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Threshing rice in Cote d'Ivoire
(Photo by S. Tobita)

CONTENTS

JIRCAS
JAPAN INTERNATIONAL RESEARCH CENTER FOR AGRICULTURAL SCIENCES

Improving Heat Tolerance of Crops

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The Okinawa Subtropical Station of Japan International Research Center for Agricultural Sciences (JIRCAS-Okinawa) is located in Japan's southernmost city, Ishigaki. By taking advantage of the subtropical climate, research is being carried out to develop techniques which will contribute to the promotion of agriculture in the tropical and subtropical areas.

Heat tolerance is one of the major themes taken up at the Station to promote and increase crop production in those areas. For example, vegetables are mainly cultivated during winter and spring in Okinawa and it is very difficult to grow temperate vegetables during the summer season.

Therefore, attempts were made to overcome this shortcoming at the Station through the evaluation of the germplasm collected from Southeast Asian countries and foreign research institutions including CIAT, USDA, etc. By screening the germplasm for heat tolerance, a heat-tolerant snap bean variety "Haibushi" was eventually developed (Haibushi means "southern star" in Okinawa dialect). We anticipate that "Southern Star, Haibushi" will pave the way for the development of crop varieties with higher heat tolerance by conducting further research on the mode of inheritance of heat tolerance and physiological mechanisms.

Therefore, the five-year research project entitled: "Inheritance and Physiological Mechanism of Crop Heat Tolerance and Development of Heat-tolerant Crops" has been initiated in collaboration with research teams from several universities since the end of September, 1998. This project is financed by Bio-oriented Technology Research Advancement Institution (BRAIN). Dr. Yoshinobu Egawa, Head of Crop Introduction and Cultivation Laboratory, JIRCAS-Okinawa is assuming the leadership of the project. This project consists of four research teams with the following themes and research contents.

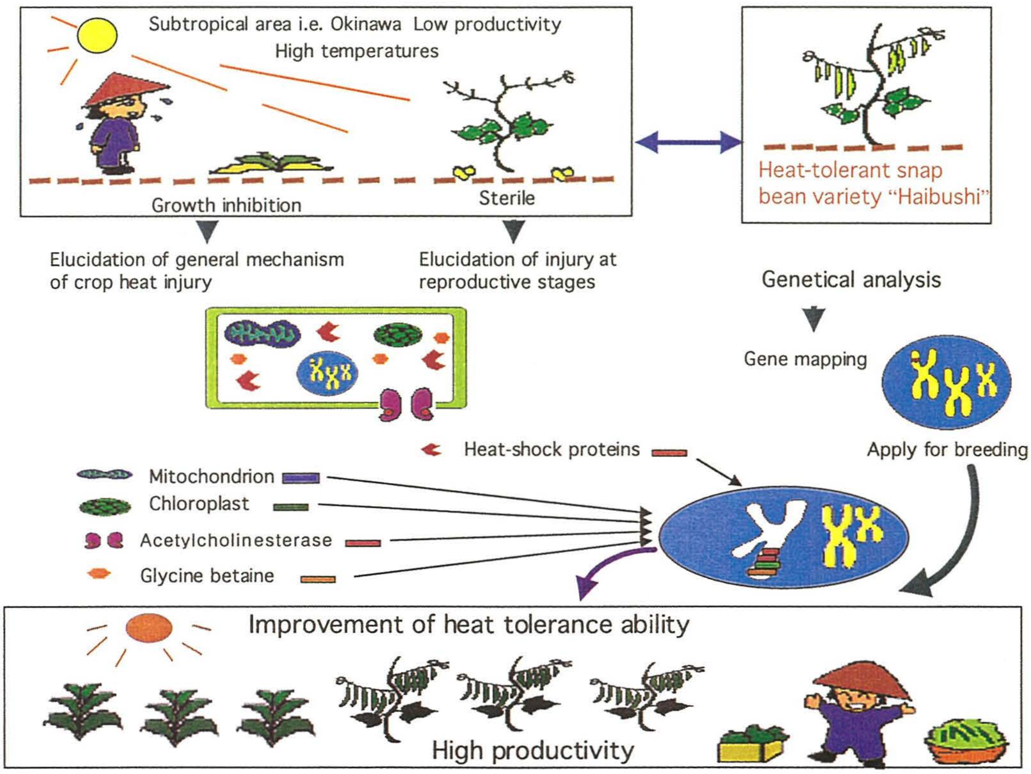
(1) Genetic and physiological mechanisms of crop heat tolerance during the reproductive process (JIRCAS-Okinawa)

By comparing the heat-tolerant genotype of a crop with heat-sensitive genotypes, the following aspects will be studied: 1) the stage at which plants are most sensitive to high temperature during the reproductive process, 2) the organs or tissues which are damaged by high temperature, 3) growth response of heat-tolerant genotypes under high temperature conditions, 4) QTL mapping and genetic regulation of heat tolerance of crops.

Under heat stress conditions, small-heat-shock-proteins (sHSP) are sometimes observed in plant. Role of sHSP and their function under heat stress are also studied by this team.

(2) Improvement of heat tolerance by functional modification of mitochondria (Ryukyu University)

Active oxygen sometimes injures organic substances inside the cell. Injury is mainly generated at the level of mitochondria and its generation is accelerated under high temperature conditions through the process of respiratory electron transport chain in mitochondria. In this research, the physiological and genetic role of mitochondria will be specified with reference to heat tolerance of crop at the mitochondria-DNA level and attempts will be made to develop heat-tolerant crops by functional modification of mitochondria.



(3) Gene transfer for heat tolerance and functional analysis of its recombinant (Nagoya University)

Under stress conditions such as cold or salinity, some of the tolerant plants show an accumulation of glycine betaine inside the cells. It is considered that this accumulation is related to some protection mechanisms from environmental stresses. Transgenic rice plants which harbor the pathway of glycine betaine synthesis are expected to be heat-tolerant due to a similar mechanism. This team will examine the transgenic plants to clarify further mechanisms of plant response to heat.

Since it is assumed that crop productivity decreases under high light intensity, gene expression of heat tolerance will also be examined by transferring related genes for controlling photo-respiration and elimination of active oxygen. Through collaboration with other teams, heat tolerance-related genes will be transferred into tomato and other plants.

(4) Functional characterization of acetylcholine-esterase for heat tolerance of plants (Tokyo University of Agriculture)

Acetylcholine (Ach) is a well-studied chemical transmitter in the synaptic junction for the opening of cation-selective channels in nerve synapses of animals. In plants, it is known that Ach is induced by environmental stimuli, such as heat stress, salinity stress and gravity, affecting calcium ion concentration and safranin transport. Especially, heat stress induces an elevation of calcium ion concentration and activity of acetylcholine-esterase (AchE) in plant cells. Further research on the function of Ach and AchE will be studied and transgenic plants will be produced by isolating the AchE gene to develop heat-tolerant plans.

Through this research project, we hope to elucidate physiological aspects of "Heat Tolerance of Crops" and to contribute to crop breeding in order to develop heat-tolerant crops.

Symposium

5th JIRCAS International Symposium on Postharvest Technology in Asia Was Held in Tsukuba

The 5th JIRCAS International Symposium on "Postharvest Technology in Asia — A step forward to stable supply of food products" organized by Japan International Research Center for Agricultural Sciences (JIRCAS) in cooperation with the National Food Research Institute (NFRI), National Agriculture Research Center (NARC), Food Forum Tsukuba and Nestle Science Promotion Committee was held in Tsukuba during the period 9-10 September, 1998.

Although the development of technology for increasing crop production, remains essential, "postharvest technology" was selected as theme for the symposium by JIRCAS for the first time, since the development of postharvest technology is becoming increasingly important.

Indeed in Asia, it is anticipated that, in the near future, the food supply problems will become more complex as patterns of food consumption have improved in the region along with the increase of income and social development. Postharvest technology should be more emphasized to prevent crop losses and for utilizing agricultural products efficiently to meet the changes in the food demand, especially the demand for processed foods. Food industry is closely related to crop production in the region but due to the lack of proper technology, sound progress of the food industry can not be fully achieved. In this Symposium, the development of the food industry in Asia was reviewed and the basic technical problems involved in future progress were outlined.

From 21 countries including African countries, nearly 230 scientists and administrators, (59 from abroad) gathered and exchanged views on the main priorities and constraints of postharvest technology in Asia, and on the current situation and future orientation of technology for grain storage/preservation and food industries in Asia.

Mr. Sakue Matsumoto, Chairman, Agriculture, Forestry and Fisheries Research Council gave the welcome address. A keynote speech was delivered by Dr. Keiji Kainuma (former Director General of JIRCAS), Vice President, Bio-oriented Technology Research Advancement Institute (BRAIN), Japan, and 18 papers were presented during the three sessions.

JIRCAS is implementing collaborative projects in Asian countries and "postharvest technology" should receive more attention in future research collaboration. Development of "postharvest technology" could contribute to securing a stable supply of food products in addition to the increase of agricultural income and improvement of the diet in the developing regions of Asia.

The Proceedings of the Symposium will be published by JIRCAS.

(Yoshihiko Nawa)

Photo: Speakers with other participants (from right to left, the sixth person in the front line: Mr. Sakue Matsumoto, the fifth person in the next line: Dr. Keiji Kainuma, the fourth person in the same line: Dr. Nobuyoshi Maeno)

Photosynthesis in Longan and Mango as Influenced by High Temperatures under High Irradiance

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The combination of high temperature and irradiance is a common characteristic in the tropical and subtropical regions. Fruit species originating in those regions may be adapted to these climatic conditions, but the differences in adaptability to these conditions are not well-known. The objectives of our studies were 1) to compare gas exchange characteristics of longan and mango leaves upon exposure to high temperature and irradiance and 2) to examine the mechanisms contributing to the variation in the response of the net photosynthetic rate to high temperature and irradiance.

Leaves of 2-year-old longan and mango seedlings were exposed to high irradiance (2000μ mol·m^{-2}·sec^{-1}) provided by metal halide lamps at 70±5% RH. Temperature was raised by increments of 3°C every 1.5 h, from 30 to 36°C. Net assimilation rates decreased for both species as temperature increased, but the decline was greater in longans than in mangoes (Table 1). As temperature increased, stomatal conductance decreased and intercellular CO_2 concentration increased for both species, especially longans. A significant positive correlation was found in longan between stomatal conductance and net assimilation rate at 30 and 33°C, but not at 36°C. In mangoes, the correlation coefficient was significant at all 3 temperatures. Intercellular CO_2 concentration and net assimilation rate were not closely related at 30 and 33°C, but showed a strong negative correlation at 36°C in both species. These results indicate that the decline in net assimilation rate was caused by non-stomatal limitations at high temperatures. In further experiments, the extent of decline in the chlorophyll fluorescence ratio (variable (Fv) to maximum fluorescence (Fm)) caused by high temperature treatment (up to 45°C) was greater in longans than in mangoes (Table 2), suggesting that mango leaves are more tolerant of high temperatures than are longan leaves.

Table 1. Gas exchange characteristics in longan and mango leaves exposed to 2000 μ mol·m^{-2}·s^{-1} of irradiance with stepwise increase of temperature

Species	A (μ mol·m^{-2}·s^{-1})			gs (mol·m^{-2}·s^{-1})		
	30°C	33°C	36°C	30°C	33°C	36°C
Longan (L)	6.72±0.34[Z] (100)[Y]	4.18±0.43 (62)	1.30±0.30 (19)	0.204±0.019 (100)	0.157±0.018 (77)	0.127±0.008 (62)
Mango (M)	9.64±0.33 (100)	7.71±0.50 (80)	5.87±0.51 (60)	0.241±0.016 (100)	0.203±0.015 (84)	0.180±0.013 (75)
Ratio (L/M)	0.7	0.54	0.22	0.85	0.77	0.71

Species	Ci (ppm)			E (mmol·m^{-2}·s^{-1})		
	30°C	33°C	36°C	30°C	33°C	36°C
Longan (L)	262±3 (100)	278±3 (106)	308±4 (118)	5.68±0.31 (100)	5.37±0.37 (95)	5.83±0.32 (103)
Mango (M)	246±2 (100)	254±2 (103)	265±4 (108)	6.33±0.24 (100)	6.44±0.29 (102)	7.16±0.32 (113)
Ratio (L/M)	1.07	1.09	1.16	0.9	0.83	0.81

[Z] Mean±SE (n = 26)

[Y] Numbers in parenthesis indicate percentage to the value at 30°C

Table 2. Chlorophyll fluorescence ratio (Fv/Fm) in leaves of longan and mango exposed to high temperature

Species	Duration	25°C (Control)	40°C	42.5°C	45°C
Longan	20 min.	0.827±0.004[Z] (100%)[Y]	0.808±0.013 (98%)	0.797±0.007 (96%)	0.592±0.030 (64%)
	40 min.	0.820±0.005 (100%)	0.810±0.005 (99%)	0.599±0.028 (73%)	0.481±0.029 (59%)
Mango	20 min.	0.819±0.007 (100%)	0.823±0.009 (100%)	0.808±0.009 (99%)	0.743±0.011 (91%)
	40 min.	0.827±0.005 (100%)	0.807±0.013 (98%)	0.756±0.017 (91%)	0.642±0.007 (78%)

[Z] Mean±SE (n = 4)

[Y] The percentage to the value at 25°C

Antioxidant and Antimutagenic Properties of Some Edible Thai Plants

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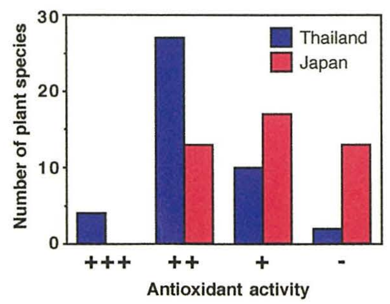
Several hundreds of native plant species are used for daily diet in Thailand as vegetables, fruits, spices and condiments. Many of them are also employed for traditional medicine. Edible plants in Thailand are rich in phytochemicals such as vitamins, terpenoids, flavonoids, alkaloids, organosulfur compounds, pigments and other phenolics. Since such phytochemicals exhibit various biological activities, it has been suggested that edible plants in Thailand may play a role in the prevention of human chronic diseases as well as cancer. In this regard, the "functionality" of such edible plants is being emphasized. However, since this aspect has been investigated in only a few Thai plants, under the JIRCAS Visiting Research Fellowship Program at Tsukuba, a total of 47 species (48 samples) of edible plants from Thailand were collected and their methanolic extracts were analyzed in relation to two important functions, antioxidant activity and antimutagenicity. The results are summarized as follows.

1. Antioxidant activity

Molecular oxygen is easily converted to reactive free radicals called "oxygen free radicals" (ORF) *in vivo*. ORFs are formed by the transfer of one electron to the oxygen molecule during various physiological processes such as respiration chain, oxygenase reactions and cellular immunization reaction. ORFs damage the cell membrane, cytosolic molecules and genes, and they are associated with the incidence of various chronic diseases, *i.e.* heart diseases, thrombosis, hypertension and cancer. It has been suggested that the ingestion of dietary antioxidants that suppress the ORF production or scavenge ORFs may prevent harmful effect of ORFs. Some phytochemicals, *i.e.* flavonoids, pigments and antioxidative vitamins, are known to be potent antioxidants. We determined the antioxidant activity of methanolic extracts from edible Thai plants by the β-carotene bleaching method. The data obtained are summarized in Fig. 1. We found that more than 70% of Thai plant species showed a very high (+++) and high (++) antioxidant activity, while only one-third of common edible plants in Japan exhibited a high activity. Especially, three plant species (4 samples) showed a significantly potent activity. "Pak kra doon" (*Photo 1; Careya sphaerica* Roxb.), a leafy vegetable, exhibited the highest activity (121.0 mg BHA equivalent /g dry weight). The leaf of "Pak sa meg" (*Syzygium gratum* Wall.), and the fruit and the leaf of "Kra thin" (*Acacia farnesiana* Willd.) also showed a high activity (40.2, 38.8 and 44.7 mg BHA equivalent /g, respectively).

2. Antimutagenicity

Heterocyclic amines and polyaromatic hydrocarbons are considered to be the major cause of cancer due to their potent carcinogenicity. They are formed during the daily cooking process and commonly occur in food. Burnt grilled fish or smoked foods contain a mutagenic heterocyclic amine, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1). Trp-P-1 acquires genotoxicity after being converted to N-hydroxy Trp-P-1 by hepatic enzyme cytochrome P450 monooxygenases (P450). N-hydroxy Trp-P-1 is easily transformed to its radical, which reacts with DNA to induce frame-shift mutation. In the present study, we examined the suppressive activity of Thai vegetables towards the mutagenicity of Trp-P-1 with *Salmonella typhimurium* TA98 in the presence of S9 mix. *S. typhimurium* TA98 is a mutant of *Salmonella* bacteria that requires histidine to grow (*his*$^-$), owing to mutation in a gene for histidine biosynthesis, and S9 mix is a mutagen-activating metabolic enzyme system from rat liver homogenate containing P450. The activated mutagen induces reverse mutation, and resulting wild-type revertants (*his*$^+$) can grow in the histidine-deficient medium. If a plant extract inhibits the reverse mutation, the number of wild-type revertants must decrease. By this method, we observed that several plant extracts have a potent inhibitory effect on the mutagenicity of Trp-P-1 (Fig. 1). The methanolic extracts from 1.25 mg of freeze-dried "Pak pai" (*Photo 2*; fragrant knotweed; *Polygonum odoratum* Lour.), that is a common condiment in Thailand and Vietnam, suppressed 96% of the mutagenicity of 50 ng Trp-P-1, followed by "Pak chee" (coriander; *Coriandrum sativum*; 94%), "Hoom yae" (*Trachyspermum roxburghianum* Craib; 92%) and "Pegah" (Indian trumpet flower; *Oroxylum indicum* Vent.; 90%).



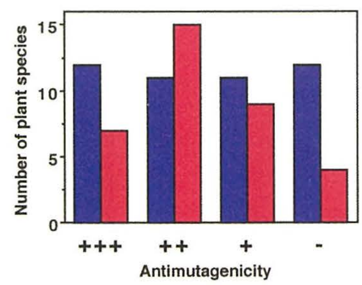


Fig. 1. Antioxidant activity (left) and antimutagenicity (right) of edible plants in Thailand and Japan.

The antioxidant activity (AA) was classified into four ranks by the inhibitory activity: +++, very high (≥ 20 mg BHA equivalent /g); ++, high (20 - 3.6 mg BHA eq./g); +, low (3.6 - 0.75 mg BHA eq./g); -, negative (≤ 0.75 mg BHA eq./g). The antimutagenicity; +++, very high (≥ 80%); ++, high (80 - 60%); +, low (60 - 40%); -, negative (≤40%).

Photo 1: "Pak kra doon", (*Careya sphaeria* Roxb)

Photo 2: "Pak pai," (*Polygonum odoratum* Lour)

Collaborative Research on Interspecific Hybrids of Rice at WARDA

Rapid demographic expansion and urbanization in Africa led to changes in food preference toward rice and bread which can be more easily prepared than traditional food-stuffs. These patterns are especially evident in West Africa where the substitution of rice for coarse grains and traditional root and tuber crops has fueled growth in demand at an annual rate of 5.6% between 1961 and 1992.

The demand for rice in sub-Saharan Africa is growing faster than for any other major food staple, with consumption increasing across all the socioeconomic classes. Rice is no longer a luxury food and the poorest urban households in West Africa derive larger shares of their cereal-based calories from rice than do higher income households, and rice purchases account for a greater part of their total cash expenditure. Rice availability and rice prices impact directly on the welfare of the poorest West African consumers who experience the lowest rate of food security.

The increase of rice production in Africa is hampered by a number of constraints, including diseases and pests, weed infestation, inadequate water management, soils with a low fertility, and lack of suitable varieties. In sub-Saharan Africa, farmers cultivate two species of rice, African rice (*Oryza glaberrima*) and Asian rice (*Oryza sativa*). Although *O. sativa* is preferred by African farmers due to its higher yield potential, *O. glaberrima*, the indigenous domesticated species, has several advantages over *O. sativa*, such as high weed competitiveness, resistance to drought, soil acidity, rice yellow mottle virus, African rice gall midge. Most of the improved varieties currently available to farmers have been developed from Asian *O. sativa* germplasm recently introduced that has yet acquired little resistance to the stresses prevailing under the environmental conditions of West Africa.

Following the initial breakthrough at WARDA in producing fertile progenies of interspecific hybrids, an Africa/Asia joint research project on interspecific hybridization of rice was initiated in January 1997, under the aegis of the Japan/United States Common Agenda for Cooperation. Funding and technical assistance were provided by the Government of Japan, the United Nations Development Program (UNDP), and the Rockefeller Foundation. The main objective of the project is to improve the welfare of rice producers and consumers by developing rice varieties adapted to low input-management through interspecific hybridization, that are well suited to resource-poor farmers in West Africa.

The new interspecific progenies include a significant percentage of intermediate types combining the seedling vigor, high tillering and weed suppression traits of *O. glaberrima* with the panicle and grain type characteristics of *O. sativa* and are considered to be promising as new breeding materials. Further efforts for combining a high-yielding ability and stress tolerance are now being emphasized at WARDA.

Within the framework of the WARDA's mid-term strategies, JIRCAS initiated a collaborative research project at WARDA from April this year to implement the hybridization project by sending senior researchers to WARDA to take up the following research topics:

1) Genetic and eco-physiological characterization of indigenous rice varieties and interspecific progenies, with emphasis placed on tolerance to drought and acid soil conditions.

2) Studies on socio-economic aspects in relation to the sustainability of lowland rice cultivation in West Africa.

(Hiroko Takagi)

Photo 2: Field for breeding of interspecific hybrids at WARDA

Photo 1: Rice fields in Cote d'Ivoire (left: irrigated field, right: rainfed upland field)

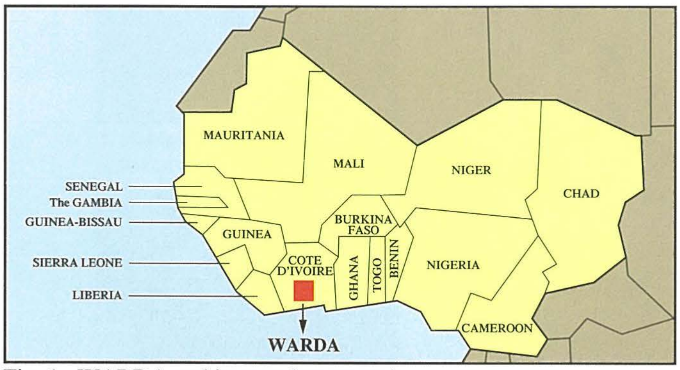


Fig. 1. WARDA and its member countries

Photo 3: A farmer and his family growing a WARDA breeding line along with maize and cassava

Workshop

International Workshop on "Cryopreservation of Tropical Plant Germplasm"

The intensive exploration and collection activities in recent years have resulted in the rapid increase of plant germplasm collections throughout the world. The development of safe and cost-effective techniques for the long-term conservation of the collections, especially recalcitrant seed- and vegetatively propagated species is considered as a priority research area.

For long-term conservation of the problem species, cryopreservation is the only method currently available. Dramatic progress has been made in recent years in the development of new cryopreservation techniques, and cryopreservation protocols have been established for over 100 different plant species. However, cryopreservation of tropical and sub-tropical species has been less extensively investigated than that of temperate species.

Against this background, Japan International Research Center for Agricultural Sciences (JIRCAS) and International Plant Genetic Resources Institute (IPGRI) jointly organized the International Workshop on "Cryopreservation of Tropical Plant Germplasm - Current research progress and application-" held at JIRCAS, Tsukuba during the period 20-23 October, 1998.

In an increasing number of National Programmes in the tropics there has been a growing awareness of the importance of cryopreservation and research activities in this field have been initiated recently. Thus the workshop was timely in providing a unique opportunity for cryopreservation researchers to get together to exchange information and to discuss numerous aspects of cryopreservation of tropical plant species. From 29 countries, 93 scientists including scientists from the International Agricultural Research Centers i.e. IPGRI, CIAT, CIP, IITA, INIBAP participated in the workshop. Dr. Nobuyoshi Maeno, Director General of JIRCAS gave the welcome address and key note addresses by Prof. Akira Sakai (Professor of Emeritus, Hokkaido University) and Dr. Florent Engelmann (IPGRI) were delivered, followed by 41 oral presentations and 51 posters.

Photo: Participants in the workshop

Session I dealt with "fundamental aspects of cryopreservation," including ultra-structural studies on cell adaptation to freezing and studies on molecular mechanisms of plant freezing tolerance. During Session II, cryopreservation techniques involving cells, pollen, embryos, meristems were outlined. During Session III, application of cryopreservation protocols to germplasm conservation was reviewed while during Session IV, the "current status of cryopreservation research and future perspectives of its application in natural programs" were considered.

The workshop was successful with active discussions to assess the current status of research, present application of and existing problems with cryopreservation of plant germplasm with a strong focus on tropical species, and to identify priority areas for collaborative research, technology development, transfer and application. It was also pointed out that the workshop could be a good start for setting up a network, even informal, in the field of germplasm conservation to facilitate international research collaboration. The proceedings of this workshop will be published early next year.

(Hiroko Takagi)

JIRCAS Fellowship

JIRCAS Fellowship Program: Welcoming New Visiting Researchers

Seventeen Visiting Researchers are participating in the JIRCAS 1998 Visiting Research Fellowship Program to carry out collaborative research at Tsukuba and Okinawa.

Tsukuba: Short-term (5 months at National Institute for Agrobiological Resources)		
Dr. Dea de Lima Vidal	Univ. of Zaragoza, Brazil	Economic and breeding evaluation of animal genetic resources in developing countries
Dr. Lawrence Misa Aboagye	Plant Genetic Resources Center, Ghana	Evaluation of diversity for a starch synthetic gene in wheat
Dr. Irina Olegovna Vvedenskaya	N. I. Vavilov, All Russian Inst. of Plant Industry, Russia	Molecular basis of protein markers in wild relatives of crops
Dr. Dennis Yeo	National Inst. of Agrobiological Resources, Singapore	Development of strategy to generate male-sterile plants using transcription

Tsukuba: Long-term (2 years at JIRCAS, Tsukuba)		
Dr. Joseph Gogo Dubouzet	Former Post Doctoral Fellow at Hokkaido National Agric. Exp. Station, Philippines	Molecular analysis of drought stress responses in rice
Dr. Vipaporn Na Thalang	Kasetsart Univ., Thailand	Chemical and biological evaluation of Thai Vegetables (Family *Leguminosae*)
Ms. Do Thi Thanh Huong	Cantho Univ., Vietnam	Physiological studies on reproduction and osmoregulation in the giant freshwater prawn, *Macrobrachium rosenbergii*
Mr. Jianjun Guo	Research Center for Rural Economy, Ministry of Agriculture, China	Economic analysis on food consumption of Chinese farm households
Okinawa: Long-term (1 year at JIRCAS, Okinawa Subtropical Station)		
Dr. Bujun Wang	Inst. of Crop Breeding and Cultivation, China	Methane emission and carbon cycling in rice fields as affected by elevated atmospheric CO_2 concentration and environmental factors
Dr. Md. Abdul Karim	Inst. of Postgraduate Studies in Agric., Bangladesh	Interactive effect of thermal and irradiance stresses on some physiological and biochemical parameters of grain legumes and mango
Dr. Narinder Pal Singh Dhillon	Punjab Agric. Univ., India	Genetic variation and relationships in sweet potato populations detected with RAPD and AFLP markers
Dr. Liu Jian	Shandong Teacher's Univ., China	Cloning the heat tolerance-related genes from tomato reproductive tissues
Dr. Armando Rubio Calderon	Instituto Nacional Autonomo de Investigtaciones Agropecuarias Iniap, Ecuador	Evaluation and characterization of oligofructan content in yacon germplasm
Dr. Xinwen Hu	Chinese Academy of Trop. Agric. Sci., China	Development of microsatellite markers in snap beans
Dr. Charuwan Bangwaek	Prachinburi Rice Research Center, Thiland	Enzymes activities related to sugar accumulation in sugarcane
Dr. M. Arumugam Pillai	Tamilnadu Agric. Univ., India	Screening of cDNA library for salt tolerance gene in rice
Ms. Maribel Regla Quintana Sanz	Inst. of Pastures and Forages Research, Cuba	Evaluation of genomic diversity of sugar-related enzyme genes in sugarcane genetic resources

Photo 1: Welcome party at JIRCAS headquarters upon the arrival of the new research fellows

Photo 2: JIRCAS visiting research fellows at Okinawa Subtropical Station

The new application form for the JIRCAS fellowship program is now available.
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