inclination factor (S) and the slope length factor (L) were obtained from digital elevation data produced by digitizing elevation contours on the 1:25,000 topographic map. In order to estimate the crop management factor (C), we analyzed three temporal remote sensing data and obtained the land cover/use data, which involved information from the cropping period. The conservation practice factor (P) was obtained by the values of texture features of aerial photos.

Fig. 3 shows the hazardous state of soil erosion represented by the 11-year averaged value for the period from 1990 to 2000. This figure represents that the erosion-prone area was concentrated in the southern part of the site. The methods employed in this study also enabled us to predict how the amount of soil loss would change if land use type or cropping pattern was altered. Table 1 summarizes the results of the calculation for the four patterns of assumed cropping, of which all the agricultural fields were cultivated simultaneously three times per year. This table indicates that the amount of soil loss differs remarkably depending on the sowing pattern. The amount of soil loss of Pattern 3 is far less than that of Pattern 1, presumably due to the effect of surface coverage by vegetation in September.

Fig. 1. A vegetable field located in the southern part of the study site.

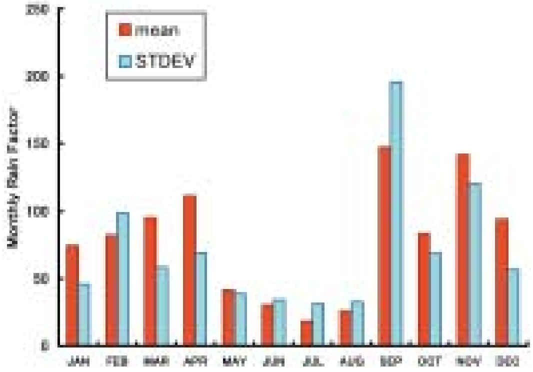

Fig. 2. Monthly rain factor showing mean and standard deviation (STDEV) estimated from daily rainfall data from 1990 to 2000.

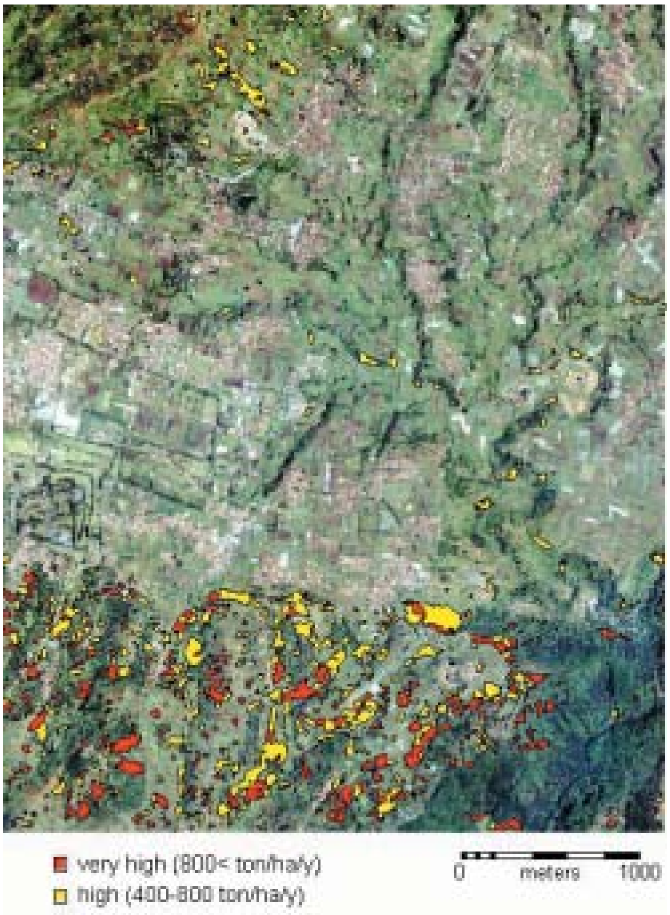

Fig. 3. Distribution of potentially hazardous areas of soil erosion expressed by the annual amount of soil loss.

Table 1. Assumed cropping period and estimated annual soil loss in the Langensari Village area (388 ha).

Pattern	Sowing TIme			Estimated Soil Loss (ton/ha/year)		
				Maximum during the period 1990-2000	Averaged over up to 3rd maximum	Averaged over up to 5th maximum
1	Jan	May	Sep	221	178	157
2	Feb	Jun	Oct	182	160	147
3	Mar	Jul	Nov	*155*	*147*	*140*
4	Apr	Aug	Dec	168	158	149
Present Land Use	Feb Apr	(Jun) (Aug)	Oct Dec	171	158	148

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E-mail address: uchidas@jircas.affrc.go.jp

Impact on food supply and demand due to changing water resources in Shandong Province, China

H. P. Chien

Development Research Division, JIRCAS

Key wards: water, food, supply and demand, Shandong

Objectives

The diversification and modernization of farm production in China have increased the dependence on irrigation water. At the same time, growing demand for water from cities and township enterprises severely restrains the available water supply needed to improve farm production. While this water resource problem may not be easily solved, a quantitative analysis on the variation of water resources and its relation to grain production might elucidate an approach for improving the situation.

Shandong Province, with a population of 90 million and a high economic growth rate, is one of the major agricultural production areas in China, and is suffering from a severe shortage of rainfall. Rainfall in the provincial capital Jinan in 2002 was only 382 mm, or merely 60 percent of the yearly average, causing grain production to decrease by as much as 20 percent from the previous year (Fig. 1). The present study employs a simulation model to analyze how the changing of water resources influences the grain production.

Results

In Shandong Province, the rate of drought-affected farm land during 1980's (averaged among the most severe drought years with a yearly damaged area above 3 million hectares) was approximately 3.45 million hectares per year. During 1990's, the rate increased to 4.16 million hectares per year. As a result, the yearly supply-demand (production and consumption) gap of these drought years (12.87 million tons) was 3 million tons more than that of the non-damage years (Fig. 2). Taking into account the decrease of agricultural land caused by future population growth, city expansion and industrial progress, as well as the influence of irrigation on the yield of grain production, simulation analysis on how the food supply-demand gap and water supply are related, was carried out using the system dynamic model.

Assuming an average yearly water supply of 25 billion m^3, the food supply-demand gap will expand continuously and exceed 20 million tons in 2008 (Fig. 3). On the other hand, if it is assumed that the water supply will increase to 35 billion m^3 (the minimum water demand in 2030 in Shandong Province is predicted to be 33.5 billion m^3), the food supply-demand gap will then be reduced by an amount predicted not to exceed 10 million tons. If the supply-demand gap is not reduced by significantly increasing water supply, the possibility of the province losing its status as a major grain production area will continue to remain high.

Fig. 1. Corn affected by drought in Shandong Province, China.

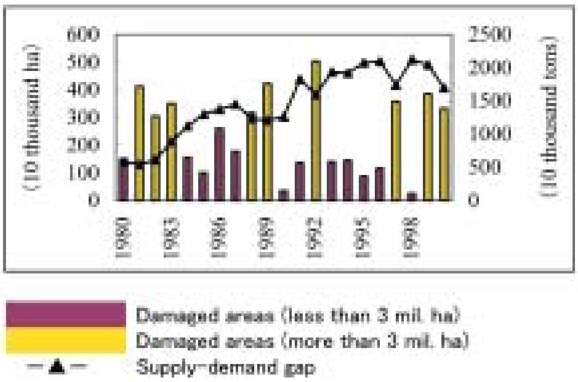

Fig. 2. Area of damage caused by drought and prediction of food supply-demand gap. Note: Grain products include cereal, tubers and beans. Colors in the demaged areas over 3 million hectares are yellow. Source: "China Statistical Yearbook (1990-2001)", "China Rural Statistical Yearbook (1985-2001)".

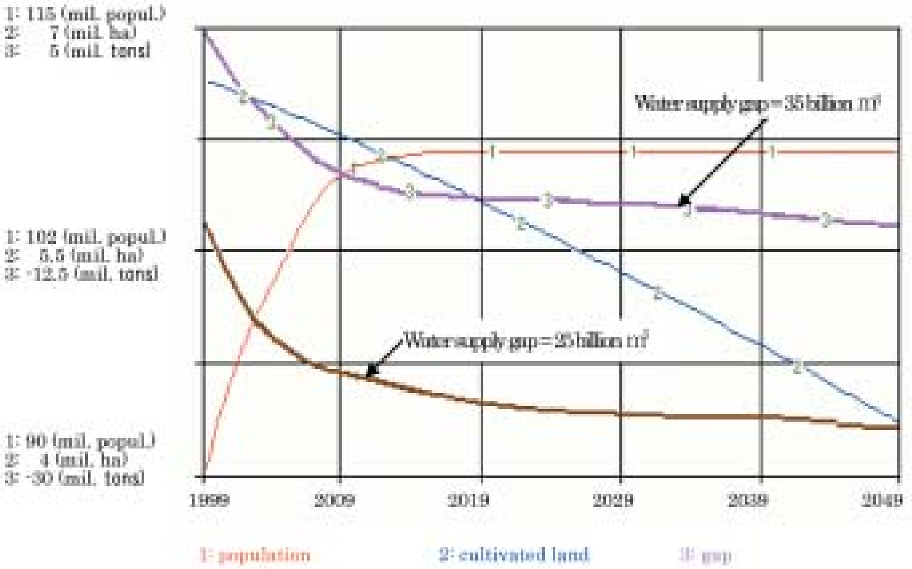

Fig. 3. Changes in the food supply-gap according to quantity of water supply.

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E-mail address: chienp@jircas.affrc.go.jp

Development of the World Forest Products Model

O. Koyama[1], J. Furuya[1], H. Oka[2] and K. Tamura[2]

[1]Development Research Division, JIRCAS
[2]Forestry and Forest Products Research Institute, Japan

Key words: forest products, global quantitative model, trade and environmental policies

Objectives

The preservation of forest resources is a critical issue in forest product trade negotiations. Many countries have analyzed the potential effects caused by liberalizing the trade of forest products on the natural environment based on the quantitative models. In this study, a project team composed of researchers from JIRCAS and the Forestry and Forest Products Research Institute developed a global quantitative model, with a particular emphasis on Pacific Rim countries in order to analyze how the reduction of tariff rates would affect the forest product markets and the sustainability of forest resources.

Results

The model, termed the "World Forest Products Model (WFPM)," is a comprehensive, yet practical model that forecasts the influence of trade and environmental policies based upon the previous leading world forest models, such as the GFPM (Global Forest Products Model) of the FAO or the GTM (Global Trade Model) of the IIASA (International Institute for Applied Systems Analysis). The WFPM is a simultaneous equation model using the same structure of the International Food Policy Simulation Model, created by JIRCAS, whereas these other models are based on mathematical programming.

The characteristics of the WFPM are as follows: 1) a partial equilibrium model focused specifically on forest products; 2) a multi-products model determining equilibrium prices of products that relate to each other simultaneously; 3) a multi-country or regional world model forecasting trade activity among regions; 4) a dynamic model using lagged variables to forecast time-series changes; 5) a synthetic model employing parameters estimated in other models; and 6) a policy simulation model analyzing the effects of policy changes such as the change of tariff rates.

The WFPM analyzes the following six products: round wood for industrial use, sawn wood, wood-derived panels, boards, pulps and paper. The data incorporated into the model include yearly time-series data that are aggregated from the FAOSTAT database. The model handles thirty-five regions and countries. Fig. 1 displays the structure of the WFPM.

The effects of tariff rate changes on supply and demand were tentatively measured during a sensitivity test of the model. In this case, the tariff rates were placed at zero for 2005. From the model, it was determined that few price changes occur at the world level (from −0.5 to 2.4%), for tariff rates are already at low rates. However, considerable effects on demand were discerned in the industrial markets, although product and regional effects vary substantially.

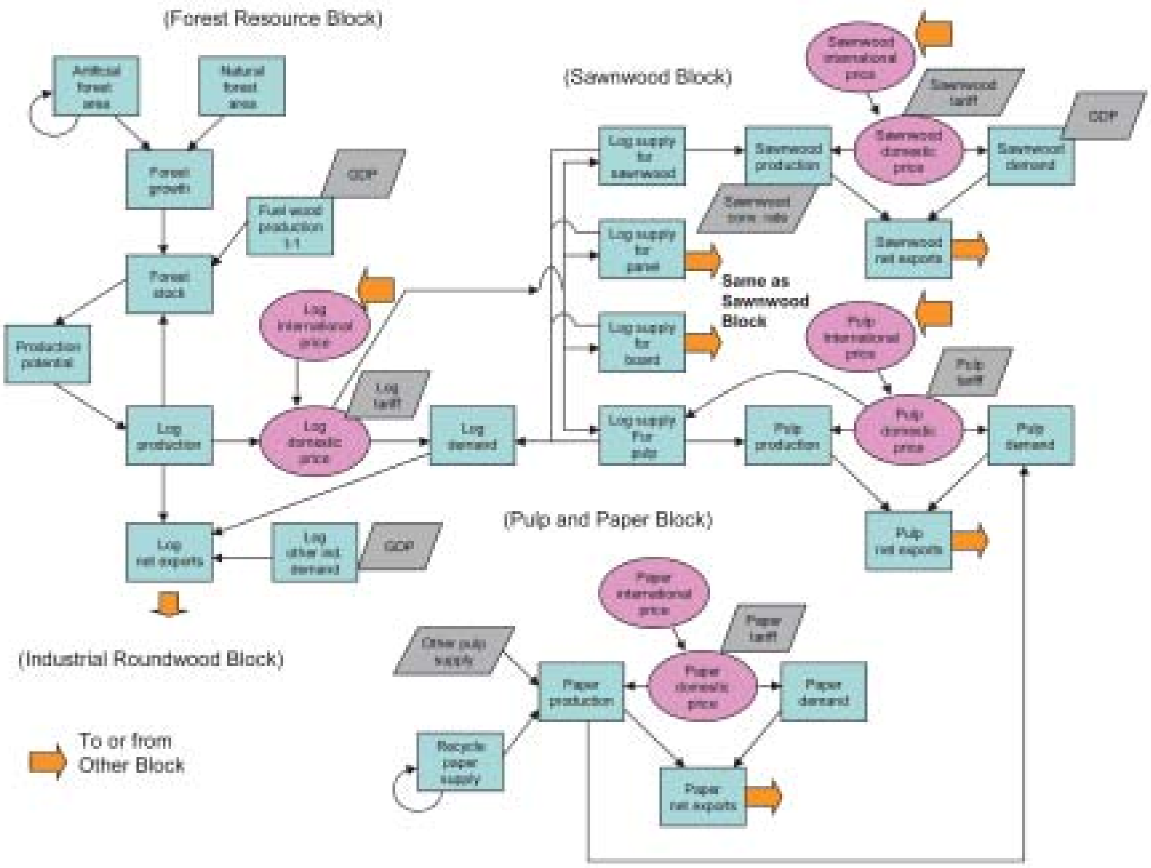

Fig. 1. Structural chart of the World Forest Products Model (WFPM).

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E-mail address: koyama@jircas.affrc.go.jp

An osmosensor as a molecular tool for the genetic improvement of drought-tolerant crops

T. Urao[1] and K. Yamaguchi-Shinozaki[2]

[1,2]Biological Resources Division, JIRCAS
[1](Present address: Research Planning and Coordination Division, JIRCAS)

Key words: *Arabidopsis*, drought stress, GM, osmosensor

Objectives

In marginal or arid lands in developing countries, environmental factors such as drought, high salinity, high temperature, and flooding are serious problems that lead to instable crop productivity. To address this problem, researchers had developed stress-tolerant plants by transferring a gene encoding protective proteins or enzymes involved in stress tolerance from various organisms. These past efforts had limited success, however, due to the genetic complexity of stress responses and adaptation. Prior shortcomings have indicated that introducing a large number of genes into a plant is necessary to confer fully improved stress tolerance. Thus, we need to manipulate a regulatory gene that controls the quantities and timing of the numerous effecter molecules described above. Under drought conditions, a change in cellular osmotic pressure caused by water loss triggers various intracellular responses. The first step in this event is perception of osmotic changes by a sensor or receptor protein(s) at plasmamembrane. Therefore, we decided to target the osmosensor as the regulatory gene to be engineered.

Results

ATHK1 contains two hydrophobic transmembrane regions adjacent to a putative extracellular domain in the N-terminal half, suggesting functional similarity with the yeast osmosensor SLN1. Overexpression of the ATHK1 cDNA suppressed the lethality of a yeast *sln1* mutant. Moreover, introduction of the ATHK1 cDNA into an yeast mutant lacking both osmosensors, SLN1 and SHO1, allowed normal growth and activation of the HOG1 MAPK cascade under the high osmolarity condition, suggesting that the ATHK1 activity changed to inactive state from active state in response to increases in external osmolarity. Thus, we demonstrated, by analyzing both sensing (input) and catalytic (output) activities of ATHK1 *in vivo* using the yeast osmosensing-defective mutants, that ATHK1 has an ability to sense and transduce a signal of external osmolarity to the downstream targets. In order to exammine the function of ATHK1 in planta, we initially screened dominant-negative ATHK1 mutants that inhibit the activity of the wild-type ATHK1 and isolated six candidates (*ATHK1-1 to 6*). We then generated transgenic *Arabidopsis* plants overexpressing the dominant-negative ATHK1 cDNAs. cDNA microarry and following northern blot analyses indicated that a number of stress-inducible genes are constitutively expressed in the dominant-negative ATHK1 overexpressors under the unstressed condition (Fig. 1). Moreover, the dominant-negative ATHK1 overexpressors were tolerant to dehydration and high salinity stresses than wild-type plants (Fig. 2). These results, together with yeast genetic analysis, suggest that ATHK1 is an osmosensor in *Arabidopsis*. This is the first evidence that a plant histidine kinase acts as an osmosensor. The ATHK1 gene could be one of the most useful molecular tools or biological resources for the genetic improvement of drought-tolerant crops.

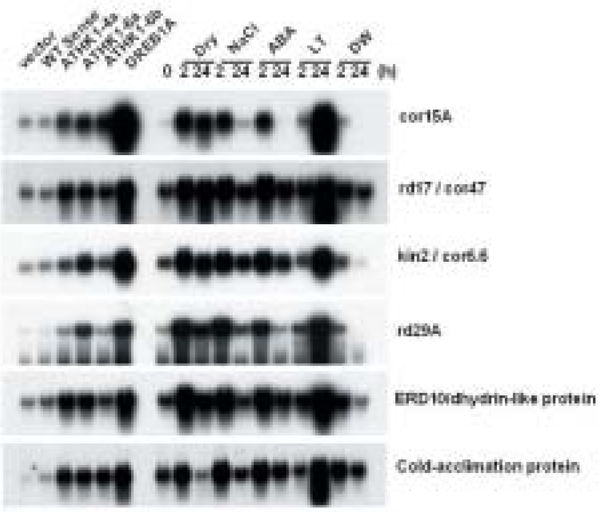

Fig. 1. Northern blot analysis of stress-inducible genes. Expression levels of a number of stress-inducible genes were higher in all of the dominant negative *ATHK1* (*1-4a, 1-6a, 1-6b*) and DREB1A overexpressors (DREB1A) than those in the control (vector) and wild-type *ATHK1* overexpressors (WT sense) under unstressed conditions. These genes were also induced by dehydration stress, high osmolarity (NaCl), absicic acid (ABA), low temperature (LT) or water (DW) in wild-type *Arabidopsis* plants.

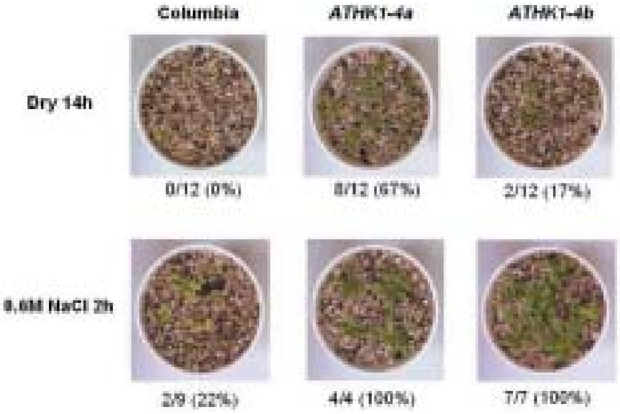

Fig. 2. Drought and high salinity tolerance of the dominant-negative *ATHK1* overexpressors. 3-week-old plants were dehydrated for 14 hours on paper and re-hydrated for 2 days with water. The plants were then transferred to a pot for 5 days. 67% and 17%, respectively, of the dominant-negative *ATHK1* overexpressors survived, but all wild-type plants died under the same conditions. 3-week-old plants were exposed to 600 mM NaCl solution for 2 hours and then grown in a pot for 5 days. The salt tolerance of the dominant-negative *ATHK1* overexpressers (100% survival, respectively) was much stronger than that of the wild-type plants (22% survival).

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E-mail address: kazukoys@jircas.affrc.go.jp

Evaluation of antioxidant activity of indigenous vegetables from South and Southeast Asia

T. SATO[1], M. NAGATA[2] and L. M. ENGLE[3]

[1]Biological Resources Division, JIRCAS
(Present address: National Institute of Vegetable and Tea Science, National Agriculture and Bio-oriented Research Organization, Japan)
[2]National Institute of Vegetable and Tea Science, National Agriculture and Bio-oriented Research Organization, Japan
[3]Asian Vegetable Research and Development Center, Taiwan

Key wards: antioxidant activity, indigenous vegetables, vitamin C, total phenol

Objectives

More than eight hundred million people currently suffer from hunger and malnourishment. Improving the production of principal food crops such as rice, wheat, corn and root crops has thus become one of the most important research subjects in the field of agriculture. Yet, sufficient levels of micronutrients and vitamins are also essential for maintaining good health, especially for pregnant women and preschool children in rural areas. Since vegetables can provide high levels of micronutrients, vitamins and fiber, producing more vegetable varieties has become an important task. To help develop vegetable variety, indigenous vegetables that are under-utilized globally but sufficiently adopted to local areas in growth and taste preferences (Fig. 1) can be of great value. The purpose of this study, conducted in collaboration with the Asian Vegetable Research and Development Center (AVRDC), has been to select the most superior indigenous leaf vegetables based on functional properties.

Results

To complete this objective, various accessions of indigenous vegetables from South and Southeast Asia were evaluated for their antioxidant activity, levels of ascorbic acid (vitamin C), and total phenol count. Antioxidant activity was assayed by a modified thiocyanate method using ethanol extracts; total phenolic compounds were determined by the Folin-Denis method using the same ethanol extracts; and ascorbic acid content was evaluated by RQflex and use of analytical test strips. These methods do not require the use of expensive analytical instruments such as high performance liquid chromatography (HPLC).

Among the twenty tested vegetable varieties, the top nine were determined to have young shoots and leaves with extremely strong antioxidant activity. These include Chinese mahogany (*Toona sinensis*), Horseradish tree (*Moringa* spp.), Feather cockscomb (*Celosia argentea*), Perilla (*Perilla frutescens*), Pigeon pea (*Cajanus cajan*), Black nightshade (*Solanum nigrum* or *S. americanum*), Ailanthus (*Zanthoxylum ailanthoides*), Capsicum pepper (*Capsicum annuum*) and White jute (*Corchorus* spp.). These vegetables also had a tendency to have higher ascorbic acid and total phenol contents (Table 1). Antioxidant activity, ascorbic acid and total phenol contents differed not only among vegetable species but also among accessions (Table 2).

For the selection of superior indigenous vegetables, evaluation of antioxidant activity, ascorbic acid and total phenol contents can be used as a novel index together with morphological and ecological characterization, and yield evaluation.

Fig. 1. Genetic diversity of Amaranth or Chinese spinach (*Amaranthus* spp.).

Table 1. The highest rated indigenous leafy vegetables in tems of antioxidant activity, ascorbic acid and total phenol contents at the AVRDC.

	Indigenous leafy vegetables/ Young shoots of fruit vegetables	Scientific name	Antioxidant activity[1]	Ascorbic acid content[2]	Total phenol content[3]
(Antioxidant activity : Strong)					
1	Chinese mahogany	*Toona sinensis*	128	(125)	(3,784)
2	Leaf of horseradish tree (Mo13)	*Moringa oleifera*	115	(287)	(691)
3	Feather cockscomb (purple)	*Celosia argentea*	114	(134)	(947)
4	Perilla	*Perilla frutescens*	114	84	(727)
5	Leaf of pigeon pea	*Cajanus cajan*	113	(259)	(833)
6	Leaf of horseradish tree	*Moringa* spp.	113	(245)	(713)
7	Leaf of black nightshade	*Solanum nigrum*	112	(146)	432
8	Ailanthus	*Zanthaxylum ailanthoides*	111	82	(2,134)
9	Leaf of capsicum pepper (purple)	*Capsicum annuum*	108	(226)	(1,158)
10	White jute	*Corchorus* spp.	107	(153)	503
11	Leaf of African eggplant	*Solanum macrocarpon*	105	120	537
12	Leaf of Chinese wolfberry	*Lycium chinense*	105	116	597
13	Parsley	*Petroselinum crispum*	104	(132)	271
14	Leaf of capsicum pepper	*Capsicum* spp.	99	(128)	(817)
15	Basil	*Ocinum basilicum*	99	28	302
16	Water convolvulus	*Ipomoea aquatica*	99	45	(726)
17	Gynura	*Gynura bicolor*	97	35	313
18	Madeira-vine	*Anredera cordifolia*	97	59	232
19	Dandelion	*Taraxacum officinale*	96	27	137
20	Sweet potato vine	*Ipomoea batatas*	96	35	684
(Antioxidant activity : Weak)					

Data in parenthese represent the top ten vegetables in terms of ascorbic acid and total phenol content.

[1] Relative value against 10 mM BHA positive control (%).

[2] Ascorbic acid content (mg/100g FW).

[3] Total phenol contant (mg chlorogenic acid equivalent/100g FW).

Table 2. Diversity of antioxidant activity, ascorbic acid and total phenol contents in young shoots and leaves of indigenous vegetables from South and Southeast Asia.

	Indigenous vegetables (Scientific name)	No. of accessions	Antioxidant activity[1] Max − Min	Ascorbic acid[2] Max − Min	Total phenol content[3] Max − Min
1	Perilla (*Perilla frutescens*)	7	118 − 110	98 − 67	1,039 − 479
2	Pigeon pea (*Cajanus cajan*)	51	116 − 86	259 − 78	1,348 − 757
3	Horseradish tree (*Moringa* spp.)	26	118 − 92	323 − 158	983 − 566
4	Black nightshade (*Solanum nigram*)	18	116 − 108	178 − 128	570 − 357
5	White jute (*Corchorus* spp.)	49	110 − 100	216 − 70	666 − 318
6	Water convolvulos (*Ipomoea aquatica*)	72	116 − 80	68 − 31	1,324 − 478
7	Capsicum pepper (*Capsicum* spp.)	22	108 − 93	226 − 82	1,158 − 428
8	Basil (*Ocimum basilicum*)	11	107 − 87	31 − 23	481 − 182
9	Spider flower (*Cleome gynandra*)	17	97 − 91	160 − 113	322 − 243
10	Garland chrysanthemum (*Chrysanthemum coronarium*)	20	96 − 89	57 − 35	343 − 210
11	Amaranth (*Amaranthus* spp.)	100	103 − 33	135 − 28	452 − 124
12	Malaber spinach (*Basella alba*)	78	94 − 35	154 − 53	547 − 211

[1] Final concentration of BHA was 40μM and that of sample was 2 mg (FW)/mL.
Antioxidant activity (AOA) : AOA (%) of sample/AOA (%) of 10mM BHA × 100.

[2] Ascorbic acid content (mg/100g FW).

[3] Total phenol contant (mg chlorogenic acid equivalent/100g FW).

E-mail address: ryutoku@affrc.go.jp

Identification of the sudden death syndrome pathogen of soybean in Argentina

Y. Homma[1], T. Aoki[2], R. Martin[3], M. J. Francioni[3] and A. Lattanzi[3]

[1]Biological Resources Division, JIRCAS
[2]Genetic Diversity Department, National Institute of Agrobiological Sciences, Japan
[3]Agricultural Experimental Station, Marcos Juarez, National Institute of Agricultural and Farming Technology, Argentina

Key words: sudden death syndrome, soybean, Argentina, *Fusarium solani* f. sp. *glycines*

Objectives

Recently, concerns have grown about the spread of and damage caused by soil-borne diseases. One of such diseases is sudden death syndrome (SDS), which has been associated with continuous cropping of soybean plants on untilled land in MERCOSUR countries. In order to bring SDS under control, identification of the pathogen that causes the disease is critical. Toward this objective, JIRCAS has conducted cooperative studies with the National Institute of Agricultural and Farming Technology (INTA) in Marcos Juarez, Argentina, entitled the “Ecology and control of major diseases of soybeans” since 1999.

Results

In Argentina, severe outbreaks of SDS have occurred in the Cordoba and Santa Fe Provinces. Infected plants were collected from the fields and the symptoms of SDS were observed and recorded. The symptoms of SDS are characterized by yellow spots on the leaves in the early stage of the disease, by the browning interveins of the leaves in the later stage, and by a loss of leaves in the final stage (Fig. 1). SDS also causes red-brown discoloration at the upper part of the main and lateral roots, and root rot, making the plant easy to unearth (Fig. 2).

While researchers are aware of the symptoms of SDS, identification of the pathogen that causes the disease has proven more difficult. Using PCR, a detection method was created that would identify the pathogen in both artificially inoculated and naturally infested plants. Tissue samples were taken from the infected plants and the isolated pathogen was observed under a microscope for its morphological characteristics.

A strain of the pathogen was cultured on a grain of sorghum, then dried and macerated to inoculate into soil. Soybean plants were grown for 40 days in a greenhouse for evaluation of the symptoms of SDS. The pathogen displayed a very slow growth rate on the potato dextrose agar (PDA) with one half to a third of the saprophytic *Fusarium* producing more than 50 μm of macroconidia, while the rest produced none. Sporodochya of the pathogen were frequently observed on the surface of the lesion of the roots. *Fusarium* was more frequently isolated from the red-brown colored tissues of the upper main roots and the lower hypocotyls than from the lateral roots (Table 1). The symptoms of the leaves and roots tested in greenhouse conditions were similar to those observed in fields. Yellow spots formed on the leaves two weeks after inoculation and the interveins subsequently began to brown.

Based on the observations and the application of Koch’s Postulates, it was concluded that the SDS pathogen in Argentina could be identified as *Fusarium solani* f. sp. *glycines*. The identification of the SDS pathogen is expected to help in the process of cultivating SDS-resistant soybean plants in Argentina. Recently, these strains of SDS from Argentina were reported as a new species Fusarium tucumaniae by Aoki et al. (2003).

Fig. 1. Leaves and roots displaying symptoms of sudden death syndrome in the field.

Fig. 2. Leaves displaying symptoms of sudden death syndrome in greenhouse test.

Table 1. Re-isolation of the MJ161 strain of *Fusarium solani* f. sp. *glycines* from roots infected in a greenhouse test.

Roots	Pieces tested	*Re-isolated	%
Upper main root	25	19	76.0
Lower main root	25	4	16.0
Lateral root	25	12	48.0

*Confirmation of the characteristics of the MJ161 strain on test tube culture of PDA.

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E-mail address: hommay@jircas.affrc.go.jp

Development of a small unhulled rice-dryer suitable for use in the Mekong Delta

M. Daikoku[1], H. Kobayashi[2], K. Kobayashi[3], Y. Kanetani[3] and Y. Oshita[4]

[1]National Agriculture Research Center for Western Region, National Agriculture and Bio-oriented Research Organization, Japan
[2]Crop Production and Environment Division, JIRCAS
[3]National Agriculture Research Center, National Agriculture and Bio-oriented Research Organization, Japan
[4]National Research Center for Hokkaido Region, National Agriculture and Bio-oriented Research Organization, Japan

Key words: rice dryer, Mekong Delta, all-weather, quality, cost

Objectives

The Mekong Delta is Vietnam's largest rice producing region. During the rainy season harvest time, conventional sun-drying methods can cause the quality of unhulled rice to deteriorate. Problems also arise during days of intense sunlight, when drying occurs too quickly and causes grains to crack. To assist cultivators in drying their rice in a more efficient and consistent fashion, researchers developed an all-weather, easy-to-use compact dryer that can dry approximately two tons of unhulled rice without deterioration and cracking.

Results

This dryer operates using a honeycomb coal furnace, air blower fan, and driving engine with slanted drying layers on both sides. Since the drying layers are located at a low position, it is easy to hold, mix and take out unhulled rice. An easily prepared weather protection tent allows users to continue to conduct drying even during sudden rainfall (Fig. 1 and Table 1). When the dryer is installed outdoors on an uneven surface, users must ensure that air does not leak from the ground plane.

Two tons of uhulled rice can be dried in approximately 13 hours with 25 percent initial moisture content without adverse effects. Drying can generate uneven moisture on the upper and lower layers of dry unhulled rice. However, this moisture can be reduced if the drying speed is increased by mixing and agitating the rice after five hours of operation. Since four people and approximately four hours are needed to complete the entire process of assembling, drying, and removal, one-time mixing and agitation is sufficient.

Fuel costs for honeycomb coal and light oil are approximately 74,000 VND (about 620 yen) per one ton of dry unhulled rice. The dryer is priced at 6,500,000 VND (about 54,000 yen), excluding the weather tent. Forty units are currently in use, primarily in the Mekong Delta area.

Fig. 1. Unfulled rice dryer.

Table 1. Dryer specifications.

Drying layer	width (mm)	2,900
	length (mm)	4,000
	height (mm)	700
	depth (mm)	280
Fan blower	fan diameter (mm)	450
	blowing capacity (m^3/s)	2.0
Heat source	honeycomb coal	2 sets
Driving engine		diesel
Rainout tent	width × length (m)	4 × 6

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E-mail address: daikoku@affrc.go.jp

Employing one-year crop rotations with three vegetable combinations to control clubroot damage in the West Java highlands

M. YAMADA[1], A. A. ASANDHI[2] and E. PURWATI[2]

[1]Crop Production and Environment Division, JIRCAS
[2]Indonesian Vegetable Research Institute, Indonesia

Key words: clubroot, crop rotation, cabbage, tropical highland, continuous cropping

Objectives

Indonesia is a typical tropical island country having a mountainous and volcanic terrain. Such geographical conditions are well suited for temperate vegetable cultivation such vegetables as cabbage, potatoes and carrots throughout the year. The highland farmers are eager to grow lucrative temperate vegetables including *Culciferae* family plants in continuous and/or highly frequent cropping and has now been brought the emergence of clubroot damage. In addressing the problem, short-term crop rotation systems that rotate three crop cycles within a year were tried in the tropical highland conditions.

Results

The continuous cropping (C) of cabbage produced the yield trend shown in Fig. 1. The C plot yields showed large fluctuations, but from the second season to sixth season, all values were lower than those produced from all the crop rotation cropping (R) plots, indicating that considerable yield reduction will occur when cabbage are grown continuously.

Since the third season, many cabbage plants in C plots died from clubroot disease and thereafter, severe clubroot damage was observed in C plots, the yield reduction was caused by clubroot disease. The effectiveness of the crop rotations was confirmed in the fourth season, after one year, namely one cycle of the rotations. The C plots were attacked by clubroot disease from early stage as shown by wilting symptoms (Fig. 2) and the yield was 2.57 kg/m^2. On the other hand, those of the R plots ranged from 7.07 to 7.39 kg/m^2 indicating the importance of crop rotation but no importance of sequence.

In the second season, early stage growth and the yields in plots kept in fallow during the last season were significantly better than those of the C plots. In the sixth season, these were compared again and a clubroot damage scores were 7 and 93 respectively suggesting the importance of removal of host plants for brief periods of time under moderate temperature conditions.

For the carrots which were combined in the crop rotations, no significant yield differences were not observed between any crop rotations suggesting less damage of continuous cropping at least until 6 times. Injury of continuous cropping of potatoes was also recovered by the crop rotations because of suppressing bacteria wilt damage.

Clubroot disease is a common in the West Java Highlands. However, the potential damage caused by clubroot disease can be mitigated by short-term crop rotations and these are a practical way to maintain cabbage and other vegetable production in ways that do not threaten the environment in these areas.

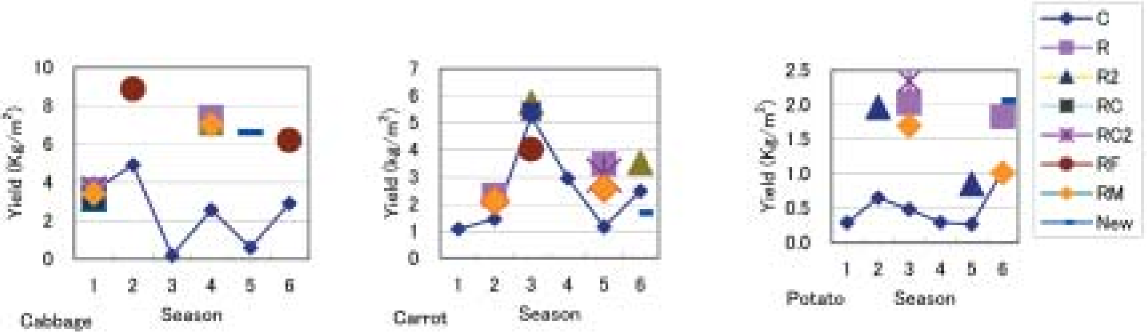

Fig. 1. Effects of continuous cropping on vegetable yield.

C, continuous cropping; R, Cabbage-carrot-potato rotation; R2, cabbage-potato-carrot rotation; RF, R rotation inserted with fallow cropping every two seasons; RC, R rotation, but inserted with corn after every vegetable; RC2, R2 rotation inserted with corn after every vegetable planting; RM, R rotation with mixed vegetables; New, cabbege plants were not cultivated for more than two years.

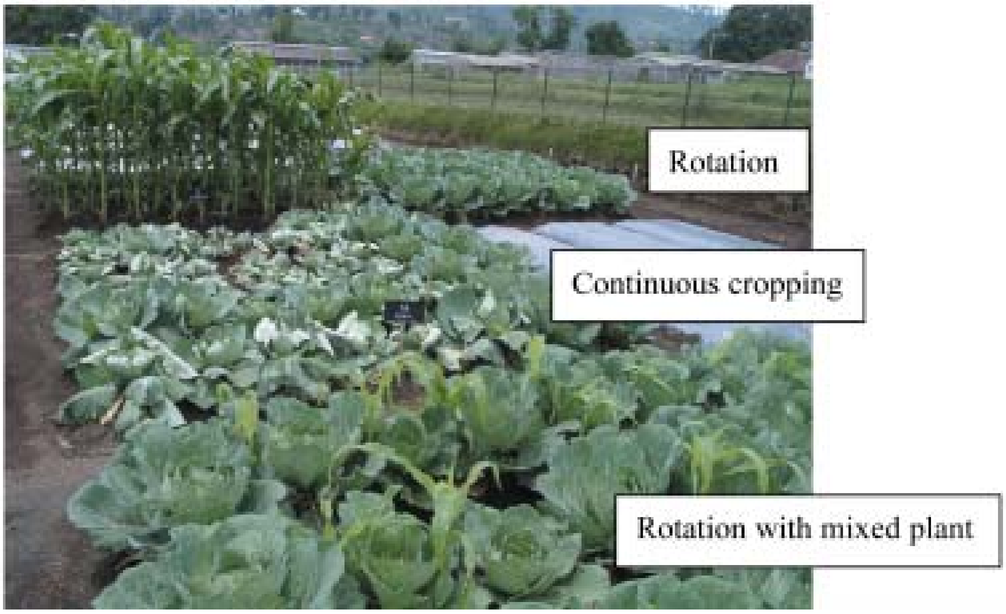

Fig. 2. Damage caused by clubroot disease in a continuous cropping plot.

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E-mail address: mitate@jircas.affrc.go.jp

Paddy field ammonia volatilization with double cropping and its control in the Red Soil Region of China

Y. Hosen[1], K. Yagi[2], M. Xu[3], D. Li[4], D. Qin[4], H. Shen[4] and J. Li[3]

[1]Crop Production and Environment Division, JIRCAS
[2]National Institute of Agro-Environmental Sciences, Japan
[3]Soil and Fertilizer Institute, Chinese Academy of Agricultural Sciences, China
[4]Red Soil Experimental Station, Chinese Academy of Agricultural Sciences, China

Key words: ammonia volatilization, polyolefin-coated urea, double rice cropping, Red Soil Region of China

Objectives

The People's Republic of China is the world's largest producer and consumer of food. New technologies and reform policies have substantially improved the conditions necessary to meet China's growing demand for grain. Yet the adverse environmental effects of intensive modern agriculture have recently raised concerns about land resource sustainability and the environment. Intensive farming has caused significant land degradation, irrigation water shortages, and air and water pollution, all of which are now serious issues in China. Many of these problems stem from farmlands being overloaded with nutrients, particularly nitrogen. China's use of nitrogen fertilizer has increased to 27.4% of world consumption, and a total of 22.4×10^6 tons of nitrogen were used in 2001 alone. Excess nitrogen in the environment poses numerous health and environmental problems.

A nitrogen cycling field experiment in the Red Soil Region, one of the most important double rice cropping hill areas in China, was carried out at the Red Soil Experimental Station, Chinese Academy of Agricultural Sciences, Qiyang County, Hunan Province (26º45'21"N, 111º52'22"E, Fig. 1). The goal was to quantify the present conditions of non-point source N pollution in the region and to develop methods to mitigate that pollution.

Results

Using a polyolefin-coated urea (MEISTER S9 for early rice, MEISTER 10 (40%) + MEISTER S15 (60%) for late rice) at a rate of 150 kg N/ha (conventional level) or 75 kg N/ha for each crop just after flooding (keeping other conditions the same), five treatments were prepared with triplicates. These were R150 (readily available urea at a rate of 150 kg N/ha), R75 (readily available urea at a rate of 75 kg N/ha), C150 (coated urea at a rate of 150 kg N/ha), C75 (coated urea at a rate of 75 kg N/ha) and a control (no nitrogen). By using an open-chamber method and monitoring other environmental data, ammonia volatilization potential for each plot was determined. The total ammonia volatilization potential during the early rice and late rice cropping period reached 35 percent of applied N at R150, decreasing to 21, 11 and 10 percent at R75, C150 and C75, respectively (Fig. 2, top). High ammonia concentrations in flooding water (Fig. 2, middle) and high pH (Fig. 2, bottom) were observed for one week after applying readily available urea. This period corresponded well to the period when high ammonia volatilization potential was observed. C75 produced a high yield that did not differ significantly from that of R150, but was significantly higher than that of C150, which suffered damage from a blast and sheath blight (Fig. 3).

It was concluded that the high ammonia volatilization potential observed from conventional management of paddy fields in this region can be mitigated. To do so, controlled-release fertilizer can be used such that a reduced fertilizer application rate is made compatible with a high yield.

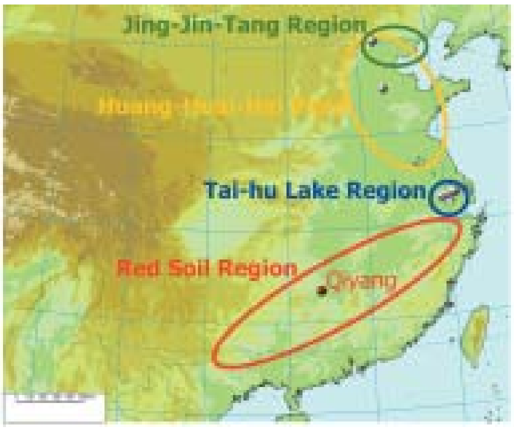

Fig. 1. The Red Soil Experimental Station (a red dot), which is located in the Red Soil Region, one of four major agricultural regions in the People's Republic of China. Dots indicate joint research sites.

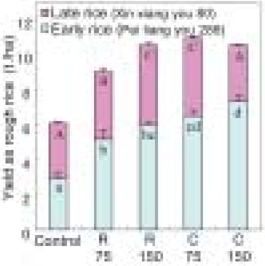

Fig. 3. Yields of early and late rice in 2002 in terms of rough rice. Vertical bars indicate the SD's of triplicate measurements. Means in a crop followed by the same letter are not significantly different at $P < 0.05$ by the Student's T-test. Abbreviations as in Fig. 2.

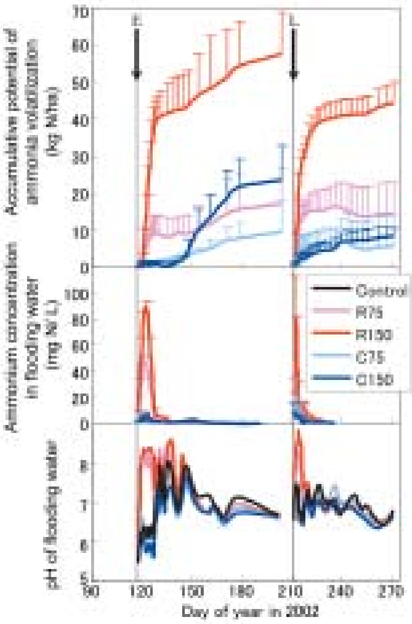

Fig. 2. Top, accumulative potentials of ammonia volatilization (control value subtracted from the original); middle, ammonium N concentrations; bottom, pH of flooding water. Measurements were carried out around sunset. R75, readily available urea at a rate of 75 kg N/ha; R150, readily available urea at a rate of 150 kg N/ha; C75, coated urea at a rate of 75 kg N/ha; C150, coated urea at a rate of 150 kg N/ha; E, early rice transplanting and fertilizer application on April 26, 2002; L, late rice transplanting and fertilizer application on July 30, 2002. Vertical bars indicate the SD's of triplicate measurements (average only for flooding water pH).

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E-mail address: yhosen@affrc.go.jp

Characterization of nitrogen utilization by tropical grasses (*Brachiaria* species) in the Brazilian savannas

T. Nakamura[1], T. Kanno[2], C. H. B. Miranda[3], Y. Ohwaki[4] and M. C. M. Macedo[3]

[1]Crop Production and Environment Research Division, JIRCAS
(Present address: National Institute of Crop Science, National Agriculture and Bio-oriented Research Organization, Japan)
[2]Agriculture, Forestry and Fisheries Research Council, Japan
[3]Embrapa, Gado de Corte, Brazil
[4]National Agricultural Research Center, National Agriculture and Bio-oriented Research Organization, Japan

Key words: *Brachiaria* grasses, Brazilian savannas (Cerrado), nitrogen utilization

Objectives

Since the 1970's, *Brachiaria* species have been introduced to 40 million hectares, or 85 percent, of grassland area in the Brazilian Cerrados. The three most common species cultivated in the region are *Brachiaria decumbens* (BD), *Brachiaria brizantha* (BB), and *Brachiaria humidicola* (BH). Poor nutrient status of the soil, especially related to nitrogen (N), is a main factor limiting grass productivity in low fertility and acid soil areas in Brazilian Cerrados. However, interspecific differences regarding nutrient uptake and efficiency of use are not still clear in these grasses. In this study, researchers thoroughly analyzed how the grasses responded to different N supplies with a particular emphasis on how they differed in terms of nutrient uptake and efficiency of use.

Results

During that analysis, the N utilization characteristics of BB, BD, and BH, grown under different N conditions, were compared, as well as the grass absorption and utilization mechanisms. BB, BD, and BH were grown under different levels of N (0, 50, and 150 kg N/ha) in a pot experiment. The dry matter weight of BH was higher than those of BD and BB when no N was applied (Fig. 1). In BD and BB, dry matter weight increased when applied N levels were increased, and after sufficient N conditions, surpassed the dry matter weight of BH. The dry matter weight of BH did not increase with N application, suggesting that BH is better adapted to limited N conditions than the other two grasses. The relative N absorption rate (RAR) was of BH was higher than those of the other two grasses in the treatment without N application (Fig. 2). The smaller reduction of BH's dry matter weight under low-N conditions could be due to a high-N absorption rate.

In a field experiment, researchers estimated the contribution ratio of fixed N derived from air in the plants (%Ndfa) under no N application using the ^{15}N natural abundance technique. *Panicum maximum* (PM) was considered to be suitable as a reference crop for the estimation because $\delta\ ^{15}N$ of PM was highest among the four plants (Table 1). The %Ndfa of BH was lower than those of BD and BB. This indicated that the contribution ratio of soil N would be higher in BH than in BD and BB.

To evaluate root nitrate uptake capacity, a kinetic experiment was conducted. Nitrate uptake rate can be expressed according to Michaelis-Menten kinetics (Equation 1). The kinetic parameters for nitrate uptake were calculated using the depletion-method (Table 2). Km in BH was lower than BB and BD, suggesting that BH has a high affinity to nitrate and can maintain a high nitrate uptake rate under limited N conditions. BH can maintain dry matter production under limited N conditions because its roots have a high nitrogen uptake ability. BD and BB, on the other hand, can achieve high productivity under N-sufficient conditions because of their high response to applied N.

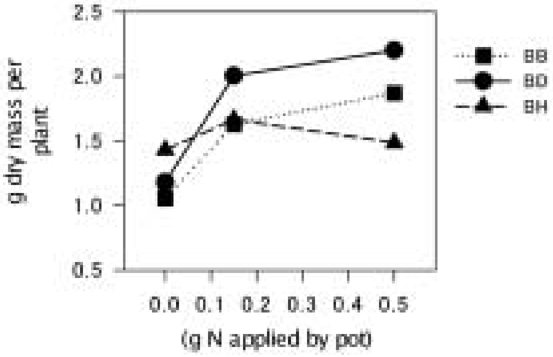

Fig. 1. Dry mass in plants under different levels of N.

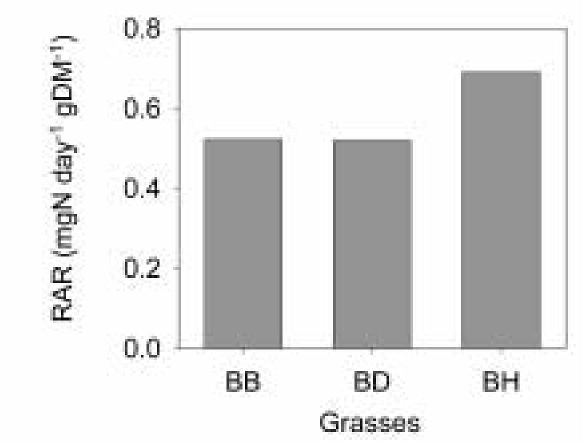

Fig. 2. Relative N absorption rates in plants with no N application.

Table 1. Contribution ratio of fixed N derived from air in plants. (%Ndfa)

	N content (mgN g^{-1}DM)	δ ^{15}N (‰)	%Ndfa (%)
BB	13.5	5.7	26.8
BD	11.4	6.0	24.0
BH	8.7	7.0	9.2
PM	12.1	7.9	

Table 2. Kinetic constants of NO$_3^-$ uptake in *B. brizantha* (BB), *B. decumbens* (BD), and *B. humidicola* (BH).

	BB	BD	BH
Vmax (μmol m^{-2} h^{-1})	152.4	146.4	160.6
Km (μM)	11.2	7.5	4.4

<Equation 1>

$$v = \frac{Vmax \times C}{Km + C}$$

v is the uptake rate of nitrate,

Vmax is the maximum nitrate uptake rate,

Km represents the Michaelis constant

C is the concentration of nitrate in the solution.

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E-mail address: takuwan@naro.affrc.go.jp

Estimation of changes in nitrogen flow accompanying agricultural development in Cantho Province, Vietnam predicted for 2010

T. WATANABE[1], T. NAGUMO[2] and N. N. HUNG[3]

[1]Crop Production and Environment Division, JIRCAS
[2]College of Agriculture, Hokkaido University, Japan
(Present address: College of Agriculture, Shizuoka University, Japan)
[3]College of Agriculture, Cantho University, Vietnam

Key words: nitrogen cycling, farming system, waste water from animal husbandry, Mekong Delta

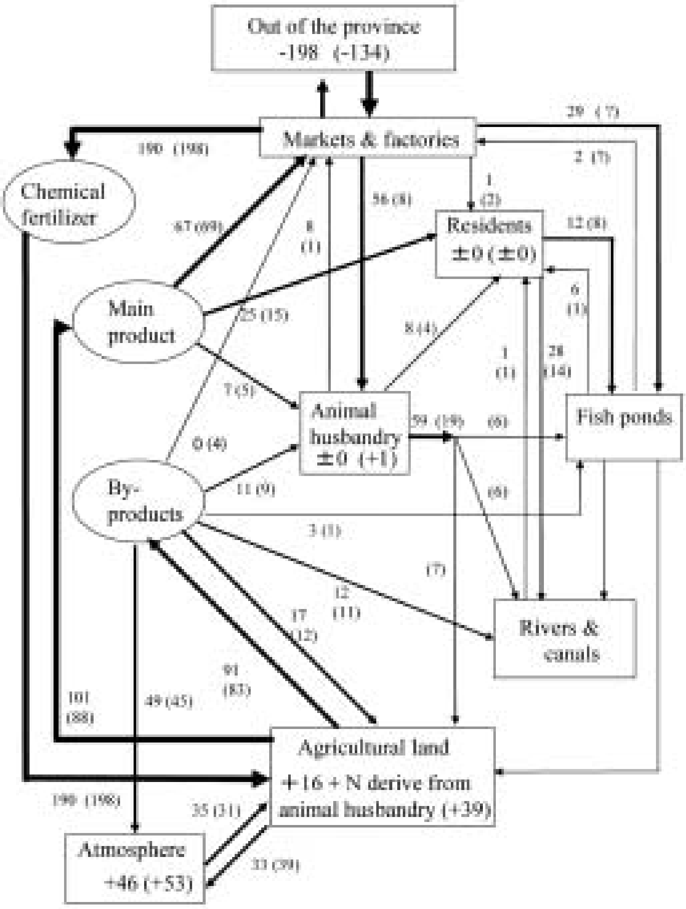
Objectives

Farming systems in Vietnam's Mekong Delta have been subjected to increased agro-chemical input in order to greatly intensify agricultural activity, and thereby significantly raise profits. As a result, however, material flows of various chemicals, and in particular, nitrogen, have been disturbed, a process that could lead to an increase in water pollution. The goal of this study was to estimate nitrogen flow in 1999 in order to determine how agricultural development will impact nitrogen flow and water pollution through the year 2010 in Cantho Province, Vietnam.

Results

Cantho Province lies on the right bank of the Hau River in the central part of the Mekong Delta, and supports a population of more than 1,815,272 over 296,422 ha of land. A model was constructed to calculate nitrogen flow during 1999 and estimate nitrogen flows during 2010. The unit nitrogen flow (kg N/ha/ year) refers to the nitrogen flow (kg N/year) divided by the area of agricultural land or planned agricultural land of Cantho Province in 1999 and 2010, respectively. To determine the volume of nitrogen flows, researchers used the official agricultural development plan authorized by the People's Committee of Cantho Province, entitled "Agricultural Planning for Cantho Province to 2010," in order to provide the predicted figures for agricultural production, cultivated area, livestock, food consumption by residents, and population for the year 2010. Where information was not provided, researchers compensated by using data from related reports published in Japan and other foreign countries.

The estimated nitrogen flow in Cantho Province in 1999 and 2010 is shown in Fig. 1. The plan predicts rapid development in the areas of animal husbandry and aquaculture (Table 1), which will bolster the demand for animal and fish feeds. If the number of livestock increases as reported, livestock excreta per unit area of agricultural land will increase from 19 to 59 kg N/ha/year. Although material cycling in Cantho Province has been intensified with the development of agriculture since the 1980s, the nitrogen balance in the agricultural land was +39 kg N/ha/year in 1999, and it seems that this balance will not change significantly towards 2010. These results indicated that the nitrogen balance will be in relatively good condition through 2010. Therefore, since the agricultural land of Cantho Province still maintains a capacity to absorb nitrogenous wastes from animal husbandry and aquaculture, it is essential that more livestock excreta be applied to agricultural land, rather than be discarded directly into rivers, in order to protect public health and the environment. To this end, the use of biogas digesters (Fig. 2) and the composting of livestock feces should be promoted in this region.



Unit: kg N / ha (agricultural land) / year

Fig. 1. Predicted nitrogen flow in Cantho Province (2010).

Table 1. Agricultural data for Cantho Province.

	unit	1999	2010
Pigs	10^3 heads	243	700
Milk cows	10^3 heads	0	23
Poultry	10^3 heads	2,940	6,000
Freshwater fish	t	8,040	64,400
Freshwater prawns	t	60	3,000
Agricultural lands	ha	249,995	239,513

Fig. 2. Plastic biogas digester.

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E-mail address: watatake@jircas.affrc.go.jp

Drought tolerance characteristics of Brazilian soybean cultivars

T. Oya[1], A.L. Nepomuceno[2], N. Neumaier[2], J.R.B. Farias[2], S. Tobita[3] and O. Ito[3]

[1,3]Crop Production and Environment Division, JIRCAS
[1](Present address: Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan)
[2]National Soybean Research Center, Brazilian Agricultural Research Corporation, Embrapa, Brazil

Key words: Brazil, cultivar difference, drought tolerance, relative growth rate, seed yield, soybean

Objectives

Soybean production in South America is highly productive but severely diminished if drought occurs during the reproductive growth stages of soybean crops. In order to illuminate the physiological and morphological characteristics associated with drought tolerance as measured by a yield index, scientists artificially measured drought stress during the reproductive growth stage of soybean cultivars from southern Brazil using rainout shelter treatment from 1999 to 2000.

Results

Researchers determined that yield ranking among cultivars in the rainout shelter (RS) plots was stable across the two-year period with a correlation coefficient for yield ranking significant at the 1% level (r=0.78). Although the irrigated plot showed the yield rankings in a different order, there was a significant correlation between the rankings for two seasons, suggesting that drought tolerance should be detectable using rainout treatment.

On the basis the RS plot yield figures, cultivar BRS 183 displayed high drought tolerance, while BR-16, Embrapa 59, and BRS 134 showed low drought tolerance (Table 1). The results of growth analysis (Table 2) in the RS plots during the rainout treatment period illustrated that the BRS 183 had a high relative growth rate (RGR), and thus, a high drought tolerance. BR-16, Embrapa 59, and BRS 134 displayed low RGRs and low drought tolerance. RGR differences reflected a difference in net assimilation rate (NAR), but the leaf area ratio (LAR) did not differ among cultivars. BRS 183 had large root dry weight during the drought period compared with the low drought tolerance cultivars, namely BR-16, Embrapa 59, and BRS 134 (Fig. 1).

Based on these results, it was concluded that relative growth rate during drought conditions for a one-month period following flowering can serve as a good index for analyzing how drought tolerance differs among cultivars. However, if drought occurs during other periods, drought response may not necessarily be the same as reported in this study.

Table 1. Seed yield of ten Brazilian soybean cultivars under drought conditions using rainout shelters.

No.	Cultivar	Seed yield (t ha^{-1}) 1999/2000	Seed yield (t ha^{-1}) 2000/2001
1	BR-16	1.01	1.38
2	BR-37	1.24	1.76
3	Embrapa 48	1.07	1.72
4	Embrapa 59	0.86	1.34
5	BRS 132	1.05	1.68
6	BRS 133	1.08	2.02
7	BRS 134	1.06	1.20
8	BRS 183	1.23	2.38
9	BRS 184	1.10	1.99
10	BRS 185	0.88	1.57
	LSD (5%)	0.70	0.84

After first flowering soybeans received no rains for one month under rain-out shelters. Cultivars with high drought tolerance are represented in blue and those with low tolerance in red.

Table 2. Growth parameters of soybean cultivars in rainout-treated (RS) plots during the drought stress period (1999/2000).

No.	Cultivar	RGR g g^{-1}d^{-1}	NAR g m^{-2}d^{-1}	LAR m^2 g^{-1}
1	BR-16	0.015	1.3	0.012
2	BR-37	0.035	3.1	0.012
3	Embrapa 48	0.006	0.6	0.010
4	Embrapa 59	0.010	0.8	0.012
5	BRS 132	0.030	2.8	0.011
6	BRS 133	0.034	2.9	0.012
7	BRS 134	0.019	1.6	0.012
8	BRS 183	0.046	3.9	0.012
9	BRS 184	0.031	2.7	0.011
10	BRS 185	0.031	3.0	0.011

After first flowering soybeans received no rains for one month under rain-out shelters. RGR=NAR × LAR. Cultivars with high drought tolerance are represented in blue and those with low tolerance in red.

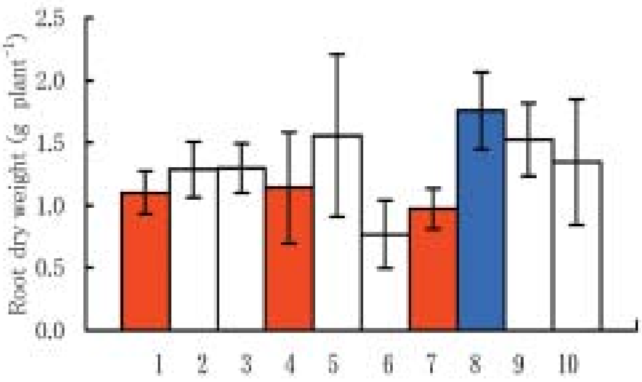

Fig. 1. Root dry weight of soybean plants 20 days after first flowering (2000/2001). Means ± SD of 3 replicates. Cultivars with high drought tolerance are represented in blue and those with low tolerance in red.

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E-mail address: oyatet@affrc.go.jp

Modification of the pouch method to evaluate the ability of lactic acid bacteria (LAB) strains for improving silage quality

S. Ohmomo[1], S. Nitisinprasert[2] and S. Hiranpradit[3]

[1]Animal Production and Grassland Division, JIRCAS
[2]Faculty of Agro-Industry, Kasetsart University, Thailand
[3]Department of Agriculture, Ministry of Agriculture and Co-operatives, Thailand

Key words: animal industry, silage, lactic acid bacteria

Objectives

In Thailand, most cows are fed with fresh pasture crops, grasses, and rice straw, and produce an average amount of raw milk at about 3,100 kg/head/lactation. However model farms and innovative farmer groups produce a significantly greater volume of raw milk, 4,200–4,500 kg/head/lactation, by feeding superior quality silage. Silage-making in Thailand is not a common technique, and improving the quality of silage is not easy. One major reason for this involves the complexity of bacteria effects during silage fermentation. It is well known that lactic acid bacteria (LAB) is essential for silage-making, and thus, a silage fermentation model was constructed to evaluate LAB specifically adapted to the tropical environments of Thailand by modifying the pouch method, a simple and easy system used to evaluate LAB in Japan.

Results

To modify the pouch method, microbial floras of silages prepared in Thailand were examined (Table 1). The LAB, aerobic bacteria, enterobacteria (coli form bacteria: CFB) and yeast were major microbes, and the counts of each microbes were different very much between grass silage and corn silage. For in grass silages, the counts of CFB and yeast were relatively abundant, but counts of LAB were low.

A silage fermentation model was constructed by modifying the pouch method in which napiergrass was used as silage material and yeast strain *Saccharomyces serevisiae* SG2-1Y and CFB strain *Enterobacter* sp. SG1-1T were used as typical silage microbes. Napiergrass was harvested after 30 days of cultivation, dried at 105℃, and then autoclaved. After inoculation with the yeast, and CFB and LAB strains, napiergrass (with moisture content adjusted to 75% and 1.5% of glucose added) was placed into a bag made from air-tight plastic film (oxygen permeability: 1 ml/m^2/day), sealed by vacuum sealer and cultured at 45℃ for 2 days.

The inoculation size greatly affected the growth of each silage microbe in the pouch. For example as shown in Table 2, when the inoculation size of LAB strain LG2-1 was 10^3 times higher than that of yeast and CFB, the LAB growth was accelerated while the CFB growth was repressed. However, LAB strain N-22 never repressed the growth of yeast and CFB in its early growth stage. Therefore, the mixture of LAB strain LG2-1 and N-22 with a high inoculation size causes the quick LAB growth. This in turn causes pH levels to drop quickly from the early stage.

The modified pouch method can evaluate a substantial quantity of LAB strains easily and quickly, helping researchers to screen practical-use LAB strains that can contribute to improving the quality of silage in tropical regions, including Thailand.

Table 1. Fermentation quality and microbial flora of silages prepared in Thailand.

Silage sample[1]	pH	MC[2] (%)	Microorganism count[3] (cfu/g)				
			TVC	LAB	CFB	Yeast	Mold
SS 1	7.71	60.4	5.0×10^{6}	$< 10^{1}$	9.0×10^{3}	6.9×10^{4}	2.0×10^{4}
SS 2	4.35	48.8	9.0×10^{4}	2.0×10^{6}	1.3×10^{3}	4.0×10^{5}	2.0×10^{4}
CS 1	3.82	78.0	5.1×10^{6}	3.3×10^{6}	$< 10^{1}$	5.4×10^{4}	$< 10^{1}$
KS 1	5.10	28.1	4.6×10^{7}	2.8×10^{7}	$< 10^{1}$	2.0×10^{2}	$< 10^{1}$

[1] SS 1, Grass silage in bunker silo (surface); SS 2, Grass silage in bunker silo (inside); CS 1 and KS 1, Corn silage in bagged silo.
[2] MC, Moisture content.
[3] TVC, Aerobic bacteria; LAB, Lactic acid bacteria; CFB, Coli form bacteria (Enterobacteria).

Table 2. Effects of inoculum size on the growth of typical silage microorganisms in a pouch.

IS[1]	Culture time (h)	*Lactobacillus* sp. LG 2-1				*Lactococci* sp. N-22			
		pH	Microbe count (cfu/g)			pH	Microbe count (cfu/g)		
			LAB	CFB	Yeast		LAB	CFB	Yeast
A	6	5.69	1.1×10^{6}	1.2×10^{6}	1.1×10^{6}	5.89	1.0×10^{5}	1.1×10^{6}	1.0×10^{6}
	24	5.47	2.8×10^{6}	3.2×10^{6}	2.0×10^{6}	5.59	1.0×10^{5}	9.0×10^{5}	1.0×10^{5}
B	6	5.69	3.2×10^{7}	2.0×10^{5}	1.0×10^{6}	5.79	1.9×10^{8}	1.3×10^{6}	7.0×10^{5}
	24	5.34	1.2×10^{8}	2.2×10^{6}	4.0×10^{6}	5.66	5.0×10^{5}	1.2×10^{6}	2.0×10^{5}
C	6	5.86	1.1×10^{6}	2.8×10^{7}	1.0×10^{6}	5.91	1.0×10^{5}	7.8×10^{7}	1.0×10^{6}
	24	5.55	2.0×10^{6}	2.0×10^{6}	2.4×10^{6}	5.44	1.0×10^{6}	8.0×10^{4}	2.0×10^{6}
D	6	5.51	1.2×10^{6}	3.2×10^{7}	2.4×10^{7}	5.77	4.4×10^{7}	6.0×10^{7}	1.4×10^{7}
	24	5.52	2.8×10^{6}	1.0×10^{8}	1.5×10^{8}	5.55	2.8×10^{6}	6.2×10^{7}	9.2×10^{6}

[1] IS, Inoculum size (cfu/g): A (LAB 10^{2}, CFB 10^{2}, yeast 10^{2}); B (LAB 10^{5}, CFB 10^{2}, yeast 10^{2}); C (LAB 10^{2}, CFB 10^{5}, yeast 10^{2}); D (LAB 10^{2}, CFB 10^{2}, yeast 10^{5}).

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E-mail address: bupmomo@jircas.affrc,go.jp

Screening of candidate genes for trypanotolerance in mice livers using a DNA microarray

Y. NAKAMURA[1], J. NAESSENS[2], S. KIERSTEIN[2], J. GIBSON[2], F. IRAQI[2], Y. YAGI[3],
H. KITANI[4] and Y. TSUCHIYA[3]

[1]Animal Production and Grassland Division, JIRCAS
(Present address: National Institute of Animal Health, National Agriculture and Bio-oriented Research Organization, Japan)
[2]International Livestock Research Institute, Kenya
[3]National Institute of Animal Health, National Agriculture and Bio-oriented Research Organization, Japan
[4]National Institute of Agrobiological Sciences, Japan

Key words: DNA microarray, gene expression, resistance, trypanosomosis

Objectives

African trypanosomosis, a protozoan disease transmitted by tsetse flies, is among the most important disease constraints to livestock production, as well as human health, in sub-Saharan Africa. It has been shown that breeds of cattle and inbred strains of mice exhibit genetically based differences in the degree to which they are resistant to the disease. Classical analyses have been made to understand the relation between resistance and host factors specified through characteristic signs and alterations during infection. However, such analyses have failed to elucidate fully the mechanisms of trypanotolerance. In the present study, gene expression levels between mouse strains were comprehensively compared to screen candidate genes for trypanotolerance using a DNA microarray carrying thousands of cDNA fragments.

Results

A DNA microarray was prepared by spotting 7,445 species of 65-mer mouse oligonucleotide probes onto a slide glass using an automatic gene arrayer. Resistant C57BL/6 and susceptible A/J mice were infected with trypanosome parasites, and liver samples were obtained on days 0, 4, 7, 10 and 17 after infection. Total RNA was extracted from each tissue, and four pooled RNA samples, each containing five individual samples, were prepared from each mouse strain at each sampling time point. They were then used to synthesize cDNA fragments labeled with red or green fluorescent dye. Red-labeled cDNA of one strain and green-labeled cDNA of the other strain derived from the same sampling time point were mixed and hybridized with the microarray. Following hybridization, signal intensities of both fluorescent dyes bound to each probe were scanned using a laser-confocal array scanner. Differentially expressed genes between C57BL/6 and A/J mice were detected by calculating a ratio of signal intensities from the two mouse strains. Fig. 1 shows a conceptual diagram of DNA microarray analysis.

A total of 169 genes were differentially expressed in the livers between the two mouse strains during the course of trypanosome infection (Fig. 2). These included genes encoding important factors involved in a variety of host responses, such as acute phase proteins, complement factors, cytokines and chemokines, electron transporters, intracellular signaling factors, apoptosis factors, ion channels and metabolic enzymes. These genes should be further studied for their roles in conferring genetically based differences in resistance and susceptibility to trypanosome infection among mouse strains.

The present study was conducted under collaboration with the University of Liverpool and the Medical Research Council-UK.

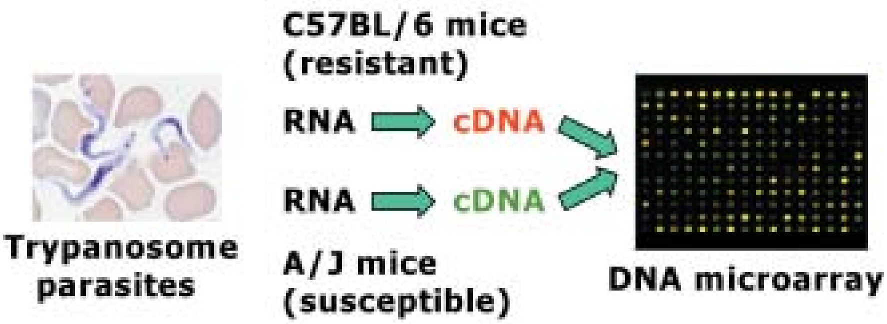

Fig. 1. Conceptual diagram of DNA microarray analysis used to screen candidate genes for trypanotolerance.

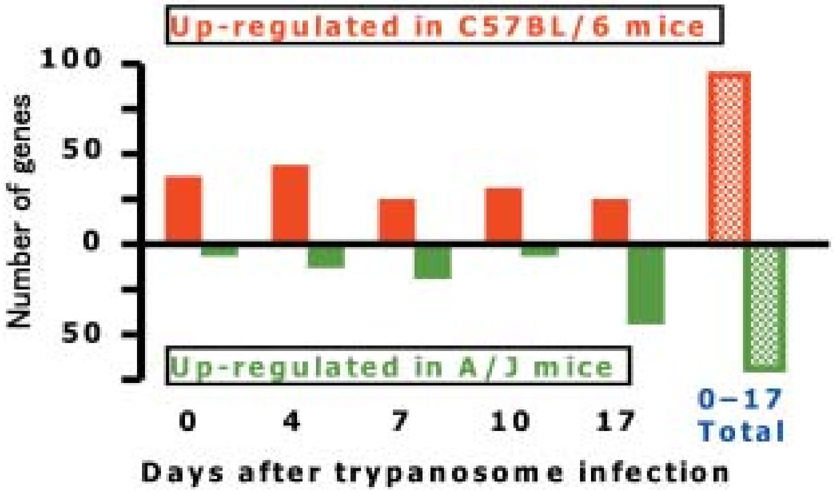

Fig. 2. Number of differentially expressed genes in mice liver compared between strains examined using a DNA microarray.

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E-mail address: yoshion@affrc.go.jp

Construction of a molecular linkage map in soybean

N. Yamanaka[1], Z. Xia[2], M. Hoshi[2], Y. Tsubokura[2] and K. Harada[2]

[1,2]Department of Horticulture, Chiba University, Japan
[1](Present address: Biological Resources Division, JIRCAS)

Key words: AFLP, cDNA, molecular linkage map, RFLP, soybean, SSR

Objectives

A molecular linkage map covering a large region of the genome with informative DNA markers is very useful for effective and efficient plant selection in breeding programs. It is also essential for identifying and isolating the genes responsible for various quantitative traits. Recently, some soybean linkage maps have been developed with restriction fragment length polymorphism (RFLP) markers based on soybean genomic DNA, and simple sequence repeat (SSR) markers. DNA markers based on polymerase chain reaction (PCR), such as SSR markers, are suitable for the rapid selection of plants in breeding programs. However, DNA markers derived from expressed genes are important for the identification of gene-rich regions as well as quantitative trait loci (QTLs). In addition, since DNA sequences that code for genes are conserved among many plant families, this kind of marker can also be used in other species. Therefore, we constructed a molecular linkage map of soybean by using complementary DNA (cDNA) markers derived from expressed genes in addition to DNA markers based on PCR.

Results

The soybean (*Glycine max*) varieties Misuzudaizu and Moshidou Gong 503 were crossed to develop a segregated population of 190 F_2 plants. Based on this population, linkages were calculated among RFLP markers derived from cDNA and genomic DNA, SSR markers, amplified fragment length polymorphism (AFLP) markers, random amplified polymorphic DNA (RAPD) markers, and qualitative trait loci.

A portion of the constructed soybean molecular linkage map is shown in Fig. 1. This molecular linkage map has 724 markers, including 412 RFLP markers (223 cDNA and 189 genomic DNA), 106 SSR markers, 218 AFLP markers, and one RAPD marker. These markers contain 472 DNA markers developed originally (unpublished data for AFLP markers). This map is the first soybean linkage map having a large number of cDNA markers.

This linkage map also consists of 20 major linkage groups that may correspond to the 20 pairs of soybean chromosomes. The total length of the linkage groups is 3,221 cM of the soybean genome according to the Kosambi function, indicating that this map covers the soybean genome almost completely. This linkage map may thus be used for various aspects of soybean genetic study and breeding.

This study was conducted at Chiba University under partial support from the JIRCAS research project entitled “Comprehensive studies on soybean improvement, production and utilization in South America”.

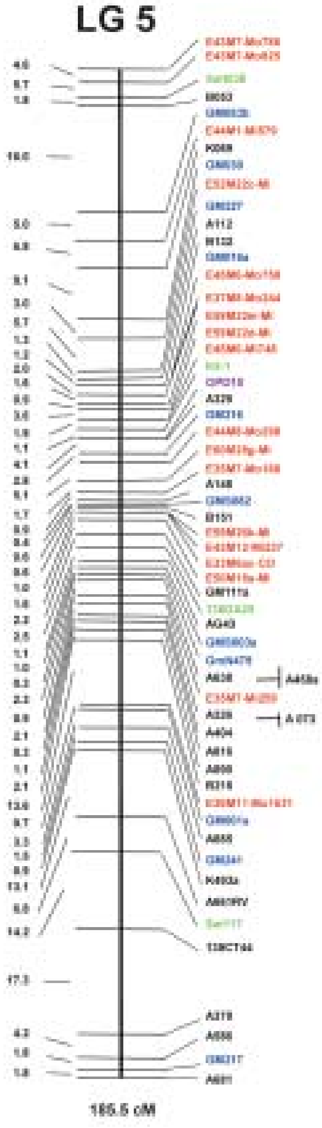

Fig. 1. Soybean molecular linkage map of linkage group 5. Distances according to the Kosambi function and markers are shown on the left and right of the bar, respectively. The total length of this linkage group is shown on the bottom. Color separation indicates marker type (i.e. black, RFLP markers derived from genomic DNA; blue, RFLP markers derived from cDNA; green, SSR markers; red, AFLP markers; and purple, RAPD markers).

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E-mail address: haradaq@faculty.chiba-u.jp

Formation of flavor compound in aromatic rice and its fluctuations with drought stress

T. YOSHIHASHI[1], N. KABAKI[2], T. T. H. NGUYEN[1] and H. INATOMI[3]

[1]Food Science and Technology Division, JIRCAS
[2]Crop Production and Environment Division, JIRCAS
(Present address: National Agriculture Research Center, National Agriculture and Bio-oriented Research Organization, Japan)
[3]Faculty of Agriculture, Meiji University, Japan

Key words: 2-acetyl-1-pyrroline, aromatic rice, Khao Dawk Mali 105, drought stress

Objectives

With consumer demand for rice surging, farmers throughout the world face substantial pressure to increase productivity while maintaining the quality of their product. Such pressure is particularly severe for farmers cultivating Khao Dawk Mali 105, a major aromatic rice variety usually marketed as "Jasmine," in Northeast Thailand, where drought-stricken sandy soils and other constraints have caused infertility and restricted sufficient cultivation. What is more, cultivation conditions and postharvest practices are compromising the aromatic quality of the rice.

2-acetyl-1-pyrroline, a compound having a "popcorn"-like flavor, is a characteristic flavor component of aromatic rice, and has a lower odor threshold (less than 0.1 ppb in water) than other volatile compounds found in aromatic rice varieties. This compound has also been isolated and identified from pandan (*Pandanus amalyrofolys* Roxb.) plant leaves and popcorn, and contributes to the aroma of roast beef and wheat and rye bread crusts. There have been no reports thus far elucidating the formation of 2-acetyl-1-pyrroline in aromatic rice varieties, yet formation of this compound in cocoa fermented products and baked products such as breads have been reported. In bread this compound is formed from ornithine and triose phosphates during baking through the Strecker degradation of ornithine. In cocoa, this compound is produced from both L-proline and L-ornithine by *Bacillus cereus* during fermentation.

Results

The formation of these compounds was investigated using a stable isotope dilution method and tracer analysis with ^{15}N amino acids to determine the structure of the precursor (Fig. 1). Analysis confirmed that 2-acetyl-1-pyrroline formed at normal temperatures in the aerial part of aromatic rice, and that L-proline was the nitrogen precursor (Fig. 2). Storage tests also indicated that this compound in aromatic rice kernels can exist as a complex within the hydrophobic region of crystalline starch. These results suggested that certain pre-harvest factors control the quality of aromatic rice, particularly aroma. It was therefore hypothesized that osmotic stress and nitrogen fertilizer application would increase L-proline concentration in rice kernels during ripening. Researchers tested these factors on Khao Dawk Mali 105 and found that osmotic stress during the milky ripening stage increased 2-acetyl-1-pyrroline content, whereas stress after the yellow ripening stage did not. Experiments on nitrogen fertilizer application indicated that application slightly increased 2-acetyl-1-pyrroline content (Fig. 3). These results indicate the possibility of controlling the quality of aromatic rice during cultivation, particularly through ripening and postharvest practices.

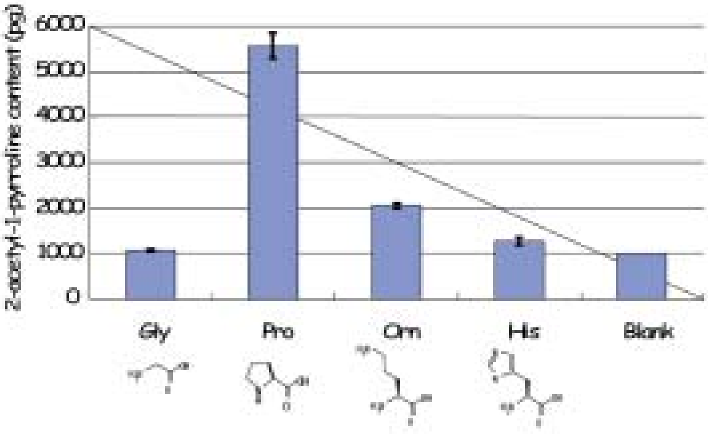

Fig. 1. Effects of amino acid addition to rice seedlings on 2-acetyl-l-pyrroline formation. When proline (Pro) was added to the solution for rice seedlings, 2-acetyl-1-pyrroline increased the concentration by more than 3-fold in comparison to the control. Addition of glycine (Gly) and histidine (His) did not increase the content in rice seedlings.

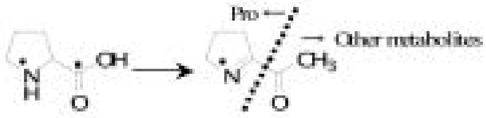

Fig. 2. Possible pathway for 2-acetyl-l-pyrroline formation from proline. Dots show nitrogen in the pyrrolidine ring on proline, and squares show the carbonyl carbon on proline. Carbonyl carbon was not derived from proline, although the nitrogen source was proline.

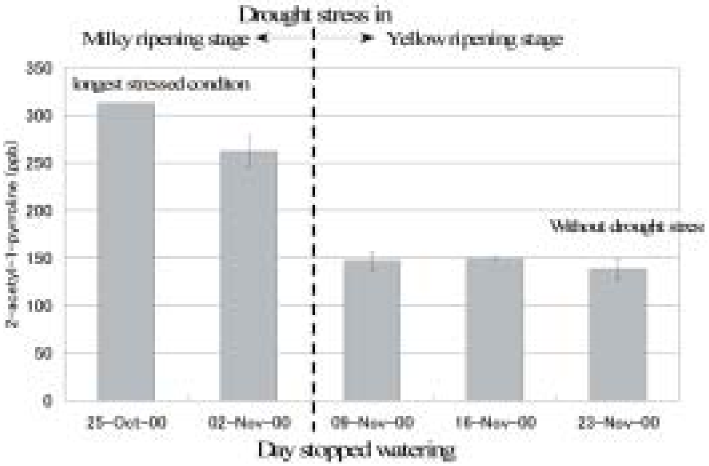

Fig. 3. Drought stress induced 2-acetyl-1-pyrroline content when rice was in milky ripening stage, though the stress in yellow ripening stage did not affect the content as the result of pot-based experiment of Thai aromatic variety Khao Dawk Mali 105.

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E-mail address: tadashi@jircas.affrc.go.jp

Antimutagenicity of local vegetables in Thailand

K. NAKAHARA[1], G. TRAKOONTIVAKORN[2], H. ONO[3],
M. KAMEYAMA-OHNISHI[3] and M. YOSHIDA[3]

[1]Food Science and Technology Division, JIRCAS
[2]Institute of Food Research and Product Development, Kasetsart University, Thailand
[3]National Food Research Institute, Japan

Key words: antimutagenicity, vegetable, Thailand, *Micromelum minutum*, *Oroxylum indicum*

Objectives

Epidemiological studies have revealed that high consumption of fruits and vegetables reduces the risks of chronic diseases and cancer. In fact, cancer incidence rates in several Thai cities, where many indigenous plants and minor crops are utilized as food and medicine, are significantly lower than those of northern Europe and North America (IARC/ WHO). In this research, indigenous plants from Thailand were analyzed to determine their antimutagenic and/or anticancer effects.

Results

The antimutagenic activity against Trp-P-1 (3-amino-1,4-dimethyl-*5H*-pyrido[*4,3-b*]indole) of methanolic extracts of 108 species of edible Thai plants was examined by the Ames Test. The activity was evaluated by the amount of plant extracts which suppressed 90% of the mutagenesis (ED_{90}). Five plants, *Micromelum minutum* (Thai name: *yod mui*), *Oroxylum indicum* (*pheka*), *Cuscuta chinensis* (*sai mai*), *Azadirachta indica* (*sadao*) and *Litsea petiolata* (*thammang*) exhibited significant activity with antimutagenic ED_{90} values lower than 5 μL/plate (0.1 mg of dry plant material equivalent). *M. minutum* (Rutaceae) which showed the highest activity in the antimutagenic screening, is consumed mainly in the southern area of Thailand as a fresh vegetable served with thin rice noodles and spicy sauce, a meal commonly referred to as *kanom-chin-nam-ya* or *kanom-chin-keang-tai-pla*. Various parts of *M. minutum*, including edible twigs (Fig. 1), are also used as folk medicine to treat fever or dizziness. The activity-guided fractionation of the extracts resulted in the isolation of an active principle, which was identified as mahanine (Fig. 1) based on its physicochemical properties. Mahanine is a carbazole alkaloid derivative previously found in *Murraya koenigii* (curry leaf, consumed in South Asia), which is an allied species of *M. minutum*. Mahanine exhibited various bioactivities, including antimutagenicity against some heterocyclic amines (ED_{50} against Trp-P-1 was 5.2 μM), cytotoxicity against a tumor cell line HL-60, and antimicrobial activity against *Bacillus cereus* and *Staphylococcus aureus*. *Oroxylum indicum* (Bignoniaceae), is a deciduous tree distributed throughout South Asia, Southeast Asia and China. The tree is used as a crude ingredient in traditional medicine for curing stomach disorders, diarrhea and rheumatic swelling. In Thailand, the fruits and flowers of the plant are consumed as a vegetable in the north and northeastern areas. The active constituent of the fruit of *O. indicum* responsible for antimutagenicity was baicalein (Fig. 2). The antimutagenic ED_{50} of baicalein against Trp-P-1 was 2.78 μM. The high content of baicalein (4.0%, dry weight basis) is associated with the potent antimutagenicity of the original extract.

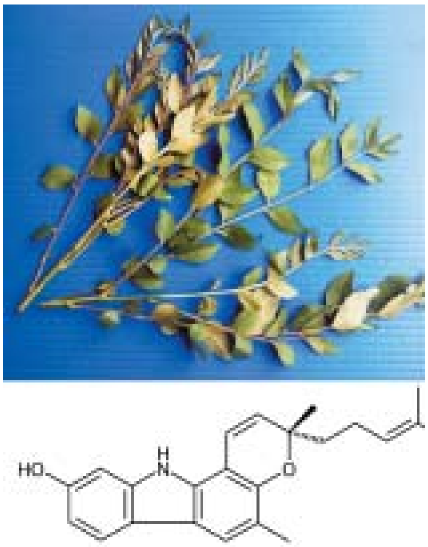

Fig. 1. *Micromelum minutum* twigs, and structure of the isolated antimutagen, mahanine.

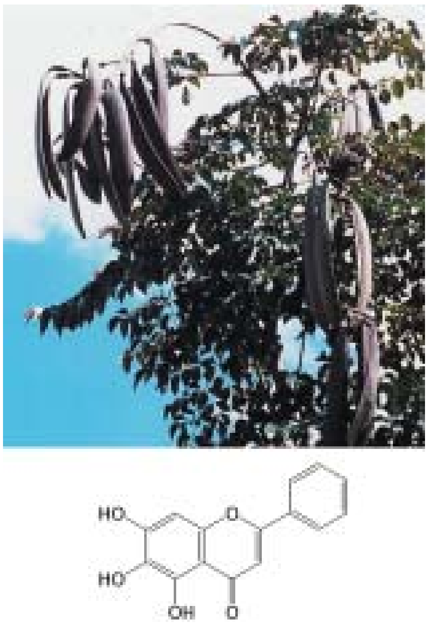

Fig. 2. *Oroxylum indicum,*and structure of the isolated antimutagen, baicalein.

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E-mail address: knakahar@jircas.affrc.go.jp

Antioxidative and angiotensin I-converting enzyme inhibitory activities of sufu (fermented tofu) extracts

M. SAITO[1], E. TATSUMI[1], L. WANG[2] and L. LI[2]

[1]Food Science and Technology Division, JIRCAS
[2]College of Food Science and Nutritional Engineering, China Agricultural University, China

Key words: sufu, peptide, antioxidative activity, angiotensin I-converting enzyme

Objectives

Traditional fermented soybean foods have long been an important nutritional staple in Asian societies. Recently, researchers have become increasingly interested in the physiological functionality of these foods. Some research has shown that many types of fermented soybean foods exhibit significantly stronger antioxidative activity than unfermented ones, yet there have been few investigations associated with such functions in sufu and tofuyo. Sufu is a fermented tofu product popular throughout China, while tofuyo, a similarly fermented tofu product considered to have originated in China, is commonly produced in Okinawa. Understanding the physiological functionality of sufu may help to improve the fermentation process required to produce a highly functional food product. In our study, antioxidative activity and angiotensin I-converting enzyme (ACE) inhibitory activity was analyzed in water extracts from four types of tofuyo and five types of sufu. ACE is an enzyme that increases blood pressure, and thus materials that inhibit ACE are considered to be useful for preventing hypertension.

Results

The antioxidative activities of the tofuyo and sufu extracts determined by the DPPH radical scavenging method are shown in Fig. 1. Antioxidative activity varied depending on the conditions of production, but all the sufu extracts except one sample showed higher antioxidative activities than the four tofuyo extracts. ACE inhibitory activities of the tofuyo and sufu extracts are shown in Fig. 2. All samples exhibited ACE inhibitory activity, and sufu displayed higher ACE inhibitory activity than did tofuyo.

Research showed a positive correlation between antioxidative activity and ACE inhibitory activity of the extracts from the nine samples. The SDS-polyacrylamide gel electrophoresis patterns in the extracts indicated that the molecular weights of most peptides were less than 10 kDa. Researchers estimated that samples with high antioxidative and ACE inhibitory activities also contained high quantities of small peptides. Significant variations in these activities of the extracts from several types of tofuyo and sufu were observed. Such variations might be closely related to the conditions of processing, *e.g.* the kind of microorganism used or the duration of fermentation. Thus by changing the fermentation conditions, researchers are able to produce many kinds of peptides with different activities.

It was shown that sufu contained highly active components and could be used as a functional food. Division researchers are continuing research to analyze the relationship between the conditions of processing and function. Through continued research, researchers will be able to improve processing methods to produce highly active, functional sufu that will ultimately increase the demand for sufu products.

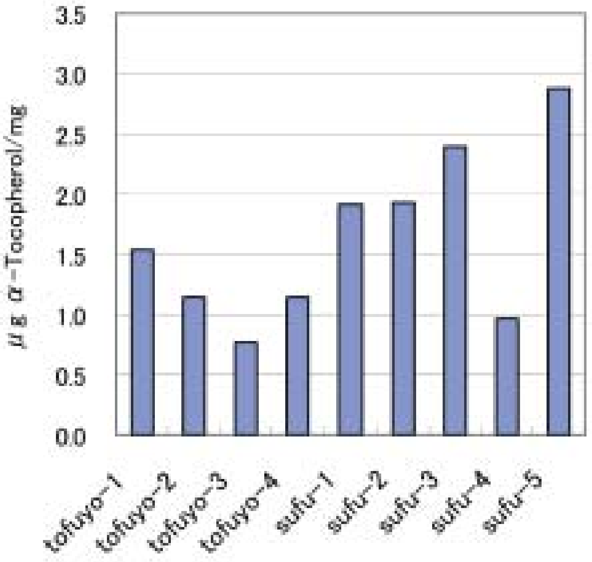

Fig. 1. Antioxidative activities of tofuyo and sufu extracts.
Tofuyo 1 to tofuyo 4, various types of tofuyo from Okinawa, Japan; sufu 1 to sufu 5, various types of sufu from China.

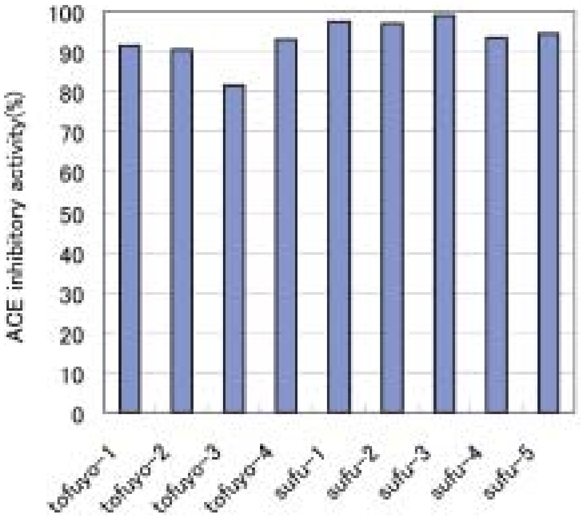

Fig. 2. ACE inhibitory activities of tofuyo and sufu extracts.
Tofuyo 1 to tofuyo 4, sufu 1 to sufu 5, same as in Fig. 1.

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E-mail address: masaito@jircas.affrc.go.jp

Water conservation practices and how they differ between tropical rainforest and rubber plantation sites

S. NOGUCHI[1], K. BAHARUDDIN[2], Y. ZULKIFLI[2,3], Y. TSUBOYAMA[4] and M. TANI[5]

[1]Forestry Division, JIRCAS
[2]Forest Research Institute Malaysia, Malaysia
[3]University Technology Malaysia, Malaysia
[4]Forestry and Forest Products Research Institute, Japan
[5]Kyoto University, Japan

Key words: saturated hydraulic conductivity, soil water retention, soil depth, water conservation

Objectives

The construction of agricultural plantations for oil palm and rubber production has devastated a significant proportion of Malaysia's natural forests during the early 20th century, and the conversion of forests to cultivate crops has caused major soil disturbances which have led to an increase of stormflow. The primary objective of this research was to compare the physical properties of forested and rubber plantation sites in order to determine how soil disruption impacts water conservation and how available water resources might differ depending on land-use qualification.

Results

Soil depth and physical properties were investigated at the Bukit Tarek Experimental Watershed in a tropical rain forest (Photo 1) and an adjacent rubber plantation (Photo 2) in Peninsular Malaysia. Using a portable dynamic cone penetrometer, it was discovered that total soil depth in the rubber plantation was shallower than the 277 cm mean observed in the tropical rain forest. Moreover, total soil depth at the rubber plantation terrace bench, with a mean of 119 cm, was shallower than the 141 cm mean of the rubber plantation riser bank.

Saturated hydraulic conductivities (Ks) were measured using vertical undisturbed soil cores from the tropical rain forest and the rubber plantation site (Table 1). Ks values decreased with increasing soil depth at both sites, however, the average Ks values in the tropical rain forest were larger than the prevailing rainfall intensity in this region, illustrating that rainwater infiltrates the soil. Though the average Ks values at the rubber plantation riser bank were similar to those in the tropical rain forest, the average Ks values at terrace bench were smaller. Soil porosities decreased with increasing soil depth with the following order of magnitude: the tropical rain forest > riser bank > bench terrace (Table 1).

Mechanical establishment of rubber plantations causes considerable topsoil removal and compaction. Plantation workers further compact terrace bench topsoil when trapping and collecting latex. Thus, terrace bench soils display low permeability and low water storage capacity, characteristics that could explain the frequent overland flow of rainfall during storms (Photo 3), while forest soils display high permeability and water storage capacity, which leads to higher rainfall absorption and baseflow production. These results should help policy makers and land managers understand how water conservation differs in forest and rubber plantation sites.

Photo 1. Example of a tropical rain forest.
Photo 2. Example of a rubber plantation.
Photo 3. Overland flow and surface detention at terrace benches in a rubber plantation during a heavy storm.

Table 1. Physical properties of soil in a tropical rain forest and rubber plantation.

Site	Depth (cm)	Macro - porosity (%)	Meso - porosity (%)	Total porosity (%)	Saturated hydraulic conductivity (mm h^{-1})
Tropical rain forest	10	8.5	35.2	72.1	1573.0
	20	9.7	30.2	69.5	1498.0
	40	8.6	16.7	61.9	706.0
	80	8.6	15.9	55.5	378.0
Rubber plantation bench terrace	10	5.7	8.4	45.4	29.9
	20	4.0	11.1	39.9	19.8
	40	5.2	5.9	29.2	18.4
Rubber plantation riser bank	10	5.0	21.1	60.4	921.0
	20	5.4	20.5	65.2	838.0
	40	2.7	9.7	51.1	1062.0
	80	3.8	6.7	51.7	260.0

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E-mail address: noguchi@jircas.affrc.go.jp

Development of freshwater prawn seed production technology suitable for use in the Mekong Delta region of Vietnam

M. N. WILDER[1], N. T. PHUONG[2], T. T. T. HIEN[2], N. T. HAI[2] and D. T. T. HUONG[2]

[1]Fisheries Division, JIRCAS
[2]College of Aquaculture and Fisheries, Cantho University, Vietnam

Key words: giant freshwater prawn, Mekong Delta, seed production, technology transfer

Objectives

The giant freshwater prawn, *Macrobrachium rosenbergii* (Fig. 1), has been pinpointed by the Vietnamese Government as one of the major target species of the aquaculture sector. Until the beginning of 2000, the supply of hatchery reared *M. rosenbergii* depended on a few previously established national hatcheries, and did not meet the growing needs of commercial prawn culture. Recognizing this problem as a major factor limiting the development of *M. rosenbergii* culture in the Mekong Delta, JIRCAS and Vietnam's Cantho University initiated collaborative research in 1995.

Results

The "green water" and the "re-circulating water" models of seed production were initially compared. Densities of 60 to 120 larvae per liter were recommended for both models, but the "green water" model yielded more post-larvae (PL) per liter, varying from 27.8–41.7 PL/liter compared with 18.6–32.9 PL/liter for the "re-circulating water" model (Table 1). Moreover, the "green water" model requires less labor, and is easier to implement in "back-yard" hatching facilities that are likely to be adopted by farmers engaging in prawn-rice culture (Cantho University's mini-hatchery shown in Fig. 2). Based on these results, a modified static "green water system" suitable for the conditions of seed production in the Mekong Delta was developed. In this system, super-saturated seawater from salt fields in the southernmost coastal parts of the Mekong Delta and freshwater are mixed in appropriate quantities to obtain the desired salinity concentration, and the water is treated for several days with chlorine. Next, in order to create "green water", tilapia are stocked for a week in a separate tank to create an environment where planktonic algae bloom and the water turns green. *Chlorella* is obtained selectively by filtration, and the water is then transferred to tanks to be used for prawn seed production. After larval prawn hatch, they are reared for nearly 30 days without the exchange of water until they metamorphose into post-larvae. Since the beginning of 2000, the "green water" model has been introduced to various users (including provincial authorities and the private sector), and the number of hatcheries and quantity of post-larvae produced rapidly increased. In fact, the production of post-larvae reached over fifty million by the end of 2001 or about 50-fold compared to the 1990s.

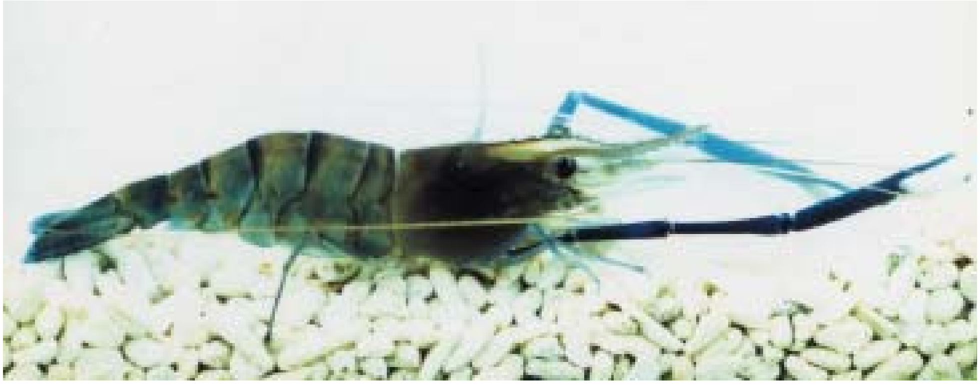

Fig. 1. The target species, the giant freshwater prawn, *Macrobrachium rosenbergii*.

Table 1. Survival rates of larvae and final number of post-larvae (PL) produced under the "re-circulating water" and "green water" systems.

Treatment	PL Density	Survival rate (%)
"Re-circulating water" system		
30 larvae/l	19.5 PL/l	52.5
60 larvae/l	18.6 PL/l	28.8
90 larvae/l	28.4 PL/l	31.7
120 larvae/l	32.9 PL/l	27.4
"Green water" system		
30 larvae/l	27.7 PL/l	92.3
60 larvae/l	27.8 PL/l	46.3
90 larvae/l	41.7 PL/l	46.4
120 larvae/l	38.8 PL/l	32.3

Fig. 2. Cantho University's mini-hatchery.

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E-mail address: marwil@jircas.affrc.go.jp

Development of technology to determine maturity in important prawn species

M. N. WILDER[1], V. JAYASANKAR[1], S. JASMANI[1], N. TSUTSUI[1] and N. T. PHUONG[2]

[1]Fisheries Division, JIRCAS
[2]College of Aquaculture and Fisheries, Cantho University, Vietnam

Key words: shrimp aquaculture, ovarian maturity, yolk protein, vitellogenin

Objectives

In recent years, shrimp aquaculture has rapidly expanded and developed as an important commercial enterprise in Southeast Asian regions. However, in hatchery operations, lack of reliable methods for accurately determining maturation stage of spawners makes broodstock selection a difficult process, often leading to eggs and larvae of poor quality, and unstable production. Given this background, in order to enable selection of suitable female spawners for purposes of stable seed production, it has been necessary to develop a technology to determine ovarian maturity based on studies elucidating yolk protein structure and processing in economically important species of shrimp.

Results

A full-length cDNA encoding vitellogenin (Vg) was firstly cloned and its complete amino acid sequence deduced in the giant freshwater prawn, *Macrobrachium rosenbergii*; subsequently, similar methods were employed to examine Vg in the kuruma prawn, *Penaeus japonicus* and the coonstriped shrimp, *Pandalus hypsinotus*. These results and those obtained by other authors thereafter have demonstrated that a high degree of identity exists among yolk proteins in various shrimp and prawn species (Fig. 1). Results obtained from electrophoretic and immunological analyses of the subunit composition of yolk proteins in the hemolymph and ovary in *M. rosenbergii* have clarified the site of synthesis and processing mechanisms of Vg. After being synthesized in the hepatopancreas as a precursor molecule, Vg is proteolytically cleaved into two subunits, VgA and VgB-C which are released into the hemolymph. In the hemolymph, the VgB-C subunit is further cleaved, forming two subunits VgB and VgC. The three subunits Vgs A, B and C are then sequestered by the ovary to give rise to Vn (Fig. 2). It is very likely that these processing mechanisms are common features among many prawn species. On the basis of the structural similarities of Vg in prawn species, an anti-serum against shrimp yolk protein has been developed and used in quantitative determination of hemolymph Vg levels in prawn and shrimp species by dot-blots and enzyme immunoassays. This thus-developed technology, "A process for determining maturing by using anti-serum against shrimp egg yolk protein," can therefore be used in the development of immunological kits to measure yolk protein levels. In combination with morphological observations, it will prove to be a useful and reliable tool for assessing maturity in aquaculture-important prawn species.

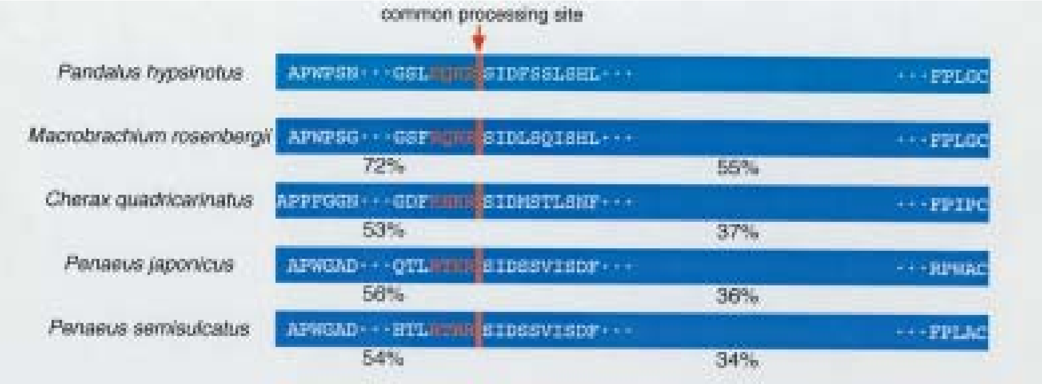

Fig. 1. Representation of vitellogenin primary structure in significant shrimp and prawn species. Degree of amino acid identity compared to *Pandalus hypsinotus* is indicated by percent. Location of a common processing site (see also Fig. 2) is indicated by the arrow.

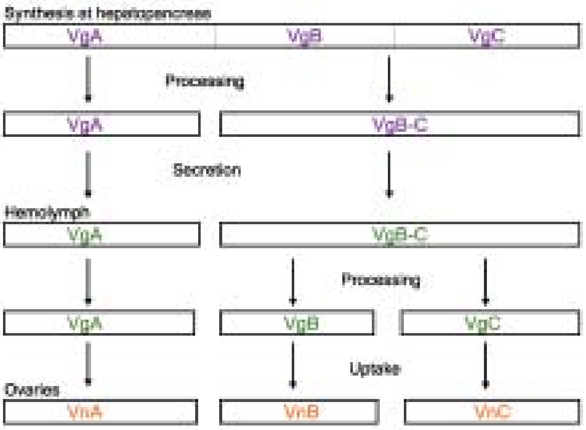

Fig. 2. Processing of vitellogenin (Vg) in *Macrobrachium rosenbergii*. Vg is initially produced at the hepatopancreas, undergoes processing at two different locations, and is taken into the ovaries as vitellin (Vn).

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E-mail address: marwil@jircas.affrc.go.jp

Mass mortalities associated with viral nervous necrosis in hatchery-reared orange-spotted grouper in the Philippines

Y. MAENO[1], L. D. de la PENA[2] and E. R. CRUZ-LACIERDA[2]

[1]Fisheries Division, JIRCAS
(Present address: Seikai National Fisheries Research Institute, Fisheries Research Agency, Japan)
[2]Southeast Asian Fisheries Development Center, Philippines

Key words: piscine nodavirus, grouper, viral nervous necrosis (VNN), Philippines

Objectives

In 2001, severe acute mortality characterized by anorexia and abnormal swimming behavior was observed among hatchery-reared larvae of the orange-spotted grouper *Epinephelus coioides* in the Philippines. Mortality rates of 5 to 10 percent per day were observed with 100 percent morality being reached within 10 days. A viral etiological study on this mass mortality was conducted using histopathological techniques, cell culture utilizing SSN-1 cell lines (established cells derived from striped snakehead whole fry), reverse transcription-PCR (RT-PCR) and electron microscopy.

Results

The initial clinical signs of affected larvae were reduced feeding activity followed by darkening pigmentation. Diseased fish became lethargic, often lying with their abdomen-up and rising to the surface of water. Abnormal swimming behaviors, such as rotating, spinning, and horizontal looping were observed (Fig. 1). Histopathological examination by light microscopy revealed heavy vacuolation in the brain and retina (Fig. 2). Cytopathic effects were observed in SSN-1 cells two days after inoculation with the filtrate of the affected grouper characterized by cytoplasmic vacuole formation. By PCR technique using primers based on the sequence of the striped jack nervous necrosis virus RNA 2 gene, an amplified product of about 430 base pairs was observed in samples from naturally infected fish and the culture supernatant of SSN-1 cells inoculated with the tissue filtrate of affected fish. The size of the PCR product was consistent with that of other piscine nodaviruses using the same primer set. Small spherical, non-enveloped virus particles 20–25 nm in diameter arranged in paracrystalline arrays or in membrane-bounded vesicles were abundantly observed in the cytoplasm of the brain (Fig. 3) and retina.

The histopathological lesions and other clinical signs observed in moribund orange-spotted grouper were very similar to those described in other viral nervous necrosis (VNN)-affected fish species. The isolated virus was identified as a piscine nodavirus based on the results of RT-PCR and electron microscopy performed on infected cell cultures. Furthermore, the experimental infection trial reproduced the same clinical signs and the virus was re-isolated from the experimentally infected fish (Fig. 4). These results clearly indicate that the mass mortality of the larval grouper was caused by VNN. This is the first documented outbreak of VNN among hatchery-reared larvae of orange-spotted grouper in the Philippines.

The present study was carried out as part of international research project “Studies on sustainable production systems of aquatic animals in brackish mangrove areas” between JIRCAS and South East Asian Fisheries

Fig. 1. Orange spotted grouper larvae affected with viral nervous necrosis (VNN).

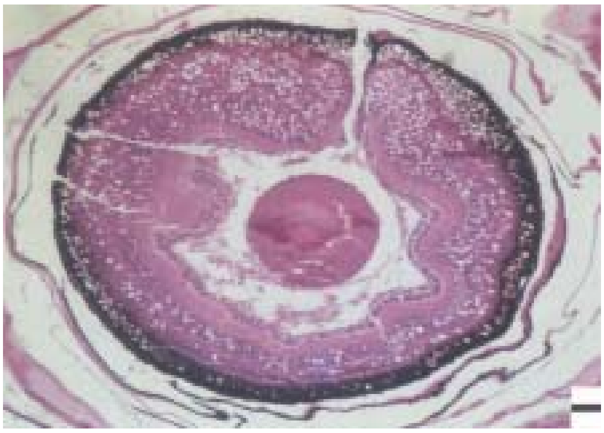

Fig. 2. Light micrograph showing vacuolation in the retina of affected orange-spotted grouper larvae. Haematoxylin and eosin staining. Scale bar = 100 μm.

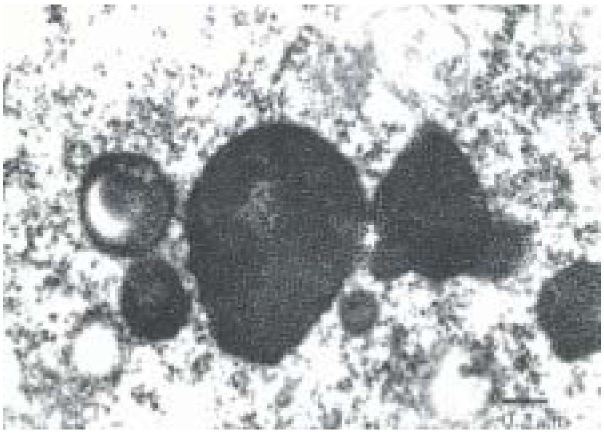

Fig. 3. Electron micrograph of virus particles in the brain cytoplasm of affected orange-spotted grouper. Scale bar = 200 nm.

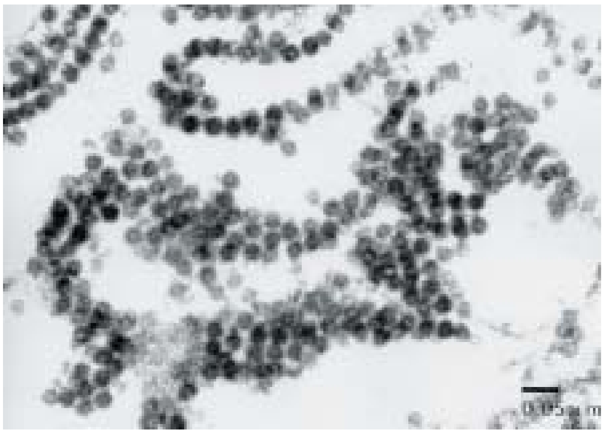

Fig. 4. Electron micrograph of virus particles in the brain cytoplasm of experimentally infected orange-spotted grouper. Scale bar = 50 nm.

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E-mail address: ymaeno@affrc.go.jp

Occurrence of abnormal pods and abscission of flowers at high temperatures in snap bean

K. SUZUKI[1], M. SHONO[2] and Y. EGAWA[3]

[1, 2, 3]Okinawa Subtropical Station, JIRCAS
[1](Present address: National Institute of Vegetable and Tea Science, National Agriculture and Bio-oriented Research Organization, Japan)
[3](Present address: Research Planning and Coordination Division, JIRCAS)

Key words: green bean, high temperature, pollen fertility, pollen tube growth, fertilization, abnormal pod

Objectives

As the result of high temperature stress, which causes damage to plants during the reproductive growth stage, production of temperate vegetables in tropical and subtropical areas has become unstable. However, research has thus far not completely discerned how reproductive organs are damaged under conditions of high temperature stress.

Results

Under high temperature stress, the yield reduction in snap bean (*Phaseolus vulgaris*) was closely related to the abscission of flowers and occurrence of abnormal pods (Fig. 1). To understand how this occurs, researchers investigated how the organs that maintain pollen viability, which is critical to fertilization, might be impaired by heat. It was discovered that high atmospheric temperatures alter the structure of the endoplasmic reticulum in the tapetum and disenable the tapetum to supply nutrients to microspores in the anther (Fig. 2). The tapetum degenerates earlier than usual, resulting in high pollen sterility, and anther indehiscence occurs when pollen stainability falls below 20 percent.

Pollen tubes were stained with aniline blue to make the ovule more observable under optimum conditions, although heat treatment still limits visibility. Researchers were still able to determine that heat stress at the flowering stage blocked pollen tube elongation in the style, particularly high temperatures exactly occurring one day before flowering (Fig. 3). Both normal pollen development in the anther and normal pollen tube elongation in the style are necessary for successful fertilization under high temperature conditions.

Abnormal pods occurred along with failure of fertilization because the pollen tubes did not reach the ovules on the peduncle side under high temperatures. Even when fertilization occurred successfully, abnormal pods appeared with poor ovule development (Fig. 4).

These effects also occurred in the field when air temperatures exceeded 28℃. Pollen fertility, pollen tube elongation, and ovule development after fertilization are important factors allowing the production of normal pods in snap bean.

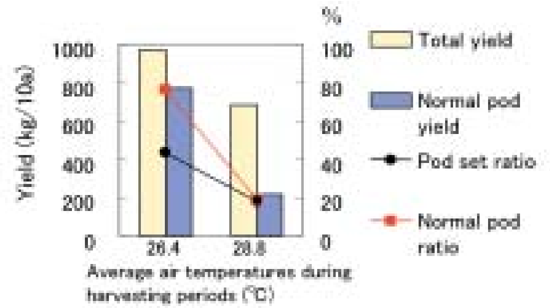

Fig. 1. Relationship between average air temperatures, yield and pod set ratio (cv. ‘Kentucky wonder’).

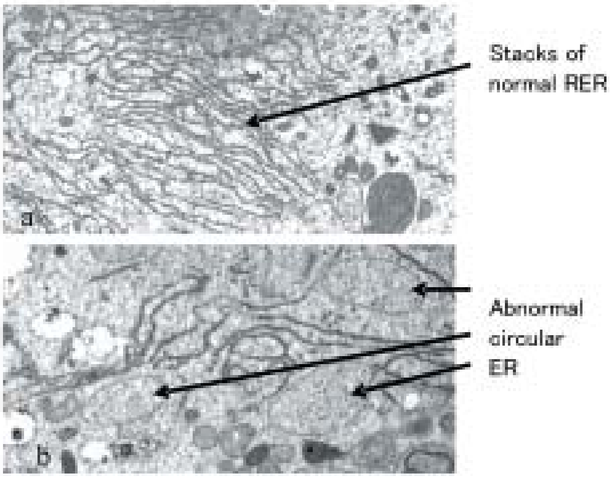

Fig. 2. Endoplasmic reticulum (ER) in tapetum at the uninucleate pollen stage.
a, normal conditions (24℃);
b, high temperature conditions (29℃).

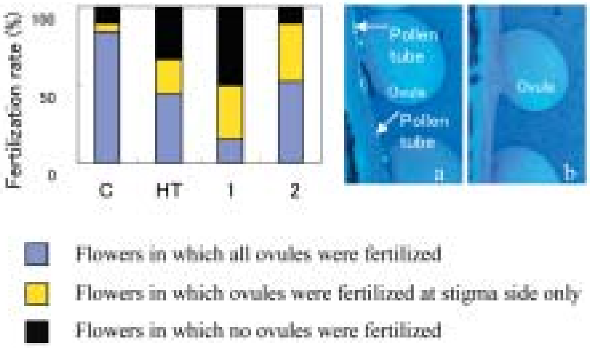

Fig. 3. Left, Changes in ovule fertilization rate after heat treatment; right, fertilized ovules (a) and non-fertilized ovules (b).
C, Control (27/23℃); HT, Heat treatment day (29 ～ 34 ℃, 18h); 1, 1 day after HT (27/23℃); 2, 2 days after HT (27/23℃).

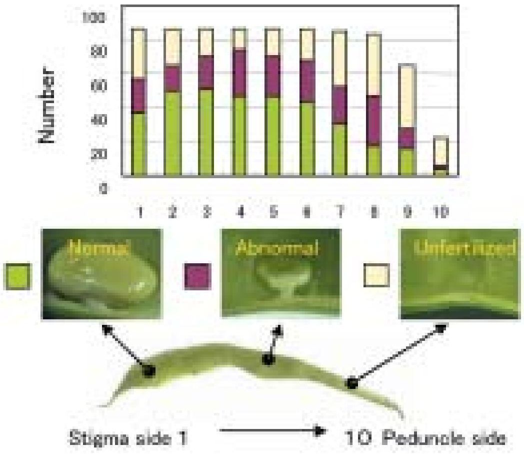

Fig. 4. Growth of ovules at different positions in abnormal pods.

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E-mail address: mshono@jircas.affrc.go.jp

Thermotolerance of transgenic tobacco with altered expression of mitochondrial small heat shock proteins

K. SANMIYA[1], J. LIU[2], K. SUZUKI[1], Y. EGAWA[3] and M. SHONO[4]

[1, 3, 4]Okinawa Subtropical Station, JIRCAS
[1](Present address: National Institute of Vegetable and Tea Science, National Agriculture and Bio-oriented Research Organization, Japan)
[2]Department of Biology, Shandong Teacher's University, China
[3](Present address: Research Planning and Coordination Division, JIRCAS)

Key words: mitochondrial small heat-shock protein, thermotolerance, tobacco, transformation

Objectives

Plants respond to heat stress by repressing the expression of mRNAs for most normal proteins that induce the synthesis of heat shock proteins (HSPs). Heat-stressed plant cells accumulate mitochondria-located small heat shock proteins (MT-sHSP) and the accumulation is synchronized with the thermotolerance of mitochondria. Under heat stress, mitochondrial metabolic pathways breakdown and function abnormally, thus diminishing cell viability. While Division researchers have already shown that tomato MT-sHSP has a molecular chaperone function *in vitro*, the goal of this study was to clarify the role of MT-sHSP *in vivo* during heat-shock response using MT-sHSP transgenic tobacco.

Results

Researchers introduced the tomato MT-sHSP gene into tobacco plants (*Nicotiana tabacum* L. cv. SR1) with sense and antisense constructs under the control of the CaMV 35S promoter, and then examined the thermotolerance of the seedlings. Sense plants overexpressed MT-sHSP even under normal growth temperatures, while antisense transformants accumulated less MT-sHSP than control plants under heat stressed conditions (Fig. 1). Irrespective of gene orientation, sense or antisense, transgenic plants exhibited a normal morphology and growth rate in the vegetative growth stage (Fig. 2). Thus, MT-sHSP does not have a pleiotropic effect on vegetative growth under normal conditions.

When the 4-week-old seedlings (T2 progeny) were exposed to sudden high temperature stress for two hours, sense plants exhibited thermotolerance and survived at 48℃, whereas control tobacco did not survive. On the other hand, antisense plants were susceptible to stress at 46℃, whereas control tobacco can survive under such conditions (Fig. 3). These results indicate that MT-sHSP plays a pivotal role in heat-shock response.

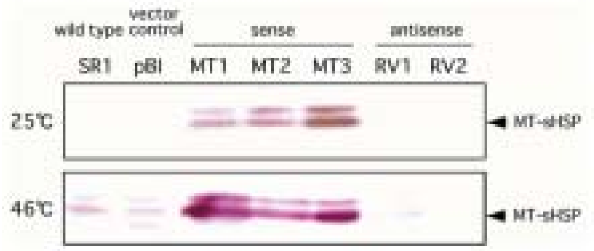

Fig. 1. Expression of the gene for MT-sHSP in transgenic tobacco plants. Four-week-old seedlings of the wild type (SR1) and T_2 homozygous lines into which a vector without an insert (pBI) or the MT-sHSP gene was introduced (sense: MT1, 2 and 3, antisense: RV1 and 2) were subjected to Western blot analysis with anti-MT-sHSP antibody. Mitochondrial fractions for the analysis were prepared using seedlings sampled before (25℃) and after (46℃) the heat stress treatment for 2 hours.

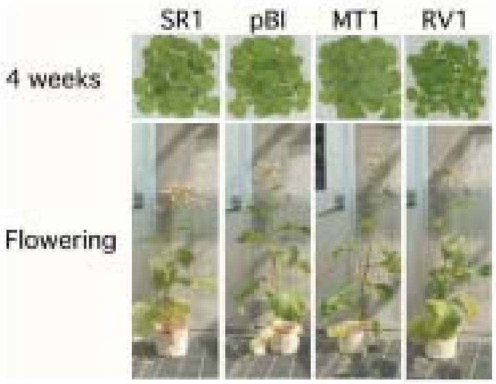

Fig. 2. Transgenic tobacco plants having the tomato MT-sHSP gene. Tobacco plants of the wild type, SR1 and T_2 transgenic lines without an insert or with the MT-sHSP sense or antisense gene (pBI, MT1 and RV1, respectively) were germinated and grown on MS medium for 4 weeks. The young seedlings were then transferred to soil in a greenhouse and kept at 25℃. The morphology and growth rate of the plants were observed 4 weeks after seeding and on the day of flowering.

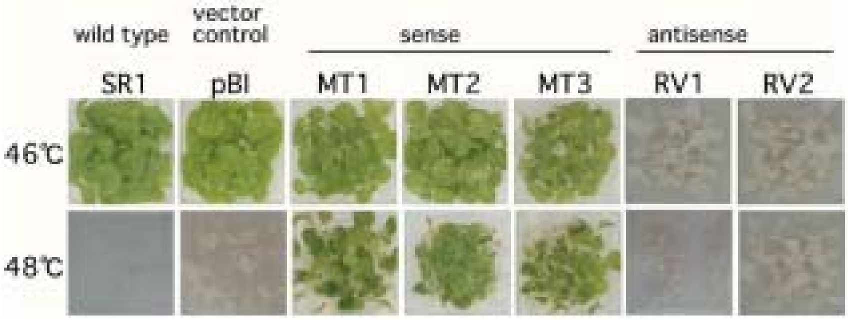

Fig. 3. Thermotolerance of the transgenic plants with the MT-sHSP gene. Four-week-old seedlings of the wild type and T_2 homozygous lines grown as shown in Fig. 2 were exposed to heat stress at 46℃ or 48℃ for 2 hrs. The seedlings were photographed 7 days after treatment.

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E-mail address: mshono@jircas.affrc.go.jp

Development of high biomass forage crops tolerant to water stress by interspecific and intergeneric crossing

M. MATSUOKA[1], W. PONRAGDEE[2], T. SANSAYAWICHAI[2], S. FUKUHARA[3], Y. TERAJIMA[3],
K. UJIHARA[3] and A. SUGIMOTO[3]

[1]Okinawa Subtropical Station, JIRCAS
[2]Khon Kaen Field Crops Research Center, Thailand
[3]National Agriculture Research Center for Kyusyu Okinawa Region, National Agriculture and Bio-oriented Research Organization, Japan

Key words: sugarcane, wild relatives, interspecific hybrids

Objectives

The lack of sufficient feed resources during the dry season has substantially limited ruminant production in Northeast Thailand. Forage crops of high yield and nutritive value must be produced in order to address these problems. It has been reported that some interspecific and intergeneric hybrids of *Saccharum* complex have shown good ratooning ability, as well as markedly vigorous growth and high yield. It is expected that new, highly adaptable forage crops can be bred in Northeast Thailand using such hybrids and a series of new criteria selection processes. The purpose of this study was to screen breeding materials for high biomass and adaptability to the environmental conditions of Northeast Thailand, and then to breed new types of forage crops using interspecific and intergeneric hybridization among sugarcane and their wild relatives. This project has been conducted in collaboration with the Khon Kaen Field Crops Research Center (KKFCRC).

Results

In 2002, researchers evaluated the wild relatives of sugarcane collected in Thailand (Fig. 1) and characterized some of these as breeding materials using cytological analysis (Fig. 2). In addition, researchers modified methods for crossing and pollen cryopreservation. As a result, about 3,000 seedlings were obtained from the 16 combinations of cross between commercial sugarcane and selected *S. spontaneum*. The evaluation of these progenies is in progress in the field. In addition, to evaluate the productivity of these breeding lines as a forage crop, field experiment to compare the harvest interval and re-growth rate after harvest are also being investigated. The stem and leaves of these breeding lines will be accessed nutrient value, silage adoptability, etc. by animal husbandry group.

Fig. 1. Sugarcane germplasm conservation field at the KKFCRC. Researchers are shown evaluating and characterizing the wild relatives of sugarcane.

Fig. 2. Metaphase chromosome spread on root tips of accession 98T-1 (2n=70).

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E-mail address: m19597@jircas.affrc.go.jp

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Japan International Research Center for Agricultural Sciences
1-1 Owashi, Tsukuba, Ibaraki 305-8686, Japan
Tel.　+81-29-838-6330
Fax　+81-29-838-6316
e-mail　head@jircas.affrc.go.jp
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