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Research Highlights 2003

Major Research Results from April 2003 to March 2004

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Major Research Results from April 2003 to March 2004

Transition in cropping systems from lowland rice-based to temperate vegetable-based systems between 800 and 1,800 m elevation in West Java, Indonesia

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Key words: cole crops, intercropping, plant protection, transects, white potato

Objectives

Agriculture in West Java, Indonesia, is characterized by rice paddies in the lowlands and temperate vegetables in the highlands. Intercropping is widely practiced for vegetables (Fig. 1). We developed the “focused transect survey” method and applied it in two production areas to understand the diversity of cropping systems depending on elevation. This method adapts agro-ecosystems analysis for more quantitative assessment, by combining GPS measurement, field transects, farmer participatory methods, and household surveys in a comprehensive rapid survey method. Transect and household surveys were conducted in two temperate vegetable-producing regions in West Java from 2001 to 2002. Horizontal transects of 120 m were placed perpendicular to a vertical transect at intervals of 200 m (Garut) or 100 m (Bandung) elevation from 800 m to 1,800 m and cropping patterns recorded. Cropping patterns over 1999–2001 and reasons for each pattern were obtained by respondent surveys of three farmers for each elevation level.

Results

In Garut, over three years, cole crops, white potato, and tomato predominated, with a range of other types of vegetables taken together also forming an important percentage of plantings. On transects in 2001, the most important vegetables were cabbage, carrot, onion, white potato, and other types of vegetables taken together. In Bandung, both over three years and on transects in 2002, celery was more important, followed by onion, cole crops, white potato, and other types of vegetables taken together (Fig. 2). During the three-year period from 1999 until 2001, the transition from rice to vegetables occurred between 1,100 m and 1,200 m in both sites.

Paddy was selected primarily due to experience and economics, especially lack of capital. Experience, rotation, and environmental factors were predominant reasons for selection of cole crops and white potato in both sites. Environment and rotation were more important selection factors at the highest altitudes (≥ 1400 m in Garut, ≥ 1300 m in Bandung). Costs and capital were more important at lower levels (800 m in Garut, 800–1000 m in Bandung). The proportion of transect length in sole cropping was 3–4 times the proportion in intercropping and trellis culture. In both sites, intercropping was concentrated in the 1,100–1,600 m zone. Border intercropping and in-row intercropping comprised over half of the intercropped plantings in Garut, while trellis culture was the most important type of intercropping in Bandung. Reasons for intercropping were based on horticultural considerations and economic motivations rather than tradition. Plant protection comprised 60–83% of farmer production constraint responses, with insects and diseases equally important.



Fig. 1. An example of intercropping.

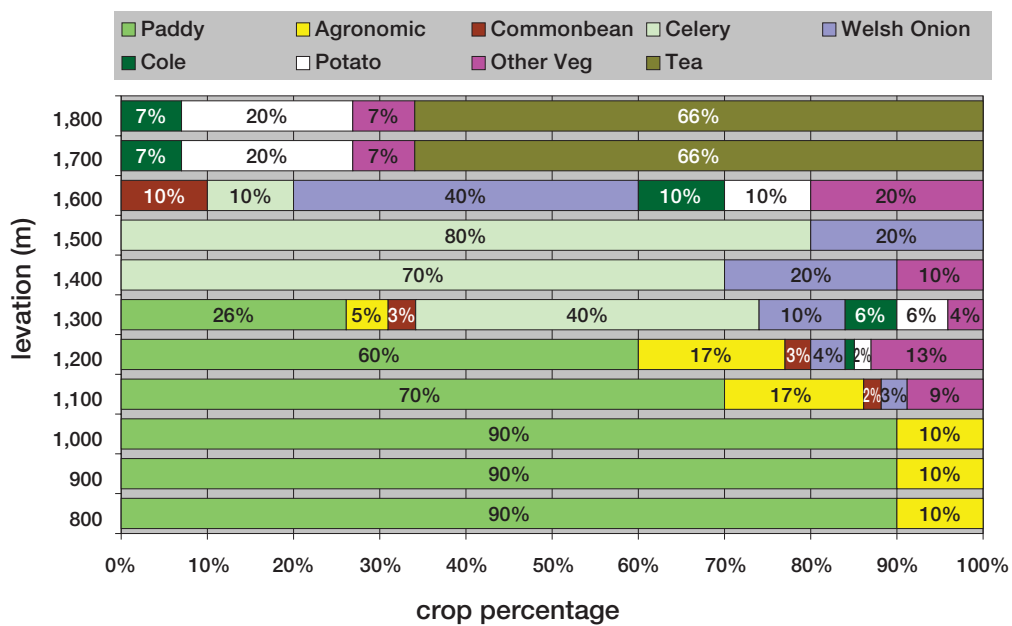


Fig. 2. Types of crops at different elevations, 2002.

References

Caldwell, J., Ishaq, I., Sastraatkadia, S., Ruswandi, A., Yamada, M. and Bachrein, S. (2005): Transition in cropping systems from lowland rice-based to temperate vegetable-based systems between 800 and 1,800 m elevation in West Java, Indonesia. JIRCAS Working Paper, No.43 (in press).

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Interlinkage in the rice market of Ghana: Money-lending millers enhance efficiency

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Key wards: interlinkage, rice miller, capacity utilization, money lending, Ghana

Objectives

The development of local agricultural markets plays a crucial role in the agricultural development of developing countries. Due to recent trade liberalization, the importance of local agricultural markets has further increased. One of the significant aspects of rural economies is interlinked transaction. By studying the nature of interlinkages in a particular region, we will be able to identify the constraints of market development and implement new policies to facilitate further development. Following along these lines, this study has analyzed rice markets in Ghana, particularly focusing on rice millers.

Millers are key players in Ghana's rice market. The millers mill the paddy and charge producers for milling fees. After milling, the producers sell milled rice to traders through those millers. Not only do they serve as middlemen, but some also provide rice producers with funds on a loan basis under the agreement that they will bring paddy only to that miller; surprisingly, the millers do not charge interest in most cases. In order to investigate whether this interlinkage between producers and millers, known as "Pareto," is improving and evolving, a study was conducted to examine the effects of the millers' no-interest loans on the efficiency of their milling operations.

To this end, we estimated a short-run quadratic cost function for the millers and evaluated the impact of money-lending on the operating rate. As experimental subjects, 61 millers were randomly selected from the Kumasi area in Ghana. We interviewed miller owners and their operators to obtain information on each mill and their milling activities in 2001. For this study, we used "capacity utilization" as the operating rate index and a two-stage regression method to control "self-selection bias."

Results

Table 1 shows the estimated costs and capacity utilization of money-lending and non-lending cases, and Fig. 1 indicates the relationship between capital input and average variable cost for each case. The results suggest that the operation rate of the mills increases by 24% if farmers are provided with loans. In addition, the total cost should decrease by 17.1% when compared to situations in which farmers are not provided with loans, and thus increase efficiency. Based on this interlinkage transaction, we can conclude as the following: (1) the amount of paddy collection increases by providing farmers with loans; (2) the operation rate and efficiency of millers increase; and (3) the farmers are able to obtain credit with little or no interest.

Nevertheless, the emergence of this interlinkage underlines the inefficiency of the Ghanaian rice market. Although our data suggests that relatively larger mills are more technically efficient, the millers cannot fully

Table 1. Costs and capacity utilization.

	Estimates and averages	
	Non-lending	Lending
Unit variable cost (cedi/kg)	66.811	51.971
Unit total cost (cedi/kg)	86.600	71.760
Capacity utilization	0.683	0.925

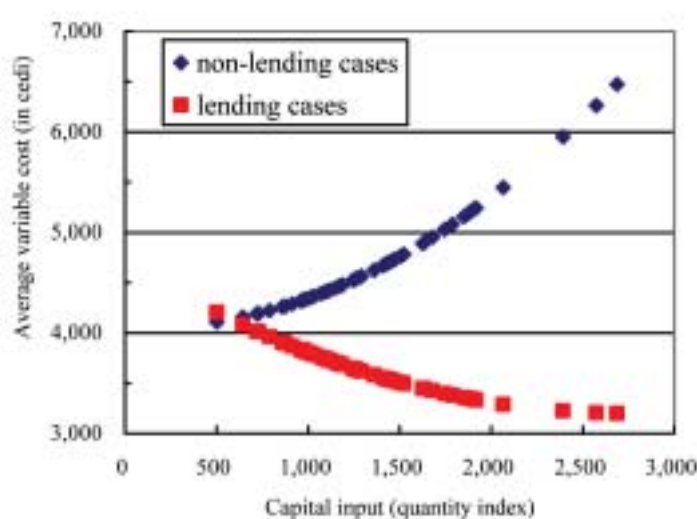


Fig. 1. Simulation results of capital input and average variable costs. (8,600 cedi = 1 US\$).

optimize their utilization capacity due to the underdevelopment of transportation and paddy storage, as well as the difficulty of obtaining market information. Large-scale mills should be promoted in Ghana, not only because of their superior efficiency, but also because of their better milling potential, both of which will enhance the competitiveness of local rice production. Efficiency of the rice markets may not improve as expected without complementary policies to develop efficient transportation, storage facilities to alleviate seasonal fluctuations of operation efficiency rates, and information systems to relay current demands for paddy milling.

References

- Furuya, J. and Sakurai, T. (2003): "Interlinkage in the Rice Market of Ghana: Money-lending Millers Enhance Efficiency." Contributed paper selected for presentation at the 25th International Conference of Agricultural Economists, http://www.iaae-agecon.org/conf/durban_papers/papers/088.pdf.
- Furuya, J. and Sakurai, T. (2002): "Efficiency Gains by Money Lending: The Case Rice Millers in Ghana." Program and abstract of 9th JIRCAS International Symposium, 25.

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Monitoring expression profiles of rice genes under cold, drought and high-salinity stresses, and ABA application using both cDNA microarray and RNA gel blot analyses

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Key words: rice, environmental stress, gene expression, microarray

Objectives

Drought, high salinity, and low temperature are the most common environmental stress factors that influence plant growth and productivity. To improve crop yield under conditions of stress, it is important to increase stress tolerance in crops. Genetic engineering can be used as a fast and precise means of achieving enhanced stress tolerance. The identification of novel genes, determination of their expression patterns in response to the stresses, and an improved understanding of their functions in stress adaptation will provide us with the basis for effective engineering strategies to increase stress tolerance. Rice is not only an important crop but also a model monocot crop. Determination of the biological functions of rice genes is one of the greatest challenges of post-genomic research. We have initiated transcriptional monitoring of stress-inducible rice genes in response to dehydration, high salinity, low temperature and abscisic acid (ABA) application.

Result

To identify cold, drought, high-salinity and/or ABA-inducible genes in rice, we prepared a rice cDNA microarray including about 1,700 independent cDNAs derived from cDNA libraries prepared from drought-, cold- and high-salinity-treated rice plants. We confirmed stress-inducible expression of the candidate genes selected by microarray analysis using RNA gel blot analysis, and finally identified a total of 73 genes as stress-inducible including 57 novel unreported genes in rice. Among them, 36, 62, 57 and 43 genes were induced by cold, drought, high salinity and ABA, respectively (Fig. 1). We observed a strong association in the expression of stress-responsive genes and found 15 genes that responded to all four treatments. Venn diagram analysis revealed greater cross-talk between signaling pathways for drought, ABA and high-salinity stresses than between signaling pathways for cold and ABA stresses, or cold and high-salinity stresses in rice. The rice genome database search enabled us not only to identify possible known cis-acting elements in the promoter regions of several stress-inducible genes but also to expect the existence of novel cis-acting elements involved in stress-responsive gene expression in rice stress-inducible promoters. Comparative analysis of Arabidopsis and rice showed that among the 73 stress-inducible rice genes, 62 have already been reported in Arabidopsis with similar functions or gene names, 38 of which have been documented as stress-inducible in Arabidopsis. Transcriptome analysis revealed novel stress-inducible genes suggesting some differences between Arabidopsis and rice in their response to stress.

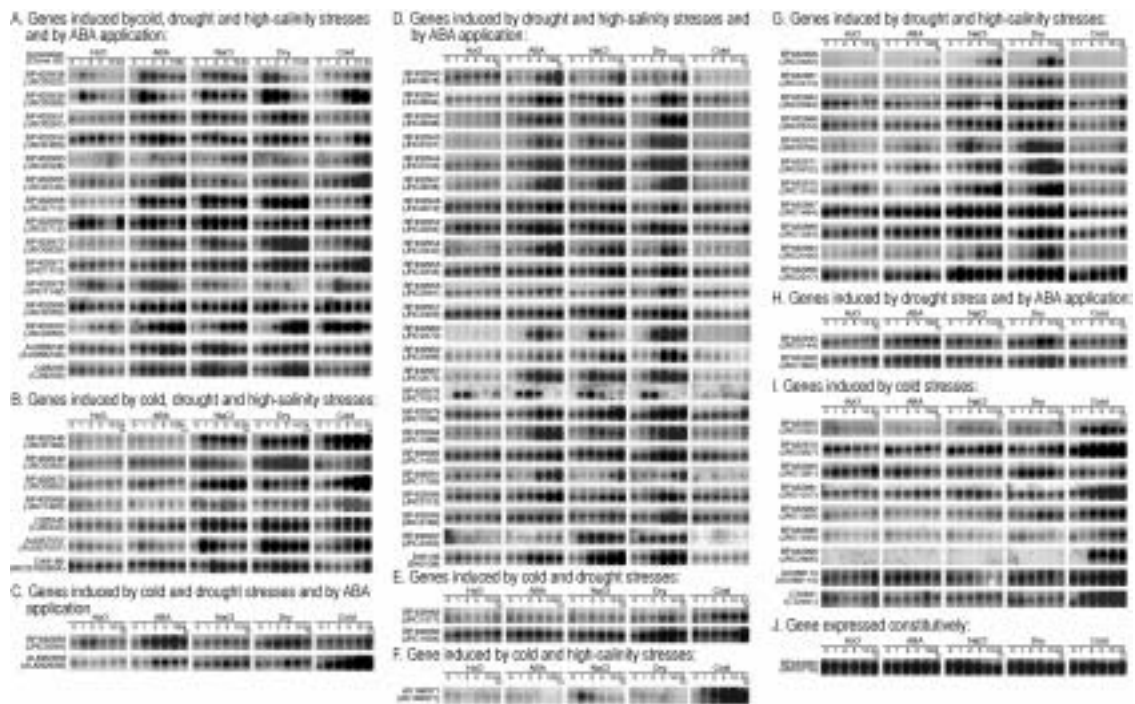


Fig. 1. RNA gel blot analysis of stress-inducible genes. Each lane was loaded with 10 μg of total RNA isolated from two-week-old rice seedlings that were each exposed to H₂O, dehydration, 250 mM NaCl, 100 μM ABA and 4°C cold treatment for 1, 2, 5, 10 and 24 hours. RNA was analyzed by gel-blot hybridization using gene-specific probes based on selected stress-inducible clones obtained via rice cDNA microarray analysis. Stress-inducible clones were classified into various groups on the basis of their expression patterns in RNA gel blot analysis under each stress treatment. Some of the inducible genes were induced by all four stress-treatments while some were upregulated by cold, drought and high salinity, and others were induced by drought and high salinity. Some genes were induced by singular conditions such as cold only.

References

- Dubouzet, J.G., Sakuma, Y., Ito, Y., Kasuga, M., Dubouzet, E.G., Miura, S., Seki, M., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2003): OsDREB genes in rice, *Oryza sativa* L, encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. *Plant J.*, 33, 751–763.
- Rabbani, M.A., Maruyama, K., Abe, H., Khan, M.A., Katsura, K., Ito, Y., Yoshiwara, K., Seki, M., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2003): Monitoring expression profiles of rice (*Oryza sativa* L.) genes under cold, drought and high-salinity stresses, and ABA application using both cDNA microarray and RNA gel blot analyses. *Plant Physiol.*, 133, 1755–1767.
- Shinozaki, K., Yamaguchi-Shinozaki, K. and Seki, M. (2003): Regulatory network of gene expression in the drought and cold stress responses. *Curr. Opin. Plant Biol.*, 6, 410–417.

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Genetic variation of accessions within *Fusarium* head blight resistant wheat cultivars revealed by SSR markers

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Key words: *Triticum aestivum*, wheat germplasm, disease resistance, DNA marker

Objectives

Fusarium head blight (FHB) is one of the most destructive diseases that afflict wheat. Repeated screening of genetic resources has led to the identification of several resistant cultivars of spring wheat, such as Frontana from Brazil, Sumai 3 from China and Nobeokabouzu-komugi from Japan. It is known, however, that several morphological and ecological variations with different responses to FHB exist within global accessions of Frontana and Sumai 3, and differing sources of Sumai 3 have been identified by DNA markers. In this study, we revealed genetic variation within accessions of Frontana (Brazil, the United States, Canada and Japan) and Sumai 3 (China, the United States, Canada, Iran, Austria and Japan) that had been sent to the International Maize and Wheat Improvement Center (CIMMYT) by using 242 SSR markers (Fig. 1) which encompass the entire wheat genome.

Results

Frontana-USA and -CAN were found to be identical with, or to be variants of, the original Brazilian accession. In the case of Sumai 3, the American, Canadian and Iranian accessions were of the same genotype as the Chinese accession. Sumai 3-JIR may possibly be a derivative of these accessions. The Austrian accession was determined to be a derivative of an original Chinese accession following out-crossing and selection with additional resistance genes to FHB (Table 1). The results of genetic variation analysis within accessions of Frontana and Sumai 3 reveal that we must always consider the source and genotype of such accessions when evaluating the results of QTL analysis and utilizing them in breeding programs with marker-assisted selection.

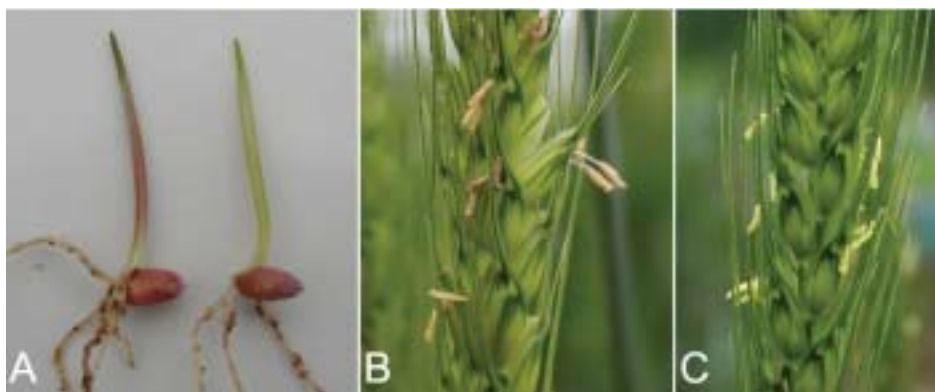


Fig. 1. Morphological variations within Sumai 3 accessions. Anthocyanin pigmentation of coleoptiles (A-left) and anthers (B) vs. yellow ones (A-right and C).

Table 1. Number of SSR markers showing polymorphism within Frontana and Sumai 3 accessions compared to the Brazilian cv. Frontana-BRZ and the Chinese cv. Sumai 3-CHN.

Chromosome	No. of markers	Frontana accessions compared with Frontana-BRZ					Sumai 3 accessions compared with Sumai 3-CHN							
		USA	CAN	JPN	Nobeoka	Gamenya	USA	CAN	IRN	AUT	JIR	JPN	Nobeoka	Gamenya
1A	8	–	–	2	4	3	–	–	–	2	–	1	2	4
1B	13	–	–	4	4	7	–	–	–	1	–	–	2	9
1D	9	–	–	1	4	4	–	–	–	–	–	1	3	4
2A	18	–	–	4	5	11	–	–	–	6	–	6	3	12
2B	18	–	–	3	8	11	–	–	–	1	–	3	5	11
2D	18	–	–	4	4	7	–	–	–	6	–	7	6	7
3A	10	–	–	2	6	5	–	–	–	2	–	3	5	6
3B	19	–	2	1	10	9	–	–	–	1	–	2	2	12
3D	9	–	–	1	3	3	–	–	–	–	–	–	3	4
4A	10	–	–	3	5	4	–	–	–	1	–	–	4	5
4B	4	–	–	–	–	–	–	–	–	–	–	–	1	–
4D	2	–	–	–	–	–	–	–	–	–	–	–	–	–
5A	21	–	–	3	8	11	–	–	–	2	1	11	8	13
5B	22	–	2	3	15	13	–	–	–	6	–	–	11	13
5D	7	–	–	3	4	4	–	–	–	2	–	1	–	4
6A	8	–	–	–	2	5	–	–	–	1	–	2	2	5
6B	8	–	–	1	2	1	–	–	–	–	–	4	–	3
6D	14	–	–	1	1	4	–	–	–	1	–	4	3	5
7A	12	–	–	–	5	5	–	–	–	–	–	1	4	7
7B	7	–	–	1	1	2	–	–	–	–	–	1	2	3
7D	5	–	–	–	–	2	–	–	–	–	–	–	1	3
Total	242	0	4	37	92	111	0	0	0	32	1	47	67	130
Polymorph (%)		0.0	1.7	15.3	38.0	45.9	0.0	0.0	0.0	13.2	0.4	19.4	27.7	53.7

–, no polymorphism; polymorph (%) = (no. of polymorphic markers/Total no. of markers) x 100.

References

Ban, T., Cong, H., Sukegawa, K. and Gilchrist, L. (2003): Genetic variation of accessions within Fusarium head blight resistance wheat cultivars revealed by SSR markers. Proceedings of 10th International Wheat Genetic Symposium, September 1–7, Paestum, 551–553.

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Development of extension-AFLP method for conversion of AFLP markers into STS markers in wheat

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Key words: *Triticum aestivum*, Fusarium head blight, marker-assistant selection, DNA marker

Objectives

Amplified fragment length polymorphism (AFLP) analysis has proven to be a powerful tool for tagging genes or QTLs of interest in plants. However, conversion of AFLP markers into sequence-tagged site (STS) markers is technically challenging in the case of wheat due to the complicated nature of its genome. In this study, we developed an 'extension-AFLP' method to convert AFLP markers into STS markers. When an AFLP marker of interest was detected with an *EcoRI*+3/*MseI*+4-selective primer combination, the PCR product was used as a template for an additional selective amplification with four primer pairs in which one base (either A, C, G, or T) was added to the 3'-end of one of the two primers. The extended primer-pair that produced the targeted band was further extended by adding each of the four selective bases for the next round of selective amplification (Fig. 1).

Results

By using the extension-AFLP method, we successfully converted two AFLP markers which are located on chromosome 3BS and are associated with FHB resistance into STS markers (Fig. 2). Our results indicated that the extension-AFLP method is an efficient technique for converting AFLP markers into STS markers in wheat. The developed STS markers may be used for marker-assistant selection (MAS) for FHB resistance in wheat breeding programs.

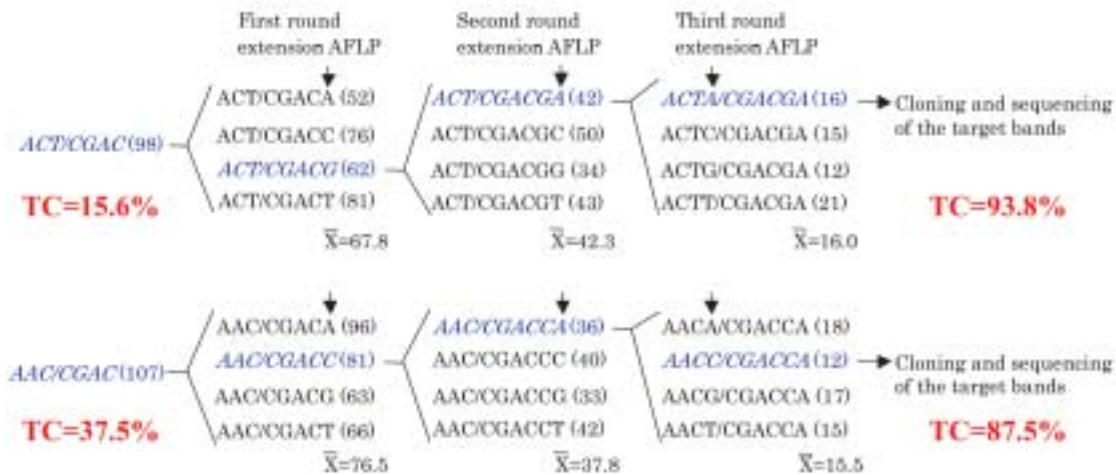


Fig. 1. Flow chart of extension-AFLP for the two AFLP markers, E-ACT/M-CGAC118-120 (upper), and E-AAC/M-CGAC285 (lower). Primer pairs that amplified the target bands are indicated in italics. Numbers in parentheses indicate the total number of AFLP bands produced by each primer combination.

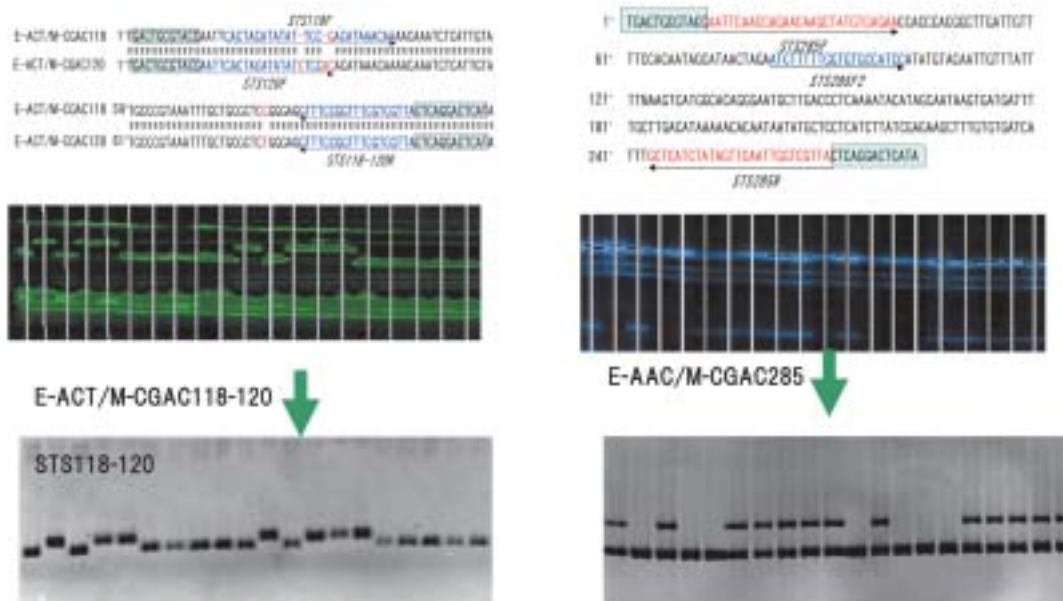


Fig. 2. Sequence alignment between two allelic fragments of AFLP marker E-ACT/M-CGAC118-120. STS primers are indicated with arrows (upper). Adaptor sequences of the two sides are shown in boxes. Band patterns of the two AFLP markers, E-ACT/M-CGAC118-120 and E-AAC/M-CGAC285, and their corresponding STS markers in a deoxycholate hydrogen sulfide lactose (DHL) population derived from ‘Sumai 3’ and ‘Gamenya.’ Lane 1, ‘Sumai 3’; Lane 2, ‘Gamenya’; and Lanes 3-20, DHLs.

References

Xu, D.H. and Ban, T. (2004): Conversion of AFLP markers associated with FHB resistance in wheat into STS markers with an extension-AFLP method. *Genome*, 47, 660–665.

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Characteristics and genetic diversity of soybean genetic resources in Northeast China

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Key words: soybean, genetic resources, Northeast China, data base, SSR marker

Objectives

Northeast China is thought to be one of the regions from which soybean originated, due to the existence of a number of valuable soybean varieties in this region. Although these soybean varieties are important as genetic resources, their primary characteristics and genetic diversity have not yet been clarified. Therefore, we studied the main characteristics of some 3,000 soybean genetic resources and constructed a database, aiming to improve the efficiency of their utilization as breeding stock. In addition, we evaluated their diversity to clarify the usefulness of northeast Chinese soybean genetic resources.

Results

In evaluation of primary characteristics, considerable variation was found in the quantitative and qualitative characteristics. For example, a large variation was found in main stem length, ranging from 7 to 277 cm, with a mean value of 86 cm. This measurement was somewhat longer than that of Japanese varieties, but the incidence of lodging tends to be small, except for climbing varieties. There were some varieties that were rich in protein content and low in oil content, while others had high oil content and low protein content. Highest and lowest protein contents were 59.4 and 11.5%, and highest and lowest oil contents were 23.6 and 10.3% , respectively. Chinese soybean genetic resources clearly showed a wide range of variation in protein and oil content. It is considered that many varieties would be useful as materials for various types of industrial processing. As these results show, soybean genetic resources in Northeast China harbor great diversity and differ in some traits from Japanese varieties. They may thus hold great potential as breeding materials.

In order to evaluate soybean genetic resources at the DNA level, utilization of soybean SSR markers to classify varieties and investigation of genetic diversity is necessitated. The plant materials that were used as subject matter for this research totaled 253 types: 194 varieties of Northeast Chinese soybean genetic resources and, for comparison, 59 Japanese soybean varieties. The evaluation of genetic diversity and classification of varieties were conducted through cluster analysis. Almost all varieties and most Japanese varieties can be classified into one cluster, as a subgroup of Chinese varieties (Fig. 1). These results indicate that the Northeast Chinese and Japanese soybean genetic resources are distantly related genetically and that Chinese genetic resources are clearly rich in diversity at the DNA level; thus, the incorporation of both Japanese and Chinese genetic resources into new soybean varieties will be effective for promoting enhanced breeding strategies.

The database of Chinese soybean genetic resources is available via Internet (<http://www.jircas.affrc.go.jp/DB/guide-eng.html>).

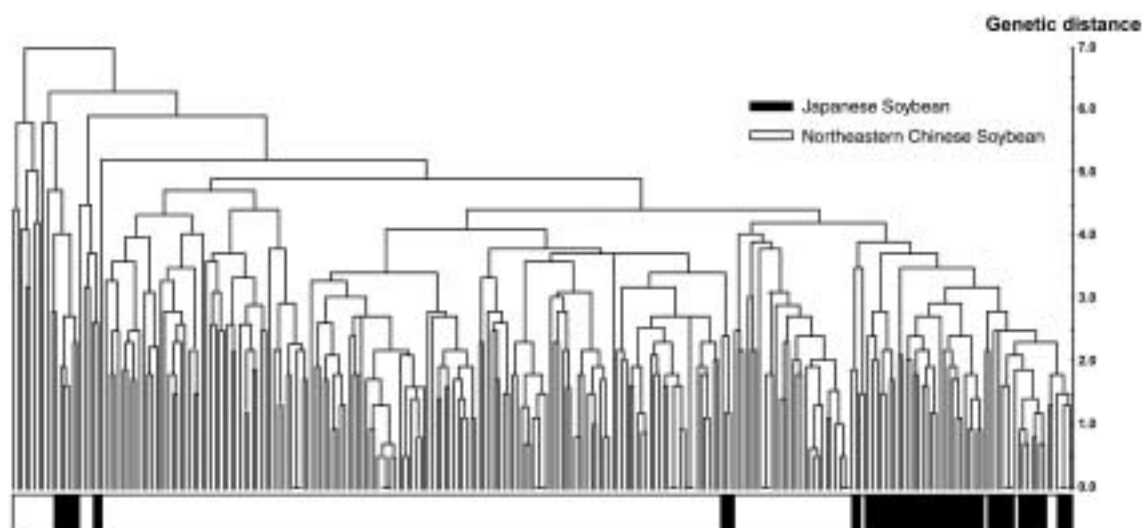


Fig. 1. A dendrogram (UPGMA) of Japanese and northeastern Chinese soybean varieties based on SSR markers. Black and white boxes denote Japanese and northeastern Chinese soybean groups, respectively.

References

- Yamanaka, N., Sato, H., Yang, Z., Xu, D.H., Adachi, T. and Ban, T. (2003): Genetic diversity of northeastern Chinese soybean germplasm in SSR markers. *Breed. Res.*, 5, Suppl. 1, 221 (in Japanese).
- Okabe, A., Yang, C., Yang, G., Kikuchi, A. and Saruta, M. (2003): Evaluation of soybean genetic resources in northeastern region of China. *Breed. Res.*, 5, Suppl. 1, 236 (in Japanese).
- Okabe, A., Yamanaka, N., Adachi, T., Yang, C., Yang, G., Yang, Z., Ma, X., Cai, L. and Song, Z. (2004): Data base of soybean genetic resources in northeastern region of China. *Breed. Res.*, 6, Suppl. 2, 252 (in Japanese).

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Genetic relationship and diversity between and within *Mangifera* species revealed by AFLP analysis

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Key words: AFLP, dendrogram, *Mangifera*, mango

Objectives

Mangifera indica L. (common mango) and its relatives of the genus *Mangifera* are important crops and can be found throughout tropical Asia. The origin and the center of diversity are thought to be located in Southeast Asia with the greatest diversity occurring in Peninsular Malaysia, Borneo and Sumatra. Currently, classical methods are applied to identify *Mangifera* species based on phenotypic characteristics such as leaves, fruits and seeds of the plant. However, this method is a slow process due to the long cultivation periods and the evaluation itself, which is easily affected by environmental factors. The objectives were to clarify the effectiveness of AFLP analysis for the identification of *Mangifera* accessions and to explore the genetic relationship and diversity within and among 4 *Mangifera* species.

Results

Amplified fragment length polymorphism (AFLP) analysis was utilized to investigate the genetic relationship and diversity among and within 4 *Mangifera* species. As shown in Fig. 1, we analyzed 35 accessions consisting of 11 accessions (8 cultivars and 3 landraces) of *M. indica* L., 11 accessions (landraces) of *M. odorata* Griff., 7 accessions (landraces) of *M. foetida* Lour., and 6 accessions (landraces) of *M. caesia* Jack. We carried out AFLP analysis using 8 kinds of primer combinations and 16% polyacrylamide gel. Polymorphic bands among accessions were recorded to clarify diversity and genetic relationship. Eight combinations of primers produced a total of 518 reliable bands in all 35 accessions and a pair of primer combinations yielded 64.8 bands on average. Four-hundred and ninety-nine bands were polymorphic and thus accounted for 96.3% of the total bands. Clustering analysis showed that all 35 accessions were basically classified into 4 groups corresponding to the 4 *Mangifera* species (Fig. 2). However, the accession in *M. indica* L., Mi 3, was distantly related to the other *M. indica* L. accessions and closely related to the *M. odorata* Griff. group. Although *M. odorata* Griff. accessions showed very little diversity compared to other *Mangifera* accessions, this was a suitable method for differentiating between other *Mangifera* accessions. In the *M. caesia* Jack accession, Mc 5 was clustered into a group of *M. caesia* Jack accessions, but this accession was distantly related to the other *M. caesia* Jack accessions. *M. caesia* Jack is grouped out from the other species, indicating that its genetic variation is different within the taxa. Our results indicate that the morphological differences and genetic relationship of *Mangifera* accessions revealed by AFLP analysis are in a good agreement, thus AFLP analysis is an applicable and effective means of conducting taxonomic studies in *Mangifera* species.

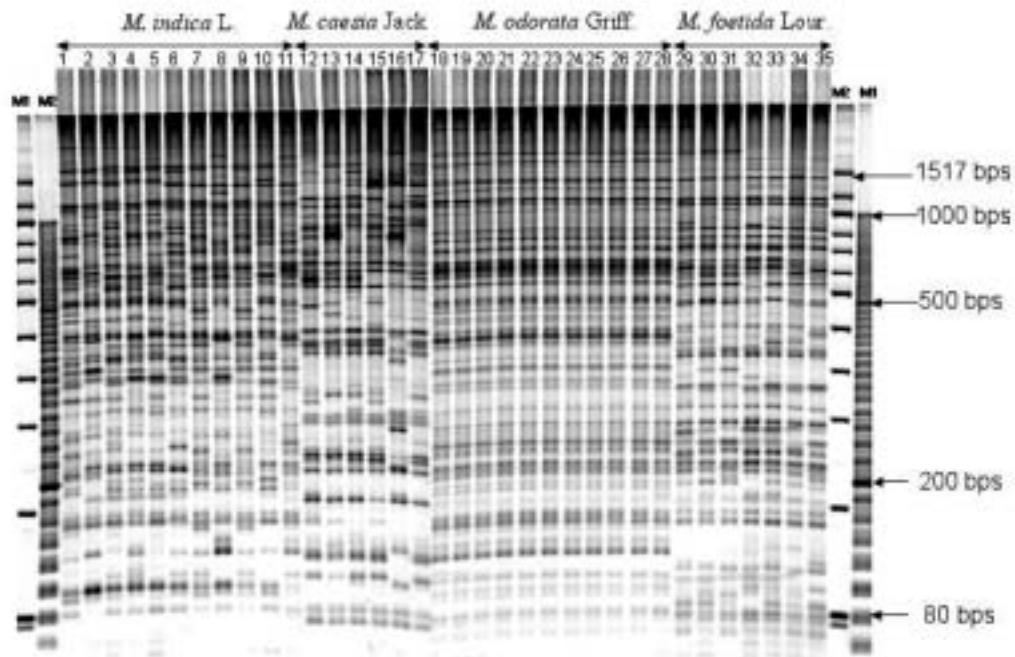


Fig. 1. AFLP analysis banding patterns of 35 *Mangifera* accessions. DNA markers M1 and M2 as well as the E-ACC/M-CTA primer combination were used.

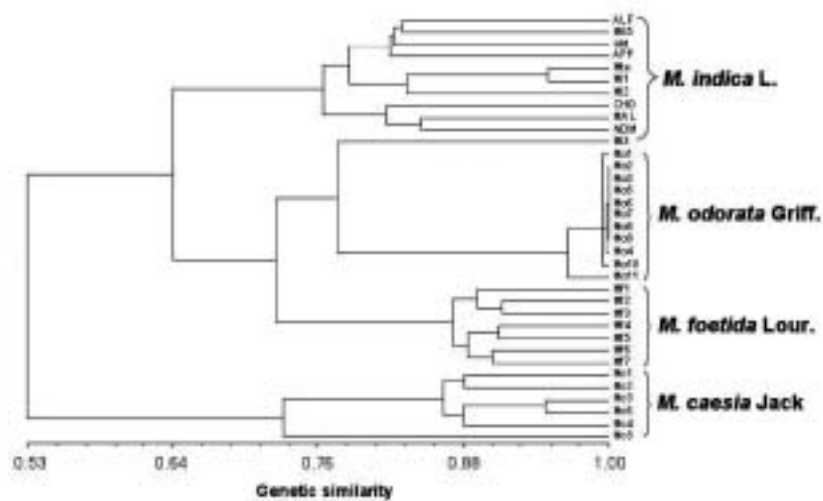


Fig. 2. A dendrogram of *Mangifera* accessions displaying the results of AFLP analysis.

References

- Yamanaka, N., Hasran, M., Xu, D.H., Tsunematsu, H., Idris, S. and Ban, T.(2005): Genetic relationship and diversity of four *Mangifera* species revealed through AFLP analysis. Genetic Resources and Crop Evolution (in press).
- Hasran, M., Yamanaka, N., Xu, D.H., Tsunematsu, H. and Ban, T.(2003): Genetic relationship of *Mangifera* accessions revealed by AFLP analysis. Breed. Res., 5, Suppl. 1, 222.

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Development of new international standard differential variety series to integrate blast resistance of rice (*Oryza sativa* L.)

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Key words: differential variety, resistance, blast (*Pyricularia grisea* Sacc.), rice (*Oryza sativa* L.)

Objectives

Blast is one of serious diseases in rice cultivation, and the differential system using differential varieties based on gene-for-gene theory were applied to identify the pathogenicities of blast races and resistance gene(s) of rice in a few countries. Unfortunately, there have not been developed the differential varieties which covered a lot of resistance genes and could use in all temperate and tropical areas. To build up the differential system in all countries, IRRI-Japan Collaborative Research Project (IRRI-Japan Project) has developed some differential varieties' sets, which have been accepted worldwide since 1994.

Results

The first international standard differential variety (ISDV) set composed of monogenic lines (MLs) for targeting 24 blast (*Pyricularia grisea*) resistance genes, was recently developed in rice (*Oryza sativa* L.) through the IRRI-Japan Project entitled "Physio-genetic studies on yield determination and ecological adaptability for sustainable agriculture". Additionally, new ISDVs, near isogenic lines (NILs), for targeting 14 blast resistance genes having an Indica-type rice CO39 genetic background were also developed. CO39 NILs are the first ISDVs for Indica-type rice. Two other kinds of differential variety sets, NILs with Chinese Japonica-type susceptible variety Lijiangxintuanheigu (LTH) and US-2 genetic backgrounds are also being developed. These ISDVs will be available worldwide, for use in pathological analysis of blast races and as the gene sources of resistance genes in breeding programs.

MLs were developed by several backcrosses between donor varieties of resistance gene and LTH as a recurrent parent. MLs including only a single gene in each genetic background targeting for 24 kinds of genes, e.g., *Pia*, *Pib*, *Pii*, *Pik-s*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pish*, *Pit*, *Pita* (*Pi4*), *Pita-2*, *Piz*, *Piz-5* (*Pi2*), *Piz-t*, *Pi1*, *Pi-3*, *Pi5*, *Pi7(t)*, *Pi9*, *Pi11(t)*, *Pi12(t)*, *Pi19*, and *Pi20*. These lines have already been distributed to more than 15 countries and have been used in pathogenic analysis and rice breeding as sources of resistance genes under the IRRI-Japan Project.

The other three NILs were backcrossed at least six times with recurrent parents, LTH, CO39, and a high-yield type rice US-2. In each backcrossed and selfed generation, the resistant plants with targeted genes were selected using avirulent isolates. Inoculation and selection of resistance genes were continued from BC_nF₄ to BC_nF₁₁ in order to purify the lines and fix their morphological traits.

A total of 31 NILs for targeting 14 kinds of resistance genes - *Pib*, *Pik-s*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pi1*,

Table 1. New international standard differential variety series used to evaluate rice blast resistance.

Target gene	Designation	
	Monogenic lines	CO39 near isogenic lines (including <i>Pia</i>)
<i>Pia</i>	IRBLa-A, IRBLa-C	–
<i>Pib</i>	IRBLb-B	IRBLb-IT13/CO, IRBLb-W/CO
<i>Pii</i>	IRBLi-F5	–
<i>Pik</i>	IRBLk-Ka	IRBLk-Ka/CO, IRBLk-Ku/CO
<i>Pik-h</i>	IRBLkh-K3	IRBLkh-K3/CO
<i>Pik-m</i>	IRBLkm-Ts	IRBLkm-Ts/CO
<i>Pik-p</i>	IRBLkp-K60	IRBLkp-K60/CO
<i>Pik-s</i>	IRBLks-F5, IRBLks-S	IRBLks-CO/CO
<i>Pish</i>	IRBLsh-S, IRBLsh-B	IRBLsh-S/CO, IRBLsh-B/CO, IRBLsh-Ku/CO
<i>Pit</i>	IRBLi-K59	–
<i>Pita (Pi4)</i>	IRBLta-K, IRBLta-CT2, IRBLta-CP1	IRBLta-Ya/CO
<i>Pita-2</i>	IRBLta2-Pi, IRBLta2-Re	IRBLta2-Pi/CO, IRBLta2-Re/CO, IRBLta2-IR64/CO
<i>Piz</i>	IRBLz-Fu	–
<i>Piz5 (Pi2)</i>	IRBLz5-CA	IRBLz5-CA/CO
<i>Piz-t</i>	IRBLzt-T	IRBLzt-IR56/CO
<i>Pi1</i>	IRBL1-CL	IRBL1-CL/CO
<i>Pi3</i>	IRBL3-CP4	–
<i>Pi5 (t)</i>	IRBL5-M	IRBL5-M/CO
<i>Pi7 (t)</i>	IRBL7-M	IRBL7-M/CO
<i>Pi9</i>	IRBL9-W	–
<i>Pi11 (t)</i>	IRBL11-Zh	–
<i>Pi12 (t)</i>	IRBL12-M	–
<i>Pi19</i>	IRBL19-A	–
<i>Pi20</i>	IRBL20-IR24	–

Pi7(t), *Pish*, *Pita*, *Pita-2*, *Piz-5*, *Piz-t* and *Pi5(t)* - were developed and characterized. Each NIL harbored only the major target gene for resistance with the exception of *Pia*, as confirmed by the stability of their reaction patterns of monogenic lines to standard isolates. In addition, each NIL was very similar to recurrent parent CO39 in morphological terms. Among them, 21 NILs will be made available as a new set of ISDVs having Indica-type genetic background.

The other two kinds of NILs having two genetic backgrounds, LTH and US-2, are being developed as the targets of 17 and 16 resistant genes, respectively. However, it is necessary to generate and select fixed lines within each line, because segregation of some morphological traits were still observed in LTH NILs. In US-2 NILs, several lines will be backcrossed one or two more times and the selection and fixation for resistance and morphological traits will be continued until at least the F₉ generation. The designation and target resistance genes of developed monogenic lines and CO39 NILs are shown in Table 1. Monogenic lines and NILs are labeled as IRBL, which is followed by the type of resistance gene and an abbreviation of the donor variety having the initials of the recurrent parent.

References

- Fukuta, Y., Araki, E., Yanoria, M.J.T., Imbe, T., Tsunematsu, H., Kato, H., Ebron, L.A., Mercado-Escueta, D. and Khush, G.S. (2004): Development of differential varieties for blast resistance in IRRI-Japan Collaborative Research Project. Rice Blast: Interaction with Rice and Control. Proceeding of the 3rd International Rice Blast Conference. ed. Shinji Kawasaki, Kluwer Academic, Netherlands, 229–233.
- Tsunematsu, H., Yanoria, M.J.T., Ebron, L.A., Hayashi, N., Ando, I., Kato, H., Imbe, T. and Khush, G.S. (2000): Development of monogenic lines of rice for blast resistance. *Breed. Sci.*, 50, 229–234.
- Tsunematsu, H., Yanoria, M.J.T., Ebron, L.A., Hayashi, N., Ando, I., Mercado-Escueta, D., Kato, H., Fukuta, Y.H. and Imbe, T. (2003): Developing near-isogenic lines for rice blast resistance. *Advances in Rice Genetics*, 39–41.
- Yanoria, M.J., Fukuta, Y., Imbe, T., Tsunematsu, H., Ban, T., Escuta, D., Ebron, L., Araki, E. and Khush, G.S. (2004): The first of Indica type differential variety for blast resistance in rice (*Oryza sativa* L.). World Rice Research Conference abstract, 353.

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Identification of the low-tillering gene in rice (*Oryza sativa* L.)

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Key words: low-tillering, rice (*Oryza sativa* L.), Aikawa 1, Shuho, DNA marker

Objectives

The genetic mechanism and allelic relationship between the low-tillering in two Japonica-type rice varieties Aikawa 1 and Shuho were clarified, and the chromosomal location was also identified with molecular (DNA) markers, to apply for the breeding program targeting to develop the new plant type as one of gene sources.

Results

The genetic mechanisms of low-tillering in two Japonica-type rice, Aikawa 1 and Shuho, were clarified and the controlling gene was identified on rice chromosomes using molecular markers. In these two Japonica varieties, the single dominant gene controlling low-tillering was confirmed on the basis of segregation analyses in each BC₁F₂ population derived from the crosses with Indica-type varieties IR64 and IR72 as recurrent parents, respectively.

Aikawa 1 and Shuho were found to have the same low-tiller gene based on results of allelism tests in F₂ populations derived from reciprocal crosses between Aikawa 1 and Shuho. Transgressive segregation was not observed in any of the distributions of 378 Aikawa 1/Shuho and 425 Shuho/Aikawa 1 F₂ plants.

The low-tiller gene was mapped between the SSR marker, *RM149*, and the RFLP marker, *XNpb56*, on the long arm of chromosome 8 using DNA markers (Fig. 1). As no gene related to low-tillering has been reported yet in this region, the genes found in Aikawa 1 and Shuho plants were tentatively designated as *Ltn* (t).

The near isogenic line (NIL) that introduced *Ltn* (t) into the elite Indica-type varieties IR64 and IR72 were developed from the advanced recurrent backcrosses of progenies to clarify yield ability of low-tiller plant types and to incorporate more detailed genetic and physiological analyses (Fig. 2). Partial sterility and malformed palea in the panicles of NILs were observed; this may have occurred due to the multiple effects of *Ltn* (t).

Information on genetic mechanisms and mapping of low-tiller genes will be used for breeding programs using marker-aided selection or map-based cloning of *Ltn* (t); NILs will also be useful as breeding materials for Indica-type varieties.

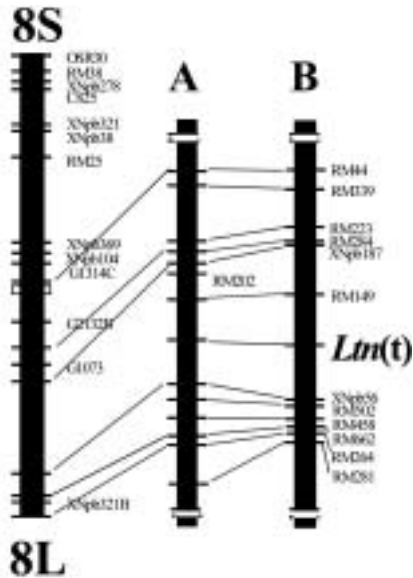


Fig. 1. Identification of *Ltn(t)* locus using DNA markers.
 A: BC₁F₂ (IR64/Aikawa1//IR64) family lines (n=33).
 B: BC₁F₂ (IR72/Shuho//IR72) family lines (n=74).



Fig. 2. Near isogenic lines introduced with the low-tiller gene, *Ltn(t)*, into the IR64 and IR72 genetic backgrounds.
 A, IR64; B, IR64 NIL (IR64/Aikawa1/5/IR64, BC₆F₃); C, Aikawa 1; D, IR72; E, IR72 NIL (IR72/Shuho/5/IR72, BC₆F₃); F, Shuho.

References

- Araki, A., Ebron, L.A., Cuevas, R.P., Mercado-Escueta, D., Khush, G.S., Sheehy, J.E., Kato, H. and Fukuta, Y. (2003a): Identification of low tiller gene in rice cultivar Aikawa 1. *Breed. Res.*, 5, Suppl. 1, 95.
- Araki, E., Ebron, L.A., Cuevas, R.P., Mercado-Escueta, D., Khush, G.S., Sheehy, J.E., Kato, H. and Fukuta, Y. (2003b): Genetic analysis of low tillering in Japonica-type rice (*Oryza sativa* L.) cultivar Aikawa 1. *Philippine journal of Crop Science*, 28 (1), 40.
- Fukuta, Y., Araki, E., Ebron, L.A., Cuevas, P.R., Mercado-Escueta, D., Khush, G.S., Sheehy, J., Tsunematsu, H. and Kato, H. (2004): Identification of low tiller gene in rice two varieties, Aikawa 1 and Shuho in rice (*Oryza sativa* L.). *Plant & Animal genome XII*, Abstract, 167.
- Araki, E., Fukuta, Y., Ebron, L.A., Cuevas, P.R., Mercado-Escueta, D., Khush, G.S., Sheehy, J.E., Tsunematsu, H. and Kato, H. (2004): Fine-mapping of a low tillering gene in a Japonica-type rice (*Oryza sativa* L.), Aikawa 1. *World Rice Research Conference abstract*, 138.

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Nitrogen export via surface water from an agriculture watershed in the Taihu Lake area, China

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Key words: non-point source pollution, rice-wheat cropping region, land use, soil erosion, sediment/water-associated nitrogen loss

Objectives

Situated in the center of the Yangtze River Delta, one of China's most important rice-wheat cropping areas as well as most developed economic zones, Taihu Lake has become severely eutrophicated. The Chinese government has resolved to combat this pollution by closing a large number of heavy-polluting enterprises, and by banning the distribution and use of P-bearing detergents in its catchment area. Nevertheless, water quality of the lake has not shown any significant improvement in recent years. Increased nutrient loss from arable land due to the nutrient surplus in agricultural systems was suggested as its main reason. However, that information in the area had been very limited. To address this problem, in one of typical watersheds in the area, the Meilin Watershed, Yixing City, Jiangsu Province (31°20'N, 119°51'E; 1.22 km²; Fig. 1), temporal changes in N concentrations in stream water (Fig. 2) and stream discharge, as well as sediment and N losses from erosion plots with different land uses (Fig. 3), were investigated.

Results

Much higher runoff, sediment and N losses were observed under upland cropping and vegetable fields than under chestnut orchards and bamboo thickets (Fig. 3). Sediment-associated N losses accounted for 8–43.5% of total N discharge via overland runoff. N lost in dissolved inorganic N forms accounted for less than 50% of total water-associated N discharge. Agricultural practices and weather-driven fluctuation in discharge were main reasons for the temporal variations in nutrient losses via stream discharge (Fig. 2). Significant correlation between the total N concentration and stream discharge load was monitored. N losses from the studied watershed via stream discharge between mid-May 2002 and mid-May 2003 were estimated to be 20.3 kg N/ha. From farmers' interviews, it was estimated to be equivalent to 8.5% of the applied N in this watershed. This indicates that 16 mg of N was loaded into each liter of Taihu Lake water per year, if we assume that there was the same intensity of N load from the whole catchment area and that all the N was accumulated in the lake.

Combined with the results of township-level investigation obtained at Xuyan Town, Wujin City, Jiangsu Province (33 km²), about 27 km East-Northeast from the Meilin, the intensities of N load from agricultural land, village, town centers and poultry farming were estimated at 48, 40, 10 and 2%, respectively, suggesting that agricultural activity was the leading source of N pollution in this region.

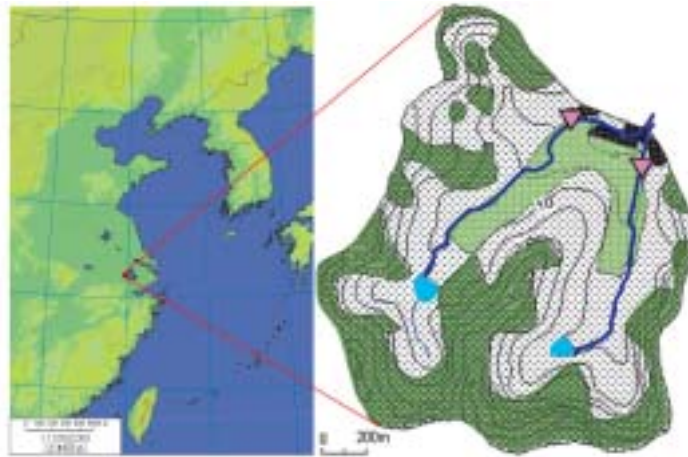


Fig. 1. Location, land use and topographical map of the Meilin Watershed, Yixing City, Jiangsu Province, China. A typical agriculture watershed on the west coast of Taihu Lake was targeted, with paddy fields (green) at the bottom of valleys around two channels (blue); upland fields and orchards (brown), and bamboo and other woods (dark green) near the slopes. The lower reaches of channels are populated areas (grey), but almost no habitation can be found above the weirs (red triangle) where water flux and quality were investigated.

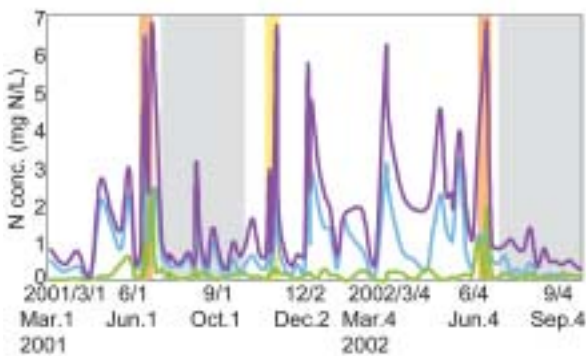


Fig. 2. Nitrate (blue), ammonium (green) and total (purple) nitrogen concentrations in outlet channel water from the Meilin Watershed (see Fig. 1). Channel water was sampled at the weirs on a weekly basis during baseflow, and more intensively for 14 hours after significant rainfall events. Grey: July-September when rice was in its full growing season, orange: basal fertilizer application for rice, yellow: basal fertilizer application for rape and wheat.

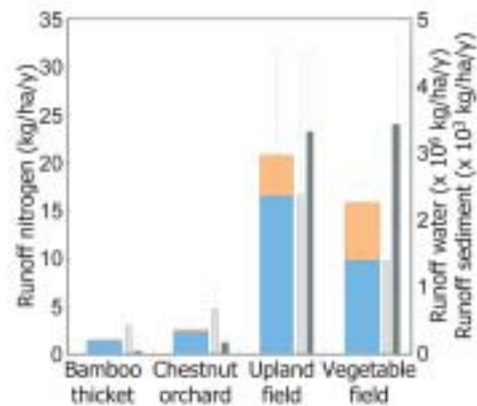


Fig. 3. Effects of typical upland water usage for bamboo thickets, chestnut orchards, upland fields and vegetable fields, at slope areas showing amounts of runoff water (white), sediment (grey), sediment-associated nitrogen (orange) and water-associated nitrogen (blue) observed for a year in 2002 when remarkable runoff events were observed 12 times following heavy rainfall events. Vertical bars indicate the SDs of duplicate measurements.

References

- Gao, C., Zhu, J.G., Zhu, J.Y., Gao, X., Dou, Y. and Hosen, Y. (2004): Nitrogen export from an agriculture watershed in the Taihu Lake area, China. *Environmental Geochemistry and Health*, 26, 199–207.
- Guo, H., Gao, C., Liu, G., Zhang, Y., Han, Y., Cai, Z., Wang, X., Dou, Y., Zhu, J., Yagi, K. and Hosen, Y. (2004): Evaluation of the environmental impact of non-point sources in Taihu Region Catchment. Workshop on Evaluation and Development of Methods for Sustainable Agriculture and Environmental Conservation, February 2004, Beijing, 7, 70–111.
- Hosen, Y., Yagi, K., Gao, C., Bai, X., Zhu, J. and Cai, Z. (2002): Nitrogen and phosphorus outflow from an agricultural watershed in Taihu Region, China. Abstract of annual meeting of the Japanese Society of Limnology, September 2002, Tokyo, 67, 82 (in Japanese).

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Growth suppression of rice caused by water drainage during fallow season in the tropics

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Key words: fallow season, growth-suppression, P deficiency, pH. Rice

Objectives

Intensive rice ecosystems producing two crops of rice per year are important agricultural improvements that increase rice yield in tropical areas. In a long-term rice cultivation experiment at the International Rice Research Institute (IRRI), water drainage during the fallow season suppressed the growth of paddy rice. In this study, the mechanisms underlying this suppression were analyzed.

Results

Research was conducted on four subplots within the research site. At the W0 plot, the field was not supplied with irrigation water, while at W1 plot, the field was flooded throughout the fallow season. In S0 subplot, all rice straw was removed from the field after harvesting crops of the previous season, whereas in S1 subplot, rice straw was left on the field, and was incorporated into the soil one month before transplanting. In the latter field, 50 kg/ha of nitrogen (N) was applied irrespective of season; neither phosphorus (P) nor potassium (K) was applied. The growth suppression that was observed during both the wet and dry seasons became more prominent in the W0S0 plots (Fig. 1). This tendency was same as those in 2001 wet season (WS), 2002 dry season (DS), and 2002WS (data not shown). The rice yields in the W0S0 plot were significantly lower than those of the other plots indicating that growth suppression during the early stages of cultivation at the W0S0 plots lead to the decrease in yield of rice (Fig. 2). Phosphorus content in the growth-suppressed rice (W0S0 plot) was lower than that of the rice not subjected to growth suppression (Fig. 3a) suggesting that suppression of rice growth was caused by phosphorus deficiency in the plants. From the data for the growth-suppressed plots, the P deficiency of growth-inhibited rice was associated with a small amount of available P due to the low Fe(II) content in the soil (Fig. 3b) and/or the high soil pH (Fig. 3c).

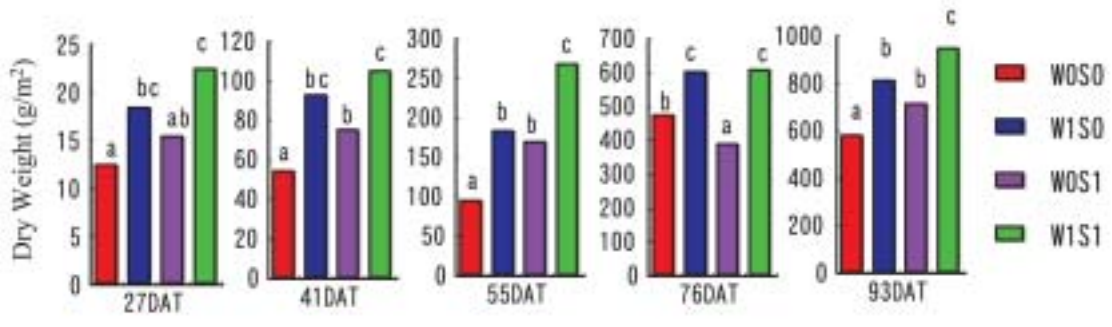


Fig. 1. Changes in dry weight (2001 wet season). Symbols with differing letters denote significant differences at the 5% level. (Same as below)

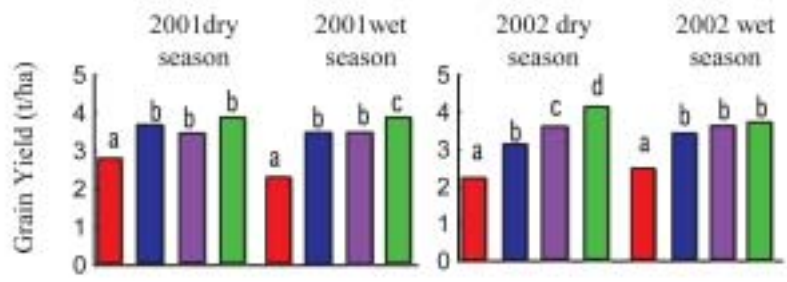


Fig. 2. Grain yield. The colors for each plot are as given in the legend for Fig. 1.

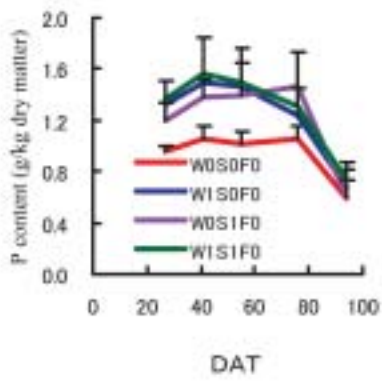


Fig. 3a. Changes in P content in leaves.

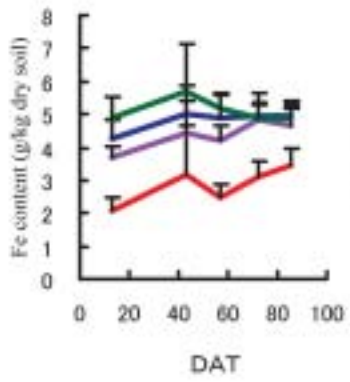


Fig. 3b. Changes in Fe (II) content in soil.

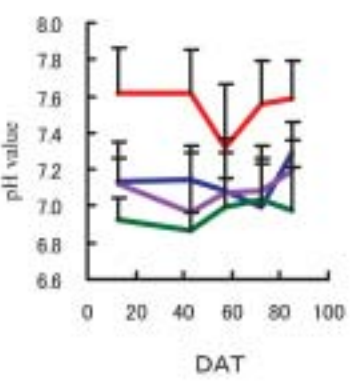


Fig. 3c. Changes in pH levels.

Vertical bars indicate standard deviation (same as above).

QTL analysis of lodging resistance-related properties in paddy rice

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Key words: QTL, lodging resistance, rice, pushing resistance force, root traits

Objectives

In the Jiangnan Region of China, direct sowing is rapidly spreading due to changes in industrial structure in recent years, and root lodging has become a serious problem. Therefore, it is necessary to investigate the lodging resistance mechanisms in relation to the root system and shoot morphology, for breeding lodging-resistant varieties. In this study, we performed QTL analysis of lodging resistance-related traits, including pushing resistance force and morphological characteristics of the root system, using 83 double haploid lines derived from ZYQ 8 (indica variety) / JX 17 (japonica variety) crosses.

Results

The lodging degree was most highly correlated with the traits of the aboveground parts, followed by pushing resistance force per shoot weight, and finally TR rate. The correlations between the lodging degree and the root morphology or root activity were low. Pushing resistance was most highly correlated with heading date, followed by aboveground morphology and then roots morphology.

QTLs for the lodging degree resided on chromosomes 2 and 6. QTLs for pushing resistance were located on chromosomes 7 and 10. QTLs for plant height were located on chromosomes 4, 8, 10, and 12, and QTLs for height of the center of gravity were located on chromosomes 4, 8, 9, and 10. With regard to the other characters, more than one QTL was found for each character (Fig. 1).

These QTLs were classified by the sign of the correlation coefficient between each character and the degree of lodging into QTLs that increased lodging resistance and QTLs that decreased lodging resistance. The results clearly indicated that the QTLs that increased lodging resistance were located on chromosomes 3, 5, 6, and 7, and those that decreased lodging resistance were on chromosomes 1, 2, 4, 8, 9, 10, and 12 (Fig. 1).

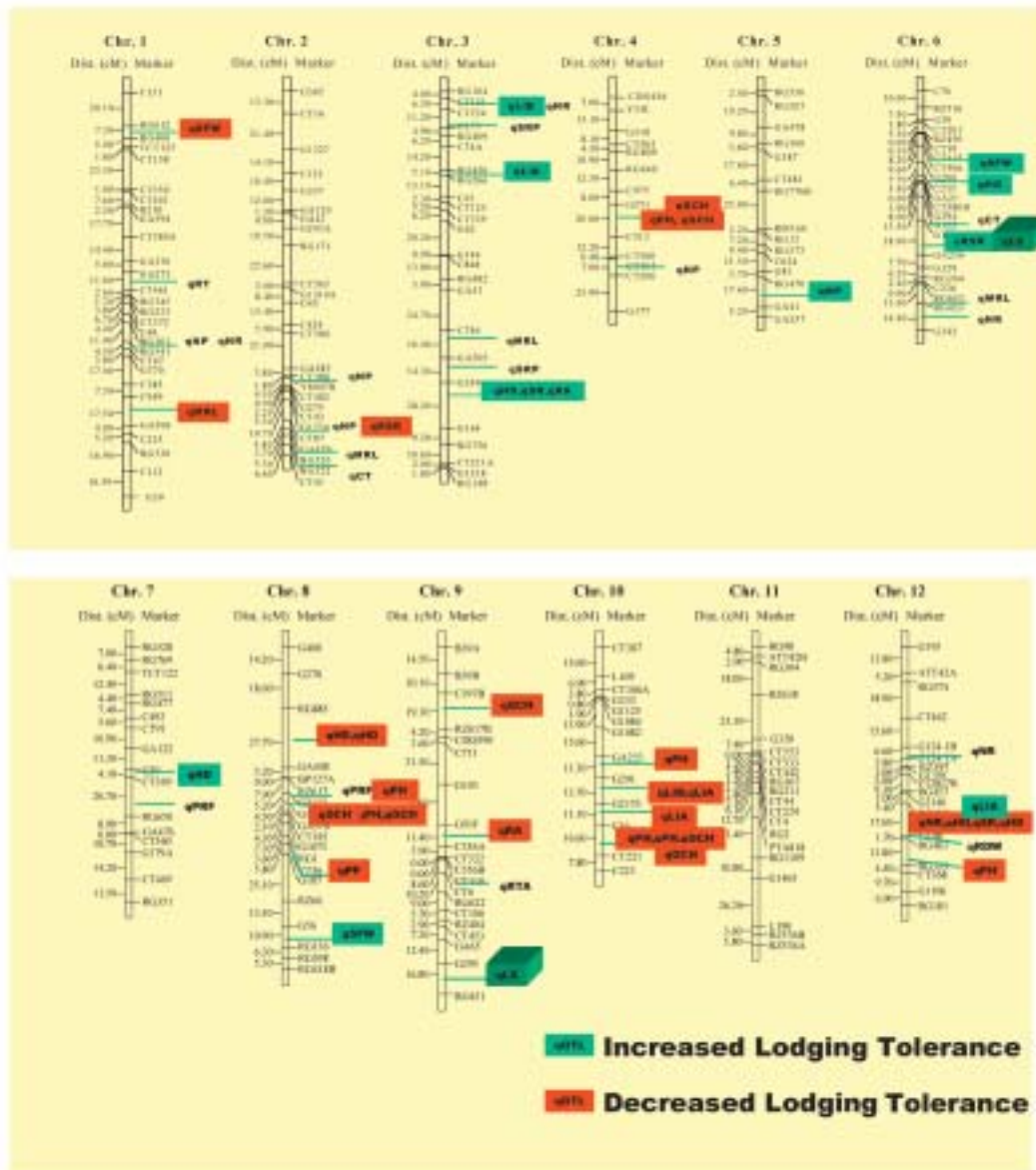


Fig. 1. QTL map in relation to lodging tolerance.

References

Fujimoto, H., Qian, Q., Wu, W., Zeng, D., Tobita, S., Dong, G. and Guo, L. (2004): QTL analysis of lodging tolerance in rice plant. *Japanese Journal of Crop Science*, 73 (Extra issue 1), 206–207 (in Japanese).

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Interspecific difference in the utilization of proteinaceous nitrogen among gramineous crops

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Key words: gramineous, organic nitrogen, proteinaceous nitrogen, sorghum

Objectives

In the developing regions such as Sub-Saharan Africa, farmers use litter chemical fertilizers and instead utilize organic materials such as crop residues and manure as alternate sources of nitrogen (N). When organic N is applied to soil, the amount of proteinaceous N, the most common form of available N in soil, temporarily increases. In order to develop the integrated use of organic and inorganic materials, it is necessary to understand the mechanisms behind the utilization of differing forms of N by crop species. Thus, the properties of proteinaceous N utilization by four globally important gramineous crops-sorghum, upland rice, maize and pearl millet-were elucidated in this study.

Results

As a field experiment, equal amounts of N (150 kg N/ha) were applied in both inorganic (urea) and organic (a mixture of rice bran and rice straw, C/N=20) forms, and its effects on the growth of the four gramineous crops were monitored. The four crops were divided into two groups in terms of their response to organic N application. Sorghum and upland rice displayed more prominent growth under organic N treatment than under inorganic N treatment, whereas maize and pearl millet did not show significant difference in growth under the two treatments. A similar pattern was also observed in the N uptake of these crop species. Sensitivity towards organic N in upland rice had previously been revealed, but that of sorghum was newly discovered through this experiment.

In pot experiments, the same amount of N (500 mg N/kg) was applied in the form of (1) ammonium nitrate, (2) rice bran (C/N=12), and (3) a mixture of rice bran and rice straw (C/N=20). N uptake of each of the four crops was compared 21 days after transplanting. The four crops were again divided into the same two groups. Sorghum and upland rice absorbed similar quantities of N from both organic and inorganic N treatments, while maize and pearl millet absorbed more N from inorganic N treatment than from organic N treatment (Fig. 1). These results reflected the proteinaceous N and inorganic N content in the soils under each treatment.

Uptake rates of proteinaceous N were directly measured from a solution culture system. Proteinaceous N, extracted from field soil treated with a neutral phosphate buffer and purified through dialysis, was added to the solution; decreases in levels of proteinaceous N in the solution was used as a means of measuring N uptake by the plant roots. Absorption rates of proteinaceous N was much higher in sorghum and upland rice than in maize, and no proteinaceous N was absorbed by pearl millet roots (Fig. 2). These results suggest that the above-mentioned responses to organic N application among crops are due differing absorption rates of proteinaceous N from the soil.

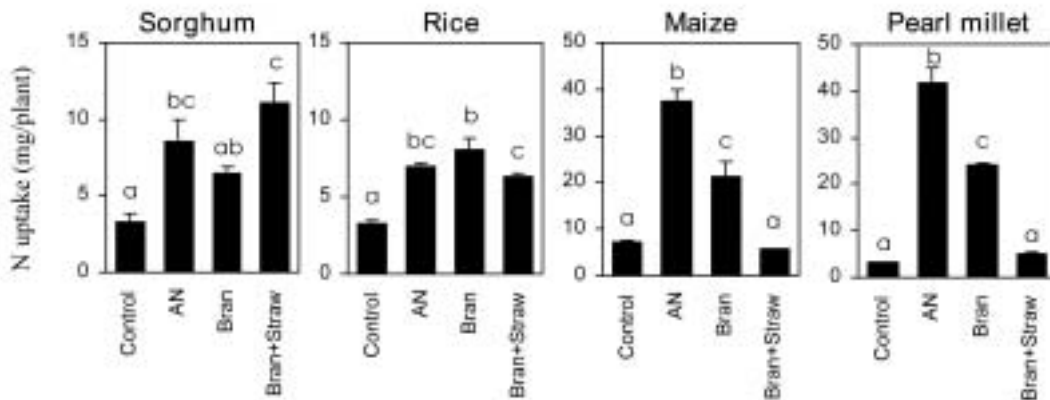


Fig. 1. N uptake by the four gramineous crops under differing N treatments (pot experiment). Differing letters indicate significant differences ($p < 0.05$). (AN: ammonium nitrate)

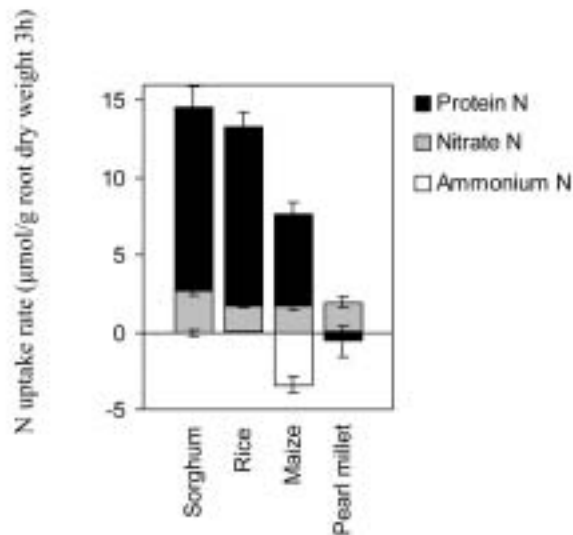


Fig. 2. Nitrogen uptake rates of the four crop species when N was applied to the root bathing solution in the form of proteinaceous N. Vertical lines indicate standard deviations.

References

- Okamoto, M., Okada, K., Watanabe, T. and Ae, N. (2003): Growth responses of cereal crops to organic nitrogen in the field. *Soil Sci. Plant Nutr.*, 49, 445–452.
- Okamoto, M. and Okada, K. (2004): Differential responses of growth and nitrogen uptake to organic nitrogen in four gramineous crops. *J. Exp. Bot.* 55, 1577–1585.

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Physiological mechanisms of aluminum resistance of rice varieties and the development of a rapid screening method for resistant genotypes

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Key words: acid soil, aluminum, calcium, resistance, rice

Objectives

Upland rice is an important crop for small farmers in developing countries. The growth of upland rice is often reduced by the soil acidity of highly weathered soils (Oxisols and Ultisols) in tropical regions. Since many small farmers cannot use enough lime, the genotypes resistant to soil acidity should be developed. Fewer studies have been conducted for the acid-soil resistance of upland rice compared with the other crops such as wheat. A previous study by JIRCAS in South American savannas revealed that calcium (Ca) plays a major role in the genotypic differences in the resistance to soil acidity in Oxisols. In this study, the mechanisms of resistance to acid soils in rice was elucidated in relation to Ca in comparison to cases of wheat, and a new efficient screening method was proposed.

Results

The major inhibitory factor in acid soils is Al. The first symptom by Al is the inhibition of root elongation. Thus, the degree of Al resistance of rice was measured by the relative root growth in a culture solution containing Al. The degree of Al resistance was as follows: Toyohatamochi (73%) > Oryzica Sabana 6 (53%) > IR72 (28%) > IR36 (19%) \geq Kasalath (16%) (Fig. 1). Increasing Ca concentration in the solution recovered root growth in all genotypes, and the recovery rates were greater in susceptible genotypes (Fig. 1).

In the case of rice, the addition of barium (Ba), the congeneric element to Ca, to Al-affected roots further reduced root elongation (Fig. 1). However, in the case of wheat (Atlas cultivar 66), the addition of Ba recovered Al-affected roots (data not shown). These results suggest that the mechanisms of the resistance to Al differ between two crops.

For wheat, the hematoxylin staining which determines Al content in root tissue, has been used as a proven screening method for Al resistance. However, it is not possible to apply this methodology to rice, most likely due to the differing mechanisms of Al resistance in the two plant species as mentioned above. Keeping in mind that the relative adsorption of Al and Ca on root cell walls is directly related to the mechanisms of Al resistance in rice, a rapid screening method using pyrocatechol violet (PCV) was developed. In this method, the roots are first immersed in Al solution for 15 min, and then treated with Ca solution. The easily exchangeable Al is displaced by Ca in this process, allowing the detection of the hardly exchangeable Al at the root surface by PCV staining. Under this method, the root tips of susceptible varieties were more strongly stained than the three resistant varieties (Fig. 2). These results coincide with the results of root elongation (Fig. 1). When the Ca solution which displaces adsorbed Al was omitted from the process, differences in the degree of staining among the varieties

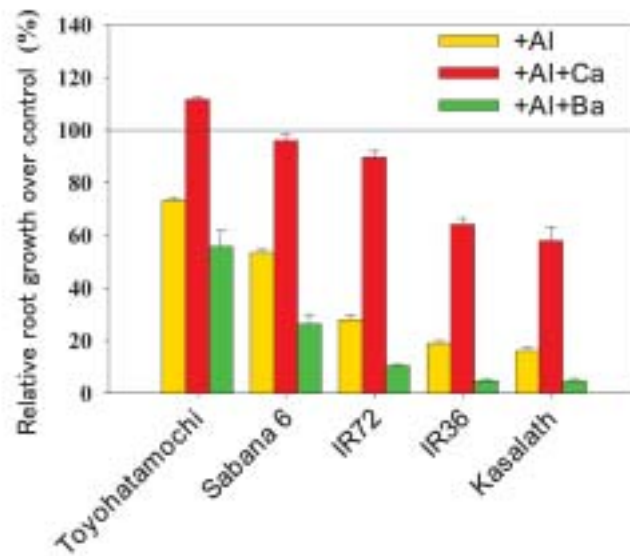


Fig. 1. Effects of the addition of Ca and Ba on the relative root growth of Al-affected rice roots. [Control], 50 μ M Ca; [+Al], 50 μ M Ca + 20 μ M Al; [+Al+Ca], 500 μ M Ca + 20 μ M Al; [+Al+Ba], 50 μ M Ca + 450 μ M Ba + 20 μ M Al.

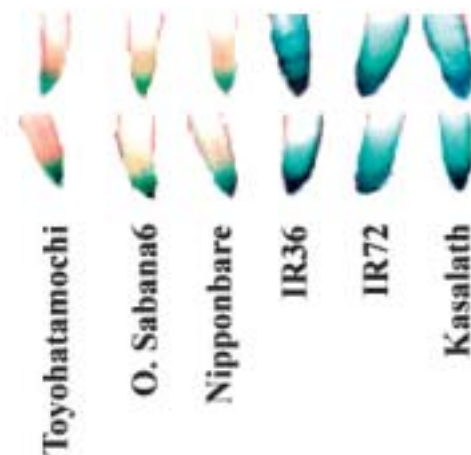


Fig. 2. Results of PVC staining of the roots of rice varieties after they were treated for 15 min with solutions containing 50 μ M Ca + 100 μ M Al, followed by a 45 min treatment with solutions containing 500 μ M Ca. Two replications of each variety is shown.

became unclear (data not shown). In addition, the use of this method allows the rapid evaluation of Al resistance in rice varieties. Further tests should be carried out for the validation of this technique, through comparison of results with the growth rates of rice varieties planted in acid-soil fields.

References

- Okada, K., Fischer, A.J., Perez-Salazar, F.A. and Canon-Romero, Y. (2003): Difference in the retention of Ca and Al as possible mechanisms of Al resistance in upland rice. *Soil Sci. Plant Nutr.*, 49, 889–895.
- Watanabe, T. and Okada, K. (2005): Interactive effects of Al, Ca and other cations on root elongation of rice cultivars under low pH. *Ann. Bot.*, 95, 379–385.

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Quantitative analysis of groundwater effluent and reservoir water influent in a pond using ^{222}Rn - and water-balance equations

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Key words: Radon-222, water balance, groundwater

Objectives

It is essential to quantify groundwater effluent and reservoir water influent in ponds in order to use water resources effectively and to predict water quality. However, conventional methods measure only the amount of surface water inflow and outflow, and can only reveal differences between groundwater effluent and reservoir water influent. It is not possible to quantify both parameters simultaneously. In this study, we concentrated on the radon-222 (^{222}Rn) content in the environment and developed an analytical method using ^{222}Rn - and water-balance equations.

Results

Fig. 1 shows ^{222}Rn - and water-balance in a pond. Although groundwater influent and reservoir water influent are unknown quantities, it is possible to quantify them both by constructing ^{222}Rn - and water-balance equations. The dispersion of ^{222}Rn into the atmosphere, an important factor contributing to ^{222}Rn loss, is calculated assuming that there is a stagnant film between water and air. The thickness of this stagnant film was empirically estimated to be $830\ \mu\text{m}$.

The developed method was applied to a pond near a landslide-prone area in Japan, where it is considered that reservoir water influent is a major cause of landslides. Table 1 shows the results of the field investigation. Using these data, we constructed ^{222}Rn - and water-balance equations, and groundwater effluent and reservoir water influent were calculated to be $0.67\ \text{L/s}$ and $0.41\ \text{L/s}$, respectively. As stated above, conventional methods could not quantify groundwater effluent and reservoir water influent simultaneously, but our new method has made this possible.

The on-site application of the method is expected to facilitate effective water use, predict water quality, and prevent landslides.

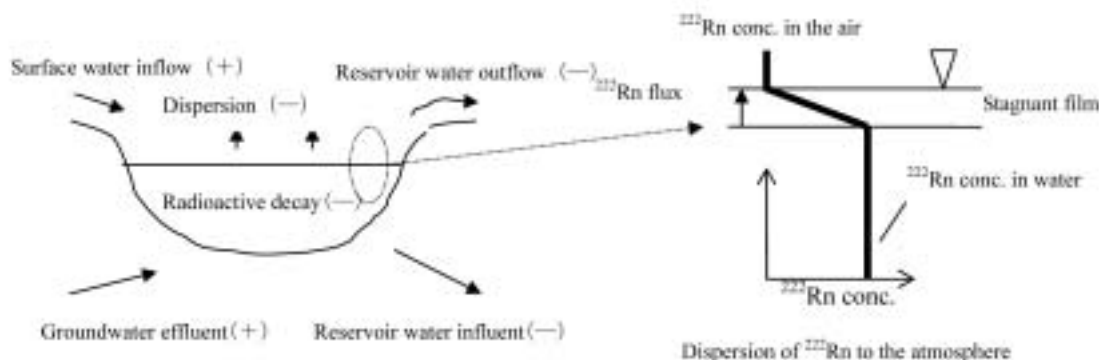


Fig. 1. ^{222}Rn - and water balance in a pond (+ : supply, - : loss.). Water balance was maintained (water level remained constant) when the amount of surface water inflow and groundwater effluent was equal to the amount of reservoir water outflow, evaporation, and reservoir water influent. ^{222}Rn balance was maintained (concentration remained constant) when the amount of surface water inflow and groundwater effluent equaled the amount of radioactive decay, dispersion, reservoir water outflow, and reservoir water influent.

Table 1. Results of field investigation.

	Discharge (L/s)	^{222}Rn conc. (Bq/L)	Amount of ^{222}Rn (Bq/s)
Spring	0.11	6.54	0.72 (discharge \times conc.)
Surface water	0.15	0.00	0.00
Groundwater effluent	x	6.54	6.54x (discharge \times conc.)
Radioactive decay			3.74 (decay const. \times volume \times conc.)
Dispersion			1.00 (area \times diffusivity \times conc./thickness of stagnant film)
Reservoir water outflow	0.43	0.41	0.18 (discharge \times conc.)
Reservoir water influent	y	0.41	0.41y (discharge \times conc.)
Evaporation	0.09		

The area of the pond is 1550 m². The average depth is 2.8 m. The thickness of stagnant film is 830 μm . ^{222}Rn concentration in groundwater equals that of the spring.

References

Hamada, H. and Kishi, S. (2004): Quantitative analysis of groundwater effluent and reservoir-water influent in a small pond using ^{222}Rn - and water-balance equations. JARQ, 38, 253–258.

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Termite activities increase the productivity and nutrient value of grasses and grazing frequency by cattle around termitarias

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Key words: grazing pasture, cattle, termite, *Brachiaria decumbens*, *Cornitermes cumulans*

Objectives

Termites play leading roles in organic matter decomposition and nutrient cycling in tropical and subtropical zones. It is important, therefore, to understand the effects of termite activity on soil fertility, grass growth and cattle grazing in subtropical pastures, in order to predict the dynamics of agropastoral systems and to enhance their productivity and sustainability. Soil fertility, productivity and nutrient value of grass, *Brachiaria decumbens*, as well as the grazing behavior of cattle in areas near termite mounds populated by the species *Cornitermes cumulans* were compared with those of control areas free of mounds in subtropical pastures of Brazil.

Results

The total carbon and nitrogen content of the surrounding soil increased as the distance to the mounds decreased in the lower soil layers, whereas a clear pattern could not be deduced for upper soil layers. The rates of height elongation, dry matter production and crude protein production of grass per unit area were approximately 1.4, 1.6 and 2.1 times higher, respectively, around termite mounds than those of control areas (Fig.1). It was observed, using cameras with infrared ray sensors, that cattle grazed in these nutritious and productive grasses around the mounds with much higher frequency than in control areas (Fig. 2). As a result, the height of the grass always remained lower within a 68 cm radius, on average, of the margins of termite mounds.

In areas surrounding termite mounds, increases in productivity and nutrient value of grasses due to soil nutrient enrichment is expected to induce the frequent and preferential grazing of cattle. This suggests that the presence of termite mounds exerts significant influence on the grazing behavior and intake of nutrition, especially crude protein, of cattle and therefore affects beef quality of cattle raised in subtropical pastures. It is crucial for this to be taken into consideration when managing subtropical pastures in which C₄ grasses with low crude protein content are predominant and where supplying legumes as major nitrogen sources for cattle is often difficult.

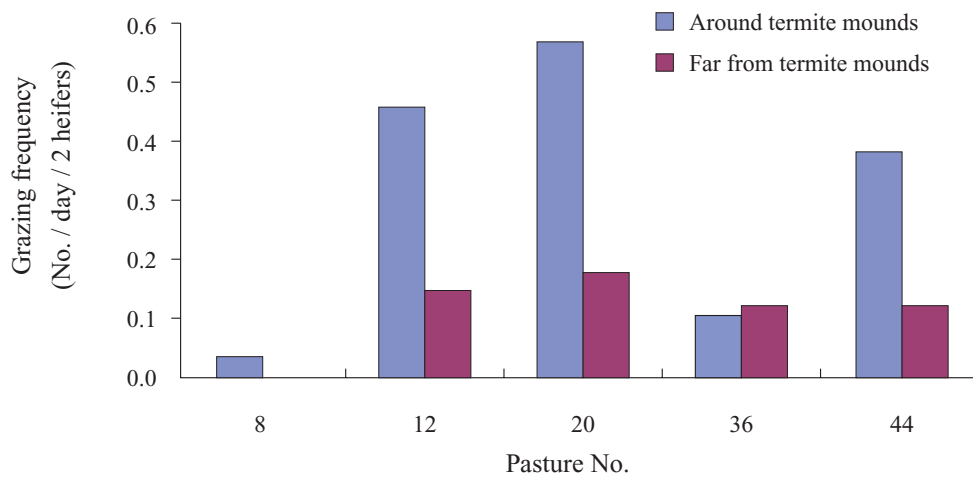


Fig. 1. Grazing frequency of two heifers per day.

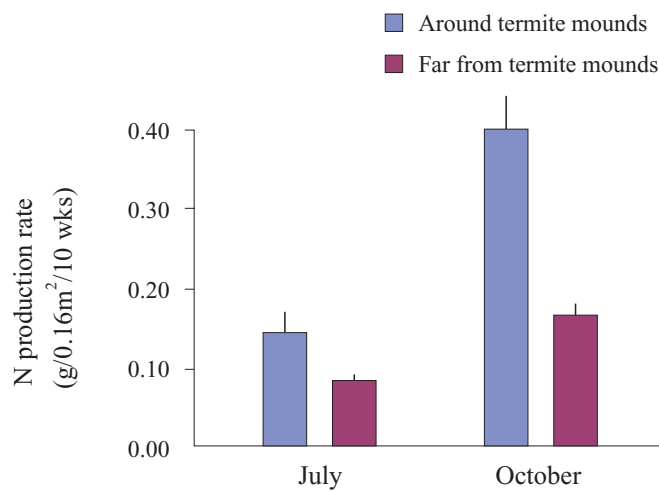


Fig. 2. Nitrogen production rate (N g/0.16 m²/10 wks) near and far from termite mounds in *Brachialia decumbens* pasture. Vertical bars indicate + 1 SE for 12 repetitions.

References

Fukuda, E., Macedo, M.C.M., Borges, M.J. and Pitaluga, G.M. (2003): Effects of mounds of a termite *Cornitermes cumulans* on grass production and behavior of grazing cattle in semitropical grasslands in Brazil. *Grassland Science* 49, 24–25.

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Development of an experimental model for the evaluation of *in planta* colonization of nitrogen-fixing endophytes in rice plants

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Key words: nitrogen-fixing bacteria, NFB, endophyte

Objectives

We have previously reported that large quantities of nitrogen-fixing endophytic bacteria symbiotically inhabit the tissue of gramineous plants. Currently, we have isolated a number of bacteria having nitrogen-fixing abilities, such as *Azospirillum* sp., *Klebsiella* sp., and *Herbaspirillum* sp. from various grasses. However, it remains necessary to establish a means of inoculation testing in order to investigate the colonization dynamics of these bacteria and elucidate how they contribute to the growth of important plant species. Hence, as an experimental model with which to verify the effectiveness of a new inoculation test method, we chose the bacteria *Herbaspirillum* sp. A46, originally isolated from *Panicum maximum* and rice plants. If successful, this method will serve as a foundation for analyzing the behavior of nitrogen-fixing bacteria within plants.

Results

Herbaspirillum sp. A46, marked with transposon-based *gusA* gene, was evaluated for its ability to colonize fodder rice. A total of three rice varieties were used as hosts: Sprice, which is a rice cultivar now used as silage, and two other traditional varieties, Tetep and Koshihikari. The bacteria, which possibly enter through cracks in the emerging lateral root of cv. Sprice, were localized in the inter- and intra-cellular areas of the cortical cells and the xylem vessels of the root; bacteria then systemically spread into the shoot portions, where they were found in the intercellular spaces of adjacent cells in the leaf vascular bundle (Figs. 1 and 2). Colonization began as early as two days after inoculation with numbers ranging from 10^5 – 10^7 per gram fresh weight. The population of isolated herbaspirilla remained constantly larger than of the bacteria isolated from the shoot 2 to 21 days after inoculation. In the case of bacteria isolated from shoots within the same sampling period, the population tapered only at 30 days after inoculation in all three varieties accompanied by a consequent increase in numbers of bacteria. Among the three cultivars, no significant differences were observed in the number of herbaspirilla isolated from the inner tissues except at seven days after inoculation, when the fodder rice, cv. Sprice, harbored the highest numbers of bacteria. The addition of $10\ \mu\text{M}$ NH_4Cl inhibited the colonization of all three rice cultivars, of which cv. Koshihikari showed the highest sensitivity. Nitrogenous activity was detected in all three varieties in the absence of carbon sources until seven days after inoculation.

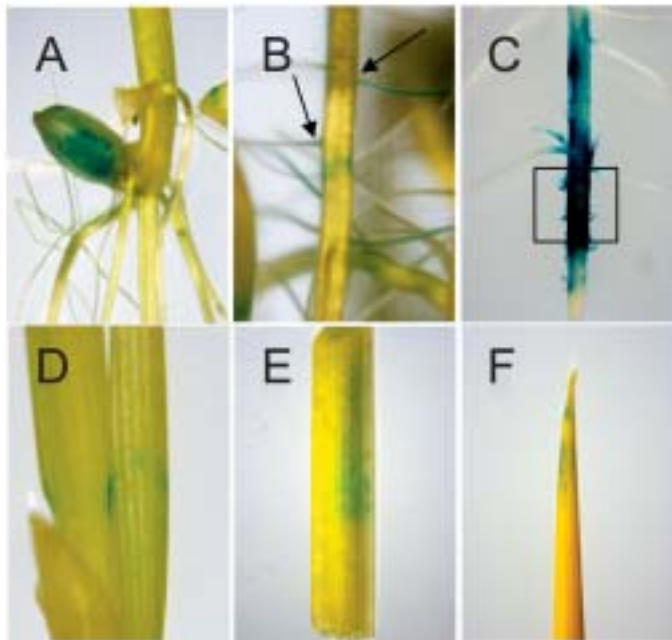


Fig. 1. Light micrographs of cv. Sprice 7 days after inoculation. Roots were inoculated with *Herbaspirillum* sp. A46 marked with transposon-based *gusA*, showing the gus staining in the seeds (A), lateral root and root junctions (B), root tip region (C), stem (D), leaf sheath (E) and tip of leaf blade (F).

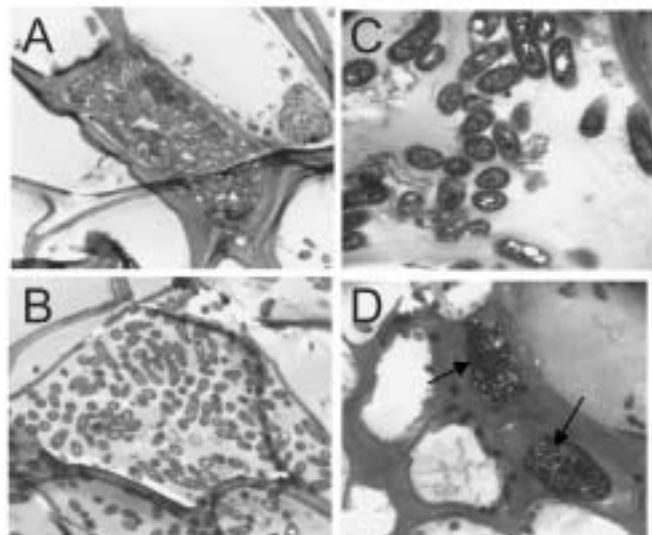


Fig. 2. Transmission electron micrographs (70 nm sections) of cv. Sprice roots 7 days after inoculation. Roots were inoculated with *Herbaspirillum* sp. A46, showing the bacteria colonizing the intracellular portion (A, B) and intercellular space (C) of cortical cells, and protoxylem vessels of the vascular bundle (D).

Isolation of lactic acid bacteria strains suitable for producing high quality silage in Thailand

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Key words: silage, lactic acid bacteria

Objectives

Silage feeding is an effective and easily adaptable technique despite its limited application in Thailand. The use of high quality silage is a highly dependable means of increasing and stabilizing the quality of raw milk production (Ohmomo et al, 2002); however, successful results are not always ensured if ensilage depends on natural fermentation. In order to address this problem, lactic acid bacteria (LAB) strains suitable for silage making in Thailand were screened.

Results

Previously, 13 LAB strains having culture filtrate pH levels less than 4.0 at 45°C were selected out of 215 strains isolated from 14 silage samples prepared in Thailand. Each strain was carefully monitored for silage fermentation inoculants, using the modified pouch method to simulate a tropical environment (Ohmomo et al, 2004a). The various profiles of lactate production in the four isolates, CS 1–8, CS 5–5, KS 1–9 and SP 1–3, were confirmed. The time courses of lactate production in the cultures using the modified pouch method with various inoculum sizes are shown in Fig. 1. The most important property of LAB strains in terms of silage making is their high ability to produce lactate during the silage fermentation process, which is a type of solid-mixed, non-sterilized fermentation. This property becomes further apparent when using the modified pouch method. Strain SP 1–3 isolated from corn silage and tentatively assigned to *Lactobacillus plantarum* exhibited an inherent tolerance for high incubation temperatures and lactate. Strains CS 5–5 and KS 1–9, isolated from corn silage and tentatively assigned to *Pediococcus* sp., also exhibited similar properties to strain SP 1–3, but they showed weaker lactate tolerance than that of strain SP 1–3. Strain CS 1–8, isolated from TMR silage and tentatively assigned to *Pediococcus* sp., displayed steady growth during the early stages of silage fermentation, but did not accumulate much lactate by the end of the long-term fermentation (Ohmomo et al, 2004b).

Based on these results, laboratory-scale silage of Napiergrass inoculated with strain SP 1–3 and/or CS 1–8 was prepared. The fermentation quality of silage inoculated with LAB strains significantly increased the quantity of lactate produced (close to double the amount) and reduced counts of coliform bacteria and yeast. From these results, both SP 1–3 and CS 1–8 were considered to be suitable strains for use as silage fermentation inoculants in tropical regions.

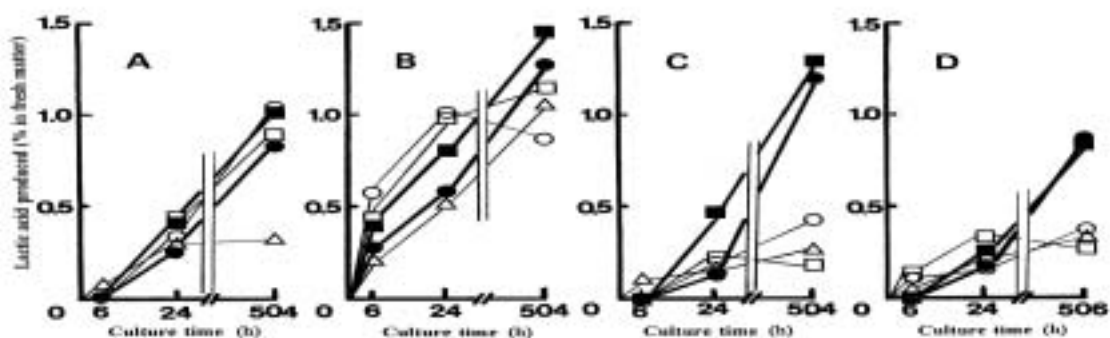


Fig. 1. Time course of lactate production by typical isolates using various modified inoculum sizes. Inoculum size (cfu/ml): A (LAB 10^2 , CFB 10^2 , Yeast 10^2); B (LAB 10^5 , CFB 10^2 , Yeast 10^2); C (LAB 10^2 , CFB 10^5 , Yeast 10^2); and D (LAB 10^2 , CFB 10^2 , Yeast 10^5). Symbols: ■ (strain SP 1-3); ● (strain CS 5-5); ○ (strain CS 1-8); △ (strain LS 2-38); and □ (strain KS 1-9).

References

- Ohmomo, S., Nitisinprasert, S. and Hiranpradit, S. (2002): Silage-making and recent trend of dairy farming in Thailand. *JARQ*, 36(4), 227-234.
- Ohmomo, S., Nitisinprasert, S., Kraykaw, D. and Hiranpradit, S. (2004a): Modification of the pouch method to evaluate the ability of lactic acid bacteria strains for making good quality silage in Thailand. *JARQ*, 38(2), 119-124.
- Ohmomo, S., Nitisinprasert, S., Kraykaw, D., Laekorn, P., Tanomwongwattana, S. and Hiranpradit, S. (2004b): Evaluation of lactic acid bacteria isolates for silage fermentation inoculant in Thailand by using a modified pouch method. *JARQ*, 38(3), 199-208.

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Improvement of feeding management and meat quality of pigs by using locally-available under-utilized feed resources in the Mekong Delta region of Vietnam

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Key words: *Eichhornia Crassipes*, *Ipomoea aquatica*, *Ipomoea batatas*, rice bran, farming systems

Objectives

In the Mekong Delta region of Vietnam, many forms of rice-based farming systems have taken root, and swine production has become an important component of these farming systems. Most swine production is carried out by small-scale farmers who depend on pork as an important means of income-generating activity. However, a nutritional imbalance in the pigs' diet has become apparent, due to the high percentage of rice bran used in their feed. To compensate for this imbalance, farmers also use commercial protein-concentrate feed (concentrate feed for short), but the additional costs of concentrate feed often become a financial burden for the producer. In addition, the meat quality of swine produced at small-scale farms tends to be lower, having higher fat content compared to that of swine produced at large-scale farms. Moreover, increasingly affluent consumers have begun to choose the higher-priced lean pork over the cheaper pork having higher fat content. Thus, in this research, a study was conducted with the aim of reducing feed costs while simultaneously improving pork quality.

Results

Ideal due to their abundance and low costs, two species of natural water plants were evaluated for their potential as alternative feed sources. Water hyacinth (*Eichhornia Crassipes*, Fig. 1) and water spinach (*Ipomoea aquatica*) were fed raw to pigs. First, the effects of their replacement in the diet on growth performance factors, such as daily weight gain and feed conversion ratio, were determined. There was enough of each plant to supply up to 5–6% of the feed and replace concentrate feed without significant reduction of growth performance. Next, the effects of the diet supplementation of each plant on pork quality and economic value were examined (Table 1). Both the fat content and meat quality were significantly improved by replacing the concentrate feed with water hyacinth. The iodine value in pig fat remarkably improved in pigs fed with water spinach, and the unit price of pork in pigs fed with water hyacinth and water spinach were higher than those of the pigs fed with the control diet. Sweet potato (*Ipomoea batatas* L.) vine was also tested using the same methods as the water plants, and also showed positive results.

It was revealed from the above results that these local feed resources could be used at the majority of farms in the regions, and that the substitution of concentrate feed with these plants lowers feed costs, and improves meat quality.



Fig. 1. Water hyacinth floating on the Mekong River.

Table 1. The effects of feeding water hyacinth and water spinach on the back-fat thickness, meat quality and economic value of pigs.

	Water hyacinth ¹⁾		Water spinach ¹⁾	
	control	test	control	test
Back-fat thickness, mm ¹⁾	18.0 ^a	15.7 ^b	—	—
Meat quality				
Crude protein, %	20.7 ^a	21.4 ^b	21.1	21.3
Iodine value of the back-fat	63.9 ^a	54.7 ^b	34.4 ^a	32.9 ^b
Economic value/head, % ²⁾				
Selling price	100	105	100	110
Feed cost	100	90	100	95
Profit ³⁾	100	110	100	127
Cost performance ⁴⁾	100	89	100	94

^{a, b} Mean values in the same row labeled with differing letters are significantly different at P<0.05.

1): The effects of each of the coupled diets, control and test, were determined. Proportions of commercial protein concentrate feed of control diets were reduced and that of water hyacinth or water spinach were increased in the test diet. 2): Relative to the controls (Control = 100). 3): The selling price subtracted by the feed cost. 4): Selling price/live weight.

References

- Manh, L.H., Dung, N.N.X., Men, L.T., Takada, R. and Yamasaki, S. (2002): Replacement of concentrate protein by water spinach (*Ipomoea aquatica*): effects on digestibility feed intake and live weight gain in growing pig production. JIRCAS Working Report, 26, 17–20.
- Son, V.V. (2003): The effect of supplement of sweet potato (*Ipomoea batatas*) vine in diet on the performance and backfat thickness of finishing pigs. Proceeding of the final workshop of JIRCAS Mekong Delta Project, 158–163.
- Men, L.T., Yamasaki, S., Caldwell, J.S., Thuan, T.K., Yamada, R., Khoa, D.V.A. and Takada, R. (2003): Effect of rice based balanced resources and the water hyacinth (*Ipomoea aquatica*) in diet on pig production in Tan Phu Thanh village, Cantho province. Proceeding of the final workshop of JIRCAS Mekong Delta Project, 164–173.

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Diagnosing and developing control measures for classical swine fever in Mekong Delta, Vietnam

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Key words: classical swine fever, viral detection, epidemiology, vaccination program

Objectives

In Mekong delta pig production plays an integral role of nutrient-recycling farming system among other crop productions such as rice, fish, vegetables and fruits through exchange of by-products, besides pig sales account for substantial percentage of cash income. However, farmers' operations are often hampered by disease outbreaks with high mortality of piglets. Diseases are named by symptoms and without any differential diagnosis, sick pigs are treated by antibiotics and even temporarily improved, relapsed into death or become stunted. We suspected outbreaks of classical swine fever (CSF), a highly contagious and fatal viral disease, but it also happened in vaccinated herd. Therefore, our objective of the study was to diagnose CSF through laboratory examination, investigate CSF vaccines and vaccination practices to finally provide appropriate control measures for the farmers.

Results

Between July 2002 and June 2003, we encountered ten fatal disease outbreaks in Cantho province. The symptoms were initially recognized in 44-day-old piglets (se 9.9, n=17 litters) and the sow was in her first parturition (median, 1 to 3). We detected CSF virus (CSFV) in piglets of seven farms with fluorescent antibody (FA) tests as well as CSFV genome in piglets of all ten farms by either analyzing amplified RT-PCR product (288 nt) of 5' NTR (Vilcek et al, 1994) with restriction fragment length polymorphism (RFLP, digestion with BgII) and/or from the sequence (240 nt) (Table 1). All four CSF vaccine viruses available in Mekong delta and viruses detected at farms belonged to different genogroups.

CSF vaccines were administered irrespective of the type of vaccine; no injections to the sow during pregnancy, and an injection for piglets near their weaning age and simultaneously for the sow. Thus boosters for gilts were often neglected and piglets from first parturition suffered from the disease in early stages. Higher weanlings' mortality was observed in the litter of sow vaccinated 60 days before pregnancy with a popular domestic N vaccine than that of 30 days, both failed in mating at the first heat. Ten farms that practiced N vaccines and had observed no fatal disease, so regarded as CSF free, had the sow pregnant within 3 weeks after simultaneous vaccination, and piglets at a-month old. Suggesting strict vaccination program of the sow before pregnancy is critical and sows in irregular reproductive cycle need to be individually re-boosted. CSF vaccines needs standardization and an applicable prescription shall accompany each product. Accurate diagnosis and correct recognition of the disease is indispensable to control CSF.

Table 1. Results of CSFV detection tests in piglets and the sows.

Test / Farm	1	2	3	4	5	6	7	8	9	10	Total
CSFvirus-FA	w/1	1/1	1/1	1/1	2/2	0/1	1/1	w/1	3/3	1/1	10/13
RT-PCR (5'NTR)	2/2	1/1	2/2	1/1	4/5*	1/2*	1/1	4/5*	3/4*	1/1	20/24*
RFLP (BglI)	1/1	1/1	1/1	1/1	2/2	1/1	1/1	3/3	n.t.	n.t.	11/11
Sequence (5'NTR)	1/1	1/1	2/2	1/1	4/4*	1/1	1/1	4/4	3/3	1/1	19/19*

n.t., not tested; w, weak/doubtful; FA, fluorescent antibody test; RT-PCR, reverse transcriptase polymerase chain reaction; RFLP, restriction fragment length polymorphism analysis.

*including sows' sera.

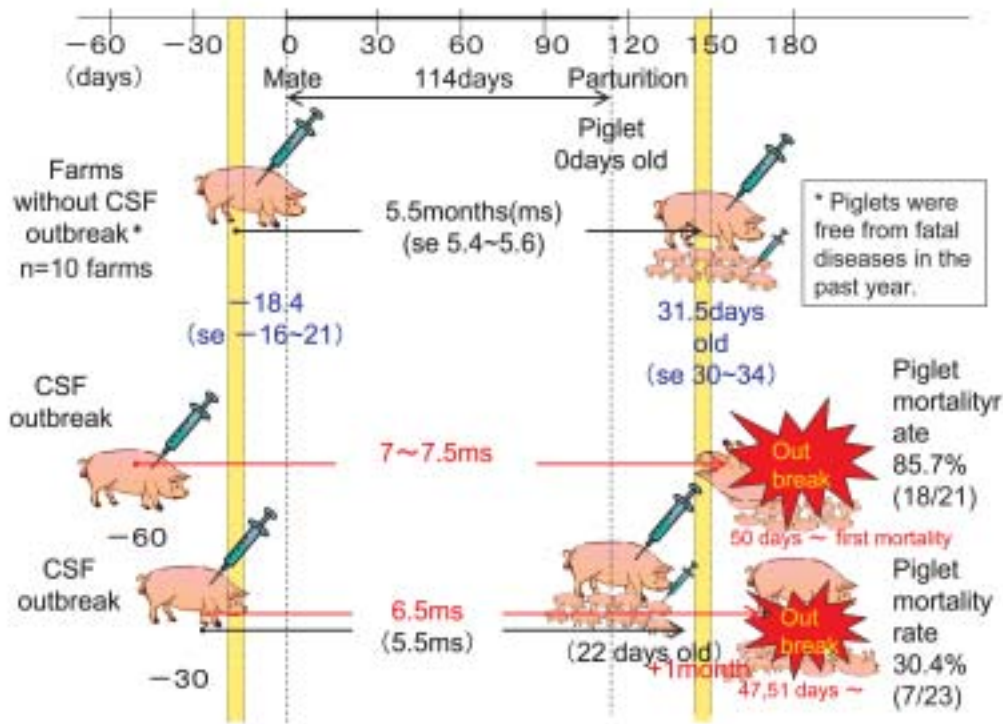


Fig. 1. CSF outbreaks and the vaccine practice (N vaccine, HCMC, Vietnam).

References

- Kamakawa, A. et al. (2003): Classical swine fever among pig herds and its control in Cantho province, Mekong delta. Proceedings of the 2003 annual workshop of JIRCAS Mekong delta project, JIRCAS-CTU-CLRRI-SOFRI, Cantho, Vietnam 2003.
- Kamakawa, A. et al. (2003): Characterization of the losses in pig production as a part of farming system in the Mekong Delta region, Vietnam. Journal of veterinary epidemiology, 7(2), 85-92 (in Japanese).
- Kamakawa, A. et al. (2002): Pig management in Tan Phu Thanh village and characterization of the losses. Proceedings of the 2002 annual workshop of JIRCAS Mekong delta project, JIRCAS-CTU-CLRRI-SOFRI, Cantho, Vietnam 2002.

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Influence of natural fermentation on the physico-chemical characteristics of rice noodles

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Key words: rice, rice noodle, fermentation

Objectives

The natural fermentation of *Indica* rice is a traditional method used in producing high-quality rice noodles having specific functional properties in China. However, rice noodles produced in small, labor-intensive factories rely heavily on the worker's skill, and quality greatly varies with processing conditions. Little research has been done on the effects of fermentation on the physico-chemical characteristics of rice starch, and hence the effects of fermentation on rice noodles have not yet been elucidated. Thus, it is necessary to conduct studies in order to understand the relationship between fermentation and the rheological properties of rice noodles, as well as to standardize and increase the efficiency of its production. In addition, testing the fermentation technology for other rice cultivars and applying it to other starchy crops such as maize would contribute to increasing the value of raw grain materials. The overall objective of this study, therefore, was to investigate the influence of fermentation of whole-milled rice granules on the physico-chemical characteristics of rice starch and the rheological properties of rice noodles.

Results

Rice granule samples were fermented at 35°C for 27 h in an incubator until the pH value of the soaking water reached 4.0; samples soaked for 3 h were used as a control, and were very similar to non-fermented rice noodles. Fig. 1 shows that the pH of the fermented supernatant decreased to a minimum of 4.0 over 18 h. The maximum acidity reached was about 1.1 mg/mL (lactic acid) when fermented for 27 h. Fermentation did not have a significant effect on the starch and amylose content of rice granules; protein, lipid and ash content decreased, whereas free fatty acid levels increased during fermentation. The rice noodles made from fermented samples had lower maximum stress of 54 kPa, and higher maximum strain of 10.8%, and had a white, transparent appearance and favorable chewiness compared to control samples (Table 1). From these results, we concluded that fermentation decreases protein, lipid and ash content, freeing complex starch and increasing its expansion, thus facilitating the formation of hydrogen bonds. In general, it is considered that rice stored previously for more than 6 months makes better rice noodles than newly-harvested rice. This may be due to a similar protein and lipid content decrease, or an increase of free fatty acid content during the storage period.

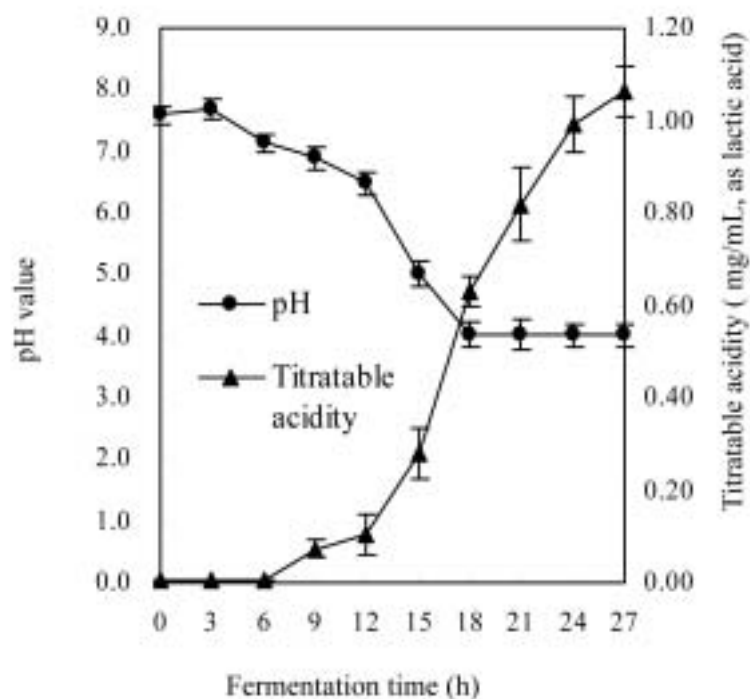


Fig. 1. Changes in pH values and titratable acidity of the fermented supernatant (n=3).

Table 1. Rheological properties and sensory evaluation of rice noodles.

Samples	Fermentation	Control
Maximum tensile stress σ (kPa)	54.0 \pm 2.0 ^b	144.9 \pm 2.3 ^a
Maximum strain ϵ (%)	10.8 \pm 0.4 ^a	8.2 \pm 0.4 ^b
Apparent elasticity index E (kPa)	4.99 $\times 10^2$ \pm 6.3 ^b	1.77 $\times 10^3$ \pm 68.3 ^a
Break power W (N·cm)	0.33 \pm 0.02 ^b	0.66 \pm 0.03 ^a
Sensory evaluation results	Softy, pliable, clear, white and chewy	Firm, opaque, crumbly and not pliable

^{a,b} mean values in each horizontal row followed by different superscripts are significantly different, as determined by Duncan's Multiple Range Test ($P < 0.05$).

All data are the means of 5 values \pm standard deviation.

References

- Lu, Z., Li, L., Min, W., Li, Z. and Tatsumi, E. (2002): Influence of natural fermentation technology on the rheological properties of rice noodle. *Journal of Chinese Institute of Food Science and Technology*, 2, 8–12 (in Chinese).
- Lu, Z., Li, L., Cao, W., Li, Z. and Tatsumi, E. (2003): Influence of natural fermentation on physicochemical characteristics of rice noodles. *International Journal of Food Science and Technology*, 38, 505–510.

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Predatory ability of *Joppeicus paradoxus* Puton, a predator of stored-product insect pests

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Key words: natural enemy, *Joppeicus paradoxus*, *Tribolium confusum*, predatory ability, stored-product insect pests

Objectives

Methyl bromide (MeBr) is the one of the most useful chemical agents for insect control in stored products. Fumigation with MeBr, however, may have negative effects in terms of human health. More importantly, the use of MeBr has been found to lead to ozone depletion, and for this reason will be banned from use in most cases by 2005 in developed countries and by 2015 in developing countries. It is therefore urgent that we develop alternative methods and adopt integrated pest management (IPM) techniques. Biological control is thus being regarded with increasing interest, due to its nontoxic, human-safe, environment-friendly properties. The use of natural enemies (predators and parasitoids) in controlling stored-product insect pests has also been studied by various researchers worldwide.

The predacious bug, *Joppeicus paradoxus* is the only member of the Joppeicidae family (Fig. 1). There have been records of *J. paradoxus* in the Middle East and northern Africa, Egypt, Sudan, Ethiopia and Israel. Recently, *J. paradoxus* was also discovered in a stored-product environment, in a bean storage area in Thailand, which was coincidentally, one of the research sites for our project.

Results

In order to utilize *J. paradoxus* as a natural enemy, feasible insect pests in food-storing environments must be examined. Feasibility was checked for nine species of insect pests of the orders Coleoptera and Lepidoptera (Table 1). According to the results, *J. paradoxus* is able to attack many species of stored-product insect pests and prefers insect pests in their young larval stages to ones in their later stages of life.

The predatory ability of *J. paradoxus* towards two stored-product insect pests, *Tribolium confusum* in its last-instar larval stage, and *Plodia interpunctella* in its second instar larval stage, were examined. *J. paradoxus* adults were left unfed for three days and then placed individually into plastic containers containing different prey densities. The assay was carried out at 30°C for 1 day. The predation index increased with the concentration and saturation of prey species (Tables 2 and 3). Especially toward the last instar larvae of *T. castaneum*, the mean predation activity index was six, which is three-fold higher than the mean predation index of *Xylocoris flavipes*, which is already on the U.S. market. These results suggest that *J. paradoxus* has a higher potential as natural enemy. A joint patent application between JIRCAS, the National Food Research Institute (NFRI), and Thai Department of Agriculture (DOA) for the elucidation of *J. paradoxus* as a bio-insecticide towards stored product insect pests and its application methodologies was submitted to the Japan Patent Office in January 2004.



Fig. 1. An adult female *Joppeicus paradoxus*, known by its common Thai name, “Puton.” Body length: 3 mm

Table 1. Prey range of *J. paradoxus* on stored-product insect pests.

Species	Developmental stages			
	Eggs	Young larvae	Older larvae	Adults
Coleoptera				
<i>Tribolium castaneum</i>	○	○	○	
<i>Tribolium confusum</i>	○	○	○	
<i>Tribolium freemani</i>	○	○	○	
<i>Oryzaephilus surinamensis</i>		○	○	
Lepidoptera				
<i>Ephestia elutella</i>		○		
<i>Ephestia kuehniella</i>		○		
<i>Ephestia cautella</i>		○		
<i>Corcyra cephalonica</i>		○		
<i>Plodia interpunctella</i>		○		

○ : feasibility as prey for *J. paradoxus*.

Table 2. Mean values of predation on last-instar larvae of *T. castaneum*.

Prey density	4	8	12	16	20	24	28
	Number of predations						
<i>J. paradoxus</i> ♀	2.7	4.5	5.6	4.3	4.1	4.8	6.2
<i>J. paradoxus</i> ♂	2.4	4.9	6.9	7.5	5.7	6.1	6.0

Table 3. Mean values of predation on second-instar larvae of *P. interpunctella*.

Prey density	4	8	12	16	20	24	28
	Number of predations						
<i>J. paradoxus</i> ♀	2.8	4.1	8.5	9.2	10.7	12.7	13.5
<i>J. paradoxus</i> ♂	3.7	4.8	6.0	7.8	10.3	10.1	13.5

References

- Brower, J.H., Smith, L., Vail, P.V. and Flinn, P.W. (1996): Biological control. Integrated Management of Insects in Stored Products, eds. B. Subramaniam and D. W. Hagstrum Merce Dekker Inc., New York, 223–283.
- China, W.E. (1955): A reconsideration of the systematic position of the family Joppeicidae Reuter (Hemiptera-Heteroptera), with notes on the phylogeny of the suborder. *Ann. Mag. Nat. Hist.* 12 (8), 353–370.
- Davis, N.T. and Usinger, R.L. (1970): The biology and relationships of the Joppeicidae (Heteroptera). *Ann. Entomol. Soc. Am.*, 63, 577–587.
- Nakakita, H. (1999): Role of biological control in grain storage in the tropics. The 5th JIRCAS International Symposium Series No.7, eds. Y. Nawa et al., Postharvest Technology in Asia. JIRCAS, Tsukuba, Japan, 73–86.
- Stys, P. (1971): Distribution and habitats of Joppeicidae (Heteroptera). *Acta faun. ent. Mus. Nat. Paragae*, 14, 199–208.
- Visarathanonth, P., Nakakita, H. and Sittisuang, P. (1994): Role of natural enemies in the regulation of stored-product insect populations in rice storages in Thailand. *JIRCAS journal*, 1, 1–7.

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The specific glutenin *Glu-1* allele frequencies of Japanese hexaploid wheat (*Triticum aestivum* L.)

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Key words: glutenin, *Glu-D1f*, allele, hexaploid wheat, electrophoresis

Objectives

The objective was to analyze this distribution in Japan that the most geographically remote region for common wheat production, concentrating mainly on HMW glutenin allelic variation within common wheat, and the factors which affect it in a worldwide context.

Results

According to a study of the worldwide distribution of *Glu-1* alleles in hexaploid wheats, the *Glu-D1f* is rare. However, the *Glu-D1f* allele was the most abundant hexaploid Japanese wheat seed storage protein allele (Table 1, Fig. 1). The present study revealed the specific differences in frequency of the *Glu-D1f* allele for Japanese hexaploid wheat varieties and landraces. The allelic frequency of this subunit was shown to have a 35% excess among improved Japanese cultivars and 25.3% among Japanese landraces, while it was found in only 1.8% of 274 Chinese wheats targeted in this study. It is well known that the Chinese wheat has contributed to the development of Japanese landraces, and Japanese landraces led to the emergence of modern varieties now found Japan. However, from this study, it has become apparent that Japanese and Chinese hexaploid wheats differ a great deal in the frequencies of the *Glu-D1f* allele (Table 1). As a consequence of its dissemination, adaptation and phenotypic refinement, Japanese hexaploid wheat has developed a unique composition of *Glu-1* glutenin alleles and a narrow hexaploid wheat genetic base (Table 2). Each of these bottlenecks is followed by a flush of rapid population growth, so once again there are optimal conditions to include genetic alterations. With this design from China, the hexaploid wheats were exposed to a selective bottleneck induced by the external environment, as well as a founder effect (since all populations went through small bottlenecks). Consequently, the selective bottleneck was extremely intense and, in fact, most ancestral populations may become extinct in Japan. It should also be pointed out that although the selective bottlenecks discussed in this study were primarily induced by genetic environment, most of the predictions made in this study would also apply if the selective bottlenecks were induced by external environments. The ease with which genetic changes occur in Japan strongly implies a lack of genetic variability in natural populations of Japanese hexaploid wheats. The theory presented here has increased the explanatory powers of the wheat genetic revolution model of speciation and, more importantly, has generated testable hypotheses that can be investigated in both natural and artificial selections using current methodologies and systems.

Table 1. Frequencies (%) of alleles for loci *Glu-A1*, *Glu-B1* and *Glu-D1* in Japanese and Chinese varieties.

Locus and Allele	Subunit	Chinese	Japanese improved	Japanese landraces	Published data distributed worldwide
<i>Glu-A1</i>					
<i>a</i>	1	5.2	12.2	4.6	32.8
<i>b</i>	2*	14.4	13.7	8.6	30.9
<i>c</i>	Null	80.4	74.1	86.8	36.3
<i>Glu-B1</i>					
<i>a</i>	7	12.3	0	1.7	12.9
<i>b</i>	7 + 8	71.9	83.2	94.1	25.2
<i>Glu-D1</i>					
<i>a</i>	2 + 12	84.6	55.0	70.1	52.9
<i>d</i>	5 + 10	10.5	1.5	3.4	40.8
<i>f</i>	2.2 + 12	1.8	35.1	25.3	rare



Fig. 1. SDS-gel electrophoresis of common wheat seed storage proteins.

Table 2. Identification of Japanese and Chinese varieties with respect to HMW glutenin allele composition.

<p>Chinese : <i>Large genetic base</i></p> <p>29 different glutenin HMW subunit compositions in <i>Glu-A1,-B1,D1</i></p> <p><i>a,a,a b,d,a c,b,f a,a,d b,f,a c,c,a a,b,a b,k,a c,c,b a,b,b c,a,a c,d,a a,b,d c,a,c c,e,a b,a,a c,a,d c,f,a b,a,d c,b,a c,g,a b,b,a c,b,b c,i,a b,b,f c,b,c c,i,f b,c,a c,b,d</i></p>
<p>↓</p>
<p>Japanese: <i>Narrow genetic base</i></p> <p>17 different glutenin HMW subunit compositions in <i>Glu-A1,-B1,D1</i></p> <p><i>a,b,a b,g,a a,b,b c,b,a a,b,c c,b,d a,b,f c,b,f a,c,c c,c,a a,d,c c,c,f a,i,a c,e,a b,b,a b,b,f b,c,d</i></p>

References

- Nakamura, H. and Fujimaki, H. (2002): The specific *Glu-D1f* allele frequency in Japanese hexaploid wheat. *Cereal Chemistry*, 79(4), 486–490.
- Nakamura, H. and Fujimaki, H. (2003): By reason of the founder effect or a selective bottleneck, the specific alleles frequency of Japanese hexaploid wheat (*Triticum aestivum* L.) compared to the worldwide distribution of *Glu-1* alleles. 10th International Wheat Genetics Symposium, 1, 459–462.
- Nakamura, H. (2004): The Specific Glutenin Allele frequencies of Japanese Common Wheat: An Overview. *Recent Research Development in Biochemistry*, 5, 81–87.

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Techniques for recovering cleared forests in Semangkok, Malaysia

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Key words: logged-over forest, recovery techniques, *Shorea cuitisii*, enrichment planting, natural regeneration

Objectives

Providing basic knowledge for promoting the recovery of logged-over forests by enrichment planting.

Results

The research site is located in Compartment 28 of the Semangkok Forest Reserve, 60 km north of Kuala Lumpur, Malaysia. The planting sites are selected along a logging road. The seedlings of six *Shorea* species were planted on a level ridge, a sloping ridge, and in the lower parts of slope in June 1997 (see Photo 1). Their scientific and common names are: *S. curtisii* or Seraya; *S. leprosula* or Meranti Tembaga; *S. macroptera* or Meranti Melantai; *S. parvifolia* or Meranti Sarang Punai; *S. ovalis* or Meranti Kepong; and *S. acuminata* or Meranti Rambai Daun.

The average height of seedlings after six years reveals that all species grow most quickly on the sloping ridge that are located slightly lower than the level ridge (Fig. 1). The results show that sites suitable for natural regeneration differ from sites that are suitable for enrichment planting. According to the results, natural regeneration is suitable on the upper slopes, whereas enrichment planting on the lower slopes.



Photo 1. Trees planted on sloping ridges.

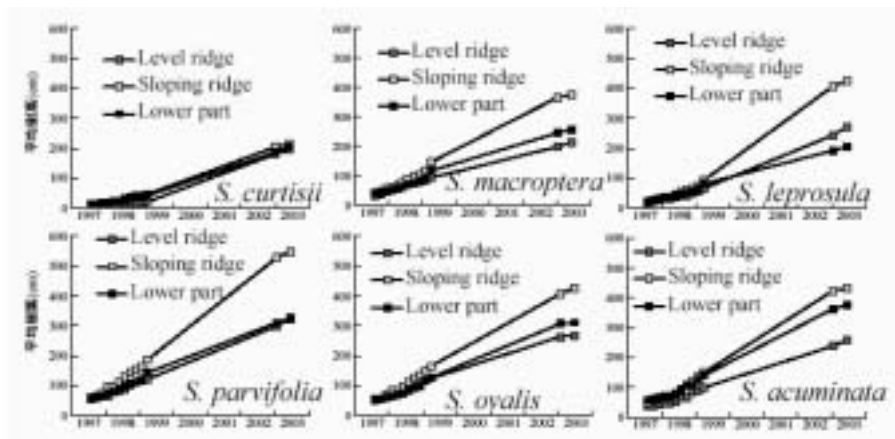


Fig. 1. Growth data for the six *Shorea* species planted in Semangkok, Malaysia.

References

- Ochiai, Y. and Azman Hassan (1997) : Regeneration of Seraya (*Shorea curtisii*) on the logging roads in a hill forest, Semangkok, Selangor, Peninsular. 4th Conference on Forestry and Forest Products Research, Malaysia, 32 (in Japanese).
- Ochiai, Y. (1999): Silvics of tropical trees (15) Meranti Sarang Punai. The tropical forestry, 44, 86–88 (in Japanese).
- Ochiai, Y. (2000): Natural regeneration in Peninsular Malaysia. The Tropical Forestry, 50, 33–36.
- Ochiai, Y. (2001): Physiological and ecological site preferences of some Dipterocarp species. TFRI extension series, 145, 210–214.

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Agroforestry experiment for rehabilitating tropical forests: Effects of forest canopies on initial seedling establishment and tending

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Key words: *Acacia mangium*, *Dipterocarpaceae*, nurse trees, seedling survival and growth, weeding

Objectives

We studied the initial survival and growth of various plant species including *Dipterocarpaceae* species, fruits trees species, medicinal plants etc. in *Acacia mangium* stand (1.6 ha) and secondary forest (2.9 ha) with thinning and non-thinning plots, and in cleared forest sites (2 ha) in eastern Sabah, Malaysia in 2002 and 2003 in order to clarify the suitable shading conditions on the initial establishment of various plant species in the process of establishing the mixed forests from *A. mangium* stand and secondary forest. The necessary degree of weeding, which is crucial for the productive growth of seedlings, was studied as well.

Results

The results obtained in the initial experiments are as follows:

1) A majority of the plant species had higher survival rates in thinning plots (RLI: 10 to 20%) and under canopies of exotic tree species than in cleared forest sites during the first 8 months after planting. In particular, *Dipterocarpaceae* species, except for *Parashorea tomentella*, showed much higher mortality rates in cleared forest plots (33 to 67%) than in thinning plots (0 to 16%) (Fig. 1).

2) Except for shade-intolerant tree species, including *Octomeles sumatrana* and some fruit tree species, most plant species including *Dipterocarpaceae* showed significantly higher growth rates in thinning plots than in cleared plots, and significantly higher growth rates in line thinning plots extending from north to south than line thinning plots extending from east to west (Fig. 2).

3) *Morinda citrifolia* (Fig. 3) came into bearing around eight months after planting in thinning plots as well as cleared plots. This medicinal plant species had a high growth rate in thinning plots as well as in cleared plots.

4) Based on the recorded amount of time spent on weeding, questionnaires on weeding conditions in various sites, and pulse rate measurements of people conducting the weeding, it was found that weeding time was around 40% shorter and weeding conditions became less harsh in thinning sites than in cleared sites.

These results suggest that moderate shade (RLI: 10–20%) cast by forest canopies may facilitate seedling establishment and reduce the amount of necessary weeding, and thus saving weeding costs in comparison with conventional planting in cleared forest sites. The results of initial monitoring also suggest that medicinal plant including *Morinda citrifolia* would be a useful companion species for sustainable medicinal fruit production in the establishment of sustainable mixed forests.

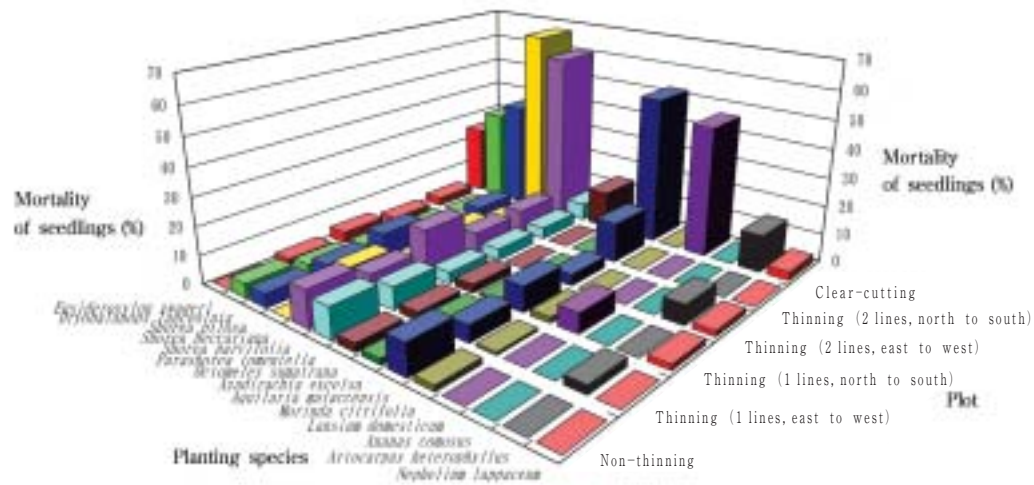


Fig. 1. Seedling mortality rates in thinning, non-thinning and cleared forest plots, eight months after planting.

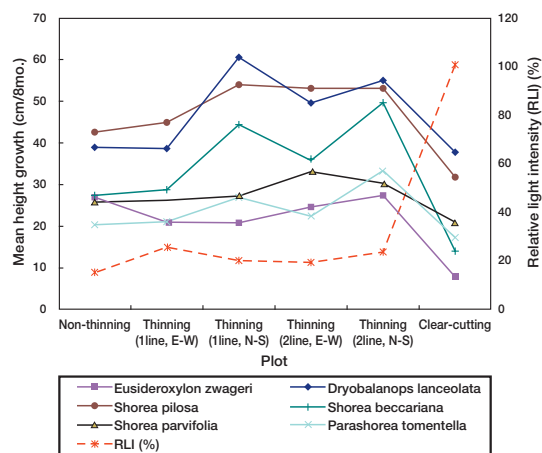


Fig. 2. Height growth of seedlings in thinning, non-thinning and cleared plots, eight months after planting.



Fig. 3. *Morinda citrifolia* fruits in a thinning plot.

References

- Kamo, K., Jamalung, L. and Lapongan, J. (2004): Agroforestry experiment for establishing mixed forests. Abstract of annual meeting of the Japanese forestry society, 115, 156.
- Kamo, K., Jamalung, L., Ota, T. and Lapongan, J. (2004): How important are nurse-trees for reforesting tropical land? Abstract of Workshop on "Development of Agroforestry Technology for the Rehabilitation of Tropical Forests", Sandakan, Sabah, Malaysia, 13–16.

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The importance of arachidonic acid in fry production of tropical/subtropical fish species

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Key words: arachidonic acid, EPA, DHA, tropical fish

Objectives

The mass production of marine fish fry used in aquaculture and aqua-ranching has progressed remarkably due to the discovery of the nutritional importance of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to marine fish, and the establishment of cultivation technologies of EPA/DHA-enriched feeds. As of now, most studies on essential fatty acids in relation to fry production have been focused on EPA and DHA. However, emphasis has not been previously placed on the importance of arachidonic acid (ArA), since ArA is found in only small quantities in cold/temperate water fish. In a preliminary study, we discovered that the eggs and larvae of the mangrove red snapper (*Lutjanus argentimaculatus*) contain relatively high ArA contents compared to fish living in cold/temperate waters. Moreover, information on the fatty acid composition of eggs and gonads of tropical and subtropical fish has been relatively limited until the present. We thus investigated ovarian fatty acid composition of aquacultural fish in the central Philippines and southern Japan, paying special attention to ArA and EPA content.

Results

Ovarian polar lipids were found to have intermediate or high ArA levels, but relatively low EPA levels; consequently, high ArA/EPA ratios (> 4.0) were observed in wild mangrove red snapper, two species of rabbitfish (*Siganus guttatus* and *S. canaliculatus*) and coral trout (*Plectropomus leopardus*), all of which were sampled in the central Philippines (Fig. 1). Similar trends were observed in wild coral trout, rabbitfish (*S. guttatus*), humpback snapper (*L. gibbus*), ornate jobfish (*Pristipomoides argyrogrammicus*) and brassy chub (*Kyphosus vaigiensis*) in southern Japan (Fig. 2). Not only ovaries, but also tendons and liver contained relatively higher ArA levels than EPA levels in all species in the present study, regardless of the sample source (data not shown). Fig. 3 shows ArA and EPA levels of polar lipids in wild-caught and hatchery-produced mangrove red snapper fry (whole). The hatchery-produced fry displayed lower ArA levels and higher EPA levels than did wild-caught fry. Overall, aquacultural species in central Philippines and southern Japan show high ArA and low EPA content, thus having high ArA/EPA ratios relative to cold/temperate water fish. The present results indicate that ArA is not a minor component of the eggs and larvae of tropical and subtropical fish, suggesting that ArA may have greater nutritional significance in relation to egg development and larval growth in tropical/subtropical fish than in cold-water fish. This information can be utilized in formulating guidelines for the future development of appropriate broodstock cultivation technology and larval diet development in tropical and subtropical areas. As

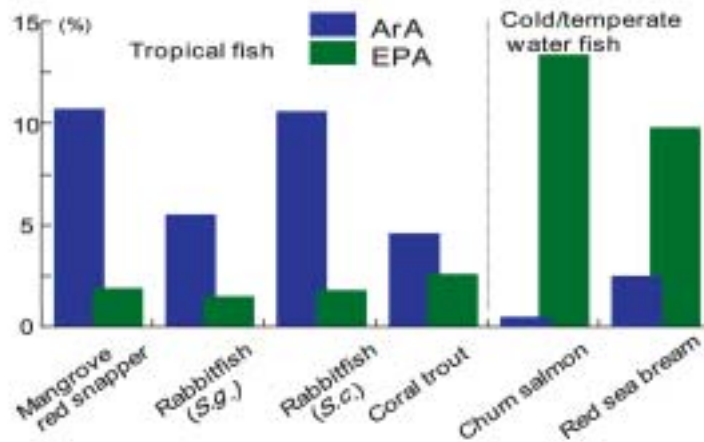


Fig. 1. ArA and EPA levels (%) of ovarian polar lipids in tropical fish in the Philippines (mangrove red snapper, rabbit fish and coral trout), cold-water fish (chum salmon) and temperate water fish (red sea bream).

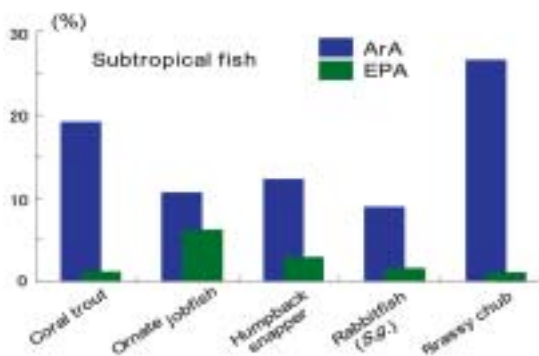


Fig. 2. ArA and EPA levels (%) of ovarian polar lipids in subtropical fish (southern Japan).

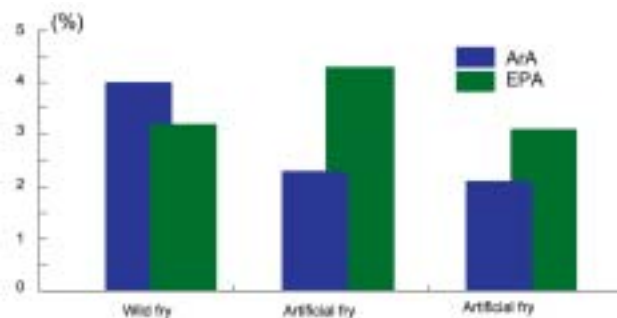


Fig. 3. ArA and EPA levels (%) of wild-caught and hatchery-produced mangrove red snapper fry.

of yet, the present findings on the potential value of ArA have not been applied to fry production technology in tropical/subtropical areas, but broodstock management and larviculture technologies based on the nutritional traits of tropical fish should be developed as rapidly as possible in order to stabilize fry supply and availability. Along these lines, we have initiated a follow-up project on the effects of dietary ArA on egg/larval quality in mangrove red snapper.

References

- Emata, A.C., Ogata, H.Y., Garibay, E.S. and Furuita, H. (2003): Advanced broodstock diets for the mangrove red snapper and a potential importance of arachidonic acid in eggs and fry. *Fish Physiol. Biochem.*, 28, 489–491.
- Ogata, H.Y., Emata, A.C., Garibay, E.S. and Furuita, H. (2004): Fatty acid composition of five candidate aquaculture species in Central Philippines. *Aquaculture*, 236, 361–375.

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Elucidation of natural purification capabilities in mangrove ecosystems

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Key words: shrimp, aquaculture, mangrove, phosphorus, budget

Objectives

In order to develop an environment-friendly aquaculture system utilizing the natural purification capabilities and high productivity of mangrove brackish waters, water was circulated between aquaculture ponds and mangrove enclosures. The continuous flow of water from aquaculture ponds maintained water and mud quality, while aquaculture ponds received plankton-rich water from the mangrove enclosures (Fig. 1).

Results

Survival rate was highest, and the feed conversion ratio (quantity of feed per unit weight increase) was lowest in Pond 5, in which larvae were stocked at low density, and the water was exchanged with mangrove water. Thus, Pond 5 achieved the most efficient level of aquacultural production.

In Pond 1, P content in the mud exceeded the amount provided in the feed (Table 1) and the P budget for Pond 1 was negative. This suggests that extra P was supplied from another source. We believe that anoxia was responsible for the release of P from the lower depths of the ponds. In Pond 2, the budget results suggest that 1.55 kg of P flowed into the mangrove-planted pond. Although the budget results were negative in Pond 5, it was higher than that of Pond 1. If the same quantity of P in Pond 1 had been supplied in Pond 5 from the lower depths of the pond, about 0.43 kg of P should have flowed into the mangrove ecosystem. As a result, deterioration in the lower pond environment was reduced in Ponds 2 and 5 via the circulation of water between the shrimp and mangrove ponds, in comparison to the control. Because the P budget was higher in Ponds 2 and 5 relative to the control, environmental burden in the ponds was reduced.

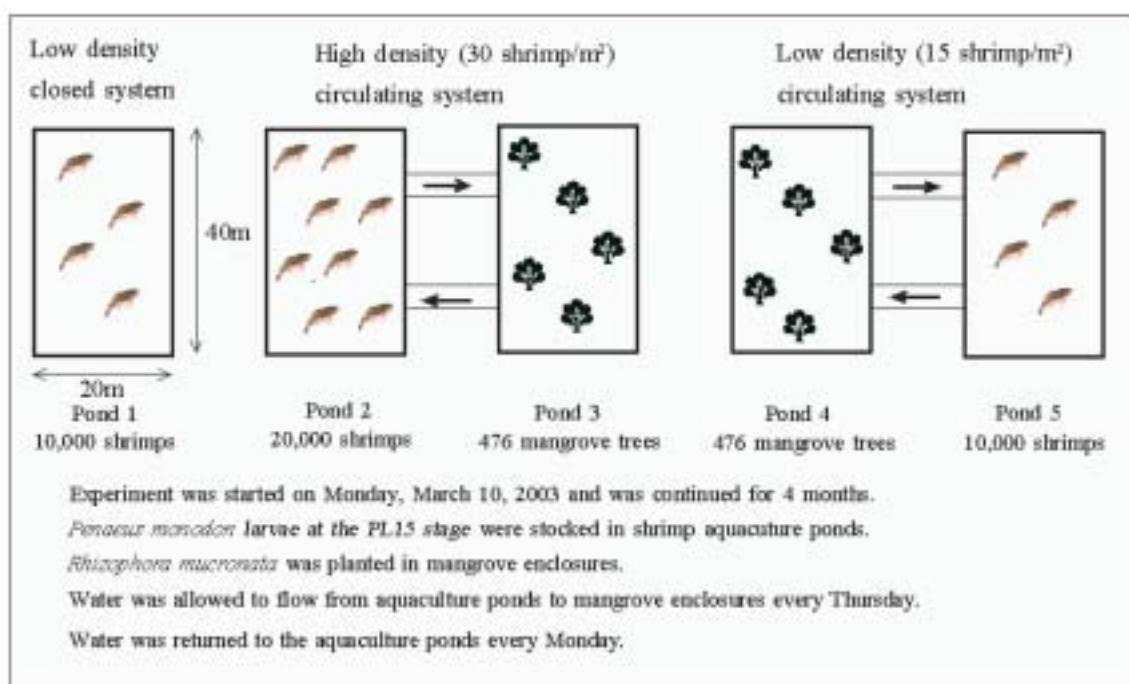


Fig. 1. Outline of the water exchange experiment.

Table 1. Phosphorus budget in each shrimp pond.

		Pond 1	Pond 2	Pond 5
Input	Phosphorus supplied from feed	2.09	4.36	2.19
	P content in stocked larvae	< 0.01	< 0.01	< 0.01
Stored	Phosphorus accumulated into shrimp at harvest	0.29	0.48	0.32
	Phosphorus content in snails	0.26	0.24	0.00
	Phosphorus content in barnacles	0.03	0.02	0.04
	Increase in phosphorus in water	0.08	0.20	0.07
	Increase in phosphorus in mud	2.97	1.86	2.86
Budget (input - stored)		-1.54	1.55	-1.11

References

- Shimoda, T., Srithong, C. and Aryuthaka, C. (2005): Attempt at purification of effluent and sediment in shrimp aquaculture ponds using mangrove Trees. JARQ, 39(2) (in press).
- Shimoda, T., Srithong, C. and Aryuthaka, C. (2004): Phosphorus budget in mangrove woods and ecosystems to which are added artificial nutrients and wastewater from the shrimp aquaculture ponds. JIRCAS working report, No. 35, 95–102.
- Shimoda, T., Srithong, C. and Aryuthaka, C. (2004): Attempt at purification of wastewater from shrimp aquaculture using mangroves. Abstract Volume of Spring Meeting of Oceanographic Society of Japan, 307 (in Japanese).

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Runoff of suspended solids, nitrogen and phosphorus estimated from catchment basins of Miyara River on Ishigaki Island

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Key words: red soil erosion, L-Q equation, SS, nitrogen, phosphorus

Objectives

It is commonly known that large amounts of eroded red soil, which flow into the seacoast via rivers from agricultural fields and other various bare lands adversely affect natural ecosystems such as coral reefs and fisheries in the subtropical islands of Japan. Marine pollution, which is caused by nitrogen and phosphorus emitted from chemical fertilizers and animal wastes, have recently become an increased threat to the environment, particularly in these coastal areas. There is little data available on the actual amounts of suspended solids, nitrogen and phosphorus that flow into the island ecosystems. Therefore, concentrations of these chemicals and the flow volume of the Miyara River in Ishigaki Island were monitored for 3.5 years from May 1999 to December 2002.

Results

Total nitrogen concentrations were low in the upper reaches of the river and rose gradually with the decrease in distance from the river mouth (Fig. 1), most likely because many forests are located upstream, and the main sources of nitrogen, such as agricultural fields, are located downstream. In addition, the data in Fig. 1 implies that nitrogen pollution was brought about by animal husbandry practices; the results show higher total nitrogen concentrations at the Shinko Bridge near the river tributary, where a large livestock breeding complex is located, in comparison to the lower concentrations recorded at the Nakamizu Bridge, located upstream near paddy fields.

According to the results, there are direct correlations between the flow rate of suspended solids and river flow rate (Fig. 2), and between nitrogen flow rate and river flow rate. Thus, we were able to calculate the flow rates of suspended solids and nitrogen based on river flow rates and regression equations. Two regression equations were derived from data for the flow rates of suspended solids, with an upper threshold value of 5.5 mm/day rainfall.

The quantity of suspended solids, nitrogen and phosphorus, which flow through the Miyara River, into the seacoast per year were estimated to be 1882, 68 and 7 tons, respectively. Based on this data, 0.2 mm of surface soil are calculated to erode from agricultural fields in a period of one year, and 25 and 6% of the total amounts of nitrogen and phosphorus used in agriculture and discharged from livestock excretions will flow into Miyara River in one year.

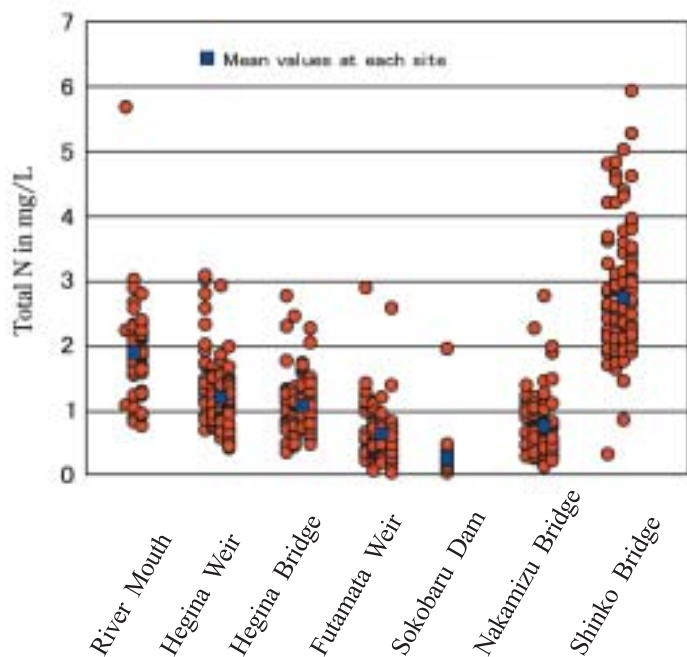


Fig. 1. Changes in total N concentrations from the river mouth to the upper reaches of the Miyara River.

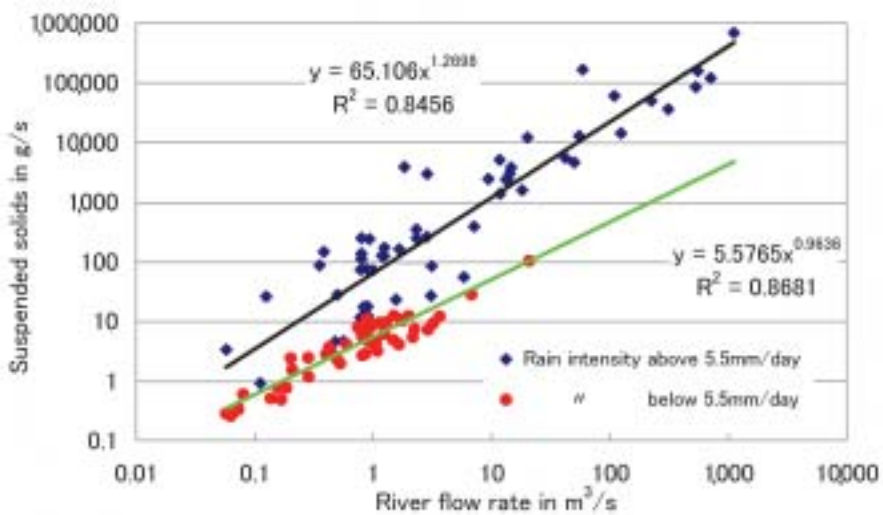


Fig. 2. Relation between river flow rates and quantity of suspended solids.

References

Banzai, K. and Nakamura, K. (2003): Soil particles runoff from cultivated land water quality loading in river basin. *Applies Hydrology (JSIDRE)*, 16, 38–45 (in Japanese).

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Improved sugarcane cultivation in the subtropical islands of Japan using controlled-release N-fertilizers

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Key words: nitrate-nitrogen pollution, sugarcane, LPS160, labeled ¹⁵N

Objectives

Nitrate pollution in ground water has become a serious problem worldwide; one of its causes is known to be the excessive use of nitrogen fertilizers in agricultural production (Fig. 1). Using water containing nitrate-nitrogen concentrations above the standards of the World Health Organization's (WHO) (10 mg/L) leads to agricultural products becoming unsuitable for human consumption, particularly for infants ("blue baby disease"). Nitrate pollution has become much more serious in the past few years at Miyako Island, Okinawa Prefecture, where people are entirely dependent upon subsurface dam water, which is currently polluted with nitrate.

To solve the nitrate-nitrogen pollution of subsurface dam water in Okinawa Prefecture, we applied controlled-release N-fertilizer (LPS160) to sugarcane.

Results

The trials showed that about 40% of nitrogen fertilizer usage could be cut down without causing reduction in expected sugar yield (Table 1). The weight of sugarcane stalks, Pol percent cane (sucrose content of juice) and expected sugar yield did not decrease in the LPS160 treatment (40% reduced nitrogen) compared to those of plants that underwent conventional fertilizer treatments.

The apparent nitrogen absorption rates were estimated as 57.7% in conventional and 90.9% in LPS160 treatments (Fig. 2), and labeled ¹⁵N absorption rates were calculated as 22.4 and 38.8%, respectively. Judging from these results, it seems possible to reduce nitrate-nitrogen leaching to ground water by application of LPS160.

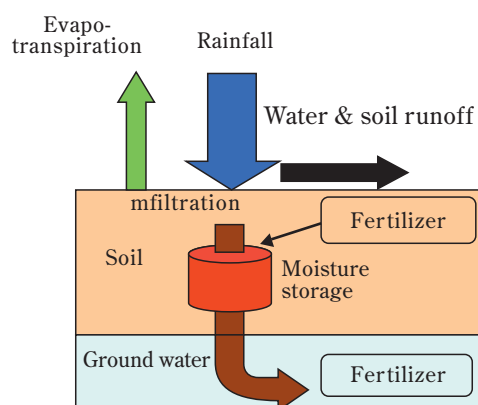


Fig. 1. Water movement in the field.

Table 1. Comparison of sugarcane yields between plants grown with conventional and LPS160 (40%-reduced nitrogen) treatments in spring planting.

Treatment	Nitrogen dose (kgN/ha)	Weight of talks (ton/ha)	Pol percent cane (%)	Expected sugar yield (ton/ha)
Conventional	Basal dressing (60) + Top dressing (60) + Top dressing (80)	94.3	15.05	13.2
LPS160 (40% reduced nitrogen)	Basal dressing (60) + LPS(60)	89.5	14.96	12.5

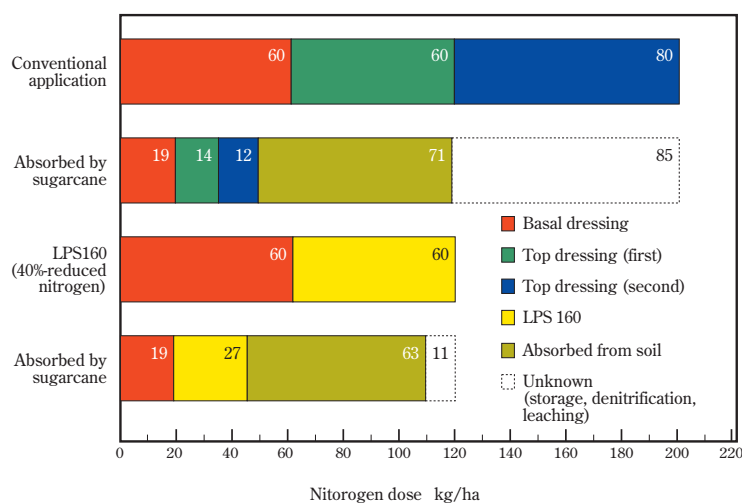


Fig. 2. Sources of nitrogen absorbed by sugarcane.

References

Masuda, T., Katsuta, Y., Hiramatsu, K. and Sugahara, K. (2002): Application of slow release fertilizer to sugarcane in spring planting. Abstracts of the Annual Meeting, Japanese Society of Soil Science and Plant Nutrition, 48,123 (in Japanese).

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Development of powdery mildew-resistant adzuki bean using wild adzuki bean germplasm

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Key words: powdery mildew, adzuki bean, *Vigna angularis*, wild adzuki bean germplasm

Objectives

Powdery mildew, or *Podosphaera phaseoli*, is a serious fungal disease threatening the cultivation of adzuki bean in tropical and subtropical regions. At present, resistant genotypes have not yet been detected in cultivated adzuki bean germplasm. In a greenhouse screening trial at the Okinawa Subtropical Station, we inoculated wild adzuki bean germplasm accessions (mainly collected by JIRCAS) with powdery mildew during their seedling stages by dusting them with spores of infected leaves using a soft brush.

Results

Cultivated and wild ancestral forms of adzuki bean (*Vigna angularis* var. *angularis* and var. *nipponensis*, respectively), *V. nakashimae* and *V. riukiensis* were highly susceptible to the disease, whereas *V. hirtella* exhibited resistance (hypersensitivity). *V. hirtella* was discovered to have high cross-compatibility with adzuki bean, and we have successfully obtained fertile F₁ hybrids from them. These F₁ hybrids exhibited resistance like that of *V. hirtella* when artificially inoculated (Fig. 1). Types of reactions to powdery mildew pathogens on primary leaves of 8 day-old BC₁ plants obtained from adzuki bean and *V. hirtella* were segregated into 24 resistant types (R) and 21 susceptible types (S), with the χ^2 value for 1:1 segregation being 0.2 (0.7 > p > 0.5) (Fig. 2).

Judging from this ratio and the resistance found in the F₁ plants, it was concluded that a single dominant gene is responsible for the resistance exhibited by *V. hirtella*. Since the resistance was verified by artificially inoculating all progeny plants, it is anticipated that resistant adzuki bean varieties will be easily obtained through successive backcrossing to commercial cultivars. Currently, we are recurrently backcrossing to cv. 'Erimo shozu' to develop new powdery mildew-resistant adzuki bean varieties.



Fig. 1. Types of reactions to the powdery mildew pathogen, *Podosphaera phaseoli* on F₁ plants derived from adzuki bean and *V. hirtella* (left: resistant) and adzuki bean cv. 'Erimo shozu' (right: susceptible).

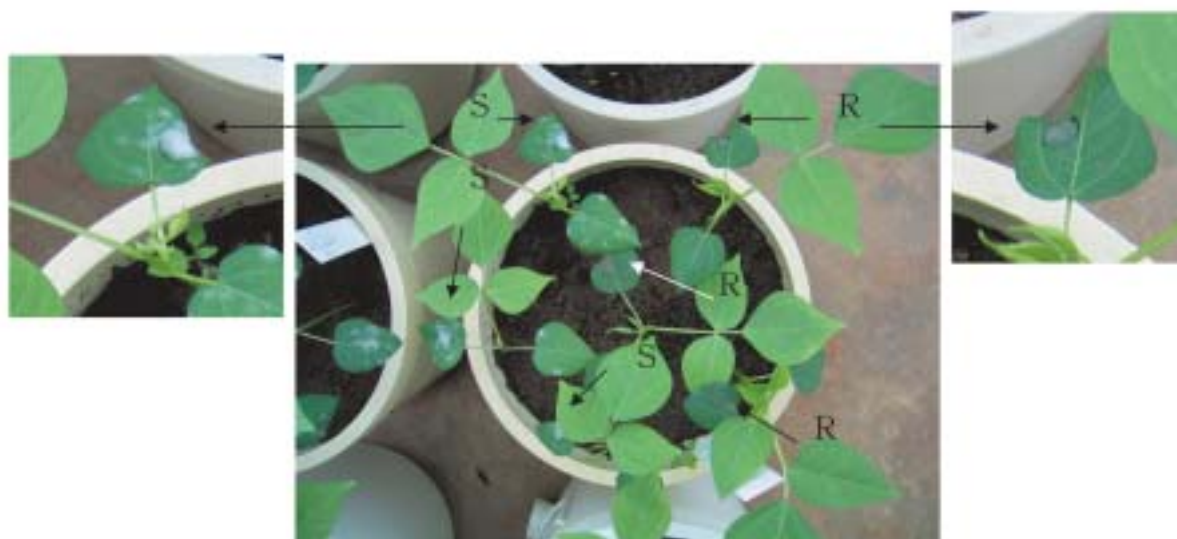


Fig. 2. Types of reactions to the powdery mildew pathogen, *Podosphaera phaseoli* on primary leaves of 8-day-old BC₁ plants obtained from adzuki bean and *V. hirtella* 5 days after inoculation. Resistant leaves are indicated by the letter "R" while susceptible leaves are marked with the letter "S".

References

Egawa, Y., K. Kashiwaba, H. Ohmae and M. Shono (2004): Resistance to powdery mildew pathogens of wild adzuki bean germplasm. *Jpn. J. Trop. Agric.*, 48, Suppl. 1, 63–64.

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Effects on the growth of the cotton stainer bug *Dysdercus cingulatus* when fed various host plant seeds including those of wild species

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Key words: *Dysdercus cingulatus*, cotton plant, okra plant, alternative host plant, development

Objectives

Cotton is the world's most widely-used natural fiber. One of the major obstacles hindering cotton production is insect pest infestation; cotton stainers (*Dysdercus* spp.; Heteroptera: Pyrrhocoridae), in particular, are difficult to control by insecticide application in cotton fields because they are very mobile and have many alternative host plants. Among them, *Dysdercus cingulatus* (Fig. 1) is the most serious pest species of cotton in southeast Asian countries, having many alternative host plants species, including malvaceous and bombacaceous plant species. Therefore, investigation of the developmental properties of bugs fed with host plants other than cotton is crucial for the prediction of the time of invasion into cotton fields and for effective pest control utilizing insecticide application. In addition, the growth of *D. cingulatus* including its temperature dependency was examined when fed with seeds of cotton, okra, and several other wild and ornamental plant species which are common host plants of *D. cingulatus* in southeast Asia and on Ishigaki island.

Results

Seeds of commercially-cultivated species (cotton plant *Gossypium arboreum* and okra plant *Abelmoschus esculentus*), wildy-grown species (musk-mallow *A. moschatus*, portia tree *Thespesia populnea*, Sakishima cotton-rose *Hibiscus makinoi*, sea hibiscus *H. tiliaceus* and Indian mallow *Abutilon indicum*), and ornamental species (wood cotton *Chorisia speciosa*) were provided as experimental feed to examine the growth properties of *D. cingulatus*. The survival rates of bugs in their nymphal stages and developmental rates when fed respective plant species displayed significant correlations ($p < 0.05$ by Kendall's τ); species that exhibited faster nymphal development also exhibited higher nymphal survival rates and vice versa. Thermal requirements for the development of *D. cingulatus* were estimated from data for 20, 22.5, 25, 27.5 and 30°C and 14L–10D when fed with seeds of eight different plant species are shown in Table 1. These results suggest that ornamental species (*Ch. speciosa*) and a few wild species, *Th. populnea* and *H. makinoi* in particular, are good host plant species for *D. cingulatus* as well as commercially cultivated species. Since all examined wild plant species excluding *H. makinoi* are commonly distributed in southeast Asian countries, of which *Th. Populnea* is judged to be an especially suitable host plant species for *D. cingulatus*, the results of this study and information on the occurrence of *D. cingulatus* on these plant species will form a solid foundation for the determination of insecticide application in cotton fields of southeast Asian countries.



Fig. 1. Cotton stainer bugs (*Dysdercus cingulatus*) sucking ripe cotton bolls.

Table 1. Thermal requirements for the development of *Dysdercus cingulatus*, estimated from data for 20, 22.5, 25, 27.5 and 30°C and 14L-10D when fed seeds of eight plant species.

Stage	Host plant	Regression equation	r^2	Lower threshold temperature (°C)	Total effective temperature (day·°C)
Egg		$v = -0.21469 + 0.013629 t$	0.979	15.8	73.4
Nymph	Wood cotton (<i>Chorisia speciosa</i>)	$v = -0.04704 + 0.003260 t$	0.976	14.4	306.7
	Cotton (<i>Gossypium arboreum</i>)	$v = -0.03950 + 0.002850 t$	0.991	13.9	350.8
	Okra (<i>Abelmoschus esculentus</i>)	$v = -0.03730 + 0.002661 t$	0.957	14.0	375.8
	Musk-mallow (<i>A. moschatus</i>)	$v = -0.03632 + 0.002563 t$	0.964	14.2	390.2
	Portia tree (<i>Thespesia populnea</i>)	$v = -0.03628 + 0.002593 t$	0.984	14.0	385.6
	Sakishima cotton-rose (<i>Hibiscus makinoi</i>)	$v = -0.03507 + 0.002501 t$	0.959	14.0	399.8
	Sea hibiscus (<i>H. tiliaceus</i>)	$v = -0.03037 + 0.002174 t$	0.979	14.0	459.9
	Indian mallow (<i>Abutilon indicum</i>)*	$v = -0.02076 + 0.001556 t$	0.997	13.3	642.8

*based on data measured under conditions of 22.5, 25, 27.5 and 30°C.

References

Kohno, K. and Bui Thi N. (2004): Effects of host plant species on the development of *Dysdercus cingulatus* (Heteroptera: Pyrrhocoridae). *Applied Entomology and Zoology*, 39, 183–187.

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Quantification of citrus-greening disease pathogens using competitive PCR

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Key words: citrus-greening disease, Huanglongbing, quantification, competitive PCR

Objectives

Huanglongbing (HLB, also known as citrus greening) is one of the most severe diseases affecting citrus production in tropical and sub-tropical regions of the world, and has recently spread to citrus orchards in southern areas of Japan. Diagnosis of this disease is usually conducted through symptom observation and PCR detection, but these methods do not allow the quantification of pathogen numbers.

The quantification of pathogens is crucial for analyzing the process of disease development. Based on recent research results, we have developed a quantification system for Huanglongbing pathogens, utilizing a competitive PCR method. This system consists of conventional PCR equipment and competitor DNA, and is less expensive than equipment used in real-time PCR systems, which currently is the most popular method for quantification of PCR-detectable materials. Thus, this system will be more easily incorporated into agricultural research activities in developing countries.

Results

The conventional PCR method multiplies specific target DNA through chain reactions. The concept of the competitive PCR method is to create competition between target DNA multiplication and competitor DNA multiplication. The ratio of multiplied DNA from target DNA and competitor DNA is expected to correlate with the original ratio of each type of DNA before multiplication. We designed competitor DNA that has PCR-primer recognition sequences identical to target DNA. Using this competitive DNA, the competitive PCR reaction showed reasonable results (Fig. 1). After plotting data on a logarithmic graph, the multiplication ratios of each DNA formed nearly perfect straight lines (Fig. 2). Based on these results, the quantity of target DNA, pathogen-originated DNA, as well as the estimated quantity of disease pathogens can be calculated.

There are several substances known to interfere with PCR DNA multiplication. Quantification systems using competitive PCR are thought to be less affected by such inhibitors. Starch is known to have accumulated in Huanglongbing infected leaves, and have inhibitory effects against PCR multiplication. We tested the quantification system with soluble starch in order to examine the reliability of measured values in the presence of such inhibitory substances. Favorable results were obtained from our quantification system as expected based on the concepts of the competitive PCR method.

Infected citrus leaves collected in southern Vietnam were applied in this quantification system, and significant differences in pathogen quantity were observed in leaves between two selected areas (Table 1). Fewer amounts of pathogen-related DNA were detected from the tissue of citrus cultivars that were believed to have



Fig. 1. An example of competitive PCR. Lane 1: DNA size markers (λ - Eco T141 digest). Lane 2: Multiplication of competitor DNA. Lane 3-7: 1pM target DNA and competitor DNA, concentrations of each lane are 100pM, 10pM, 1pM, 100nM, and 10nM, respectively. Lane 8: Multiplication of target DNA.

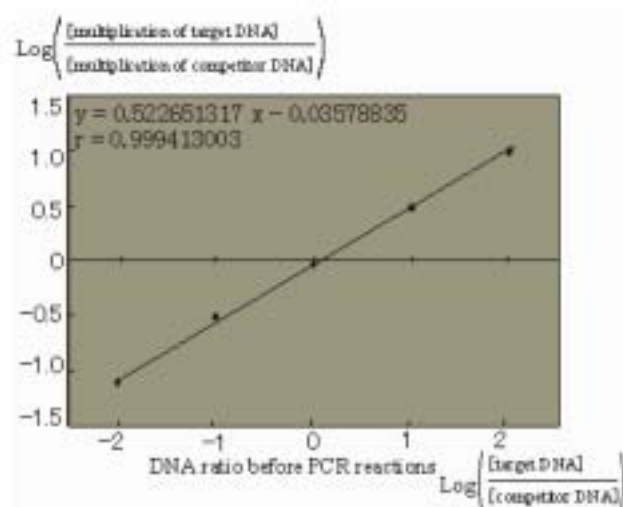


Fig. 2. Competitive reactions between target DNA and competitor DNA.

Table 1. Quantity of pathogens in infected citrus leaves.

cultivar name (in Vietnam)		quantity (fm ol/g · fw) ^{*2}			
		Max.	Min.	Avg.	S.D.
Cam Sanh	47	92.9	0.0	35.3	23.5
Nam Roi ^{*1}	33	22.0	1.1	5.6	4.1

^{*1}: "Nam Roi" is thought to be tolerant to citrus-greening disease.

^{*2}: Midlibs of infected citrus leaves were collected. DNA was extracted using CTAB method from the base parts of each midlib of fixed length, 1cm.

higher tolerance against Huanglongbing than that of susceptible cultivars. This suggests that the suppression of pathogen multiplication in tolerant citrus cultivars may enhance its tolerance.

We plan to utilize this quantification system in analyzing Huanglongbing disease prevalence, both in Japan and Vietnam, in a new international project to be initiated in April 2004 entitled, "Development of new technologies for control of Citrus Huanglongbing (HLB) in Southeast Asia."

References

Kawabe, K., Truc, N. T. N., Nhan, N. T. and Onuki, M. (2004): Pathogen quantification of citrus tissue naturally infected by Huanglongbing in southern Vietnam. *Jpn. J. Phytopathol.*, 70(3), 294.

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