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Oyster culture in Merbok mangrove estuary, Malaysia (Photo by K. Fukusho)

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JIRCAS

JAPAN INTERNATIONAL RESEARCH CENTER FOR AGRICULTURAL SCIENCES

International Collaborative Studies Undertaken by JIRCAS Fisheries Division

— Promotion of Sustainable Fisheries Industry Together with Agriculture, Animal Production, and Forestry —

Kunihiko Fukusho
Director, Fisheries Division

The Fisheries Division is in charge of research on marine and freshwater fisheries resources management, aquaculture, fisheries products processing, which are major themes for international collaboration. The researchers of the Division are engaged in the following research fields:

Dr. Yutaka Fukuda (fisheries products processing), Dr. Shigeo Hayase (squid ecology), Dr. Motoyuki Hara (fish genetics), Dr. Marcy N. Wilder (crustacean endocrinology), Dr. Satoshi Suyama (marine fish ecology), and Mr. Masashi Sekino (freshwater fish culture).

Several seminars and workshops on fisheries resources management and aquaculture were held prior to the initiation of the international collaborative studies in the field of fisheries sciences to identify priorities. The main orientation of effective collaborative studies was eventually defined as follows:

1) Development and improvement of technology for sustainable fisheries industry compatible with environmental protection
2) Japan's contribution to Asian countries as one of the Asian countries (First step; subsequently researchers will be sent to other regions such as South America, Africa, etc.)
3) Promotion of integrated and comprehensive multidisciplinary studies involving agriculture, animal production, and forestry fields
4) Collaboration with the organizations belonging to the Consultative Group on International Agricultural Research (CGIAR) such as ICLARM (International Center for Living Aquatic Resources Management) and Regional International Organizations like the SEAFDEC (Southeast Asian Fisheries Development Center)
5) Attempts to address urgent issues in the field of aquaculture

The Fisheries Division has started to implement collaborative research projects in five Asian developing countries in order to develop a technology for sustainable production of fish, methods of fish culture compatible with the preservation of the environment, and methods for efficient utilization of fisheries products. Title, duration, and counterpart organization of these collaborative studies are as follows:

1) Development of sustainable aquaculture technology in Southeast Asia (1994-1999). Faculty of Fisheries, Kasetsart University, Thailand
2) Evaluation and improvement of farming systems combining agriculture, animal husbandry, and aquaculture in the Mekong Delta (1994-1999). College of Agriculture, Cantho University, Vietnam
3) Production and sustainable utilization of tropical and subtropical brackish water ecosystems (1995-2000). Fisheries Research Institute, Forestry Research Institute, and Faculty of Science, University of Malaya, Malaysia
4) Study on the utilization and processing of freshwater fishes in China (1995-2000). College of Food, Shanghai Fisheries University, China
5) Study on the ecology and life history of coastal fishes (1995-2000). Central Research Institute for Fisheries, Indonesia

The 4th collaborative study will be incorporated into a new comprehensive

project entitled "Development of sustainable production and utilization of major food resources in China" 1997. Furthermore, a new collaborative study "Development of methods for the diagnosis and prevention of penaid viral diseases" is scheduled to start in Asian countries in 1997.

The Division also is actively participating in the JIRCAS Visiting Research Fellowship Program at Tsukuba, and has invited distinguished researchers from developing countries in the field of prevention of fish diseases. The researchers who participate in the Program are expected to be key scientists for collaborative studies in the future.

Although the Division has a small number of researchers, it is supported by nearly 430 scientists affiliated to the Fisheries Institutes of the Fisheries Agency, and will also implement joint research programs with other Divisions of JIRCAS.

Photo 1: Prawn culture, Bali Island, Indonesia

Current Research at Okinawa Subtropical Station

Tadaaki Yamashita
Director, Okinawa Subtropical Station

The Okinawa Subtropical Station is the only national research organization that carries out agricultural research under subtropical conditions in Japan. The Station is located in Japan's southernmost city, Ishigaki (2100 Km southwest from Tokyo). By taking advantage of the subtropical environment, research is carried out in order to develop techniques which should contribute to the promotion of agriculture in the tropical and subtropical areas. Research at the Station covers various fields relating to the production of tropical crops, including breeding and cultivation methods, plant nutrition, soil management, plant physiology and pathology, biotechnology, etc. At the Station, researchers from developing countries are engaged in collaborative studies for optimum utilization of bioresources for conservation of the environment and sustainable agricultural production in the tropics. Since the establishment of the Station in 1970, significant results have been obtained and emphasis has been placed on the enhancement of research activities to develop a new technology aimed at securing food production and preserving the tropical environment.

Explore, Conserve and Utilize Biodiversity

To alleviate the constraints associated with the expansion of areas with salinity problems, salt-tolerant crops are identified using rapid and simple methods. A large variation in salinity tolerance was observed among cultivated varieties of rice originating from all over the world. Salinity-tolerant varieties were geographically distributed mainly in Bangladesh, Malaysia and West African countries at higher frequencies. Isozyme analysis showed that the esterase allele *Est-2* is one of the genetic markers of salinity tolerance in rice.

For long term storage of vegetatively propagated plant germplasm, cryopreservation is a useful method. Protocols for the stable cryopreservation of germplasm of tropical crops, especially root crops such as taro, yams and sweet potato were developed. By successful cryopreservation of shoot meristems of taro (*Colocasia esculenta* (L.) Shott.), on average, 80 percent of the shoot tips could be stored in liquid nitrogen after dehydration treatment using vitrification solution. Most of the surviving shoot meristems developed shoots and roots normally.

Palatability of flour-derived food is considered to be influenced by amylose in wheat. For amylose synthesis, waxy protein is responsible and three waxy proteins (Wx-A1, Wx-B1, Wx-D1) are present in wheat. Analysis of 1,960 accessions of wheat germplasm enabled to detect a Chinese wheat variety which lacked the Wx-D1 protein. From a cross between the Chinese wheat variety and a Japanese wheat variety which was deficient in both Wx-A1 and Wx-B1 proteins, a wheat variety lacking waxy proteins was produced. This variety does not contain amylose and may become a novel material for food use.

Aiming at Sustainable Agricultural Production

In the global atmosphere, concentration of methane is increasing and methanogenic bacteria are biological producers of the gas. Application of rice straw and cellulose has resulted in a considerable increase in methane emission from subtropical paddy fields as well as an increase in the populations of methanogenic bacteria. Methanogenic colonies in cultures of the bacteria showed a blue auto-fluorescence. Currently the populations of methane-oxidizing bacteria in the soil and rice rhizosphere are being investigated and attempts are made to isolate them in order to utilize them as methane-oxidizers for biological control of methane emission.

Soil loading due to erosion in coastal marine environments is a serious problem. For the alleviation of erosion hazards in pineapple fields, the effects of organic mulch, cover crops, and nontillage planting were investigated. Results indicated that a cover crop, weeping lovegrass (*Eragrostis curvula*), on the lower edge of the slope reduced soil loss to a negligible level.

New cultivar of snap bean,

"**Haibushi**" (*Phaseolus vulgaris* L.), was released in 1995. "**Haibushi**" was bred through pure line selection from Malaysian germplasm. It shows a superior heat tolerance compared with the local cultivars and gives a high pod yield in the subtropical area of Japan where vegetable production in summer is difficult. The plant type is indeterminate and young pods are light green.

Papaya leaf distortion mosaic potyvirus (PLDMV) damages papaya plants in the subtropical zone of Japan. The DNA complementary to the 3'-terminal region of PLDMV RNA was cloned and sequenced. Papaya ringspot potyvirus (PRSV) is an other potyvirus which was isolated from papaya in Japan. Results of sequencing of cDNA of PLDMV supported the classification of PLDMV as a distinct member of the genus Potyvirus.

Physiological Studies To Overcome Environmental Constraints

Heat tolerance in tropical fruit crops was determined by chlorophyll fluorescence. This method has been widely used to examine the variation in tolerance of photosystem II to high temperature. Chlorophyll fluorescence test revealed that pineapple, cherimoya, sugar apple, and coconut palm were tolerant to heat whereas Java apple, rose apple, and longan were sensitive among 25 tropical fruit crops.

Some plants experience iron deficiency chlorosis when grown on alkaline calcareous soils. This problem can be overcome by using resistant cultivars. In chickpea (*Cicer arietinum*) and mungbean (*Vigna radiata*), some cultivars developed iron deficiency chlorosis when grown on alkaline soil while others did not exhibit visible symp-

toms. It was revealed that the resistant cultivars were able to lower the pH around the root in response to iron deficiency, resulting in the solubilization of insoluble iron.

Areas with problem soils such as saline, acid sulfate, sandy soils, etc. are continually increasing in the tropics and subtropics. Hydroponics may be a suitable alternative for crop production in such regions. We developed a new apparatus for crop production, which does not require the use of electricity. In this system, supply of nutrients is achieved through capillary action, and the amount of nutrient solution can be reduced by using a floating valve system.

Estimation of DNA Marker Effects in Marker-Quantitative Trait Loci (QTL)-Association Analysis

*Kenji Togashi**

African indigenous cattle display a high degree of resistance to many diseases including trypanosomiasis. In particular, tsetse-transmitted trypanosomiasis is the major constraint on livestock and agricultural production in Africa. Currently, tsetse infestation covers an area of 11 million km^2 in Africa, accounting for about 37% of the surface of the continent. It is considered that 7 million km^2 of the area would otherwise be suitable for livestock and mixed agriculture without detrimental effect on the environment, if trypanosomiasis could be controlled. Over a period of several thousand years, certain breeds of cattle such as N'Dama and West African Shorthorn have developed the ability to thrive in tsetse-infested areas where there is a high risk of trypanosome infection. Consideration is now being given to the use of trypanotolerant breeds of domestic animals as an approach to sustainable livestock development in tsetse-infested areas. The gene responsible for the resistance to trypanosomiasis is currently being identified at ILRI (International Livestock Research Institute) by using crosses between the trypanotolerant N'Dama and zebu cattle breeds, e.g. the Boran. The procedure to estimate the effect of DNA markers linked to the gene responsible for trypanosomiasis was developed as a joint study between JIRCAS and ILRI. Based on the procedure, the degree of effects of DNA markers was found to be highly correlated with the degree of relationship of the true and estimated marker effects. For example, the relationship was even more significant by limiting the number of markers depending on the degree of effects of DNA markers to the top 30 markers than by using all the markers (Table 1). This finding indicated that selection should be based on the use of DNA markers with more pronounced effects, since the markers with poor estimation and negligible effects only create noise for selection.

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Table 1. Correlation coefficients between the true and estimated DNA marker effects when the number of progenies per sire is 500

	Number of DNA markers per chromosome	
	3	4
Heritability		
0.2	0.7221 (0.5928)	0.6741 (0.5131)
0.4	0.7823 (0.6649)	0.7316 (0.5763)

The figures represent the correlation when the number of DNA markers was limited to the top 30 markers
The figures in the parenthesis indicate the correlation when all the DNA markers were used

Development of Sustainable Aquaculture Technology in Southeast Asia

Motoyuki Hara

Recently, in the Southeast Asian countries, especially in Thailand, an intensive form of aquaculture has been promoted in order to increase product volume in accordance with economic growth, and such an intensive form of aquaculture has rapidly spread. As a result, production from aquaculture, especially in relation to prawns, has markedly increased in a short period of time (Fig. 1). However, in some countries and regions, this intensive form of aquaculture had to be reduced or discontinued because of the occurrence of diseases affecting cultured species. It was eventually recognized that it is difficult to maintain a high productivity using intensive aquaculture. In order to achieve sustainable production for aquaculture in Southeast Asia, it is necessary to promote basic studies relating to aquaculture sciences, such as genetics, physiology, pathology and dietetics of fish and prawns. JIRCAS considers that technologies for the promotion of sustainable aquaculture can be developed though basic studies relating to aquaculture sciences.

The collaborative studies covering a five-year period were initiated in 1994 between the Japan International Research Center for Agricultural Sciences (JIRCAS) and the Faculty of Fisheries, Kasetsart University, Thailand. In the first half period of the research program, genetic studies were emphasized as one of the approaches to promote basic studies relating to aquaculture sciences.

At first, in order to develop methods for the detection of genetic variability for breeding, a manual for isozymic analysis for 20 aquatic animal species of economic importance in Thailand fisheries was prepared. The genetic variability of wild populations of catfish (*Clarias macrocephalus*) was analysed using this manual.

The wild population of catfish collected from five localities indicated in Fig. 2 was analysed by using as markers the genes at eleven loci controlling nine enzymes. As a result of isozymic analysis, it was found that the populations examined were genetically independent of each other, because most of the pairs of five populations showed significant differences in all the allele frequencies at more than one locus. Furthermore, based on the genetic distances among the six local populations of catfish, the dendrogram showed that genetic relationships of the populations could be determined (Fig. 3). The relationships between the populations of

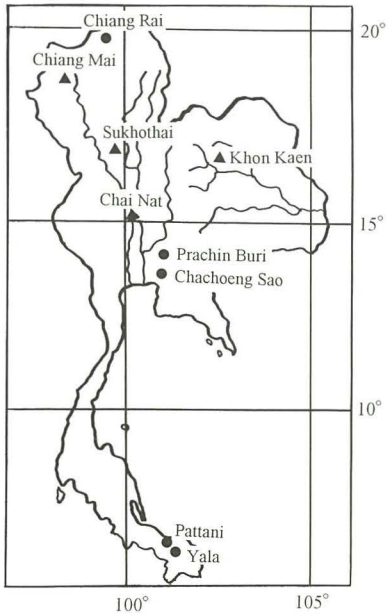

Fig. 2. Sampling sites of catfish (●) and snake-head fish (▲)

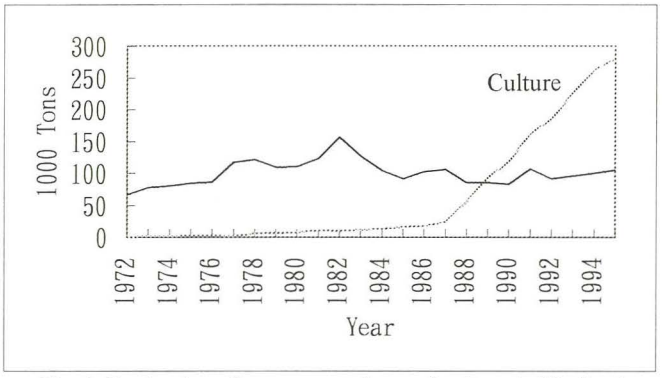

Fig. 1. Production of prawns, catches and culture in Thailand

Prachin Buri and Chachoeng Sao, or Pattani and Yala were very close genetically, whereas the relationship between the Chiang Rai and Pattani (or Yala) populations was genetically distant. A vertical dotted line was drawn across the dendrogram to delineate a group with a distance of 0.01. The genetic distance of 0.01 was considered to correspond to a local race level based on the results of analysis of many kinds of animals. Using this vertical dotted line, the five local populations were divided into two groups, which indicated the differentiation of local races into north/central and south groups.

This species was strongly related to the geographical distance or geographical regions in Thailand. Thailand consists of three regions i.e. central plain, northeastern plain and southern plain, and the three wide regions could be differentiated as a whole, which suggests the possibility of genetic segregation of wild populations.

It is important to identify a local race for breeding, since hybridization among local races can lead to heterosis, and the productivity could be increased. Thus the productivity could be increased by using breeding technologies for two species of economic importance for aquaculture.

The steady increase in the world population requires that sustainable and higher productivity be promoted. The increase of productivity through breeding may lead to sustainable production. However, to promote sustainable aquaculture, it is necessary to develop technologies adapted to the regions.

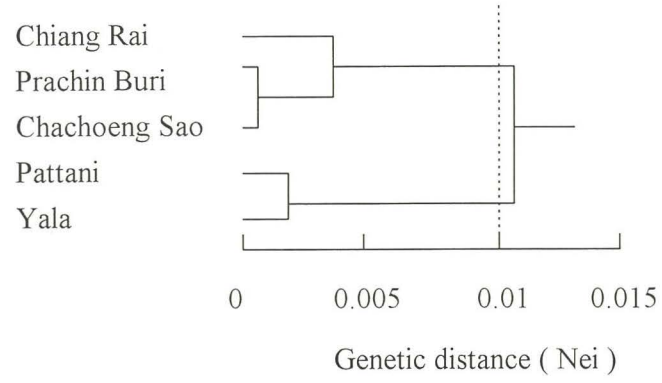

Fig. 3. Dendrogram depicting the relationship among local populations of catfish in Thailand

Ecophysiological Characteristics of Tropical Tree Species

Yutaka Maruyama

Dipterocarps are the main timber-producing trees in Malaysia. Most of the dipterocarps are slow-growing, late successional species, and require moderate shade for optimum growth at the early stage. It is generally considered that dipterocarp seedlings are not suitable for open planting because of their low adaptability to environmental stresses associated with exposed sites. On the other hand, fast-growing pioneer species require strong sunlight for their establishment. In this study, ecophysiological characteristics of these tree species were examined to compare their drought resistance and adaptability to environmental stresses such as heat and light.

The leaf water relation parameters showed that the capacity for maintaining a positive turgor was low in shaded and well-watered nursery seedlings of dipterocarps. On the other hand, leaves of exposed trees were well adapted to water stress morphologically, being smaller and thicker, as well as by their ability to maintain a positive turgor through osmotic adjustment. The rate of net photosynthesis (Pn) was lower in dipterocarps than in the fast-growing species such as acacia and teak. However, in a dipterocarp, *Hopea odorata*, Pn was similar to that of fast-growing species and the trees exhibited a very high water use efficiency (WUE). Decrease in Pn in response to non-optimum temperatures was relatively moderate in *H. odorata* and *Shorea platyclados*, suggesting that gas exchanges of these species were stable under variable temperatures. Among the dipterocarps, *H. odorata* and *S. platyclados* could be recommended as suitable species for open planting. Midday depression of stomatal conductance (Gw) was observed in most of the species examined. Gw started to decrease when the leaves were exposed to full sunlight and when the vapour pressure difference between the leaf and the ambient air (VPD) was above 0.01Pa/Pa. Since photosynthetic CO_2 uptake is strongly controlled by stomatal opening, Pn of dipterocarps was limited by the reduction of Gw. However, the reduction of the transpiration rate (Tr) was relatively small because of the increase of VPD, which stimulates evapotranspiration, with increasing temperature and decreasing air humidity. In contrast, Gw of *Acacia mangium*, a well known fast-growing and drought-tolerant species, remained relatively constant even under full sunlight and when the VPD was above 0.01Pa/Pa, resulting in a high Tr at midday. Leaf water potential (Ψw) was low, or more negative, at noon when Tr was high. In tall mature trees, the difference in the gravitational potential (Ψg) could also cause a lowering of Ψw in the upper canopy. In addition, low soil-to-leaf hydraulic conductance (L) in tall trees limited the water uptake from soil. The resulting lower minimum Ψw in tall trees can lead to enhanced water stress, and lower Gw and Pn than in smaller trees. In *A. mangium*, L was remarkably high. High L enabled this species to maintain a less negative Ψw for the same transpiration rate compared to dipterocarps. By keeping the water deficit low through high L, *A. mangium* maintained a high Gw during the day when the transpiration rate was high.

These results indicate that the slow growth of dipterocarps may be attributed to the relatively low rates of net photosynthesis and midday stomatal closure. Low L of dipterocarps can limit water availability and lower leaf water potential, especially in the mature tall trees. Nursery-grown seedlings of dipterocarps were more susceptible to water stress because of their relative inability to maintain a positive turgor. To enhance their ability to cope with water stress, it is recommended that nursery-grown dipterocarps be preconditioned to drought and direct sunlight prior to transplanting. Partial shading after transplanting is also recommended in order to avoid excessive water loss and to stabilize stomatal conductance and rates of gas exchange in the daytime.

Photo 1: Nursery

Photo 2: Drooping of leaves of a planted dipterocarp seedling

Determination of Site Factors that Promote the Regeneration of Tree Species

Hiroyuki Tanouchi

Although there is an abundance of commercial species in the hill forests of Peninsular Malaysia, the regeneration of tree species does not necessarily succeed in the forests left alone after selective logging. The more valuable of these species is *Shorea curtisii*, which is distributed on the ridges and upper slopes. To develop and further improve techniques for the regeneration of *S. curtisii*, the relationship between the regeneration dynamics and environmental factors needs first to be analysed. We set up a 4 ha (400m×100m) plot in 1994. The plot which was logged selectively five years ago was located on a west-facing slope of Semangkok area (500 m asl; 3°37'N, 101°44'E, Selangor). Dataset of an old-growth forest next to the plot was used for comparison. The density and size distribution of the saplings in both forests were not significantly different. Most saplings in the logged-over forest were established before logging, indicating that logging had not affected appreciably the mortality. However, there were few saplings along the logging roads. Though the canopy in the tree layer was not closed yet, stemless palms (*Eugeissona tristis*) formed a dense canopy in the shrub layer in the logged-over forest. Under the deep shaded conditions (relative canopy openness < 2%), most of the saplings had not developed a main shoot and they extended lateral branches (left, Photo 1). The saplings around the gap of the old-growth forest and at the site without palms showed an adequate upper growth (right, Photo 1).

The palms in a 1 ha plot were removed for improving the light conditions of the saplings (Photo 2). The relative canopy openness was 1.7% and 4.3% before and after removal, respectively. There were no morphological changes in the saplings until six months after removal, then many saplings initiated upper growth. Some saplings which lacked a main shoot elongated new shoots. Removal was estimated to be an effective method at a low cost. Moreover effects of soil compaction and erosion on the regeneration were analysed.

The techniques employed to examine the factors influencing the regeneration of the species will be adopted for studies on some of the more commercial species in the hill forests. Results from these studies will be used to develop silvicultural tending techniques for improving regeneration in logged-over hill forests. This study was carried out as part of a collaborative project between Forest Research Institute, Malaysia and JIRCAS.

Photo 1: Saplings of Shorea curtisii *in forest*
Left: the sapling does not develop a main shoot and extends lateral branches under deep shaded conditions
Right: the sapling grows upward with a main shoot in a site where more light is available

Photo 2: Removal of stemless palms forming a closed canopy in the shrub layer

≪Topics≫

2nd Seminar on "Brackish Water Project" Held in Malaysia

Shigeyuki Kawahara

The seminar is held annually as a part of the comprehensive project "Productivity and Sustainable Utilization of Tropical and Subtropical Brackish Water Mangrove Ecosystems". Tropical and subtropical brackish water areas, usually with developed mangrove forests, are an important habitat for fisheries resources. The project aims to evaluate the productivity of such areas and identify the criteria for sustainable utilization. The West coast of the Malay Peninsula was selected as the study area due to the presence of different types of mangrove forests. Counterpart organizations include Fisheries Research Institute (FRI), Forest Research Institute Malaysia (FRIM) and University of Malaya (UM).

The 2nd seminar was held in a meeting room of FRI's aquarium in Penang on December 5, 1996. Four subjects were taken up during the seminar as follows: ① Mangrove forest, meiofauna

and macrobenthos, ②Prawn and fish, ③Runoff of soil into the brackish waters, and ④Socio-economic aspects in the mangrove areas. About 30 scientists from Japan and Malaysia participated in the seminar, and nine papers were presented. Also, Dr. Kato, Director General of the Seikai National Fisheries Research Institute, introduced the Ishigaki Branch, which is the only site for studies on subtropical fisheries in Japan.

During the 1st seminar, which was held in Kuala Lumpur just one year ago, almost all the presentations dealt with reviews of previous studies (JIRCAS Working Report No. 4), because the project had just started at that time and it was considered that such reviews would contribute significantly to the execution of the project. During the 2nd seminar, the papers from the two JIRCAS researchers on long-term assignments (Dr. Hayase at FRI and Mr. Ochiai at FRIM) and seven JIRCAS researchers on short-term assignments dealt with the collaborative studies carried out with their Malaysian counterparts.

However, the comprehensive project is expected to produce integrated results in addition to the results obtained in each respective field. For integration, material/energy flow should cover the sequence from leaves in mangrove forests to crab/seashells along rivers and phyto/zooplankton and fish/shrimps in water. For such interdisciplinary studies, close collaboration between researchers in different fields is essential and it is obvious that the seminar held annually may enable to achieve this objective.

Photo1: Mr. Ibrahim Saleh (left), Director General of FRI, Drs. Kato (middle) and Hayase (right)

Photo 2: Mangrove forest and fishing vessels at Matang

≪Visiting Scientist≫

Plants Respond to Environmental Conditions

Zhu Weiming
Institute of Agrobiological Genetics and Physiology, Jiangsu Academy of Agricultural Sciences, Nanjing, China

Drought, low temperature, high salt level, insects and pathogens are some common environmental stresses to which plants are often exposed. Plants are different from animals, and since they are immobile, they have developed unique mechanisms to avoid these environmental stresses.

Plant molecular biologists have been interested in studying the mechanism(s) of plant defense systems and improving their resistance to these environmental stresses by using molecular biological techniques and plant engineering. During the past several years, I studied the resistance of transgenic plants to insects in cotton and vegetables. Now I am carrying out studies on plant response to dehydration with my Japanese colleagues. Main object of our studies is to determine how plants respond to environmental stresses, such as drought, cold and high salt at the molecular level. We have isolated several genes that respond to drought, cold and high salt stresses at the transcriptional level, and analysed their function. Now, we are trying to identify new genes that encode regulatory factors for the response to drought, cold, high salt level using yeast one-hybrid system. We are also analysing their function using transgenic tobacco and *Arabidopsis* plant system.

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