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Feature Article

Collaborative Research on Issues Relating to Environmental Resources

In developing regions, increasing the supply of agricultural products and stabilizing agricultural productivity are matters of great urgency due to the rapid increase in human population and the improvement in the standard of living. In these regions, the greatest challenge for agriculture today is to sustain agricultural production under adverse soil and climatic conditions, because most of these regions are located in unfavorable agricultural environments such as low soil fertility and/or arid and semi-arid climate, and many farmers can not afford to use enough fertilizers and agricultural chemicals due to economic constraints. Therefore, it is necessary to develop agricultural technologies, which may enable to use more efficiently the local organic resources or to maximize useful functions of plants and microorganisms, based on the characteristics of soils, climate, and socioeconomic conditions.

Recently many attempts have been made to increase food production in these regions. However, these efforts have led to a deterioration of agricultural environments and global environmental problems such as pollution of rivers, lakes and inshore waters, soil degradation, and deforestation. These environmental problems have been caused by excessive application of fertilizers and agricultural chemicals, large-scale feeding of livestock, overgrazing, inappropriate irrigation, overcropping, and expansion of agricultural lands. Accordingly, sustainable agricultural technologies compatible with environmental preservation should be developed based on the analysis of the mechanisms of material cycling in the agroecosystems.

On the other hand, agriculture is practiced under various natural and social environments in these regions. Therefore, it is necessary to analyze the actual conditions and characteristics of the environmental resources to develop agriculture compatible with environmental conservation. However, geographic information on land and environmental resources is lacking in many of these regions. Geographic information

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systems (GIS), are now expected to become an effective tool for developing methods for the analysis and evaluation of the spatial and temporal changes of environmental resources and for producing various kinds of evaluation maps on environment by overlaying multiple geographic data including remote sensing data.



To address these problems, the En-

vironmental Resources Division (ERD) is conducting research to develop technologies for the promotion of sustainable agriculture and environmental conservation. In the comprehensive research projects on sustainable agricultural technologies in Northeast Thailand and on agro-pastoral rotation systems in Brazil, EDR researchers are carrying out studies on the evaluation of sustainability of the agricultural systems from the viewpoint of material cycling and utilization of local organic resources and nitrogen-fixing plants. At the same time, in the comprehensive research projects on sustainable production and processing of major food resources in China and on the evaluation and improvement of farming systems in the Mekong Delta of Vietnam, other EDR researchers are performing studies on the evaluation and development of methods for the improvement of agricultural activities compatible with the preservation of the environment and the ecosystems.

The Division is also promoting research on GIS. Within the framework of the Northeast Thailand project and the comprehensive research project on the evaluation and improvement of regional farming systems in Indonesia, ERD researchers are analyzing the mechanisms of land use changes and physical factors for regional farming systems. Additionally, in the China project, GIS researchers are studying the spatial and temporal characteristics of environmental conditions of agricultural lands.



Sugarcane field in Northeast Thailand: sugarcane residues are used to improve soil fertility



Experimental field for rational fertilizer application to prevent excess application in China

Furthermore, one researcher is developing monitoring systems of evapo-transpiration, depth of snow and soil moisture under the harsh climatic conditions in the Mongolian plateau.

The Division is also conducting two special projects in collaboration with the international agricultural centers. With the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India, the Division has been involved in studies on the physiological and genetic adaptation of sorghum and pigeonpea to low nutrient availability in the semiarid tropical environment. Researchers dispatched by the Division to the International Rice Research Institute (IRRI) in the Philippines have been conducting studies on the stabilization of rice culture under water stress in the tropics utilizing a broader spectrum of genetic resources. These two projects will end in 1999, and the Division is planning to dispatch a researcher to initiate a new research project entitled "Physio-genetic studies on yield determination and ecological adaptability for sustainable rice culture" at IRRI scheduled to begin by the end of this fiscal year.

On the other hand, at WARDA (West Africa Rice Development Association), one researcher is carrying out studies on the genetic and eco-physiological characterization of indigenous rice varieties and interspecific progenies, with emphasis placed on tolerance to drought and acid soil conditions in the West African region.

In addition, the EDR is actively engaged in research activities within the framework of the JIRCAS Fellowship Program in the field of material cycling, GIS, photoelectron spectroscopy, etc. and frequently invites to Tsukuba counterpart researchers from the organizations with which JIRCAS carries out collaborative studies.

Research Topic Biological Control of Fish Diseases in Aquaculture

The health of fish in aquaculture, as well as in nature, depends primarily upon their inherent resistance to microbial invasion, and the biological equilibrium between competing beneficial and detrimental microorganisms at the fish/water environment interface. In fact, useful and harmful microbes in the water environment directly affect the growth of fish (Maeda, 1999).

More than one million of microorganisms per milliliter inhabit the aquatic environment and affect each other, both through the substances they produce and emit, as well as through the various ways they come into contact with one another. Fertilization of aquaculture seawater, through the addition of low concentrations of organic materials, leads to an increase in the number of microorganisms which utilize the organic materials as nutrients to construct microbial assemblages which enhance the survival rates of fish larvae (Maeda, 1999). In aquaculture, where phytoalgae are used as the main live food, the survival rates of prawns, crabs and finfish are not considered to be sufficiently high. However, if certain species of bacteria are present with the algae, the survival rate increases significantly. It is therefore preferable to feed microbes to fish along with algae (Maeda, 1999), although the control of these microbial assemblages is essential to prevent the pathogens from dominating the microbial communities. Thus microorganisms play several useful roles which help to maintain suitable environmental conditions in aquaculture and promote fish growth, as well as functioning as live food.

The methods of determining how to keep pathogenic microorganisms, mainly bacteria and viruses, away from fish rearing environments are one of the main concerns of the people engaged in aquaculture. To this end, the filtration of water, the addition of sodium chloride, ozonation, the use of ultraviolet light, and even the use of artificial compound food containing antibiotics for sterilization, are all commonly adopted techniques in aquaculture. People in aquaculture tend to consider that these procedures can eventually eliminate all

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the microbes in seawater, and produce and maintain a nearly pure water quality. However, with these treatments, pathogenic microorganisms which cause fish diseases cannot be permanently removed from the water. For example, if the antibiotic kanamycin is added to seawater, the bacterial numbers decrease for about two days, but eventually the numbers will recover to their original level. The same phenomena can be seen when seawater is sterilized by filtration, ozonation or ultraviolet light treatment. After such treatments, bacteria grow very quickly because antagonism among the bacterial populations is reduced. Furthermore, no one can anticipate what kind of bacterial species may grow in the vacant space generated by the above treatments. Penaeus prawn aquaculture in Asia and South America in part has not yet recovered from its collapse in the 1980s. In addition, many fish diseases are spreading through aquaculture facilities worldwide.

Although people who are engaged in aquaculture have started to realize that antibiotics are less effective, almost no alternative method of controlling diseases has been developed. It is therefore essential that new methods should be adopted



The bacterial strain cultured in the ZoBell 2216E medium in seawater at 25°C for 4 days was added into seawater where the larvae of striped jack were reared

wherein the antagonism of certain microorganisms is used to repress other pathogenic microbes, bacteria and viruses, in seawater.

The antagonism among microbes is a naturally occurring phenomenon through which pathogens can be killed or reduced in number in the aquaculture environment. This method, which is called biological control or biocontrol, has already been recognized in the field of agriculture. For example, the famous bacterium, *Bacillus thuringiensis*, which infects pathogenic insects orally and eventually kills them is now commercially available in Europe and North America where several thousands of tons are used. These positive results have led to further studies using viruses, fungi and protozoa as biocontrol agents to eliminate pathogenic organisms. This approach in aquaculture is described in detail herein.

Several viral diseases have seriously affected the fish rearing industry. Penaeus prawn, P. monodon and P. japonicus, have almost all been infected with Baculo-like viruses. In Taiwan, the production of *P. monodon* decreased from about 90,000 metric tons in 1987, to 30,000 in 1988, to 20,000 in 1989, and has still not recovered. In Japan since 1993, the prawn rearing industry of P. japonicus has been seriously damaged by a virus infection, and many prawn nursery ponds in the western part of Japan stopped production because all the prawns died. There are also other viruses: Infectious Hematopoietic Necrosis Virus (IHNV) and Infectious Pancreatic Necrosis Virus (IPNV) which infect salmon, Hirame Rhabdovirus (HIRRV) which infects a flounder, the Yellowtail Ascites Virus (YAV) which infects yellowtail, Striped Jack (Sima-Aji) Nervous Necrosis Virus (SJNNV) which mainly infects striped jack, the Irido Virus which infects sea-bream, and many more, all of which cause serious damage in aquaculture.

Using the direct counting method combined with electron microscopy, virus counts were reported to range from 10⁴ - 10^8 virus particles /ml, i.e. numbers which were $10^3 - 10^7$ times higher than previous reports of virus particle numbers in natural aquatic environments, that in turn were based on counts of plaque-forming units using various host bacteria. Thus viral concentrations changed from 10⁴ to 10⁸ virus particles/ ml, which indicates the possible effect of anti-viral microorganisms on the presence of virus particles in seawater. This phenomenon helps to explain why the concentration of viruses fluctuates so markedly in sea and fresh-water. In addition, viruses have the ability to be transferred from one infected organism to another through the water environment. If anti-virus bacteria dominate the water environment, virus transfer among fish communities may be repressed to a large extent. Based on this concept, anti-virus bacteria are used in rearing procedures of larvae in practical aquaculture applications.

According to one hypothesis, bacteria which are able to inhibit the growth of other bacteria, may also inhibit the growth of viruses. From this point of view, for the detection of antivirus bacteria in seawater, the first step should be to determine the anti-bacterial activity. This procedure is easier than determining the anti-viral activity directly. The bacterial strain VKM-124, *Pseudoalteromonas undina*, which has a vibriostatic activity and is used in practical aquaculture, inhibited the appearance of the cytopathic effect (CPE) on the epithelioma papillosum cyprini (EPC) cells in which about a 2-day delay was observed in the *P. undina* experimental fraction, compared to the control fraction. Thus this strain, which shows a strong antibacterial activity, can inhibit the growth of the virus.

The bacterial strain VKM-124, *Pseudoalteromonas undina*, used in aquaculture processes, is able to prevent fish larvae from being infected with SJNNV, Baculo-like viruses and Irido virus. When this useful strain was added to water at a concentration of about 10⁶ cells/ml in rearing containers of *Penaeus* prawn, Sima-aji and Sea-bream larvae, the survival rates of these larvae were much higher in the presence of the bacterium than without the bacterium. All the fish larvae died due to viral disease without the addition of the bacterium (Fig. 1) (Maeda, 1999).

Viruses in seawater spread from infected fish to others which are not yet suffering from disease. However, probiotic bacteria may inhibit the migration of viruses among fishes. In addition, if fish feed on such probiotic bacteria, the immune system of the fish may become stronger, as shown in the enhancement of immune activities of vertebrates with bacteria in many reports. With such useful effects and features, probiotic bacteria could certainly be extremely effective in protecting fish from the spread of viral diseases in aquaculture.

Note: Detailed information and references are available in the book of Maeda (1999); Microbial Processes in Aquaculture. Natl. Res. Inst. Aquaculture, 102 pp. The scientists who would like to read this book can obtain it by writing to JIRCAS.



Fig. 1. Survival of Sima-aji (Striped jack, *Caranx delicatissimus*) larvae with and without the bacterium VKM-124, *Pseudoalteromonas undina*. Larvae died due to infection with the virus, SJNNV, in the container without the addition of the bacterial strain.

> Numbers of larvae used: 254, 000 Ind. Water volume: 20 metric tonnages Bacterial numbers added every day for 17 days: about 1 million cells/ml

JIRCAS Research Highlight

Soybean Virus Diseases in Northeast China

Northeast China is the major soybean production area in China. More than 40% of soybean grains in China are produced in three provinces in the Northeast, i.e., Heilongjiang, Jilin, and Liaoning. JIRCAS has initiated a program of collaborative research on soybean breeding with the Soybean Institute of Jilin Academy of Agricultural Sciences. At first, we focused on soybean virus diseases that cause severe damage to soybean production.

In a soybean field, sometimes plants display symptoms such as curling of leaves, deep leaf color and pale green or yellow mosaic, brownish necrosis along the leaf veins, or dwarfing. These are the typical symptoms of soybean virus diseases and sometimes they lead to a severe yield reduction. Many viruses infect soybean in China. Since each virus shows a different mode of transmission, the kinds of viruses should be identified and characterized in Jilin province for control, including breeding of resistant varieties.

We investigated soybean virus diseases all over the Jilin province by applying the Dot-ELISA method using virus-specific antisera and by electron microscopy. We could detect the soybean mosaic virus (SMV), cucumber mosaic virus-soybean stunt strain (CMV-SS), alfalfa mosaic virus (AMV), and bean common mosaic virus (BCMV) during the period 1996-1998 (Table 1).

BCMV causes severe vein necrosis on some cultivars (Fig. 1). This virus infects other legume crops such as bean,

Table 1. Detection of soybean viruses by Dot-ELISA in Jilin province (1996-1998)

	*				
Region	No. of tested materials	SMV	BCMV	CMV-SS	AMV
Baicheng	35	3	2	0	0
Qianguo	22	0	2	0	0
Siping	279	128	10	9	6
Changchun	21	5	0	0	0
Jilin	52	22	0	2	2
Yanbian	174	5	0	1	5
Tonghua	10	1	0	4	0
Baishan	11	0	0	2	0
Total	604	164	14	18	13

Total



1. BCMV

Figs 1, 2, 3. Symptoms of soybean virus diseases



2. AMV

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mungbean, and adzuki bean and some of the plants may become a source of inoculum. Although severe yield reduction in individual soybean plants can be observed, the incidence is very low.

AMV causes distinct yellow mosaic symptoms on leaves (Fig. 2). Since alfalfa and some legume plant for animal feeds are a source of inoculum, AMV could be detected on soybean plants cultivated next to alfalfa fields.

Mosaic disease caused by SMV was the major virus disease in this area (Fig. 3). As SMV can be detected in soybean seeds, SMV is disseminated by seeds over a soybean-growing area and the germinated plants become inoculum sources. Then SMV is rapidly transmitted to neighboring soybean plants by aphids.

Although CMV-SS is also a seed-transmissible virus, its incidence was lower than that of SMV.

The most effective method of control of SMV is the utilization of resistant cultivars. However, many SMV strains infect various resistant cultivars. Therefore, although resistant cultivars enable to control certain SMV strains, if another SMV strain infects the resistant cultivar, a severe epidemic may occur. We investigated the SMV strains in Jilin province to select cultivars suitable for the control of SMV. Our work revealed that almost all the isolates of SMV in this area belonged to the SMV-B strain in Japan. Besides, we observed that almost all the cultivars developed at Jilin Academy of Agricultural Sciences for the past years were resistant to SMV-B. If farmers replace the old cultivars with new cultivars, SMV could be eliminated in their fields. After the introduction of resistant cultivars, the occurrence of other virulent SMV strains should be carefully monitored and the agricultural institutes are responsible for providing pathogen-free seeds.

Recently, we have isolated an unidentified virus from SMVresistant soybean plants. This virus is different from SMV and other known viruses in this area, because it does not react with the antisera used and was not transmitted by aphids. This virus must show other ecological characters. Therefore another strategy for control should be developed. We will soon identify it and will show how we can control it in the near future.



3. SMV

JIRCAS Research Highlight

Experimental Plantation of Dipterocarp Species in Luzon Island, Philippines

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Dipterocarp species are one of the main component trees of the tropical forest. Due to the high commercial value, Dipterocarp stands have been logged on a large scale, leading in some areas to a serious decline of forest resources. In the Philippines, lowland forests had been dominated by Dipterocarp species in the past, and recently logging has spread all over the country, resulting in a remarkable reduction of useful timber resources.

Reproduction of the forest generally has been achieved by natural regeneration which is too slow in some hardly disturbed cases. Planting of seedlings is more efficient to establish a targeted forest in a short period of time. In the case of Dipterocarp species, planting had been commonly carried out under canopy trees, to obtain a higher survival rate of planted seedlings. However, stagnation of growth was often observed. Light is efficient for the growth of seedlings and it is important to explore the possibility of plantation in an open environment with Dipterocarp species.

A joint experimental plantation site had been established at the campus of UPLB (University of the Philippines at Los Baños) in cooperation with the SFI Department. Seedlings of eight species belonging to the Dipterocarp family were planted. All the species were indigenous to the Philippines and seedlings from seeds collected from Mt. Makiling Forest Reserve and the surrounding area were maintained in a nursery for 6 months prior to planting. To analyze the growth response under different light conditions, the site was divided into two parts, one in a fully opened area and the other under the canopy of an *Acacia* stand (RLI about 30%).

The initial growth for two years after plantation differed

depending on the species. In the fully opened part, Anisoptera thurifera ssp. thurifera (Palosapis) and Shorea contorta (White Lauan) grew better than the other species both in terms of height and diameter. On the other hand, in the case of Dipterocarpus grandiflorus (Apiton) the values for these parameters were the lowest. The other species showed an intermediate growth between the two groups. There was no appreciable difference in growth between seedlings in the fully opened part and the part under the canopy for D. grandiflorus. However, the species that became big in the fully opened part showed only less than half growth in the part under the canopy. The survival rate showed the same trend as that of A. thurifera ssp. thurifera and S. contorta i.e., around 90%. The rate was not significantly different between the two parts of the site. The other species showed a lower rate, and particularly D. grandiflorus reached a rate of about 50% after planting.

Weed and vine growth was luxuriant in the fully opened part. This vegetation competed with the planted seedlings. To maintain higher growth and survival rates, weeding was an efficient treatment when practiced at intervals of 2, 3 months. In the non-weeded site, the survival rate decreased for all the species that had been planted.

Thus the growth and survival varied with the species and site conditions in the opened area; microclimatic factors may vary not only in terms of light intensity compared to the area under canopy, but temperature and humidity of air and soil may also be different. Therefore, light is not the only factor that accelerates growth. Further studies focused on the response of growth to other factors should be carried out to analyze the species characteristics.



A. thurifera ssp. thurifera in fully opened part



A. thurifera ssp. thurifera under the canopy of Acacia

Farming Systems Research in Indonesia – Toward the Improvement of Household Economy in Rural Areas in West Java

Natural and socio-economic conditions surrounding agriculture

Agricultural land area accounts for about 23% of the total land territory of Indonesia, while for 67% in Java Island. Agricultural production varies markedly from location to location, depending upon the natural environmental and socioeconomic conditions in each region. For instance, rapid economic growth, industrialization, and urbanization on Java have widened the gap between cities and villages and altered fundamentally the social and economic conditions in rural areas. On the outer Islands, on the contrary, generally the low standard of living reflects the slow pace of economic development including agricultural production and the delay in the development of appropriate technologies to overcome the difficulties in resources exploitation.

Agricultural production and research

Most of the agricultural research in Indonesia has been focused on lowland farming systems based on the cultivation of rice. This focus, when combined with the effects of other governmental programs to help rice farmers, allowed Indonesia to achieve its national goal of self-sufficiency in rice by 1984. On the other hand, upland crop areas have not much benefited from the modern agricultural technologies although numerous research projects have targeted upland or rainfed lands. Consequently, upland agriculture remains much less developed than lowland agriculture throughout the country. Under these conditions, in addition to the shortage of rice production experienced during the past few years, increase of food crop production, particularly of rice, maize and soybean, both in the lowland and the upland, has become the most urgent target in agricultural research and development in Indonesia (Table 1).

Implementation of farming systems research in Indonesia

The comprehensive research project entitled "Evaluation and Improvement of Regional Farming Systems in Indonesia" aims at examining, from farmers' standpoint, the socio-

economic and technical achievements of the past and on-going practices relating to farming systems research and extension in Indonesia and at suggesting ways for improvement. A "farming systems research approach" is characterized by a bottom-up participatory process and an emphasis on a multidisciplinary approach to problems in agriculture. The project started in April, 1998 based on the Memorandum of Understanding (MOU) between the Agency for Agricultural Research and Development (AARD) of Indonesia and the Japan International Research Center for Agricultural Sciences (JIRCAS), and is scheduled to last for five years. The research institutes of Indonesia with which JIRCAS is currently collaborating include the Center for Agro-Socio Economic Research (CASER), the Research Institute for Legume and Tuber Crops (RILET) of the Central Research Institute for Food Crops (CRIFC), and the Center for Soil and Agroclimate Research (CSAR) and Lembang Assessment Institute for Agricultural Technology (AIAT-Lembang).

Shuichi Asanuma

Research Information Division, JIRCAS

Research activities under progress and planning

The following research activities are in progress, 1) studies on the farmer-state linkages in upland farm development in Indonesia, 2) analysis of physical environmental resources using remotely sensed data and GIS for evaluation and improvement of regional farming systems in Indonesia, and 3) evaluation of Indonesian soybean varieties for the processing and improvement of traditional fermented foods. An additional activity consisting of the evaluation of vegetable-based farming systems and improvement of vegetable and fruit cultivation in highland regions in West Java is now under planning, the objectives of which are 1) to analyze geographic, climatic and socio-economic conditions of temperate vegetable-producing areas in West Java, 2) to analyze the demand for temperate vegetable production in Indonesia, 3) to analyze and evaluate the distribution systems of temperate vegetables and fruits in West Java, 4) to analyze and evaluate the present cultivation and protection technology of temperate vegetables in West Java, and 5) to analyze and evaluate

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Table 1. Production and	l import of	f main graiı	n crop com	modities in Indonesia	
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	I	Production		Import		
Commodities	'85	'90	'95	 '85	'90	'95
Rice	38,660	44,490	49,860	40	50	3,158
Wheat	0	0	0	1,333	1,767	4,274*
Maize	5,300	6,741	8,223	50	9	969
Soybean	825	1,427	1,689	302	541	607
Bean cake	-	-	_	175	5	682

* Including wheat flour

Source: FAO Production Yearbook; FAO Trade Yearbook

the characteristics of upland crops and fruit trees utilized in the mixed cropping systems of temperate vegetable cultivation in West Java and to suggest the incorporation of alternative varieties of upland crops and/or fruit trees into the systems.



Cabbage field in mixed cropping system on steep slope of Mt. Bromo, East Java

We expect that the collaborative research between AARD and JIRCAS will contribute to the improvement of vegetable farmers'life in West Java through the increase of household income in the near future.



Beans planted in paddy fields in Pasuruan, East Java

Topics

Mr. Matsumoto, Chairman of AFFRC, Mr. Tsuchiya, Director of International Research Division of AFFRC and Dr. Maeno, DG of JIRCAS Visited Brazil Kiyomi Kosaka

Mr. Sakue Matsumoto, Chairman of the Agriculture, Forestry and Fisheries Research Council, AFFRC, Ministry of Agriculture, Forestry and Fisheries, MAFF, Mr. Tadashi Tsuchiya, Director, International Research Division, AFFRC/ MAFF and Dr. Nobuyoshi Maeno, Director General of JIRCAS visited Brazil in August 1999. In São Paulo, they had the opportunity of meeting with Dr. Sugai, economist of the Brazilian Agricultural Research Corporation, EMBRAPA, and they discussed various aspects relating to the orientation of future collaborative research activities between Brazil and Japan, especially in the field of socio-economic studies.

Mr. Matsumoto, Mr. Tsuchiya and Dr. Maeno visited the National Center for Beef Cattle Research (CNPGC), EMBRAPA in Campo Grande and discussed with Dr. Arae



Visit of a laboratory at CNPGC. From left: Dr. Maeno, Dr. Boock, Mr. Matsumoto and Dr. Miranda

Animal Production and Grassland Division, JIRCAS

Boock, Director General of CNPGC, and his staff, in greater detail various aspects relating to the on-going collaborative research project entitled "Comprehensive Studies on the Development of Sustainable Agro-Pastoral Systems in the Subtropical Area of Brazil." In Campo Grande, they also had the opportunity of meeting with Mr. Moacir Kohl, Director General of the State Department of Agriculture and Vice President of Mato Grosso do Sul and Mr. Paulo Shiguenori Kanazawa, Deputy Director of State Finance and Science Department, and learned more about recent agricultural developments in the Cerrados Area.

On these occasions, Mr. Matsumoto, Mr. Tsuchiya and Dr. Maeno also had the opportunity of meeting with many immigrant farmers who became the leaders of the Japanese communities of Brazil in São Paulo, Campo Grande, Marilia and Guatapara, in particular with Mr. Shunji Nishimura, owner of Pompeia Agri-

culture and Industry High School, who contributed significantly to forging close links between the Brazilian and Japanese agricultural organizations for many years.

