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EXPLORATION AND COLLECTION OF PLANT GENETIC RESOURCES

PART II VEGETATIVELY PROPAGATED CROPS

TECHNICAL ASSISTANCE ACTIVITIES
FOR GENETIC RESOURCES PROJECTS

JAPAN INTERNATIONAL COOPERATION AGENCY

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JAPAN INTERNATIONAL COOPERATION AGENCY

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Ⅲ. Report from Exploration and Collection of Native Germplasm in Southeast Asia

The collection of citrus in Southeast Asia by IBPGR

by Isamu UENO
Fruit Tree Research Station

Introduction

In the recognition of vital importance of plant genetic resources for future generations international cooperation has been promoted for collection and conservation of the resources. As one of the aid programs by the Government of Japan, the Japan International Cooperation Agency (JICA) has sponsored a group training course on plant genetic resources since 1983, and provided some countries with facilities for preserving plant genetic resources. On the basis of such experiences, JICA initiated a project for providing further technical assistance to existing genebanks as well as those being planned. As a part of the new project, namely the Technical Assistance Activity for Genetic Resources Projects, GRP Newsletter has been published and distributed since 1988. In addition, it was envisaged to publish a series of manuals, which may be of help for workers in the area of plant genetic resources. Obviously, there are a number of good references, many of which have been distributed by IBPGR. As it may not make sense to issue similar text books, in the new series of manuals the emphasis is to be placed on reporting practical experiences by Japanese workers rather than on dealing with principles or general concepts. It is desired that such reports may be digested and applied to any given condition by colleagues in other countries.

The first and the second of this series have been issued with the title of "Preservation of plant genetic resources", and "Exploration and collection of plant genetic resources, seed-propagated crops", respectively. This volume is a continuation of No. 2 and covers vegetatively propagated crops under the title of Exploration and collection of plant genetic resources.

This volume is a collection of 6 reports. Dr. Y. TAKEDA reviewed the breeding of Japanese tea in relation to its genetic resources. Dr. M. GOTO outlined the role of the plant quarantine system in relation to international exchange of genetic resources.

Following four papers are reports of plant collection activities; Dr. I. TARUMOTO's paper on sweet potatoes in Okinawa Islands of Japan, Dr. I. KAJIURA's paper on a native fruit tree, *Myrica rubra*, Drs. K. TAKAYANAGI and M. HIRAI's paper on taro in Japan, and the last report by Dr. I. UENO is on a joint collection project of citrus in Southeastern Asia, which was sponsored by IBPGR.

I hope the papers in this volume may be useful for those working in the area of plant genetic resources.

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I. Theories and Practices

1. Genetic Resources of Tea in Japan

by Yoshiyuki TAKEDA
National Research Institute of Vegetables, Ornamental Plants and Tea

2. Exploration and Collection of Plant Genetic Resources and Plant Quarantine

by Masaaki GOTO
Yokohama Plant Protection Station

I -1. Genetic Resources of Tea in Japan

by

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Ornamental Plants and Tea

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1. Introduction

Tea is one of the three biggest luxury beverages ranking with coffee and cacao in the world. The area planted to tea trees in the world is 2,754,000ha with its annual production of 2,333,000tons. The main countries producing tea are China, India, Sri Lanka, Indonesia, Kenya and Turkey with the annual production of 465,000, 670,000, 214,000, 129,000, 147,000 and 131,000tons, respectively (FAO 1985).

Also in Japan, tea is one of the important crops with the area of 61,000ha and the annual production of 96,000tons (1985). At present, about 70% of Japanese tea gardens are planted to improved cultivars, which were either selected from domestic tea gardens via seed, or from the cross breeding. In relation to this trend, the propagation method of tea have drastically changed to cuttings from seedlings in these three decades. The rate of fields by clonal propagation through cutting was less than 30% in 1950, and it increased to more than 90% after 1970 (Fig.1). As the result of dissemination of clonal cultivars, Japanese tea gardens become more uniform than before, and many valuable genetic variations have been rapidly lost.

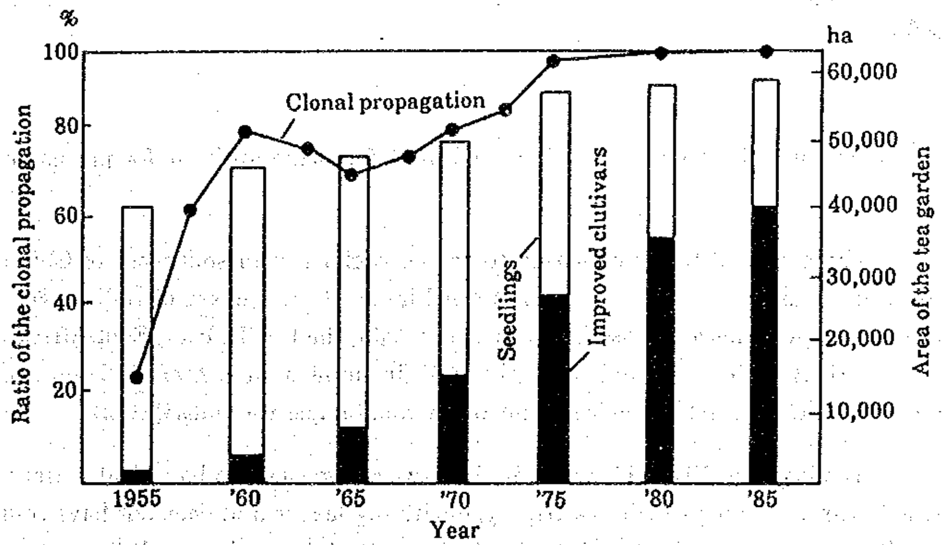


Fig.1 Changes in the propagation method and increase of the improved cultivars during the last three decades in Japan

Japanese indigenous tea plants having generally stronger cold resistance than those introduced, adapted to climatic conditions in Japan, but are inferior in quality for black tea to Assam type plants. In order to improve their quality for black tea, a cross breeding program was started to breed cultivars for black tea in 1932 in Japan. Japanese indigenous tea plants were crossed with many superior tea plants introduced by seeds from India, Sri Lanka, China, Indonesia, Myanmar, Bangladesh and Viet-Nam, and then many superior cultivars with good quality for black tea and cold hardiness were bred in 1950's and 1960's.

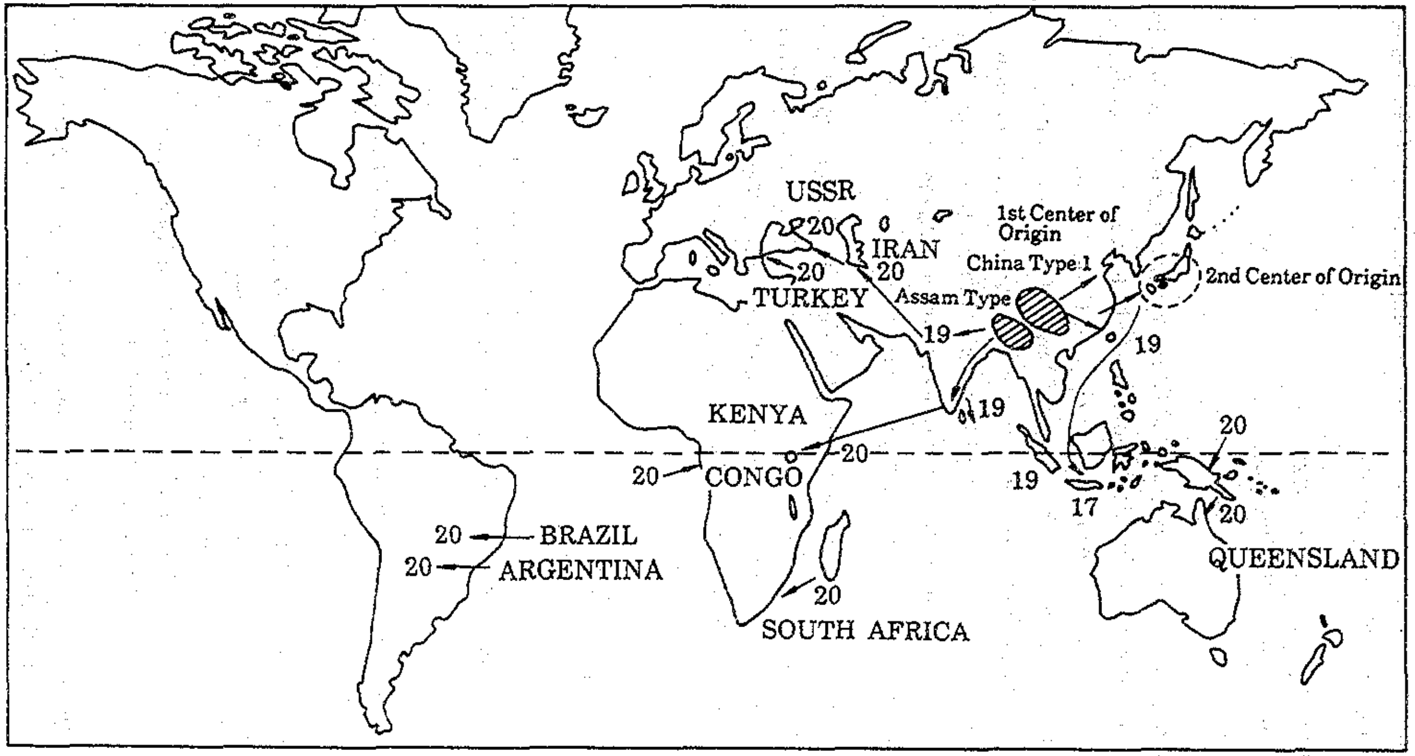
On the other hand, a systematic project of cross breeding for green tea started in 1947, and many cultivars have been developed after 1970. These newly developed cultivars have made much contribution to Japanese tea industry.

Breeding works for black tea are not undertaken now, since there is little production of black tea in Japan. But, many introduced tea plants from foreign countries have been preserved in the tea experiment stations, and are often used as the gene sources for resistance both to diseases and insects or for new taste and flavor adapted for Japanese green tea. In recent years, not only tea (*Camellia sinensis*) but also allied species belonging to the genus *Camellia* have been tested as mother plants, so that the range of genetic resources of tea become wider than before.

2. Origin and classification of tea and manufacturing aptitude for processed tea

The origin of tea is supposed to be the regions from southeast of China involving Yunnan Province and Yunkwei Plateau to northeast of India. Tea-producing countries are those in southeast of Asia, the USSR, Iran, East African and South American countries, reaching 37 in number at present. They are distributed widely in the tropical, subtropical and temperate zones (Fig.2).

Tea is generally divided broadly into two categories of a botanical variety. One is var. *assamica* which is a tree type with big leaves and does not have cold hardiness. The other is var. *sinensis* which is a bush type with small leaves and cold hardiness. These two botanical varieties of tea can be found in different regions, i.e., var. *assamica* is planted in tropical and subtropical regions and var.



Figures denote the century of the transmission of tea.

(OISHI 1983)

Fig.2 Origin and propagation of tea

sinensis in temperate regions such as China and Japan. SEALY (1958) classified tea into following two botanical varieties:

Camellia sinensis (L.) O.KUNTZE
 var. *sinensis* (China type)
 var. *assamica* (Assam type)

The differences between Assam and China types are great not only in size of plant and leaf but also in chemical components in leaves. This leads also to a discrimination of them in the manufacturing aptitude for processed tea. The leaves of Assam type have a high content of tannin (catechin) and high enzyme activity (polyphenol oxidase activity) which are important in the manufacturing process of fermented tea, especially for black tea. On the other hand, China type generally contains a less amount of tannin and lower enzyme activity than the Assam type, and it is more suitable for green tea.

Japanese indigenous cultivars which belong to China type, show the excellent quality when processed into green tea, such as 'Sencha' or 'Gyokuro'. China type varieties in southeast of mainland China and in Taiwan are suitable for 'Oolong tea' and 'Pouchung tea' which are semi-fermented tea.

3. Genetic resources of tea

Most genetic resources of tea are preserved in the following stations belonging to the Ministry of Agriculture, Forestry and Fisheries (MAFF) Gene Bank (Table 1):

Main bank station;	National Institute of Agrobiological Resources (Miyazaki)
Sub-bank station;	National Research Institute of Vegetables, Ornamental Plants and Tea (Kanaya and Makurazaki)
	National Center for Seeds and Seedlings (Kanaya, Nara and Chiran)

Besides the above MAFF Gene Bank, some tea genetic resources are preserved in several prefectural tea experiment stations.

Table 1 Number of the genetic resources of tea preserved in the National Gene Bank Network (TOYAO and TAKEDA 1987)

	Center bank (NIAR)	Sub-bank (NIVOT)		Sub-bank (NCSS)		
	Miyazaki	Kanaya	Makurazaki	Kanaya	Nara	Chiran
Total	286	872	3,162	40	20	40
Active collection	133	98	302	21	14	19
Base collection	129	488	2,675	19	6	21
Working collection	0	91	19	0	0	0
Others	24	195	166	0	0	0

Note : NIAR (National Institute of Agrobiological Resources)

NIVOT (National Research Institute of Vegetables, Ornamental Plants and Tea)

NCSS (National Center for Seeds and Seedlings)

'Others' means the collections which are counted in some more other bank stations.

The important materials are preserved in the several stations and they are often used as a parent for breeding.

The number of accessions of tea conserved as genetic resource are about 4,300 (Fig.3). More than ninety percent of the genetic resources are conserved as the breeding materials in Kanaya field (Shizuoka Pref.) and Makurazaki field (Kagoshima Pref.) of National Research Institute of Vegetables, Ornamental Plants and Tea (NIVOT).

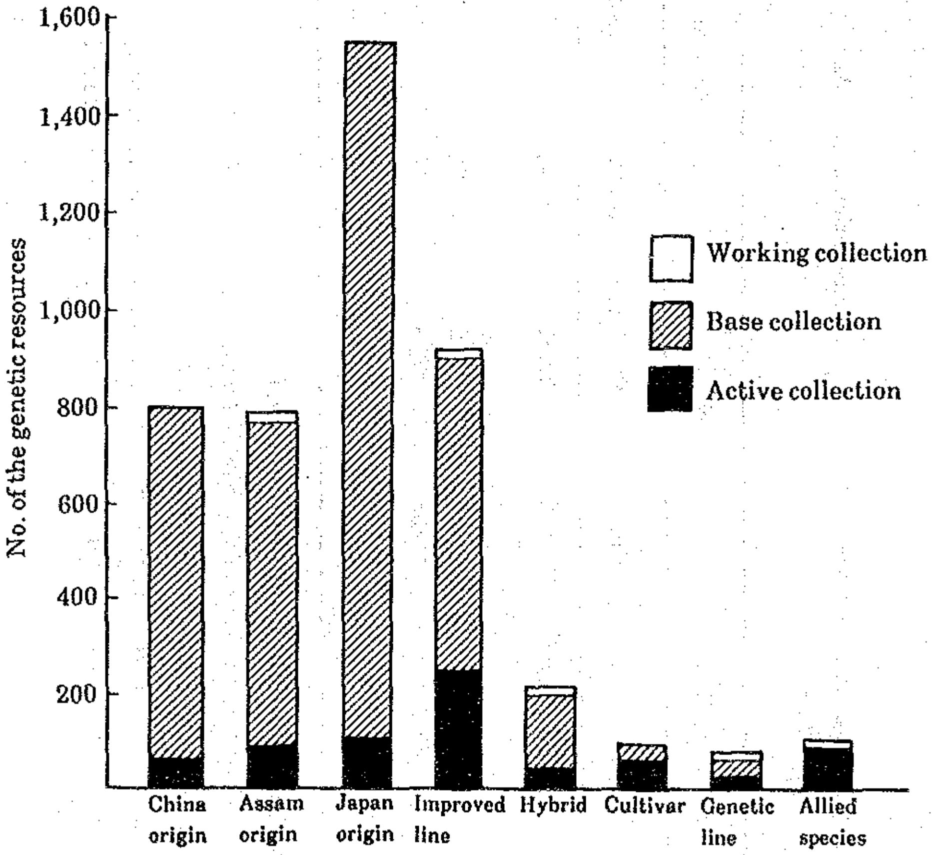


Fig.3 Genetic resources of tea preserved in NIVOT

The passport data of each plant are managed by the MAFF computer system, and accessed by terminal computers at each of National Agricultural Research Institutes related to MAFF Gene Bank. Morphological and physiological characters of tea conserved as genetic resources are recorded by a joint investigation with several stations to construct the database system for the computer.

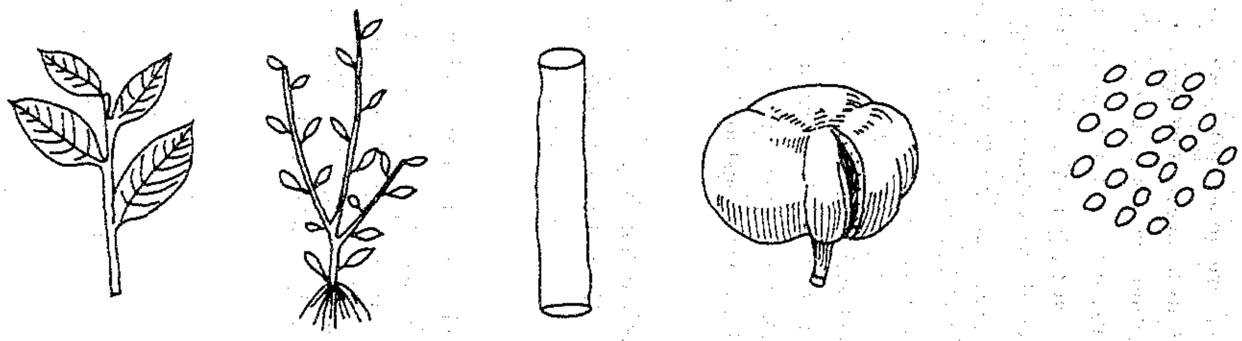
4. Conservation of vegetative organs, seeds and pollen

All of the selections and cultivars of tea are genetically heterozygotes because of their allogamous nature. Characteristics of each selection or cultivar can be maintained only by vegetative propagation such as cutting, grafting or layering. Therefore, conservation of genetic resources of tea plants is usually performed by planting in fields. Planting space for each plant in the field is 1.8m in row width and 1.0m in planting distance, then planting density is 5,000 plants per ha. As two to five plants per accession are preserved usually, it is possible to maintain about 2,500 to 1,000 accessions per ha. It means that a vast field is necessary to keep a large number of genetic resources.

This led us to try a preservation method without field. Several experiments had been conducted on the conservation of vegetative organs at a low temperature (Fig. 4). Thick roots of more than 1cm in diameter are the most suitable for conservation of organs, and it can be stored at least for 5 years (AMMA 1983).

It is also effective to preserve seed and pollen, but dehydration of tea seeds after harvest rapidly loses their germinability. Under the temperate and humid condition at a low temperature (0~5°C), tea seeds can be sustained for more than 10 years (TAKEDA 1989).

The conservation of tea pollen is relatively easy. They are viable for long term at a dry and low temperature (+5 ~ −80°C) condition. We could succeed in fertilization with pollen stored in a deep freezer at −80°C for 12 years. If pollen is stored at a super low temperature, such as in liquid nitrogen (−196°C), it might be still possible to conserve them semi permanently (TAKEDA 1989).



Germplasm	Twig	Nursery plant	Root	Seed	Pollen
Temperature of the storage (°C)	0~5	0~5	0~5	0~5	5~−80
Maximum term of the storage (Year)	1	3	5	more than 10	more than 12

Fig.4 Conservation of tea germplasm under low temperature

5. Morphological and physiological characteristics of tea preserved in Japan

1) Flower organs

Flower organs are important characters in the classification of tea as well as the leaf and plant shape. Five characters of them, that is, flower size, degree of the style length relative to the stamen, separating point of the style, constriction of the upper parts of style and hair of ovary show large variations, and they are good criteria in the classification of tea varieties (TAKEDA *et al.*1982, Fig.5 and 6).

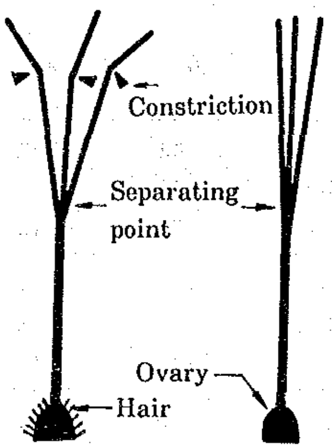


Fig.5 Shape of a pistil of tea

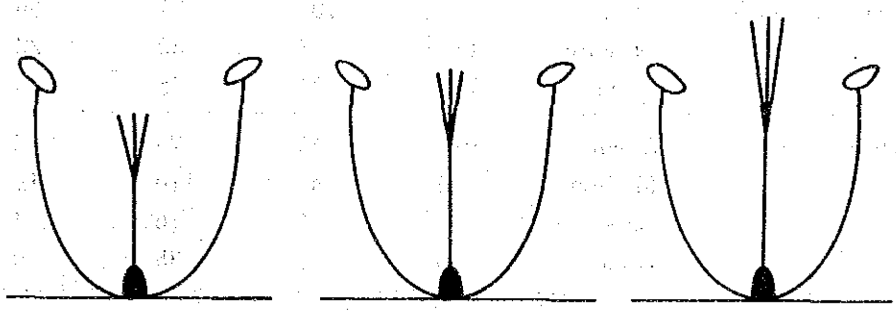


Fig.6 Degree of the style length relative to the stamen

Flower organs of Assam-type plants, especially of the materials introduced from Indo-China regions showed a large variation in comparison with those of

Japanese indigenous tea plants (Table 2). It seems to suggest that Indo-China regions are one of important places with wide genetic diversity of tea.

Table 2 Variations of the flower organs in tea genetic resources preserved in NIVOT (TOYAO and TAKEDA 1978)

Origin		Japan	China	Assam	Hybrid
Total		1,011	217	236	100
Flower size	Large	93	32	24	25
	Medium	653	172	155	61
	Small	265	13	57	14
Length of style	Long	107	131	126	80
	Medium	576	73	89	19
	Short	328	13	21	1
Separating point of style	High	380	131	213	51
	Middle	517	75	21	43
	Low	114	11	2	6
Constriction of style	Perfect	82	101	139	55
	Obscure	172	55	39	22
	None	757	61	58	23
Hair of ovary	Much	976	173	75	72
	Medium	45	41	110	18
	Less	0	0	16	4
	None	0	3	35	6

2) Cold hardiness

Tea (*C. sinensis*) has wider variation in cold hardiness than other species belonging to the genus *Camellia* (Table 3). Assam type of tea plants (var. *assamica*) is generally weak in cold resistance, while China type (var. *sinensis*) are tolerant of cold. Then, genetic resources of tea preserved in Japan showed great differences in cold hardiness according to the original areas (Table 4). The hybrids between China type and Assam type show intermediate cold resistance, since its inheritance is controlled by polygenes (TOYAO 1982).

The tea plants introduced from China into Japan were generally as hardy as Japanese indigenous plants. The original place of most of all the tea plants which are of Chinese origin and preserved in Japan are restricted to regions of the Yangtze valley. Besides, we have a few materials from the southwest of China, and it seems that they are highly susceptible to cold.

3) Disease resistance

Tea gray blight disease caused by *Pestalotia longiseta* has begun to appear in Japan since 1973. The rapid spread of this disease was closely related with dissemination of leading cultivar 'Yabukita' which is highly susceptible to the disease. Since the testing method for resistance to the disease had been established, It can be easy to evaluate the degree of disease resistance of tea plants (YANASE and TAKEDA 1987). Genetic analysis revealed that the resistance of tea plants to the disease is controlled by two independent dominant resistance gene Pl_1 and Pl_2. The gene Pl_1 which confers a high level of resistance is genetically epistatic in relation to the Pl_2 gene which confers a moderate level of resistance (TAKEDA 1988). The resistance to tea gray blight was evaluated in many tea germplasm preserved in NIVOT. It was observed that a large number of plants displayed a high level of resistance to the disease except for the indigenous cultivars (Fig. 7).

Assam type plants are commonly resistant to one more serious disease,tea anthracnose caused by *Gloeosporium teae-sinensis*, then they are very important as the gene pool of the disease resistance of tea.

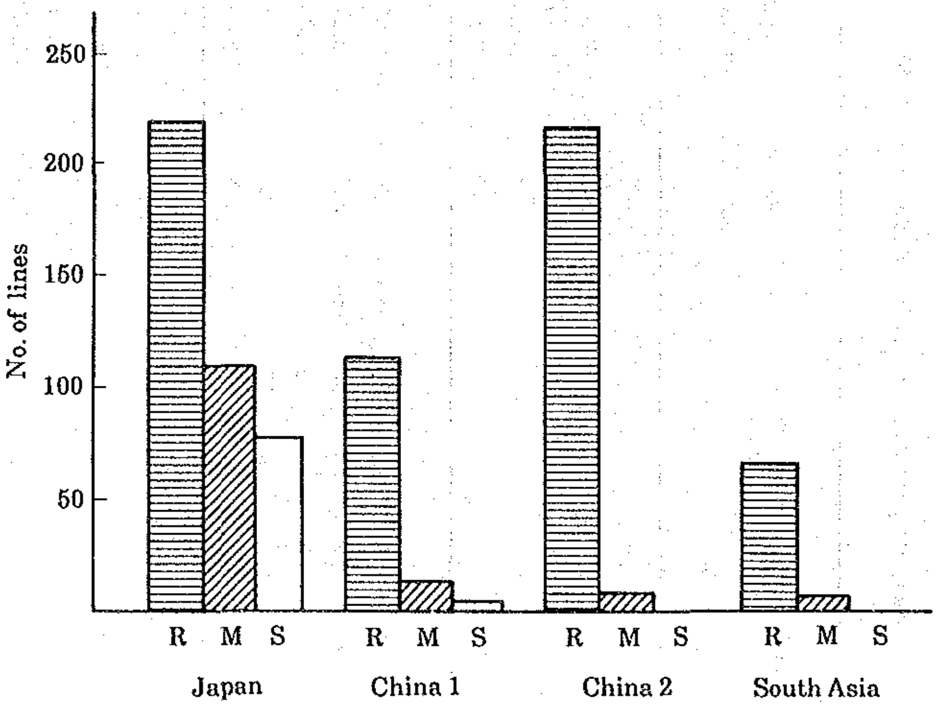
Table 3 Cold hardiness of the various species belonged to the genus *Camellia* (TOYAO *et al.* 1988)

Cold hardiness	Allied species of tea	Tea cultivars
1	*C.japonica* var. *japonica*, *C.japonica* var. *rusticana* *C.japonica* subsp. *hozanensis*	Yamatomidori
2	*C.sasanqua*, *C.tenuiflora*, *C.kissi*, *C.brevistyla*	Yabukita
3	*C.oleifera*, *C.reticulata*, *C.miyagii*	Asatuyu, Chinshin-Oolong
4	*C.rosaeflora*, *C.lutchuensis*	
5	*C.fraterna*, *C.pitardii* var. *pitardii*, *C.hongkongensis*	Hatsumomiji
6	*C.tsaii*, *C.nokoensis*	
7	*C.saliciforia*, *C.caudata*, *C.assimilis*	MAK F552
8		AI2
9	*C.taliensis*	AK176

Note: Cold hardiness score ; 1 (strong) ~9 (weak)

Table 4　Variation of the cold hardiness in tea genetic resources preserved in NIVOT (TOYAO *et al.* 1988)

Collection sites	Strong ←			Cold hardiness score			→	Weak			No. of materials	Mean value
	1	2	3	4	5	6	7	8	9	10		
China type												
Japan	122	268	113	10							513	2.02
China	71	145	12								228	1.74
(Taiwan)			3	2							5	3.40
Korea		15	30								45	2.67
Assam type												
India						3	10	21	36	80	150	9.20
Bangladesh					1	23	59	78	69	61	291	8.27
Sri Lanka					1	2	2	3	5	1	14	7.86
Myanmar								9	11	1	21	8.62
(Taiwan)							3				3	7.00
Others												
India			7	4	3						14	3.71
Iran					22	8	3				33	5.42
USSR		8	6								14	2.42
Hybrid		2	6	16	35	21	9	3			92	5.15



China 1; the mainland of China without Taiwan
China 2; Taiwan
R; Resistant, M; Moderate, S; Susceptible

Fig.7 Resistance to tea gray blight of tea plants collected from various growing areas

6. Utilization of the allied species of tea as the genetic resources for tea breeding

To improve disease resistances and cold tolerance of tea plants, interspecific cross breeding has been attempted ever since 1979. Genus *Camellia* contains many species, with 84 species being reported by SEALY (1958). As some more species were added to the genus *Camellia* in recent years, the genus *Camellia* now includes more than 100 species. These species vary in chromosome number ($2n=30\sim120$), and the representative species *C. sinensis* (tea) and *C. japonica* (camellia) have 30 chromosomes in zygote.

The interspecific cross ability is generally very low or none. The crossing rate between tea and its related species belonging to the genus *Camellia* are shown in Table 5 (TAKEDA and IKEDA 1988). Interspecific hybrids between them were usually weak, and their pollen fertility is low. Morphological characteristics of F_1 hybrids are usually intermediate between their parental species.

The hybrids between tea (*C. sinensis*) and camellia (*C. japonica*) showed a high level of disease resistance to tea gray blight and tea anthracnose. Moreover, they were highly resistant to the cold damage in winter. Regarding the chemical components in leaves, they contained very low caffeine and catechin, about 10 percent and 40 percent of those of tea, respectively (Table 6). One of them is now applied for registration under the Ministry of Agriculture, Forestry and Fisheries, both as a promising parental materials for tea breeding and as a ornamental plant (TAKEDA *et al.* 1988).

Allied species other than *C. japonica* are also greatly different from tea (*C. sinensis*) both in the morphological and physiological characteristics and in chemical components of leaves (Table 7). Thought the utilization of them for tea breeding is scarcely predictable until now, they would be used for tea breeding in the light of advance in biotechnology. At present, *C. taliensis* and *C. irrawadiensis* which belong to the same section Thea with tea (*C. sinensis*) are the most promising gene sources to improve Japanese tea cultivars.

Table 5 Results of the crosses between *C. sinensis* and its allied species in the genus *Camellia*

Cross combination Female	Male	Chromosome no. (2n)	No. of crosses	No. of fruits	Percent of fruit-bering	No. of seeds obtained	No. of seeding	No. of seedling	Cross compatibility
	(Sect. *Camellia*)								
C. sinensis	*C. japonica*	(30)	889	28	3.1 %	36	22	17	△
	C. hongkongensis	(30)	36	0	0	0	0	0	×
	C. pitardii var. *pitardii*	(30)	116	7	6.0	9	9	4	△
	C. saluenensis	(30)	92	3	3.3	4	4	0	△
	(Sect. *Paracamellia*)								
C. sinensis	*C. brevistyla*	(30)	164	10	6.1	15	8	1	△*
	C. kissi	(30)	92	19	20.7	24	18	9	**O**
	C. oleifera	(90)	253	23	9.1	34	20	5	△*
	C. sasanqua	(30,60)	122	2	1.6	2	2	2	△*
	(Sect. *Camelliopsis*)								
C. sinensis	*C. assimilis*	(30)	159	22	13.8	27	23	9	O
	C. caudata	(30)	102	24	23.5	42	42	23	**O**
	C. salicifolia	(30)	101	14	13.9	18	18	13	O
	(Sect. *Theopsis*)								
C. sinensis	*C. cuspidata*	(30)	50	21	42.0	27	27	2	**O**
	C. fraterna	(90)	166	3	1.8	3	3	0	△
	C. lutchuensis	(30)	55	0	0	0	0	0	×
	C. nokoensis	(30)	53	8	15.1	14	9	5	O
	C. rosaeflora	(90)	21	0	0	0	0	0	×
	C. transarisanensis	(-)	81	2	2.5	2	2	0	△
	C. transnokoensis	(90)	42	2	4.8	1	0	0	△
	(Sect. *Thea*)								
C. sinensis	*C. irrawadiensis*	(30)	100	29	29.0	50	48	10	**O**
	C. taliensis	(30)	123	25	20.3	36	34	24	**O**
	(Sect. *Heterogenea*)								
C. sinensis	*C. furfuracea*	(30)	84	0	0	0	0	0	×
	C. granthamiana	(60)	100	0	0	0	0	0	×
	(Sect. *Corallina*)								
C. sinensis	*C. parviflora*	(-)	26	0	0	0	0	0	?
	(*Dubiae*)								
C. sinensis	*C. drupifera*	(90)	153	2	1.3	2	2	0	△
	C. miyagii	(90)	163	27	16.6	49	49	0	O
	C. tenuiflora	(60)	109	9	8.3	10	10	5	△*

Cross compatibility; **O** High, O Medium, △ Low, × Impossible.
* It is considered that apomixis occurs in the cross combination.

Table 6 Morphological and physiological characters of F_1 hybrids between *C. sinensis* and *C. japonica*

Plant No. (Cultivar)	Flower		Pollen fertility	Cold resistance	Disease resistance		Caffein	Total amino acids	Total catechins
	Color	Size			Tea gray blight	Tea anthracnose			
Chatsubaki		cm	%				%	%	%
No. 1	Pale red	5.5	22.2	Hardy	R	R	0.34	0.963	2.10
No. 2	Pale red	4.2	28.0	Hardy	R	R	0.39	0.396	2.63
No. 3	Pale red	–	–	Hardy	R	R	0.26	0.811	4.28
No. 4	Pale red	5.4	35.5	Hardy	R	R	0.20	0.400	3.91
No. 5	Pale red	5.4	35.1	Hardy	R	R	0.46	0.898	3.56
No. 6	Pale red	4.4	47.9	Hardy	R	R	0.33	0.497	2.35
No. 7	Pale red	4.8	–	Hardy	R	R	0.47	1.860	2.00
No. 8	Pale red	5.8	28.8	Hardy	R	R	0.24	0.331	4.57
No. 9	Pale red	6.4	26.2	Hardy	R	R	0.33	1.502	2.07
C.sinensis (cv.Sayamakaori)	White	3.9	99.2	Hardy	R	R	3.15	1.290	0.93
C.japonica	Red	7.0	99.5	Hardy	R	S	0.00	0.162	0.71

Note : 'Chatsubaki's are F_1 hybrids between *C.sinensis* and *C.japonica*.
Disease resistance; R is resistant and S is susceptible to the disease, respectively.

Table 7 Chemical componets in leaves of some species belonged to the genus *Camellia* (NAGATA 1986)

Section	Species	Caffein	Theobromine	Theanin	Catechins*					Sasanquin
					EC	(+)C	EGC	ECG	EGCG	
Thea	*C.sinensis* var.*sinensis*	+++	+	+++	+++	+	+++	+++	+++	–
	C.sinensis var.*assamica*	+++		+++	+++	+	++	+++	+++	–
	C.taliensis	+++	+	+	++	++	++	++	+++	–
	C.irrawadiensis	+–	++	+	++	+	+	++	+	–
Camellia	*C.japonica* var.*japonica*	–	–	–	+++	+	–	–	–	–
	C.japonica var.*rusticana*	–		–	+++	+++	–	–	–	–
	C.saluenensis	–		–	++	+	–	–	–	+
	C.japonica var.*hozanensis*	–		–	+	+++	–	–	–	+
	C.pitardii var.*pitardii*	–		–	+++	+	++	–	–	–
Heterogenea	*C.furfracea*	–		+	+	++	+	–	–	–
	C.granthamiana	–		–	+	++	–	–	–	–
Paracamellia	*C.sasanqua*	–	–	–	+	–	–	–	–	+
	C.oleifera	–		–	–	+	–	–	–	–
	C.kissi	+–		–	+	–	–	–	–	–

Note : * EC; Epicatechin, (+)C; (+)Catechin, EGC; Epigallocatechin, ECG; Epicatechin gallate, EGCG; Epigallocatechin gallate.
–; (less than 0.01%), +; (0.01-0.3%), ++; (0.3-1.0%), +++; (more than 1.0%).
Sasanquin +; Detected, –; Not detected.

7. Breeding scheme and organizations in Japan

Tea is one of arboreal crops and needs a long breeding terms. The shortening of the breeding term is one of the most urgent projects to be solved in tea breeding.

Cross breeding is now the most common method to develop new cultivars. Standard steps of the present cross breeding scheme are shown in Fig. 8. Parents with desired characteristics, such as high quality, high yield and/or disease resistance, are chosen from the preserved genetic resources, and individuals that meet such breeding targets in each cross are selected. Another method is a direct selection from seedling gardens which have great genetic variations. The procedures are similar to the steps of the cross breeding after individual selections in Fig. 8.

Considering the tea-producing areas in Japan, the tea breeding stations are organized with a net work of breeding stations. Firstly, tea-producing areas are divided into three regions: the cool hilly region, temperate region and warm region according to the climatic conditions. Then, breeding work conducted by the national program are undertaken by the following four stations (Fig. 9).

(1) Cultivars suitable for the temperate region ('Sencha', 'Gyokuro' and 'Matcha') at National Research Institute of Vegetables, Ornamental Plants and Tea (Kanaya, Shizuoka Pref.).

(2) Cultivars suitable for the cool hilly region ('Sencha') at Saitama Prefectural Tea Experiment Station.

(3) Early budding cultivars suitable for the warm region ('Sencha' and Semi-fermented tea) at National Research Institute of Vegetables, Ornamental Plants and Tea (Makurazaki, Kagoshima Pref.).

(4) Medium and late budding cultivars suitable for the warm region ('Sencha' and 'Kamairicha') at Miyazaki Prefectural Agricultural Experiment Station, Tea Station.

In addition to the above four, two stations for testing physiological characteristics of new breeding materials and five stations for testing clonal adaptability are located in the tea planting area.

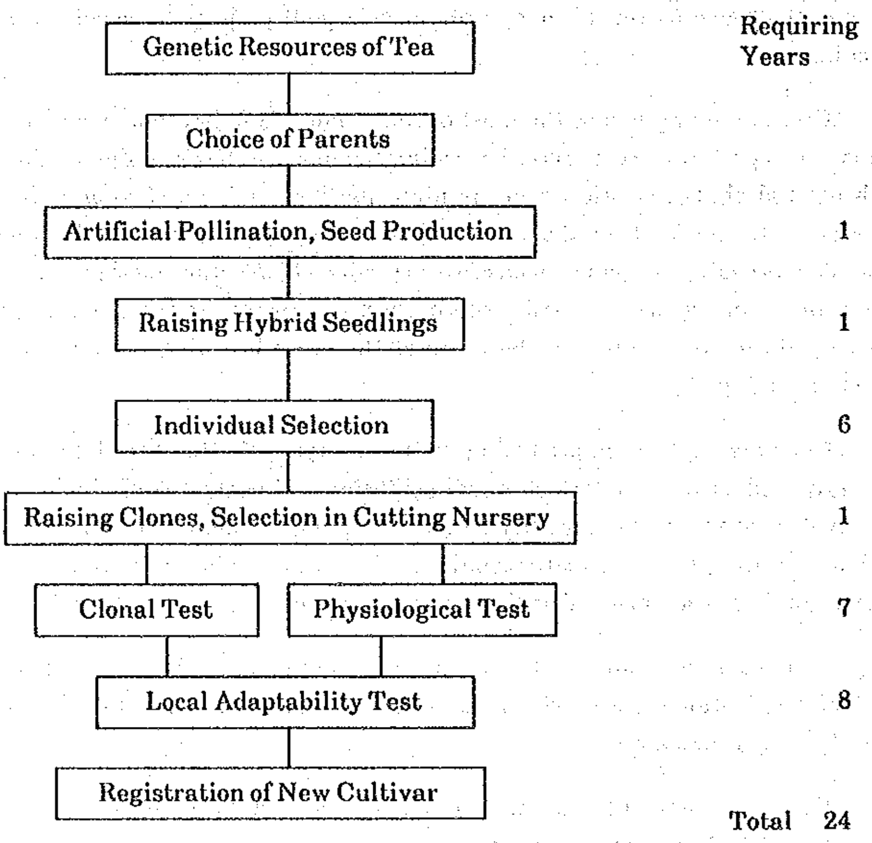


Fig.8 Steps of cross-breeding procedures and requiring years

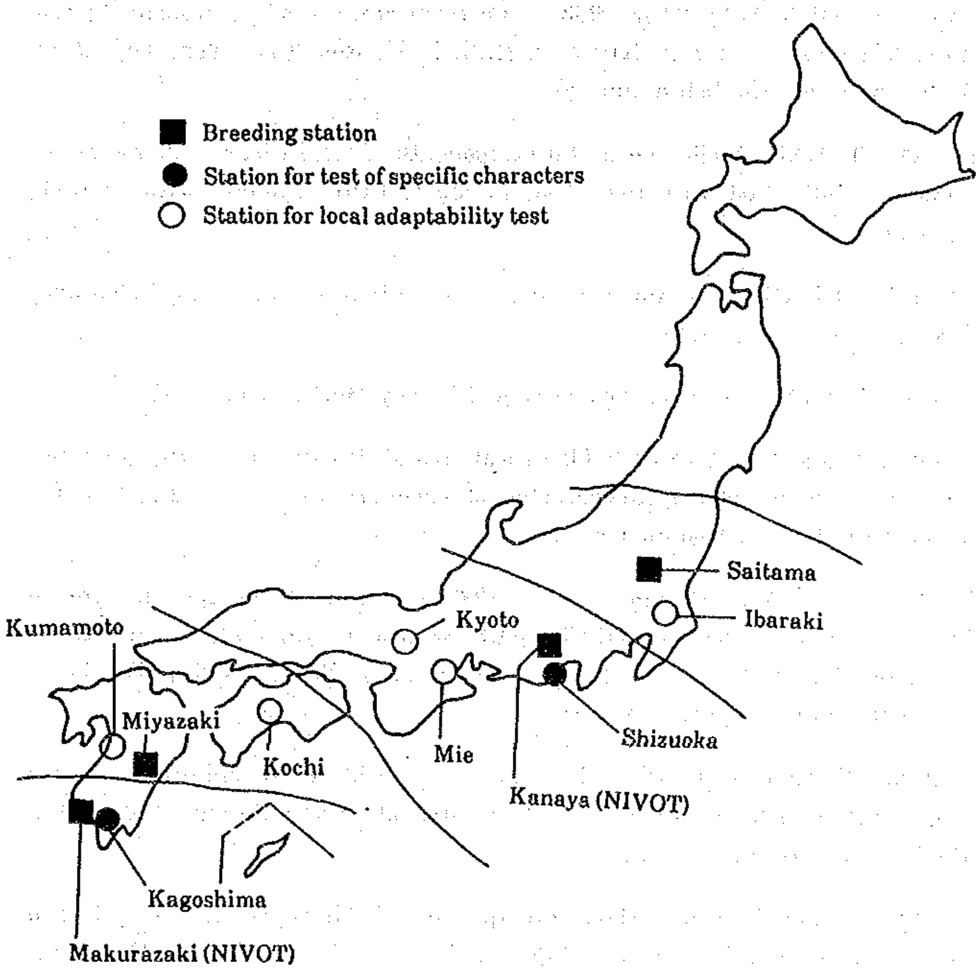


Fig.9 Organization of tea breeding in Japan

So far, twenty-two cultivars for Japanese green tea 'Sencha', 2 cultivars for 'Matcha', 6 cultivars for Japanese local green tea 'Kamairicha' and 9 cultivars for 'Black tea' have been developed by this breeding organization. More than 70% of Japanese tea gardens have been replaced by these cultivars through clonal propagation. The new cultivars have greatly contributed to an increase of the tea production and the improvement of tea quality in Japan.

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Photo 1 Genetic stocks of Assam type of tea
(Makurazaki Branch Station of NIVOT)

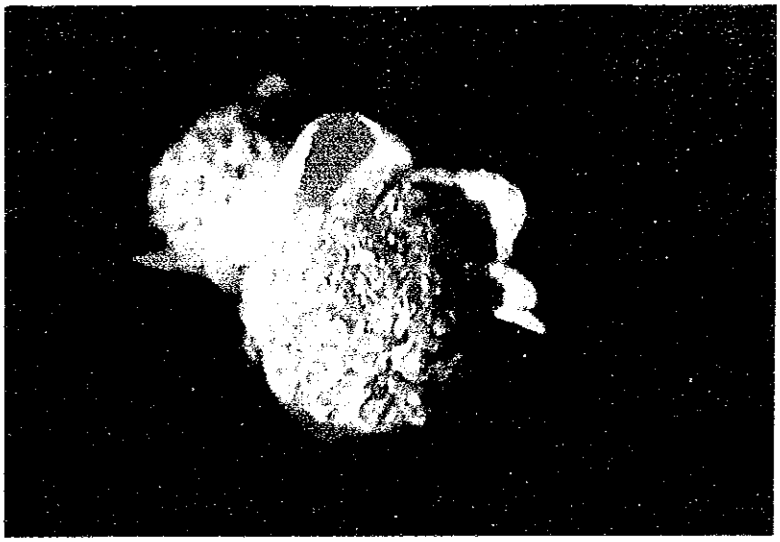

Photo 2 Flower of a mutant induced by γ-ray irradiation

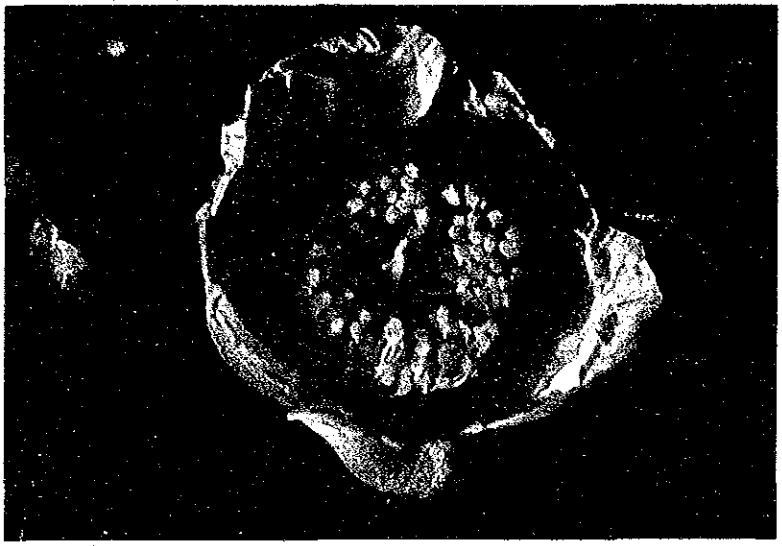

Photo 3 Flower of a interspecific hybrid between *Camellia sinensis* and *C. japonica*

Photo 4 Crossing practice in the field of genetic stock

I -2. Exploration and Collection of Plant Genetic Resources and Plant Quarantine

by

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CONTENTS

1. Introduction

If plant pests and diseases infiltrate into a new region, they often cause more damage on agricultural and green resources than the proper ones. No one can predict how the introduced pests and diseases will perform in their new location and environment. If the climate of the region would be favorite for the pests and diseases and there is no natural enemies and competitors for them and with more susceptible host plants available, the pests and diseases, which are not serious organisms in the place of origin, may have more serious effects in their new environment.

The spread of pests and diseases comes from following three ways; ① its own movement, ② delivery by rain and wind, ③ carrying together with host plants.

Recently, because of the rapid development of the transportation system, the speed of transportation as well as the chance of invasion of pests and diseases to multiply has increased. The exchange and transfer of plants and plant products has increased, not only for food supply, but also for varietal renewal and/or improvement and the introduction of genetic resources. Therefore, it is very important that these materials to be planted should be completely free from any pest and disease.

Once contaminated seeds and seedlings are transported, not only those introduced but also pre-existing favorable genes or useful genetic resources which have already been collected and preserved could be seriously damaged by infection.

When the large quantities of consumers goods, such as fresh fruits, vegetables, grain and cereals, logs are imported from definite areas and well managed in general, pests and diseases detected would be specific species, then it is possible to inspect and disinfect appropriately at the port of entry.

On the other hand, the vegetatively propagated plant material, including genetic resources, would be imported as various types such as seeds, seedlings, scions, tubers and bulbs and their origin would be unspecific and vast regions. They are including not only cultivated varieties but also wild species. So, the phase of pests and diseases are diverging. These materials will be stored and planted after importation, then the pest risk that those pests and diseases is to be established, and spreading to other plants, would be large. The significance of plant quarantine for these plant materials would be great.

2. History of plant quarantine

In 1348, Bubonic Plague was prevalent. Venice and Jenova in Italy isolated the ship arriving from countries to determine whether they would develop this disease or not for 40 days. The term "quarantine" is derived from the Latin word "quarantum" or Italian word "quaranta" meaning forty.

Plant quarantine originated from the incident in which Germany banned the import of grape seedlings for propagation by the proclamation of the "Grape Pest Insect Protection Law" of 1872, after the invasion of the grape *phylloxera* from the USA to Europe.

On the other hand, various states of the USA initiated plant quarantine measures. California's first law was passed in 1881. Federal participation began in 1912, with the passage of the Plant Quarantine Act. And many countries began prohibiting or restricting the import of plants and plant products from foreign countries, or required phytosanitary certificate from the exporting countries.

Nevertheless, as international transportation has become much more active, several problems, such as the technical difficulty of preventing the spread of pests and diseases, the disturbance of international trade by the arbitrary quarantine procedures of each country have arisen. Then the establishment of a unified agreement was discussed in order to counteract the spread of pests and diseases more effectively. In 1951, at the International Plant Protection Convention of FAO "the International Plant Protection Treaty" was agreed upon and signed by 30 countries from Europe, America, Asia and Africa for international cooperation to protect plants and plant products against the spread of pests and diseases. So far, more than 90 countries have joined or signed the treaty. This treaty is now being revised in accordance with the changed status of international trade, by a committee consisting of European and other countries. Revised points are as follows;

(1) Each countries list up and publish the quarantine pests.

(2) A phytosanitary certificate is to certify that a given consignment is entirely free from quarantine pests and substantially free from other pests.

(3) This revision also includes a model phytosanitary certificate for reexport.

3. Principles and significance

Plant quarantine is aimed at preventing the introduction and spread of hazardous organisms into new area, and is the duty of the governmental agencies to protect the agriculture and forestry of the area and the environment. It banns or restricts the entry of plants, plant products, soil, cultures of living organisms and packing materials. And also emergency control of major pests and diseases which may unfortunately have already penetrated is included.

1) Plant quarantine as a control measure

The protection of plants from pests and diseases means the elimination of the pathogen infection and pest parasitism, or at least reducing the damage caused by these incidents. That is, crop protection includes pest or disease resistant plants, chemical, cultural and biological control and plant quarantine.

According to KAHN 1977, the control measures can be classified into the following six categories; ① Avoidance, ② Exclusion, ③ Plant resistance, ④ Eradication, ⑤ Protection, ⑥ Therapy.

Number ① to ⑤ are preventive and number ⑥ is to cure. Quarantine is effective for exclusion and eradication. Under exclusion, the entry of organisms is prevented by inspection and treatment, or the host being banned or restricted. Under eradication, infested or infected plants or plant products are removed at the port of entry, or the plant material is subjected to treatment or therapy.

In this way, plant quarantine is functioning as a control measure of pests and diseases by preventing invasion and spread of hazardous organisms.

2) Pathways for the entrance of pests and diseases

The invasion of pests and diseases into new areas occurs, following either natural pathways such as hurricanes, wind, rain, running water and living vectors, or by human beings. The keystone of plant quarantine is human action as vectors or agents of pests and diseases. In fact, spores of fungi and pests are transferred for long distance by a current of air. The geographical movement of pests and diseases into new areas are carried together with infected or contaminated plant materials by man.

Before long-range transportation systems developed, the importance or necessity of plant quarantine had been minimal since the chance of human beings acting as vectors was small. Recently, however, along with the progress of the transportation system for food supply, pests and diseases have been transferred by man, making the significance of plant quarantine very great. Even scientists may act as carriers of pests and diseases when they travel by air.

3) Regional plant protection organization

One of the basic principles of plant quarantine is that the larger the landmass covered by uniform quarantine regulations, the greater the protection to any area therein. This means that the absence of coordinated action within members of a community is likely to impair the defence mechanisms aimed at preventing or retarding the entry of exotic pests and diseases that are major threat to a bio-geographical region. Then regional or international plant protection organizations are organized based on this principle and include the followings;

(1) Plant Protection Committee for South East Asia and the Pacific Region (SEAPPC).

(2) Near East Plant Protection Commission (NEPPC).

(3) Caribbean Plant Protection Commission (CPPC).

(4) European and Mediterranean Plant Protection Organization (EPPO).

(5) Organismo International Regional de Sanidad Agropecuaria (OIRSA).

(6) Comite Interamericano de Protection Agropecuaria (CIPA).

(7) North American Plant Protection Organization (NAPPO).

(8) Inter-African Phytosanitary Council (IAPSC).

(9) Junta Del Acuerao De Cartagena (JUNCA).

These organizations are organized within the framework of the FAO or cooperate with the FAO.

4) Organisms of quarantine significance

Organisms of quarantine significance may include any pest or pathogen that is considered to pose a threat to the country's agriculture and environment. Among organisms of quarantine significance, pests and pathogenes which have never occurred in an area or country are most important, and exotic strains or races, even if they have already entered into the area, are considered to be more significant than others.

NEERGAAD 1977 considered pests and pathogenes to fall within the following categories;

Category A: Dangerous plant pests and pathogenes that are not present in the region of introduction and have a high or considerable epidemic potential.

Category B: Dangerous plant pests and pathogenes that are not present in the region of introduction (or present only in more or less restricted areas under control) and have a moderate epidemic potential.

Category C: Other remarkable pests and pathogenes to the planting value of the seed (or vegetative propagules).

The 20th session of the FAO conference in 1979 proposed the following definite terms about pests and pathogenes.

The term "Pest" means any form of plant or animal life or any pathogenic agent, injurious or potentially injurious to plants or plant products. "Quarantine Pest" means a pest of potential national economic importance to the country endangered thereby and not yet present there, or present but not widely distributed and being actively controlled.

The European and Mediterranean Plant Protection Organization has a huge biological area. When EPPO defined quarantine pests, it selected those that correspond to the definition of the quarantine category and fit the following description.

① They are known to cause major damage to plant hosts that are economically important in some or all EPPO countries.

② They are unlikely to spread to EPPO countries by natural means.

③ There is a real probability of spread by current or anticipated international trade or scientific exchanges.

④ They appear capable of surviving and reproducing in some or all EPPO countries either in the field or in protected crops (under glass).

Based on these ideas, pests and pathogenes are derived into two groups (A1 and A2) and listed up.

A1 list: Pests and pathogenes not yet introduced into the EPPO region. For these quarantine organisms, a zero tolerance is required for all countries.

A2 list: Pests and pathogenes present in some but not all EPPO countries or else subject to internal quarantine regulations in some countries. A zero tolerance should normally be required for them.

In Japan, those hazardous organisms which fall into the following categories and have not been recorded in Japan are considered to be as harmful as banned noxious organisms;

① Those whose hosts are economically valuable plants for our agriculture and forestry, and may seriously damage them.

② Those that may cause a destructive hazard to plants other than ①.

③ Those that are strongly infectious in the fields.

④ Those that are adaptable to the climatic condition.

⑤ Those with a wide range of hosts.

⑥ Those that have little possibility of control and require a great amount of expense.

⑦ Those that are cautioned against entering into other countries.

5) Genetic resources and disease resistance

As for gene center of disease resistance, LEPPIK 1970 described that the primary and secondary gene centers of cultivated plants are the best places to find genuine resistance to common diseases and pests. That is, host and parasite have long been associated in their centers of origin as reciprocal selective factors in evolution. Every new and more virulent race of parasite must necessarily have eliminated most of the susceptible individuals in the local host population. Therefore the survivor will have resistant gene to them.

For example, the following vegetatively propagated plant materials are suggesting the hypothesis described above.

Grapes: The gene center of the Genus *Vitis* is in North America. Only the cultivated wine grape, *Vitis vinifera* L. and its wild progenitor, *V. vinifera* var. *sylvestris* are Eurasian with endemic gene pools of both species in Transcaucasia and Middle Asia. In the Far East there is an isolated gene center of the endemic species, *V.amurensis*. It is well known that the American species are resistant to *Plasmopara viticola* and *Phylloxera devastratix,* and that the European species are highly susceptible to these American disease and pest. Crossing of European grapes with American species, however, enable the transfer of genes for immunity and production of resistant cultivars.

Potato: The cultivated potato, *Solanum tuberosum* L. evidently originated in the Andes, but that the late blight pathogen (*Phytophthora infestans*) originated in a small area of Central America (Mexico and northern Guatemala). Blight-resistant wild potatoes are concentrated only in central Mexico, which is evidently the gene center of this pathogen. This indicates that the cultivated potatoes are susceptible to this pathogen.

Fruit trees: The gene centers of wild fruit trees of the family *Rosaceae* are scattered over a wide area on the European continent. The most important subfamily *Pomoideae* is obviously of Eastern Asiatic origin, though the original gene centers and evolution of the genera *Malus, Pyrus, Prunus, Cydonia, Mespillus* and *Amygdalus* are not yet fully established. There are, for instance in the genus *Malus*, five centers of speciation in East Asia, Middle Asia, Caucasus, Europe and North America. In connection with the geographic distribution of the genus *Malus*, disease resistance is typical of several East Asiatic species, whereas the Middle Asiatic, European and American species are susceptible to diseases. Scab(*Venturia inaequalis*) resistant species are *Malus hupehensis, M.Pumila* and *M. micromalus* from China and *M. formosana* from Taiwan. This indicates that the East Asiatic center might be the original area of the genus *Malus*.

The genera *Pyrus* and *Prunus* originated in China with resistant species such as *Pyrus serotina, P. bretschnederi, P. calleryana, P.phaeocarpa* and *P. usmatuana.*

According to these suggestion, the pest risk of invasion into new area would be great in the exploration and collection of plant genetic resources. In this meaning, plant genetic resources must be completely free from any pest and disease. And also the significance of plant quarantine would be of great.

4. Methodology

Plant quarantine regulations are enacted by state and federal governments to reduce the chances that man may transport organisms that can harm his agriculture and environment. Minimum standards for national regulations are often recommended by international plant protection organizations or commissions.

1) Plant quarantine regulations

Common features among plant quarantine regulations of many countries of the world are as follows;

(1) Specify prohibition.

(2) Grant exceptions to prohibitions for scientific purposes.

(3) Require import permits.

(4) Require phytosanitary certificates.

(5) Stipulate inspection upon arrival.

(6) Prescribe treatment upon arrival to eliminate risk.

(7) Prescribe quarantine, post entry, isolation or other safeguards.

The pest and pathogen risk associated with the international exchange of germplasm can be lowered by regulating the entry. The regulations are including the following; ①Prohibited -- the pest risk is so great that existing safeguards are not adequate: Special importation can be permitted for the purposes of scientific use. ②Post-entry quarantine -- the pest risk is high, so that post-entry quarantine would be carried out by the government quarantine station. ③Restricted, a permit is required which may stipulate certain conditions of entry -- plants are subject to inspection and treatment upon arrival. ④Restricted, although a permit is not required -- plants are subject to inspection and treatment upon arrival. ⑤Unrestricted.

And, pest risk of these germplasms can be lowered by regulating ①the volume of consignments ②the size, and sometimes age of the plant materials ③the type of plant materials imported. The types of plant materials, arranged in order to increasing pest risk are ①seeds without fruit pulp or debris ②dormant scions or cutting without roots ③plants with roots without soil and ④plants with roots and associated soil. (Soil and plants with soil attached are prohibited in Japan.)

Packing materials are regulated to lower the pest risk. Among the packing materials approved most often are charcoal, excelsior, vermiculite, sphagnum moss and so on. Packing materials usually not approved are wheat or rice straw or hulls, hay, soil and used materials. Germplasm imported as seed should be placed in unused cloth or paper bags or envelopes.

2) Inspection and detection

Germplasm, whether imported as seeds, plants, or plant parts, are inspected upon arrival by governmental plant protection service. The consignment should also be accompanied by a phytosanitary certificate issued by exporting country.

Inspection refers to visual examination, often with the aid of a microscope, for signs and symptoms of pests and pathogenes. Inspection techniques may include examination for eggs of mites and insects, fruiting bodies of fungi, hitch hiking insects, wood borers, and examination of unauthorized packing materials such as rice straw, and examination of soil-borne organisms including cyst nematodes. Observation is supplemented by soft X-ray, isolation of organisms from infected tissue in moist chambers, electron microscopic observation to detect virus particles, serological reaction and microscopic examination for bacterial ooze. These inspection methods mentioned above are not sufficient to detect viroid and virus. When any germplasm is imported for vegetative propagation, ①the host is dormant upon arrival, ②the plant has been infected only recently and the incubation period is incomplete, or ③the virus is latent. Therefor virus indexing consists of transmitting the infectious agent from a plant in which it may or may not incite symptoms to healthy indicator plants known to show symptoms. Transmission may be based on mechanical or grafting procedures or the use of insect vectors.

3) Treatment

The possibility of the entry of harmful organisms may be decreased by destroying or eradicating pests and pathogenes by a treatment of plants and plant products when they are imported.

(1) Heat treatment

Heat treatment or thermotherapy may be administered by hot water, hot air, vapor heat. The basic principle involved is that plant material must be heated to a temperature that will kill the pest or pathogen without killing the plant or plant part. The margin between the two temperatures is usually very narrow, so temperature control must be accurate.

(2) Chemical treatment

Chemical treatment may be applied to dormant or actively growing plant materials as sprays, dips, dusts or fumigants. The toxicant used to eradicate the organism must be sufficient enough to kill the organism without killing the host. In general, dormant plant materials,such as seeds and scions imported as genetic resources, are more tolerant to chemical treatment than actively growing ones.

(3) Heat therapy and meristem-tip culture

Heat therapy and/or meristem-tip culture are applied to make a pest and pathogen free plant. This technique is utilized to excise the health portions of plants from infested or infected plants.

(4) Excision of diseased parts

Excision can be used as a safeguard to remove infected parts, particularly when the incidence of infection is low. Excised portions could be destroyed in a phytosanitary manner, and the rest of the consignment is treated by heat or chemical treatments as a precaution.

5. Present status of plant quarantine in Japan

The entry into Japan of harmful organisms along natural pathways is quite rare, as the land is surrounded by the sea. But Japan ranges along distance from north to south, plants and crops in subtropics, temperate zone and northern regions are widely distributed, giving various organisms a good chance to invade. Moreover, recently a large amount of plants and plant products are imported to Japan in order to supplement insufficient agricultural production. This fact also widen artificial pathways for the entry of harmful organisms. This gives plant quarantine in Japan a great significance.

Japanese plant quarantine program was first started in 1914. "Plant Quarantine Law" on which present plant quarantine procedures are based, was enacted in 1950, after several amendments. According to the plant protection law, the objectives are to inspect exporting, importing and domestic plants to control pests and diseases, and to prevent the outbreak and spreading there of, there by to ensure stabilization and development of agricultural production. Japanese plant quarantine consists of international quarantine, that is import and export quarantine, and domestic quarantine, including quarantine of designated plants, control of plant movement and emergency control. Import quarantine aims to prevent the infiltration of pests and diseases from foreign countries. Export quarantine aims to prevent the invasion and spread of pests and diseases in response to requests by foreign countries. Domestic quarantine aims to prevent the spread of major pests and diseases within the territory. Import plant quarantine system is shown in Fig. 1 and the volume of import and export inspection in 1988 is shown in Table 1. The followings are on quarantine of seeds and vegetatively propagated materials to be imported as genetic resources.

1) Seed quarantine

All plant pests and diseases have been regarded as the objects for quarantine, and a phytosanitary certificate issued by the authorities in the exporting countries is required at the port of entry.

First, at the port of entry, the imported seed is subjected to visual inspection for such contaminants as ergots, sclerotia, nematode galls and soil, and then to sieving inspection for pests.

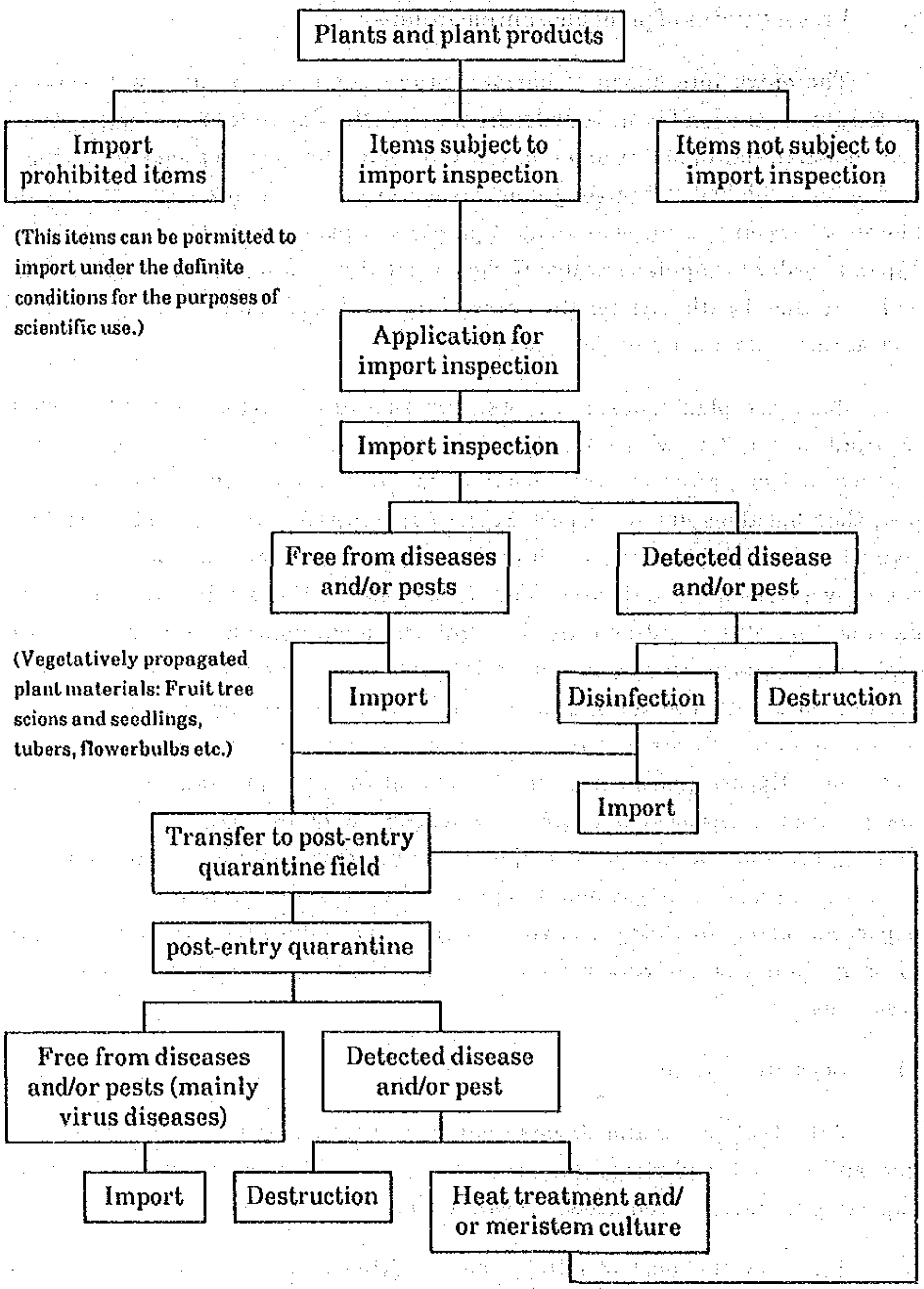

Fig.1 Import plant quarantine system in Japan

Table 1 Volume and number of import and export inspection in 1988

Items	Import	Export
Plants and plant parts to be planted	349 mil.pcs	7,364,000 pcs
Flower bulbs	114 mil.pcs	10,433,000 pcs
Seeds	26,433 tons	1,856 tons
Fruits	1,566,000 tons	16,652 tons
Vegetables	485,000 tons	1,620 tons
Grain and cereals	29,933,000 tons	30,172 tons
Beans	5,923,000 tons	370 tons
Logs	30,791,000 m^3	63,801 m^3
Others (Chinese herbs spice crops)	6,243,000 tons	6,103 tons

Secondly, representative samples are drawn from the seed lots and they may be subjected to detailed inspection, including microscopic examination, the blotter test, and inspection by FENWICK's method. A soft X-ray apparatus may be used to check for seed wasps which feed on seeds of certain trees and legumes.

A flow chart of the procedures for seeds is shown in Fig. 2.

Standardized inspection procedures are applied to seeds which may have high risk of carrying some of the listed seed-borne pathogenes. Descriptions of standardized procedures include such items as; host plants, distribution of the pathogen, detection procedure, key points for identification, and the treatments.

Rice seeds usually enter Japan only for scientific use under the Ministrial Permit. When issuing this permit, plant quarantine officials specify the safeguards necessary to prevent introduction of exotic pests and pathogenes. Progeny seeds from seed-bearing plants grown from introduced seed are released if they are found completely free from pests and diseases.

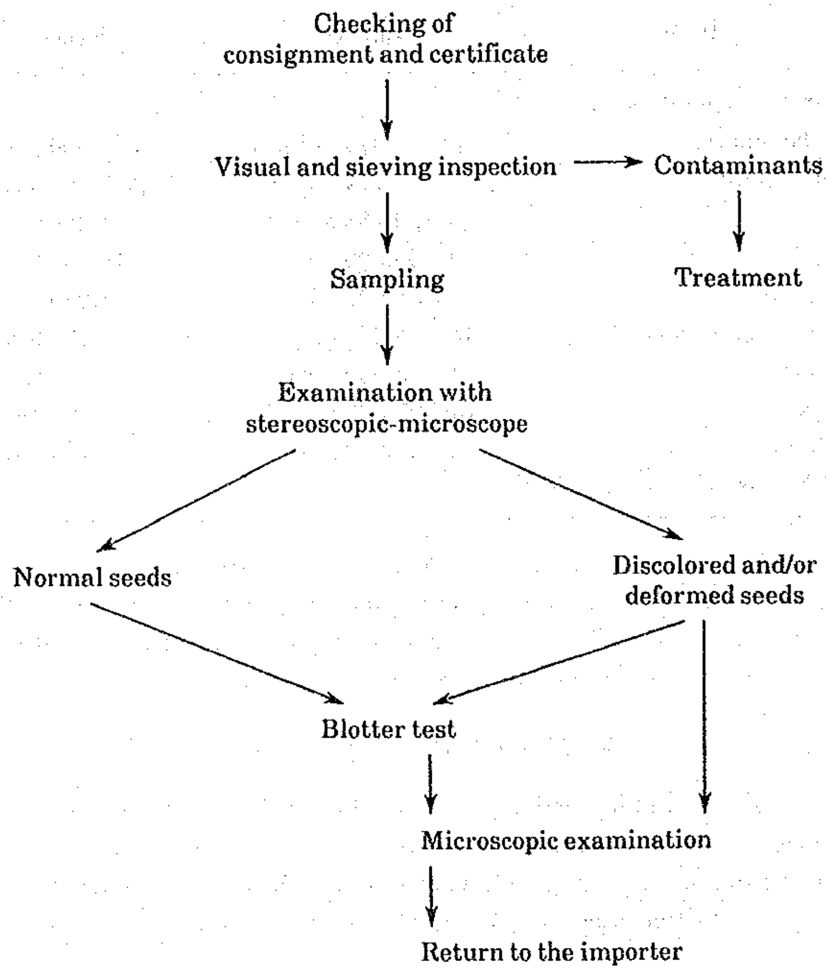

Fig.2 Flow Chart of inspection procedure for seeds

2) Post entry quarantine

In addition to routine import inspection at the seaport and airport, post-entry quarantine is required in order to check for injurious pests and diseases, mainly virus diseases which can not be detected, or are detected with difficulty at the port of entry. The subject plants of post-entry quarantine are; orchard trees (apple, pear, grape, peach, plum, citrus etc.), tubers (potato, sweet potato), sugarcane, pineapple, strawberry seedlings and flower bulbs, which are vegetatively propagated. These plants are inspected for viral infection in the post-entry quarantine field as well as for pests and diseases at the port of entry.

Procedures: After an affirmative examination at the port of entry, these seedlings are transplanted into the post-entry quarantine field. Then the viral inspections are carried out at the post-entry quarantine field, while growing. The inspections are administered by visual examination, by sap inoculation and graft inoculation to various indicator plants that develop virus symptoms, by serological reaction, electron microscopic observation to examine virus particles, by electropholesis etc. When no pests and pathogenes are found upon examination, the goods are returned to the importer. The flow chart of post-entry quarantine is shown in Fig.3. Table 2 shows the number, inspection results and intercepted virus from fruit trees and tubers imported as genetic resources during 1983-1988.

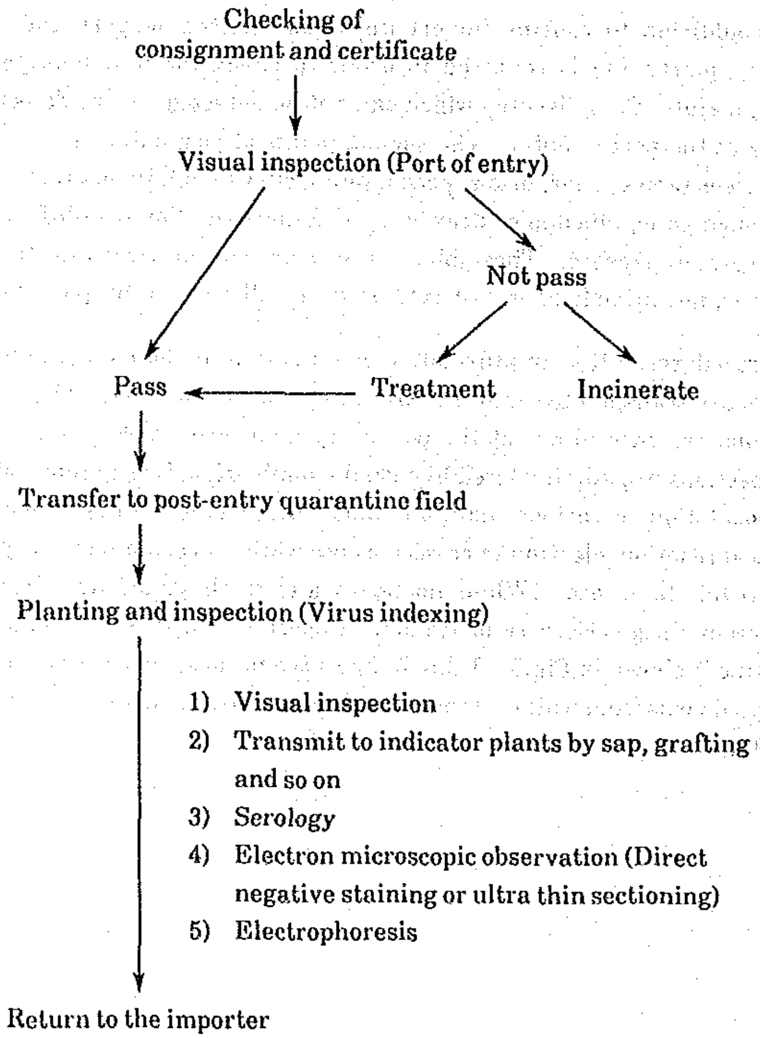

Fig.3 Flow chart of inspection procedures for vegetative propagations

Table 2 Post-entry quarantine results of plant genetic resources (1984-1988)

Plant	1984		1985		1986		1987		1988		Total		Detected viruses
	ins.	appr.	ins.	appr.	ins.	appr.	ins.	appr.	ins.	appr.	ins.	appr.	
Citrus	351	15	93	0	248	0	200	0	461	10	1,353	25	CTV, CEV, CTLV, CG, CP
Grapevine	234	103	88	30	41	29	15	8	175	insp	553	170	GLRV, GFV, FI., TomRSV, UIV
Apple	32	13	41	9	37	6	34	2	210	insp	354	30	ACLSV, ASGV, ASPV, ApMV
Pear	96	22	29	12	64	35	30	27	0	0	219	96	ASGV
Stone fruit	205	83	269	127	242	88	124	51	28	19	868	368	NRSV, PDV, ACLSV, ASGV
Small fruit	56	36	0	0	4	1	1	1	0	0	61	38	TomRSV
Other Fruit tree	158	19	34	17	23	1	13	6	0	0	228	43	SMV
Total	1,132	291	554	195	659	160	417	95	874	29	3,636	770	
Potato	90	21	109	77	166	142	20	17	31	25	416	282	PVX, PVY, PVS, PAMV, PLRV, PSTV
Sweet potato	0	0	141	29	68	19	127	39	44	40	380	127	SPFMV, SPMMV, UIV
Total	90	21	250	106	234	161	147	56	75	65	796	409	

Remarks: 1) stone fruit; peach, plum, apricot, nectarine, almond, cherry, ume.
2) small fruit; raspberry, blueberry, gooseberry.
3) other fruit trees; walnut, chestnut, myrica, sugarcane.
4) ins.; number of plants inspected. 5) appr.; number of plants approved. 6) insp; inspecting.
7) virus name; CTV-Citrus tristeza v., CEV-Citrus exocortis viroid, CTLV-Citrus tatter leaf v., CG-Citrus greening disease, CP-Citrus psorosis disease, GLRV-Grapevine leaf roll v., GFV-Grapevine fanleaf v., FI-Grapevine fleck disease, TomRSV-Tomato ring spot v., ACLSV-Apple chlorotic leaf spot v., ASGV-Apple stem grooving v., ASPV-Apple stem pitting v., ApMV-Apple mosaic v., NRSV-Prunus necrotic ring spot v., PDV-Prune dwarf v., SMV-Sugarcane mosaic v., PVX-Potato virus X, PVY-Potato virus Y, PVS-Potato virus S, PAMV-Potato aucuba mosaic v., PLRV-Potato leaf roll v., PSTV-Potato spindle tuber viroid, SPFMV-Sweet potato feathery mottle v., SPMMV-Sweet potato mild mottle v., UIV-Unidentified virus.

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II. Reports from Exploration and Collection of Native Germplasm in Japan

1. Collection of Indigenous Sweet Potato Varieties in the Okinawa Islands

   by Isao TARUMOTO
   National Agriculture Research Center

2. Genetic Diversity of Fruit Characteristics in Natural Fruit Tree Community - *Myrica rubra* forest in Japan, a marvelous sample of natural conservation of fruit crops

   by Ichiro KAJIURA
   National Institute of Agrobiological Resources

3. Collection and Identification of Taro Varieties in Japan

   by Kenji TAKAYANAGI and Masashi HIRAI
   National Research Institute of Vegetables, Ornamental Plants and Tea

II-1. Collection of Indigenous Sweet Potato Varieties in the Okinawa Islands

by

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CONTENTS

1. Background

Sweet potatoes have been cultivated all year round in the subtropical Islands of Okinawa for many years, as they have been in their original region. Since their first introduction to Miyako Island in 1594, sweet potatoes may have arrived several times from such places as China and the Philippines by way of various southeast and south Pacific islands. In modern times, they have been introduced from the main island of Japan, Taiwan, America and other countries, and hybrid varieties have been developed through cross-breeding in Okinawa Prefecture. Many types of the crop settled in the Okinawa Islands as an indigenous group and had been cultivated as a major staple food until about 1960.

Since about 20 years ago, however, the number of indigenous varieties has been reduced due to an invasion of the sweet potato weevil and to changes in the economic situation as well as in food preferences. Thus, it becomes critically important to collect, evaluate and preserve the indigenous varieties of sweet potato in the Okinawa Islands.

Until 1944, the Sweet Potato Breeding Laboratory existed in Oroku Village, Okinawa Prefecture, for producing hybrid seed by artificial crossing. At the end of the World War II, however, this laboratory was relocated to Ibusuki City, Kagoshima Prefecture (currently Kyushu National Agricultural Experiment Station). For nearly 30 years since then, Okinawa was under the administration of the American Government, so that little information was available concerning sweet potato cultivation. Since 1945, no sweet potato varieties has been brought into the main island of Japan from Okinawa Prefecture. And the sweet potatoes was added to the list of restricted crops under the Plant Protection Law when Okinawa was returned to Japan in 1972.

This survey plan was conducted to overcome the gap in the flow of information and materials by the collection and evaluation of indigenous sweet potato varieties in the Okinawa Islands, and for future utilization by the preservation of them.

Some particulars of the survey are as follows:

1) Collectors: Dr. Isao TARUMOTO and Mr. Hiromi ISHIKAWA, Head and Senior researcher of the Sweet Potato Breeding Laboratory, National Agricultural Research Center.

2) Period and locations

In 1987, 82 samples of the indigenous sweet potato varieties were collected from the northern part of Okinawa Island, the southern part of Miyako Island, all of Ishigaki Island, and the northern part of Iriomote Island.

As the value of the collected indigenous varieties proved to be greater than anticipated, the survey plan was extended. In 1988, 86 samples were collected during an 8-day survey of the southern part of Okinawa Island, the northern part of Miyako Island, the southern part of Iriomote Island, and all of Yonakuni Island.

At the same time, 53 samples were collected from three islands of Miyako County, Kuro-shima Island in Yaeyama County, and Aguni and Kume Islands in *Shimajiri County*.

3) Cooperating persons and organizations

The efforts of the following persons are acknowledged here with the many others who cooperated with the team during survey period.

Dr. Masao HOSHINO, Tropical Agriculture Research Laboratory, College of Agriculture, University of the Ryukyus

Drs. Shinjiro KATOH and Isao YAMAGUCHI, Okinawa Branch, Tropical Agriculture Research Center

Messrs. Masaki SHIMABUKURO, Jyokichi SHIMANAKA and Yoshifumi TAKAEZU, Crop Department, Okinawa Prefectural Agricultural Experiment Station

Mr. Masato ISHIMINE, Miyako Branch, Okinawa Prefectural Agricultural Experiment Station

Mr. Kenji KINA, Yonakuni Station, Yaeyama Agricultural Extension Office, Okinawa Prefecture

2. *Results of the survey*

Although the evaluation of collected materials is still underway, the size and characters of the collection can be summarized as follows:

1) Sweet potato cultivation in the Okinawa Islands

Sweet potatoes can be cultivated all year round in the Okinawa Islands which are located in the sub-tropics with a minimum temperature of 4°C. However, due to the severe damage resulting from the invasion and rapid reproduction of the sweet potato weevil and the West Indian sweet potato weevil in the 1960s, relatively good harvest can only be obtained in Autumn-Winter cropping system, in which sweet potatoes are planted in August or September and are harvested from December to February. Despite the system, the yield is low, and 20% to 30% of the harvest is still damaged by the insects. For this reason, cultivation area is limited only for the local market. During the survey period, commercial cultivation of sweet potato was seen only in some parts of Okinawa Island (Shimajiri County), Miyako Island and Ishigaki City. Nevertheless, there are a lot of small fields planted to sweet potato for domestic consumption. Except for the urban areas in the Central-north of Okinawa Island and downtown areas of Hirara City and Ishigaki City, thirty to fifty percent of farm families are found to be planting sweet potatoes in their home gardens or nearby vegetable gardens. They consumed sweet potatoes as a tuberous root crop and as well as vegetables using stems and leaves, or as a cover crop to prevent the proliferation of weeds. In flat ridge cultivation system, three to five varieties are normally cultivated in mixed planting or in separate plots, so that sweet potatoes can be harvested at various times. In many cases, tips of vine are cut as seedlings for next planting, and then transplanted to nearby fields.

2) Collecting

Fig. 1 shows the number of samples collected in the survey areas. Most of the collections were from private home gardens and nearby vegetable gardens. On the basis of the characteristics of the roots, stems and leaves of the plants, some of the samples collected were identified to be of the same varieties. Finally, 221 varieties were identified.

The followings were observed.

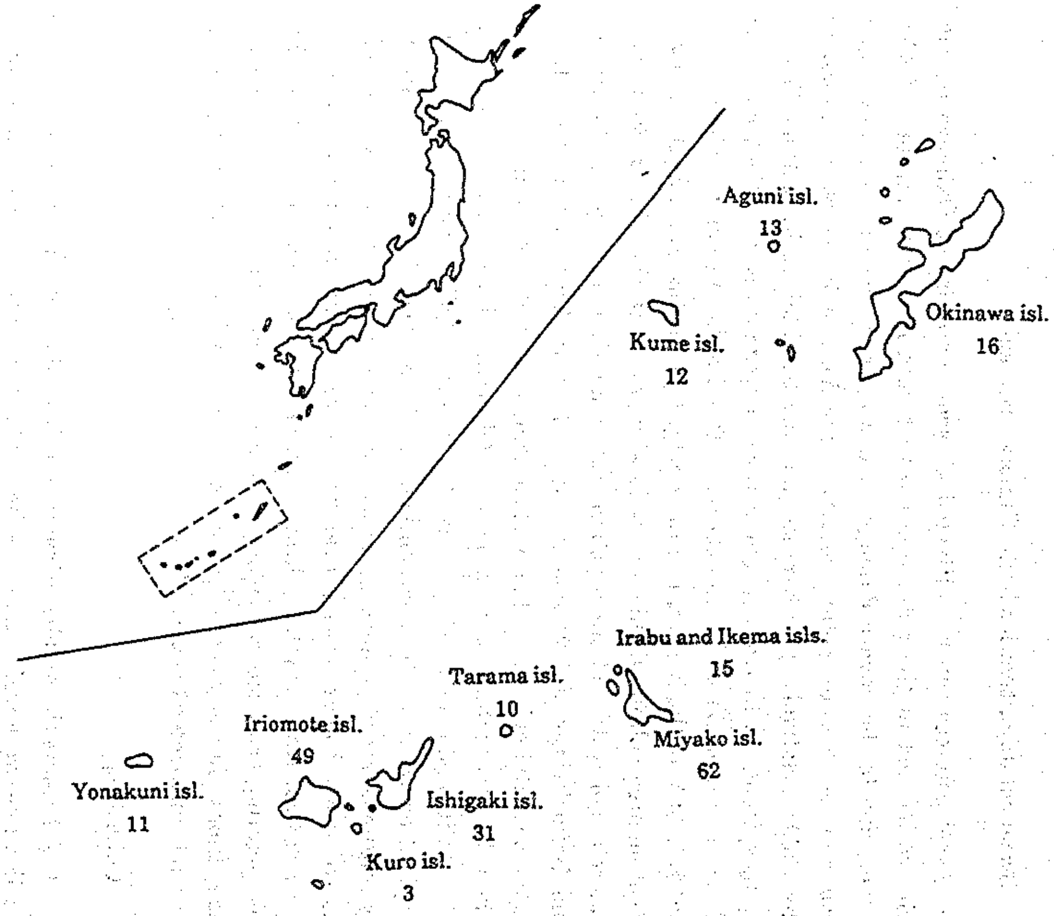

Fig. 1 Number of collections in Okinawa islands (222 accession in total)

(1) Many indigenous varieties of sweet potato are still being cultivated in the Okinawa Islands.

(2) There are many indigenous varieties with reddish-purple flesh by anthocyanin pigmentation.

(3) The largest number of varieties were collected from Miyako Island, with the second largest from Iriomote Island.

(4) Climbing-type-varieties were abundant in Ishigaki City, short-vine-type varieties were in Iriomote Island, and intermediate type varieties were in Miyako Island.

(5) Several varieties are used exclusively for their stems and leaves as vegetables.

3) Cautions related to transportation of sweet potatoes from Okinawa Prefecture.

The tuberous roots, stems and leaves of sweet potatoes from Okinawa Prefecture are always in danger of infestation by the sweet potato weevil, the West Indian sweet potato weevil and sweet potato stem borer, therefore, its transfer to the other Japanese islands is prohibited by the Plant Protection Law. Due to this restriction, there has been no record of the transportation of sweet potatoes from Okinawa Prefecture since 1945.

The transportation of prohibited plants within Japan requires special approval from the Minister of Agriculture, Forestry and Fisheries. To obtain such special approval, confirmation is necessary by the Domestic Department of the Yokohama Plant Protection Station that the objective plants are free from diseases and insects through the procedures shown below.

(1) An application for special approval to transport such plants within Japan must be submitted through a Plant Protection Station to the Minister of Agriculture, Forestry and Fisheries.

(2) After the approval has been obtained, eradication of diseases and insects should be conducted in accordance with the conditions stipulated at the time of approval. As an example, the following procedure was conducted in this survey.

① National organizations (the Okinawa Branch of the Tropical Agriculture Research Center in 1987, and the Okinawa Station of the National Center for Seeds and Seedlings in 1988 and 1989) were requested to plant the collected stocks on framed beds or pots which contain soil mixed with insecticide, and to apply insecticide to the stems and leaves during growth period, and thus objective plants were to be kept free from insects(primary eradication). This procedure ensured that good quality seedlings were obtained to minimize rejections at the time of inspection by the Okinawa Plant Protection Station.

② Tips of vine (seedlings of sweet potato) were taken from the insect-free plants and packed in bags by varieties. After inspection by the Okinawa Plant Protection Station, these were shipped to the Haneda Branch of the Yokohama Plant Protection Station.

③ After being re-inspected by the Haneda Branch of the Yokohama Plant Protection Station, the seedlings were transferred under restricted conditions to quarantine facilities at National Agriculture Research Center which was previously inspected by the Plant Quarantine official.

④ The seedlings were then transplanted to pots containing soil in which 5% of the specified insecticide powder Fenthion was applied under the supervision of the Plant Quarantine official and were cultivated in the quarantine facilities.

After being cultivated for a fixed period, the plants were dug up and the tuberous roots were inspected by the Plant Quarantine official, to determine if they were free from the prohibited insects and that eradication was complete (secondary eradication). The stems and leaves, the soil and tools used, which became unnecessary, were then steamed or incinerated.

Of the samples in the current collection, 82 and 86 samples were submitted in 1987 and 1988, respectively. In January 1989, 80 of the former samples and 78 of the latter were confirmed to be free from the insects.

3. Plan for availability of the collections

The collected indigenous sweet potato varieties from the Okinawa Islands are scheduled for propagation and evaluation of characteristics at the Sweet Potato Breeding Laboratory, National Agricultural Research Center. After the completion of prescribed procedures, the collections will then be distributed to the sweet potato breeders as breeding materials.

4. Conclusions

There were many unknown aspects concerning the varieties and cultivation methods of sweet potatoes in the Okinawa Islands, partly because Okinawa Prefecture was under the administration of the American Government for nearly thirty years following the World War II, but also because of the lack of preliminary surveys. The cooperating persons prepared several candidate sites beforehand but, due to the lack of preliminary information, meetings in each site during the 1987 visit were held in a pessimistic atmosphere.

Nevertheless, once the collection started, an enormous amount of local information was obtained, and the collectors showed increasing ability to discover new sites; therefore, results were much better than anticipated. Through the present survey it was understood that sweet potatoes have shifted from a primary food to a supplementary food, and that both the number of varieties and the cultivated area have decreased due to the damage from the sweet potato weevil. Even so, the fondness of the Okinawa people for sweet potatoes, especially those in the Miyako and Yaeyama Islands, has preserved the indigenous varieties and made it possible to obtain the fruitful results. In the words of a housewife of Iriomote Island, "These are ancestral and traditional varieties, some of which our grandmother brought when she married. This was brought by myself when I married. And this was given to us by neighbors because it is so delicious." The author think that this statement speaks for itself.

Finally, the author would like to express his appreciation to the persons who helped with the collection, the organizations that allowed the team to use their vehicles, the Tropical Agriculture Research Center and the Okinawa Station of the National Center for Seeds and Seedlings which undertook the primary eradication, and the citizens of Okinawa who allowed the team to collect sweet potato samples from there fields.

Photo 1 Monument of SUNAGAWA PEICHIN, a pioneer of sweet potato cultivation in Okinawa Islands

Photo 2 Escaped plants of sweet potato in Iriomote Island

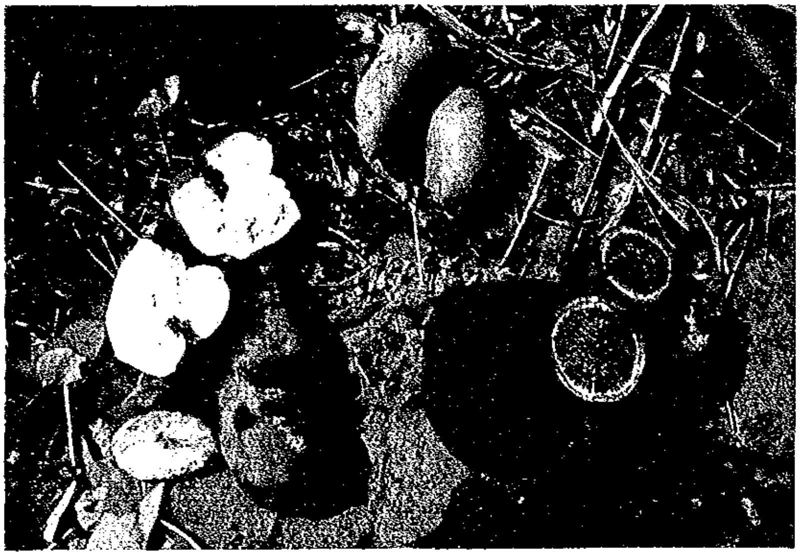

Photo 3 Typical characteristics of root tuber of samples collected in Iriomote Island

Photo 4 Different varieties are adjoining at same field in Miyako Island

II -2. Genetic Diversity of Fruit Characteristics in Natural Fruit Tree Community - *Myrica rubra* forest in Japan, a marvelous sample of natural conservation of fruit crops -

by

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CONTENTS

1. Introduction

Wide genetic diversity of deciduous pome fruits in wild forests in Cremia and Caucasus areas was reported by VAVILOV. However, most of wild forests of fruit trees have been lost in the world, and no clear result has been reported on the genetic diversity, especially on that of fruit characteristics in a wild forest.

It is difficult to precisely evaluate genetic variation of fruit characteristics in natural or primary forests, because a vegetation of fruit trees is easily disturbed by human habitation. Selection by inhabitants leads to uniformal phenotypes by changing genetic structures. Also, environmental conditions, such as soil, temperature and water stress, are likely to influence the fruit characteristics in terms of sugar contents, maturing time, fruit color, acidity and fruit weight. For a precise evaluation of fruit characteristics in natural or primary forests, the following factors should be taken into account:

1) Its genetic structures have been preserved by geographical conditions or law enforcement. 2) A fruit tree community should be located under uniform environmental conditions. For these reasons, scientific analysis of genetic diversity in a wild fruit forest, especially that of fruit characteristics, is left untouched. In Japan, most of forests are located at mountainous areas, and microclimatic differences in a forest are wide even in a small forest. Fortunately, we could find an excellent forest of *Myrica rubra* Sieb. *et* Zucc. for evaluating the genetic diversity of fruit characteristics within a wild forest.

This study was carried out as a part of a project, namely, "Fruit Genetic Resources Survey", which was sponsored by the Ministry of Agriculture, Forestry and Fisheries and supported by Grant-in-Aid for Scientific Research (B) No.62480038 by the Ministry of Education.

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2. *Myrica rubra*

Myrica rubra is dioecious with its female tree bearing jerry fruits of reddish to purple color (Photo 1). *Myrica rubra* distributes in coastal areas from southern China to central Japan. *Myrica* fruits are consumed as desert in fresh or brewed into wine or reddish liquor (Photo 2).

Maturing period of each tree varies from one week for the shortest to three weeks for the longest. Because of short maturing period of each tree, the differences of fruit characteristics within a tree are small. In the central part of Japan early maturing trees start maturing at the mid of June and terminate it at the end of June, and latest trees start maturing at the end of June. As maturing periods of the earliest and the latest tree overlap each other, only one or two sampling times can cover almost all samples from a given forest.

3. Description of the investigated forest site

The forest investigated and reported in this paper is located at Ukiyama, Yahatano, Ito City, Shizuoka Prefecture (Fig. 1), where an ancient lava stream from Mt. Iyuu into Sagami Bay developed a flat terrace of 150 ha about 2,500 years ago (Photo 3).

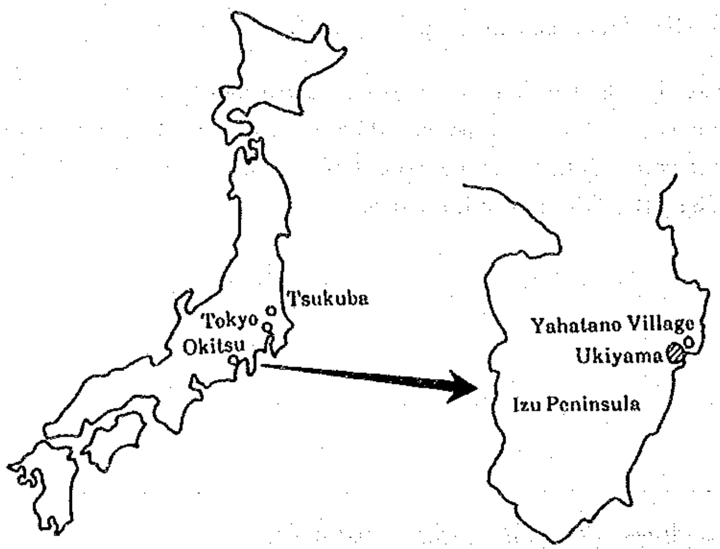


Fig. 1 Location of experimental forest of *Myrica rubra*

It was recorded that about 7,000 trees of the species had been grown and 3,000 male trees had been cut in the terrace by the order of Edo Shogunate (Samurai government) about 200 years ago. The longevity of *Myrica rubra* is estimated to be more than several hundreds years, and there still exist more than 1,000 female trees. These facts suggest that the genetic diversity of the female trees has been preserved intact in this forest from time immemorial.

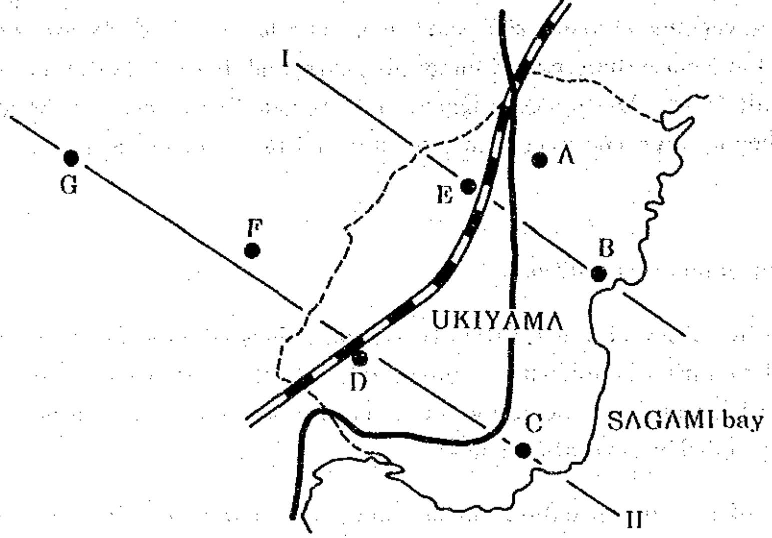
4. Environmental conditions

The investigated forest, called Ukiyama, is located on a flat lava terrace, where soil condition is uniform but unsuitable for crop cultivation. The eastern part of the Izu peninsula including Ukiyama area is a steep mountainous slope being surrounded by several volcanoes.

Therefore, human habitation has long been extremely limited. Daily change of temperature on June 24, 1988, in the fruit maturing season was measured at seven points each of which was indicated from A to G in Fig. 2. Fig. 3 shows that the temperature was always lower at F and G points in the mid of slope than that in the other points. Except the point F and G, differences in temperature among five points (A-E) were found to be small. The temperature conditions inside the sampling area seemed to be nearly uniform.

5. History of the management of the *Myrica* trees

The management of this forest had been directly controlled by Edo Shogunate. Harvesting of the matured fruits of *Myrica rubra* in the forest used to be permitted only for the inhabitants. If anyone cut the trunk or even a shoot, death penalty by decapitation was imposed by ruling officers, because *Myrica rubra* had been an important economic crop for that period for its fruit and for dye material from its bark. Since the Meiji restoration in 1868, this forest has been owned jointly by villagers, and used as a coppice until the "fuel revolution" in the 1950s. The *Myrica* trees in the forest have been preserved as common property by



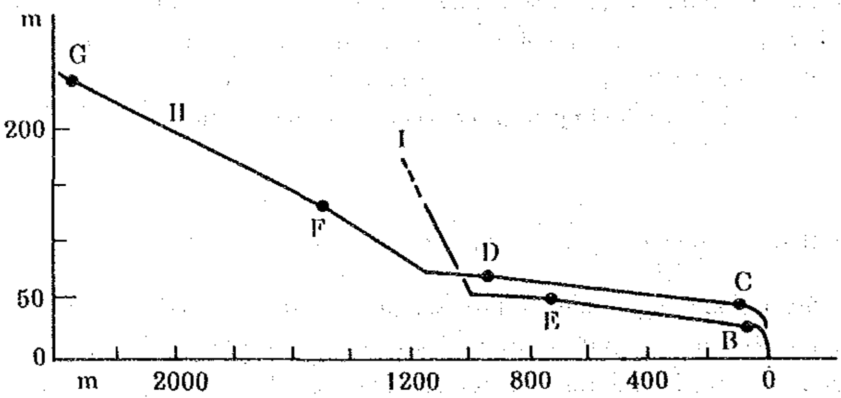


Fig. 2 *Myrica rubra* forest at Ukiyama
A-G; Seven spots where daily temperature was compared.

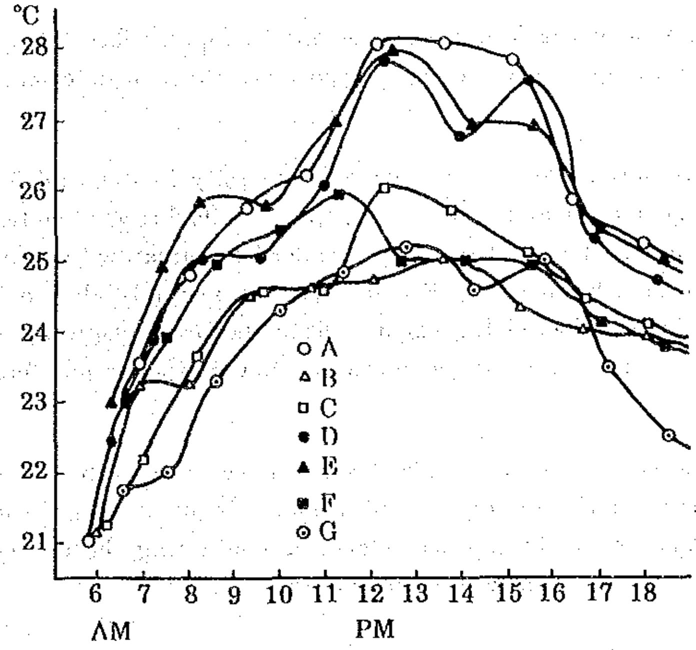


Fig. 3 Changes of temperature at seven spots in Ukiyama A-G; see in Fig. 2.

an agreement of villagers not to cut the trees. In the 1960s, a resort villa was constructed in this area (Photo 4). Fortunately, this area is located inside the Fuji-Hakone-Izu National Park. As a plant community inside the National Park is preserved by law, the *Myrica* trees in this area have been protected from cutting even in road reserves or in private gardens (Photo 5).

6. Ecological condition

In the protected forest zone along the coastal cliff, large *Myrica* trees form a part of the upper forest canopy, but young trees are not found inside the forest. On the other hand, in the inland zone forest,where management have been practiced for developing evergreen and deciduous coppice forests, young *Myrica* trees are found at a high frequency. This fact suggests that an improvement of light condition, which is caused by cutting trunks and branches for charcoal and by the removal of competitive plants through forest floor management, have made invasion and growth of *Myrica* trees possible. However, recent abandonment of the forest management as coppice forest has resulted in inhibiting the growth of young *Myrica* trees through deterioration of light intensity.

Evaluation work on the genetic diversity in the wild forest is time consuming, and needs surveyors and finance. Wild forests are often located at a long distance from an institute, and samples for evaluating the genetic diversity should cover a lot of trees. Fortunately, it takes only 4hr's drive from our institute in Tsukuba to Ukiyama, and inside the forest a network of pavement is constructed. The site of Ukiyama is a convenient experimental forest for such a survey.

The *Myrica rubra* forest at Ukiyama seems to be the excellent natural genetic reserve of fruit crops, a miraculous sample of natural conservation of fruit crops, and to be the best place for evaluating the diversity of fruit characteristics within a natural community.

7. Evaluation of genetic diversity

1) Fruit sampling

According to the result of a preliminary study on the sampling method and sample size for evaluating the genetic diversity of fruit characteristics of *Myrica rubra* (KAJIURA and HIRAI, 1987), 20 each of fully matured fruits were collected at random from 208 trees on July 9, 1986. The sampling area covered 47.2% of the flat area except the base of mountainous and cliff areas. The fruit samples were stored overnight and were evaluated on their characteristics at the laboratory.

2) Genetic diversity of fruit characteristics

Fruit color of each sample varies from pink to blackish purple. No fruit of pure white color was found. Frequency distribution of fresh weight per fruit showed a normal distribution ranging from 0.6 to 3.4g with a mean of 1.7g (Fig. 4). Those of soluble solids contents and pH value were also normal ranging from 6.5 to 13.0% with a mean of 10.0%, and from 2.3 to 3.4 with a mean of 2.8, respectively (Fig. 5 and 6).

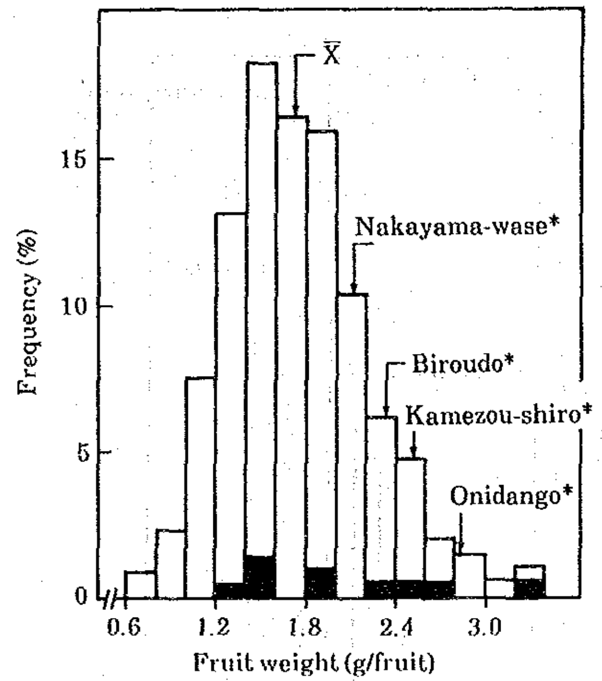


Fig. 4 Frequency distribution of *Myrica rubra* trees on fruit weight

■ Specific trees which were recognized as excellent by village peaple.

* Land races.

Twenty from 208 trees bore larger and sweeter fruits than commercial cultivars. Most of them have been recognized as such by the native villagers. Extremely early maturing type was also found.

These results indicate that the wild *Myrica* forest retains a wide genetic diversity of fruit characteristics as described by VAVILOV, that *Myrica rubra* still remains at the primitive step of cultivation process of fruit crop, and that the wild *Myrica* forest still has a potential for producing excellent cultivars.

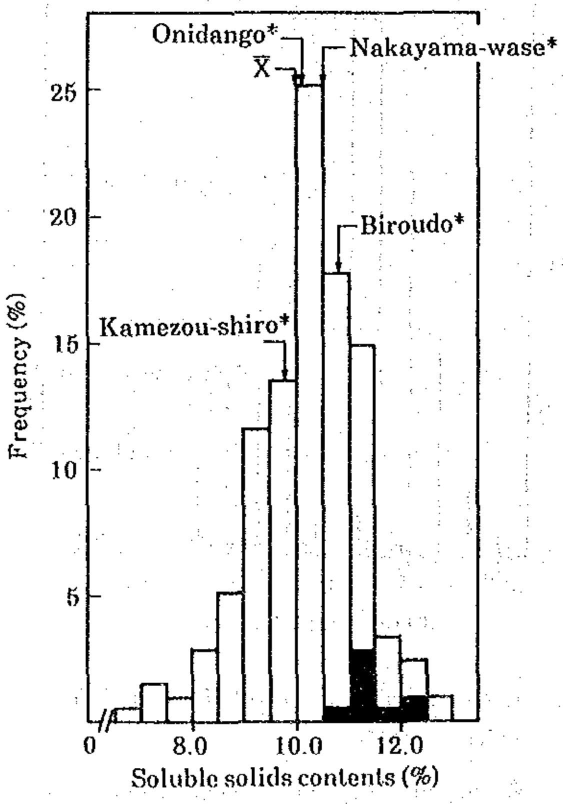


Fig. 5 Frequency distribution of *Myrica rubra* trees on soluble solids contents

■ Specific trees which were recognized as excellent by village peaple.

* Land races.

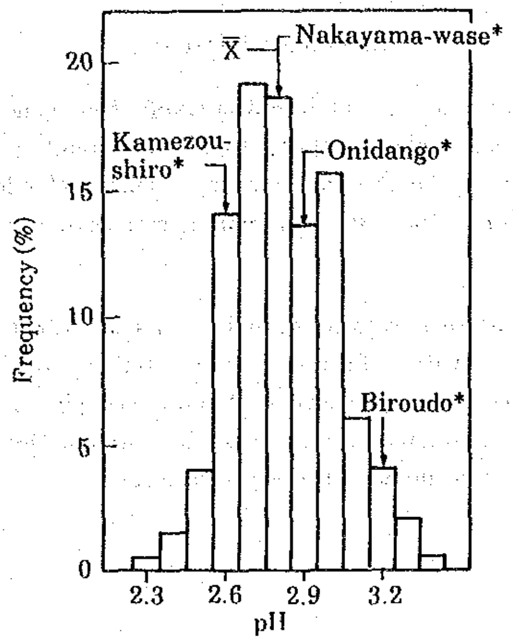


Fig. 6 Freqency distribution of *Myrica rubra* trees on fruit juice acidity (pH)

* Land races.

8. Potential of the wild forest

After the World War II, Ukiyama area has been developed as a resort village, and then people from cities became inhabitants there. They introduced new method to consume *Myrica* fruits. Although the native villagers have been eating fresh fruits directly or making a wine, new comers make liqueur by dipping fruits with sugar in spirits. For making liqueur, they selected new types of the fruits preferring to reddish liqueur color and low bitterness from their point of view different from the native villagers'.

If the native villagers had cut trees before on the basis of their own choice, this forest would have lost the potential variability and could not have supplied excellent trees for the preference of new comers. In general, as selection criteria change in the lapse of time, a natural forest which has been preserved without artificial disturbance should be preserved as a good genetic resources for future need.

9. Strategy to conserve the habitat of wild trees

Because *Myrica* trees are intolerant of shade, they generally grow in the seral stages of plant succession except for those on particular sites such as lime stone terraces. Therefore, for the maintenance of habitats of wild *Myrica rubra*, it is necessary to reorganize the vegetation management by applying the system of coppice forests.

For example, *Myrica* trees preserved in this forest suggest the people's awareness for the preservation of the genetic resources of wild fruit trees and the utilization of their habitat as open spaces. When the coppice forests are retained in private property, residents are expected to manage the forests both for preservation of wild trees and for effective use of the site.

10. References

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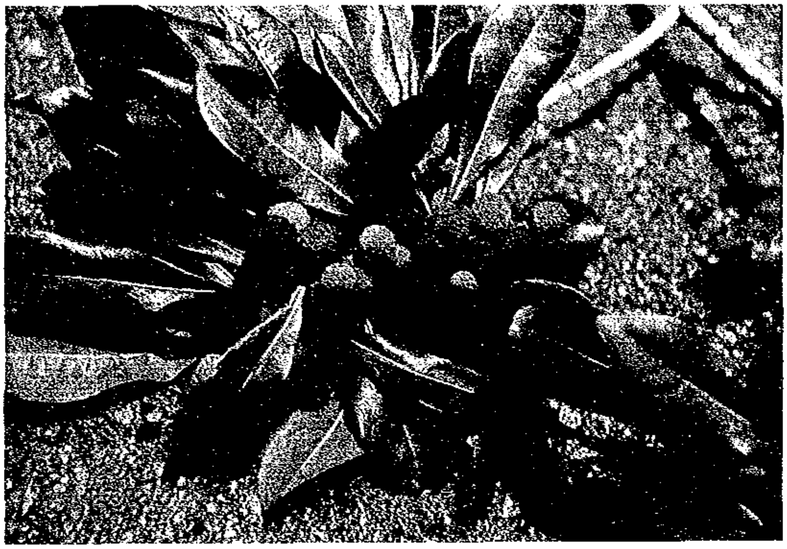

Photo 1 Fruits of *Myrica rubra* bearing on a female tree

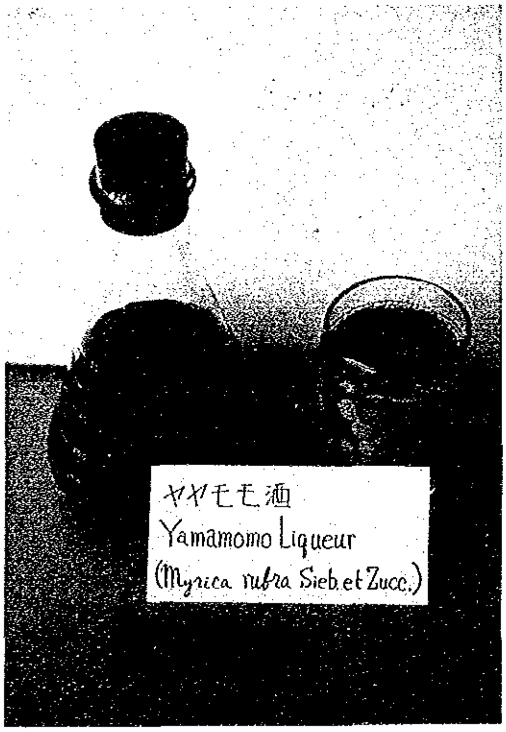

Photo 2 Liqueur made from *Myrica rubra* fruits

Photo 3 Forest of *Myrica rubra* at Ukiyama, Ito City
(Shokusan Ukiyama Hot Spring Ltd.)

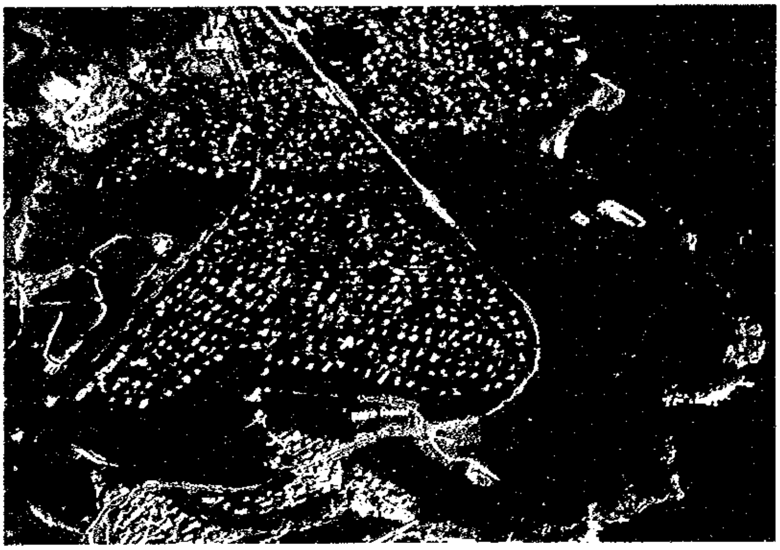

Photo 4 Resort Villa developed at Ukiyama
(Shokusan Ukiyama Hot Spring Ltd.)

Photo 5 *Myrica* trees protected in road reserves at Ukiyama

II-3. Collection and Identification of Taro Varieties in Japan

by

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CONTENTS

1. Origin and introduction to Japan

Taro is not native to Japan, and is thought to have originated somewhere in the Indo-Malay region, perhaps India or Indonesia. The cultivation of taro probably was initiated in the region of Southeast Asia. Its cultivation eventually spread southward via Indonesia, Borneo or the Philippines to Melanesia, Micronesia and Polynesia; northward to China and Japan; and westward to Africa. As shown in Table 1, taro is included among the edible aroids, being a main staple food even today in Melanesia and Polynesia.

In Japan, taro (*Colocasia esculenta* (L.) SCHOTT) is an important vegetable. *C. gigantea* is also cultivated. It is not known exactly when the taro plant was introduced to Japan. The taro plant rots so easily that no trace remains to indicate where and when the cultivation might have started. In 1966, NAKAO stated from the viewpoint of anthropology that the taro might have been introduced to Japan as long ago as 2,500 years or more as an element of the "Root Crop Cultivation Culture".

The "koboimo" of Nagano Prefecture, the "imo" mentioned in the "ishiimo" legends of various regions, and so called native species of *Colocasia* genus in Kyushu and Okinawa might be the descendants of taro that were brought to Japan more than 2,500 years ago. However, these are hardly used as food.

The earliest records of taro in Japan are found in Bungo Fudo-ki (732), Izumo Fudo-ki (733) and Man-yoshu (760), but there is no mention of their characteristics. It is presumed that the taro varieties currently cultivated in Japan have been introduced in several waves via China or Taiwan after that era.

2. Local varieties of taro in Japan

The names of taro varieties are found in Japanese literature of the 16th and 17th centuries. KUMAZAWA *et al.*(1956) compared these names with those of the taro varieties currently being cultivated (Table 2). Most of the taro varieties cultivated since ancient times correspond to descriptions found in ancient Chinese records, so it is considered that most had already been introduced to Japan by the 18th century.

According to KUMAZAWA *et al.*(1956), 158 varieties were collected from Japan, and 47 varieties from Taiwan and mainland of China, etc., they were classified into 15 groups by morphological characters. The varieties collected at that time are not preserved. In 1982, TAKAYANAGI *et al.* started collecting the varieties being cultivated in Japan. By 1985, 88 varieties has been collected (Table 2).

The same name is sometimes used for different varieties, and different names are sometimes used for the same variety, depending on the region. This makes the identification difficult, but these varieties have been classified into groups by the method proposed by KUMAZAWA *et al.* (1956). As a result, the largest group was the "dodare" group, which includes 21 varieties collected from

Table 1 Major edible aroids

Scientific name	English or local name	Distribution	Japanese
Colocasia esculenta	taro old cocoyam eddoe dasheen	China, Japan S.E. Asia Oceania, USA	satoimo
*C. gigantea**			hasuimo
Xanthosoma sagittifolium	yautia tannia new cocoyam	S. America Oceania, Africa S.E.Asia	amerika satoimo
*Alocasia macrorrhiza**	giant taro	S. America Oceania, S.E. Asia	kuwazuimo
*Cyrtosperma chamissionis**	swamp taro giant swamp taro	S.E. Asia Oceania	-
*Amorphophallus campanulatus**	elephant yam	S.E. Asia Oceania	indo kon-nyaku
*A. konjak**		Japan	kon-nyaku

*: minor crops

Table 2 Taro cultivar groups in Japan and number of collected varieties

Cultivar group*	Cv.name appeared in old literatures**	KUMAZAWA *et al.* 1956	VOCRS 1985
Eguimo	Eguimo, Aoimo	19	9
Okinawa aokuki	-	1	2
Hasubaimo	Shiroimo, Kuriimo	23	9
Ishikawa wase	-	30	16
Dodare	Tsurunoko, Hayaimo	62	21
Kurojiku	Kurokara	11	3
Akame	Akaimo, Miyakoimo, Mizuimo, Takeimo	14	8
Shougaimo	-	1	3
Binroshin	-	3	1
Tonoimo	Maimo, Murasakiimo, Akagara	17	9
Yatsugashira	Yatsugashira, Kumen-imo	6	3
Migashiki	Migashikiimo	2	2
Mizoimo	-	2	0
Takenokoimo	-	1	1
Hasuimo (*C.gigantea*)	Hasuimo, Shiroimo	1	1
Escaped type	Kuwazuimo, Ishiimo, Dokuimo, Noimo	0	0
Total		193	88

*: after KUMAZAWA *et al.* (1956).

**: appeared in Japanese old literatures, such as "Yamato Honzou (1708)", "Seikei Zusetsu (1804)" and "Honzou Zufu (1821)".

Yamagata, Kanagawa, Chiba, Gumma, Toyama, Fukui, Mie, Gifu, Tottori and Ehime. Sixteen varieties belonged to the "ishikawa wase" group, which were collected in Kanagawa,Chiba, Saitama, Gumma, Osaka, Ehime, Kagoshima and Kumamoto. These two groups included a majority of the collection. It was found that they are widely cultivated southward from Kanto area, Shikoku and Kyushu as well as in some parts of Tohoku area.

Fewer samples were collected in the remaining groups and it was found that the "eguimo" group is cultivated in the Tokai, Kinki, Chugoku and Shikoku areas; the "hasubaimo" is cultivated westward from Kanto area, Shikoku and Kyushu; the "akame" group is cultivated in Kanto, Tokai, Shikoku and Kyushu; the "tonoimo" group is cultivated in Kanto, Kansai and in Yamagata Prefecture; the "yatsugashira" group is mainly cultivated in Shikoku and Kyushu with some cultivation in Honshu, the major island; the "kurojiku" group is cultivated westward from Kanto and in Shikoku and Kyushu. Recently, some varieties belonging to the "takenokoimo" group become popular in Kansai markets, these are mostly cultivated in Kyushu. Currently, "migashiki" and "binroshin" groups are hardly cultivated. The "hasuimo" group, which belongs to *C. gigantea*, is cultivated in Kyushu for use as dried petiole. The tuber setting types of main varieties are shown in the photographs.

As mentioned above, we could collect a number of taro varieties belonging to groups other than the "mizoimo" group. It is considered that there has been no change in the main characteristics of "eguimo", "hasubaimo", "dodare", "kurojiku", "akame", "tonoimo", "yatsugashira", "migashiki" and "hasuimo" groups since the 17th century. The "takenokoimo", "binroshin" and "mizoimo" groups were introduced from Taiwan during this century but only the "takenokoimo" group is actually cultivated. The origins of the "Okinawa aoguki" and "shogaimo" groups have never been clear, but they are planted to limited farmer's fields in Okinawa and Ehime Prefecture, respectively. There is no records of the "ishikawa wase" in ancient literature and nothing resembles it among the Chinese varieties, so the "ishikawa wase" is probably resulted from a mutant occurred in Japan.

At present, many other taro varieties which are not in our collection are probably cultivated in Japan, but most of them should belong to one of the above mentioned groups. However, some taro varieties in Nepal, China and Taiwan do not belong to these 15 groups, so it is still possible to widen the genetic diversity of taro in Japan.

3. Identification of collected taro varieties

Most of taro varieties resemble each other in their morphological characters, sometimes leading to confusion in their varietal names. Such a confusion occurs even if the collected materials can be classified into groups by morphological characters. Identification of varietal names is difficult without a considerable degree of experience because of such variations as occurred within a variety.

We tried to identify and classify the collected materials by their morphological characteristics and electrophoretic patterns of stored proteins. The results showed that classification by KUMAZAWA *et al.* (1956) was appropriate. This was also very interesting from the viewpoint of clarifying phylogenic relationships among groups, which had been unclear. For example, the leaf color and the characteristics of the anthocyanin pigmentation at the petiole sheath edge of the "ishikawa wase" group are different from those of the "kurojiku" group; however, no difference is found between the groups in the electrophoretic pattern of storage protein of tuber. It means that the "kurojiku" group may be the ancestor of the "ishikawa wase" group.

The "yatsugashira" and "shogaimo" groups are morphologically unique in respect to their fasciculated growth, and of the massive tuber setting, however, the electrophoretic pattern of the storage protein is the same as the "tonoimo" and "dodare" groups, respectively. Thus, they might be morphological mutants in each group.

The "dodare" and "hasubaimo" groups can be distinguished morphologically from each other except in the electrophoretic pattern of stored protein, they might be closely related each others (HIRAI *et al.* 1989).

The materials collected are often designated by local names, so one variety is occasionally called by different names. Although we have attempted to collect and identify the taro varieties found in Japan as reported herewith, the results are still unsatisfactory, necessitating further research. Further exploration and collection survey should be planned to cover traditional taro varieties in some detail in Japan as well as wild or escaped relatives of *Colocasia* genus found in the Ryukyu islands.

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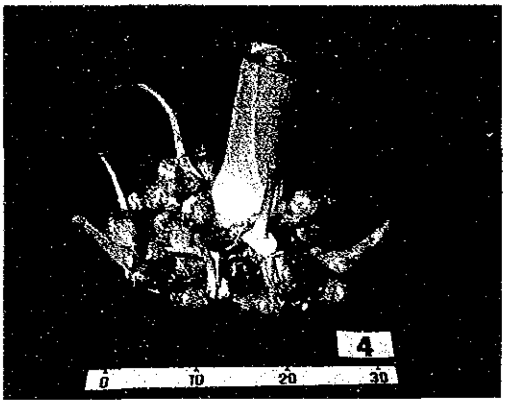
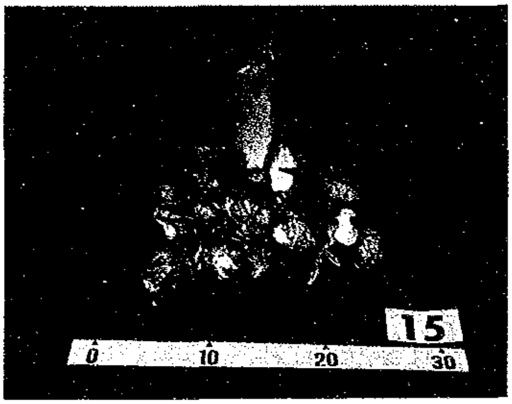
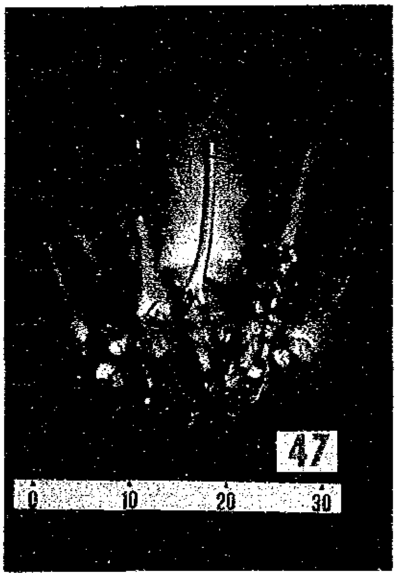


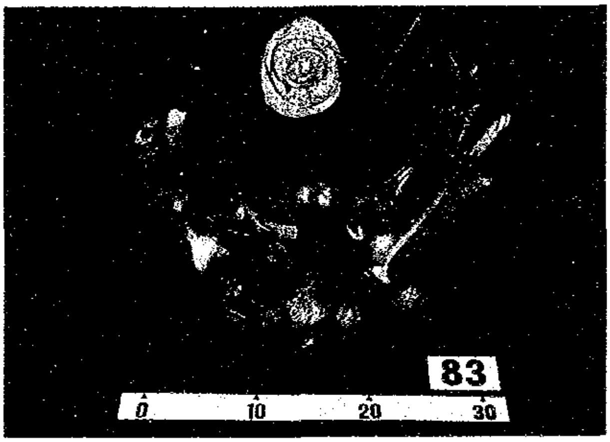
Eguimo



Ishikawa wase maru

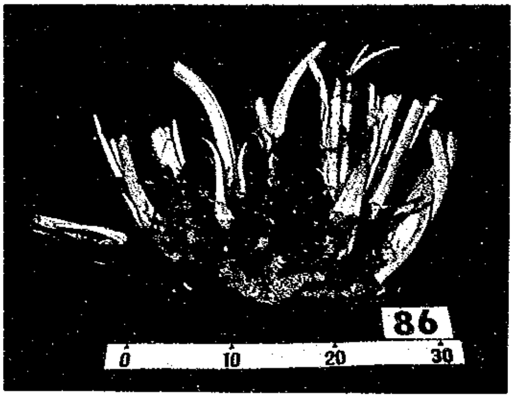


Takenokoimo

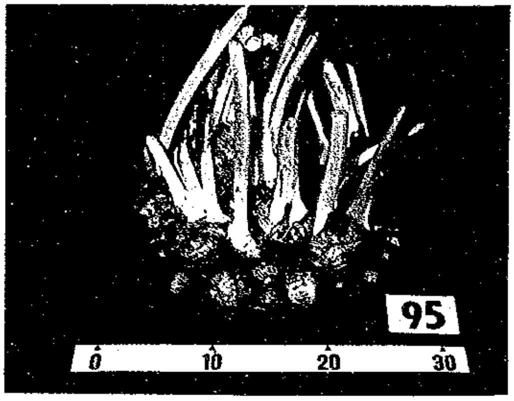


Serebesu

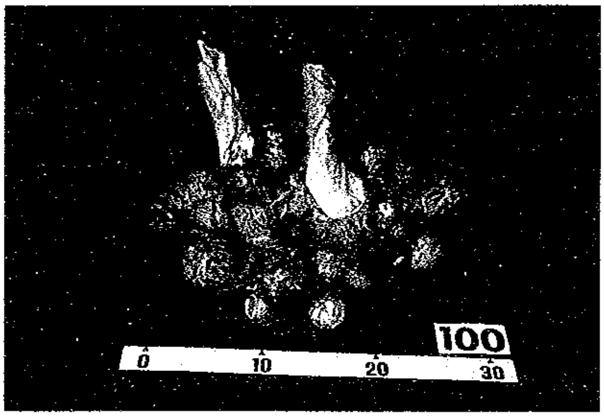
Photo 1 Corm and cormels arrangement in taro varieties (1)



Shogaimo



Yatsugashira



Dodare

Photo 1 Corm and cormels arrangement in taro varieties (2)

Ⅲ. Report from Exploration and Collection of Native Germplasm in Southeast Asia

The Collection of Citrus in Southeast Asia by IBPGR

by Isamu UENO
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III. The Collection of Citrus in Southeast Asia by IBPGR

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CONTENTS

1. Background and target areas of the project

This paper is to outline an IBPGR project for collecting citrus species following the collection of rice, wheat, soybean and other cereal crops. As is well known, citrus is a major source of vitamin C and an important fruit tree from temperate to tropical regions.

Citrus which originated in the Assam area in the Northeast of Indian sub-continent has been introduced eastward beyond the Himalayas to China where it was widely distributed, and then to Japan and other areas. The "Unshu mikan", an important Japanese citrus, must have been brought through this route. Citrus was also distributed westward through Near and Middle East, to countries bordering the Mediterranean Sea. The European citrus groups were thus formed and eventually reached America. Along its southward route, citrus was distributed through Myanmar, Thailand and Malaysia to the islands of the East Indian Ocean.

During such distribution with numerous natural mutants and natural crossing, secondary differentiations occurred so that each area has developed its own unique citrus cultivars. Typical examples of such differentiation are the pummelos of Thailand and Malaysia, the mandarin of China and Japan, and the lemons and citrons of the Mediterranean area.

To cover the diverse varietal development the citrus collection project has been continued for three years since 1983 with a collection mission once a year. In the first year, the exploration was conducted in the southern, central and northern Thailand to collect the limes and hystorix which were developed from the distribution southward, the pummelos that resulted from secondary differentiation on the Malay Peninsula, and the relatives of Citrus genera. In the second year, collections were made in the northeastern part of Thailand, Malaysia (including Borneo), and Brunei. In the third, collections were made again in northeastern Thailand as well as in the Indonesian islands of Sumatra, Java, Bali and Madra.

2. Procedure of exploration

Prior to the collection, communication between the collection team and officers of agricultural extension service in the target areas was established to get

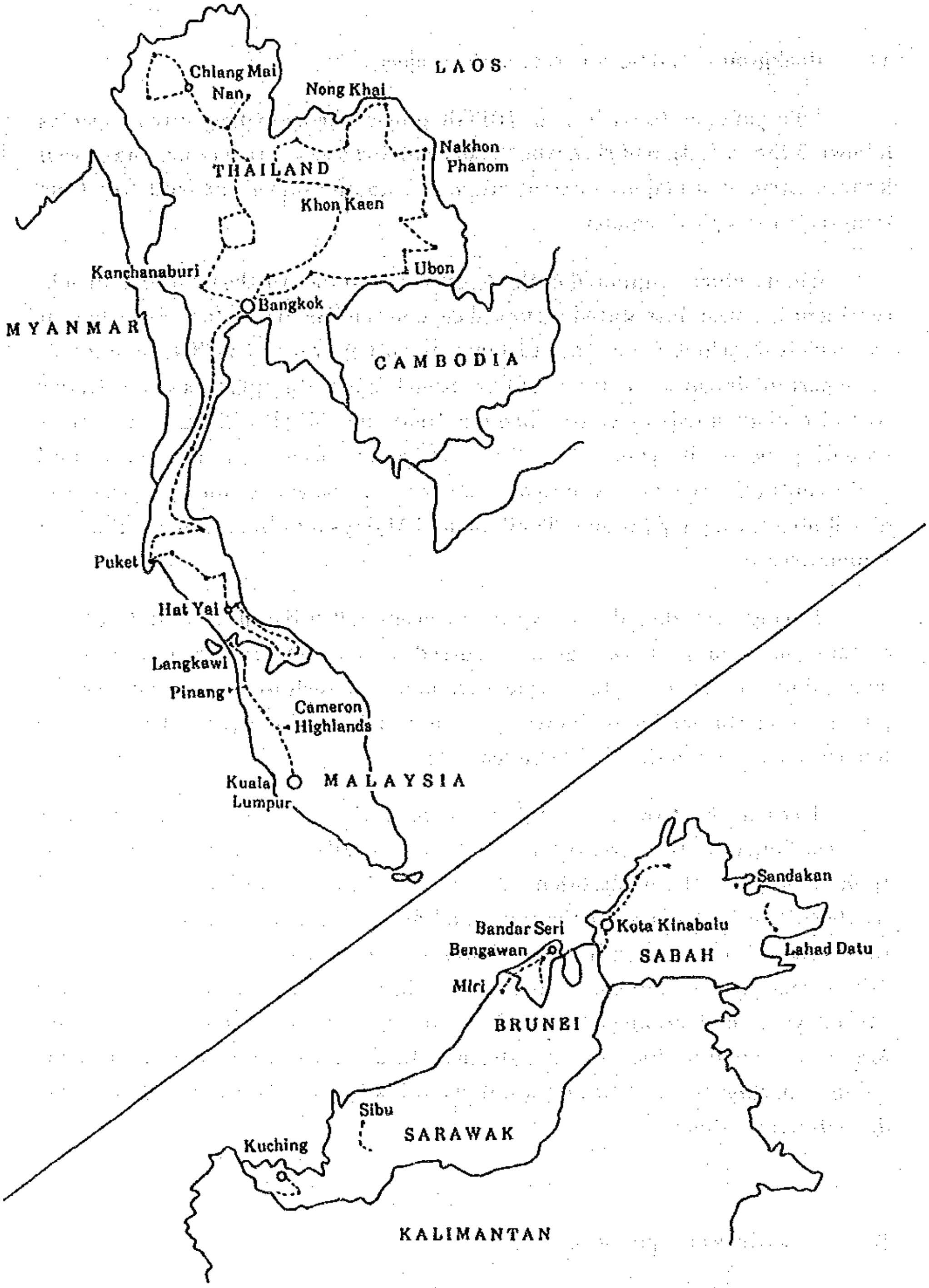

Fig. 1　Route of collection trip in Thailand and Malaysia

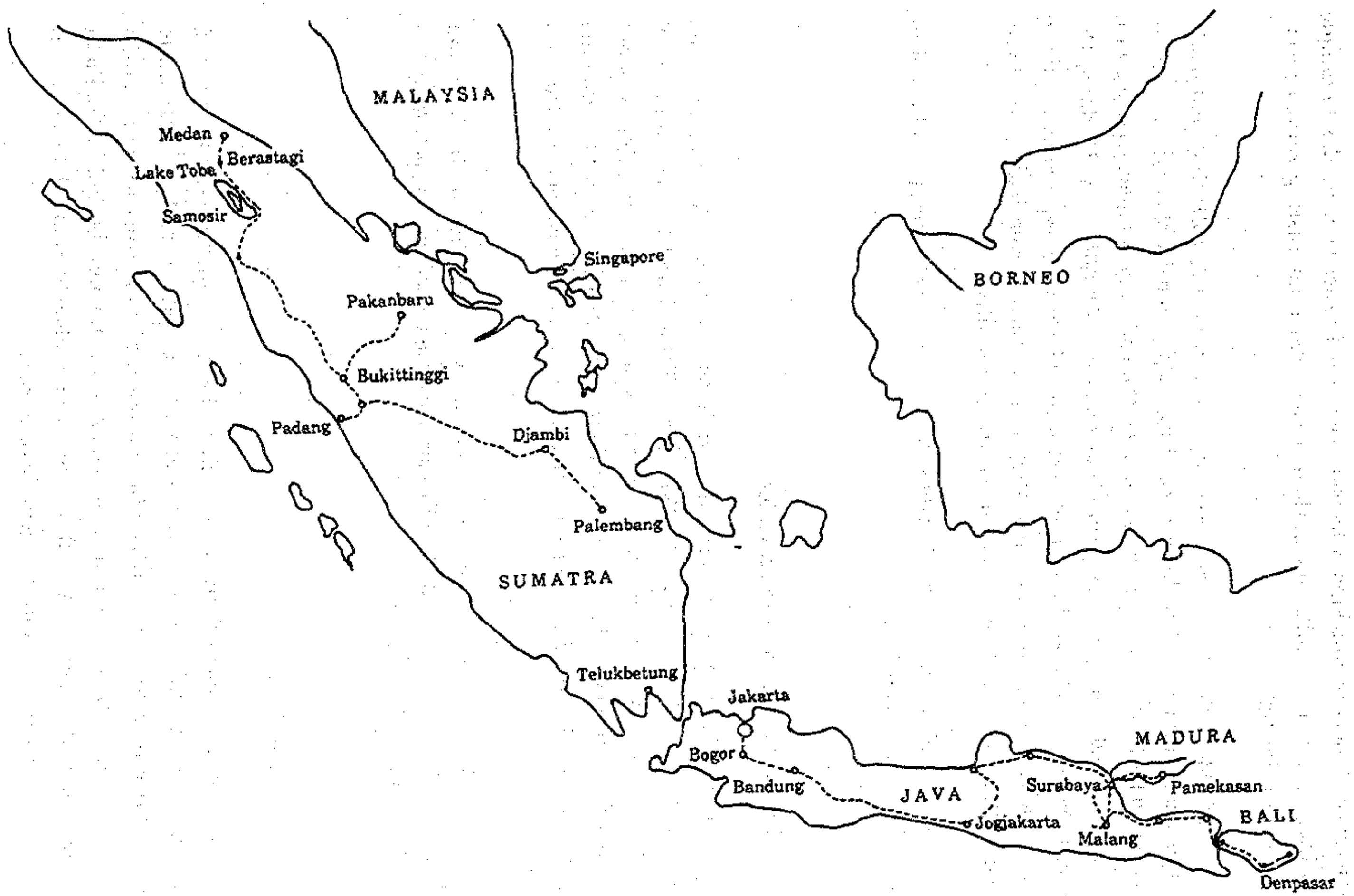

Fig. 2 Route of collection trip in Indonsia

assistance of local technicians, who were requested to serve as guides to unique and interesting citrus cultivars in their areas. Thus, interesting samples were effectively collected.

Arrangements were also necessary for preserving collected materials. As the temperature is very high exceeding 30°C even in the cooler area in northern Thailand, it was necessary to pack the collected samples (scions) with ice in insulated containers to keep them healthy. About every 10 days, the samples were transferred to a large city from where they were sent by air to Japan. When seeds were collected, they were removed from fruits, sterilized, dried and packed in cool containers to keep their viability.

We traveled approximately 200~300km daily and normally collected at two or three sites each day.

3. Numbers of the collected samples

A total of 152 samples were collected in the first year, 75 samples in the second year and 63 samples in the third year (Table 1 and 2). The number of collected samples was reduced in succeeding years, because sampling was not repeated for same materials. The numbers indicated in the tables include samples collected both via scions and seeds as well as those collected either via seeds or scions.

The scion samples introduced to Japan were planted in the isolation facilities, and sent to the Fruit Tree Research Station after the inspection by the Plant Quarantine Station Officers.

4. Local conditions

Numerous tropical fruit trees such as bananas, mangoes, durians and pineapples were found in the surveyed areas, but there were few large-scale commercial citrus groves. Although commercial varieties are not much developed in the regions, the pummelo, hystorix and the new halimi in Thailand and the Malay Peninsula, as well as the species closely related to citrus in Borneo, are of great importance as genetic resources.

Table 1. Accession number of citrus species and its related genera

Genus or species	Year		
	1983	1984	1986
Papeda	3 (3)	1 (1)	0
Lime	7 (6)	2 (2)	3
Lemon	2 (2)		0
Limonia	-	-	1
Citron	1 (1)	3 (3)	2
Pummelo	82 (28)	17 (9)	18 (7)
Sweet orange	7 (5)	1	2 (1)
Mandarin	20 (15)	4 (2)	15 (4)
Halimi	-	2 (2)	-
Hybrid of citrus	20 (18)	21 (14)	17 (10)
Other of citrus	-	-	1 (1)
Fortunella genus	-	2 (1)	-
Related genera of citrus	10 (8)	23 (19)	4
Total	152 (86)	76 (53)	63 (23)

( ): Collected by scion

Since there is little in agricultural development in the Indonesian islands of Sumatra, Madra and Bali, those which are thought to be primitive type of cultivated species were frequently observed. However, in Java where its agriculture is developed to some extent, only cultivated species were found. The characteristics observed in each area are recorded in some detail as follows.

Table 2 Accession number of citrus species and its related genera in each country

Country and sites	Year		
	1983	1984	1986
Thailand	152 (86)	28 (16)	13 (6)
Malaysia		47 (37)	
Peninsula		17 (14)	
Saba		18 (16)	
Sarawak		12 (7)	
Brunei		1	
Indonesia			50 (17)
Sumatra			34 (15)
Java			16 (2)
Total	152 (86)	76 (53)	63 (23)

( ) : Collected by scion

1) Thailand

(1) Pummelo

The most popular pummelo in markets was a medium-late, high quality cultivar "Kao Phuang". Many of this kinds were also seen in the Malay Peninsula. Pummelo belongs to such a citrus as requires high temperatures. Many high quality cultivars are being cultivated in the southern Malay Peninsula, which is an ideal place for the production, as the temperature is high all year round. In the central and northern regions, an early seedless cultivar "Kao Phuang" and a high quality cultivar "Thong Dee" were found to be planted. Though the pummelo is supposed to be originated from the Malay Peninsula, a place not near the Himalayas, it has been widely cultivated in China since ancient times, presumably introduced northward from the Malay Peninsula. There are numerous pummelo cultivars in Thailand, but little land is assigned to them. In most cases, they are sent to local markets, or grown by farmers for domestic consumption.

In the propagation of pummelo, seedlings are generally used instead of grafting. As pummelo is mono-embryonic, each individual may show different characteristics in seed propagation. Some farmers who have good quality pummelo propagate them by means of layer for their own use, but it is difficult to find the same pummelo clone at other farmer's fields even in the same village. Thus, each of farmers' gardens is like a breeding farm.

The flesh color of the pummelo ranges from white to pink or to deep red. Red albedos are also seen. Some cultivars have numerous seeds and others are seedless, and some have fuzzy skins. The fruit weight ranges from 500 to 1,500g. There are also many shapes; such as a shape of European pear, of spherical or of cylindrical.

Many cultivars of grapefruit were found, which ranged from seed types with white flesh and skin to seedless types with red or pink flesh. These variations are supposed to be resulted from mutation. The same types of mutation are also found in the pummelos that are presumed to be the ancestors of grapefruit.

In this area daily changes of temperature is so little, that the fruit skins does not seem to turn to fully matured color. Numerous fruits with pink or red albedo are discovered in this area, and the skins of these fruits would probably turn fully matured color in a area with a larger daily temperature range. Fuzzy skin characteristics was found in pummelos, but not in grapefruit. In future, there is a possibility for such a characteristic to appear in grapefruit, as was the case with the color of the flesh.

(2) Mandarin oranges

"Som Khieo Waan" is a popular cultivar in markets, and it is widely cultivated in the central plain as well as in Chengmai and Nang regions. The fruit is of spherical shape with the weight of 100 to 120g. The calyx is small, and the fruit can easily be plucked by pulling lightly by hand. The number of seeds is fairly large and good in the flavor. Both the flesh and skin are orange-colored, but the skin turns to orange only in northern regions where the temperature becomes cool during the harvest season. In the central plain, the flesh matures to an orange color, but the skin remains green.

Near the Thailand-Myanmar border, a high quality cultivar "Som Kewaan" is planted by some farmers. This cultivar has been introduced from Myanmar, and resembles the "Som Khieo Waan" except that its skin is thinner and its calyx is small. The fruit weight is approximately 100g. The number of seeds is fairly large. It seems a fair number of citrus cultivars have been introduced from Myanmar.

Farmers often plant several sour mandarin trees in their gardens to make juice and for use as seasoning. There are many cultivars of large and small sour mandarin, including "Calamondin" (30g), each of which serves as an important source of vitamins in tropical regions. There is a strong demand for sour mandarin to sugar is added to make juice. Sour mandarin have been cultivated in a small scale since ancient times, and many cultivars have arisen from natural crossing. All of such cultivars were poly-embryonic.

(3) Sweet orange

"Som Kliang" is a variety cultivated widely. Its fruit is of nearly spherical type with the weight of 200g. Its skin is thick and rough, with numerous furrows close to the peduncle. The juice contains high concentrations of sugar and acids.

"Som Chuk" has the shape of European pear, with a protruding peduncle and weight of 150g. All of sweet orange varieties are cultivated in the northern regions. The fruit does not turn fully to matured color with some remaining green. A wider fluctuation of daily temperature is required for coloration of sweet orange than that for the mandarin.

(4) Lime

In Thailand, the lime is called "manao". The fruit of most popular cultivar is 50-70g in weight, and is of lemon like shape with a protruding nipple. Its quality is good. The lime is used for lime juice and oil is squeezed from the skins. The lime juice is utilized as a source of acid to add to other fruit juice. There are some groves in the region, but normally, one or two trees are planted by a household for domestic consumption. Seeds from sources nearby are widely used for propagation, thereby the number of cultivars being large. One reason for this is that each lime fruit has only 2~3 embryos, thus the possibility of natural crossing is high. If superior cultivars can be selected from them, high yielding cultivars with excellent quality that would rival the Mexican lime would be obtained.

(5) Hystorix

Hystorix is not popular in Japan. The length of leaves is about equal to the width of leaf wing, and the shoots has spines. The fruit is about 70-100g in weight, and the skin is rough. Due to its unique aroma this plant is indispensable for Thailand cuisine. In addition to the fruit, the leaves are also used to make soup. Hystorix is poly-embryonic, but the number of embryos in one seed coat is small, and the ratio of mono-embryonic seeds is rather high; therefore, when sown, the ratio of hybrid progenies is high. Nevertheless, almost no variations were seen among individuals. Perhaps the plant has lost its genetic diversity through natural selection for generations by means of seed propagation.

(6) Relatives of *Citrus* genera

"Makwit" (*Feronia*) is used as stocks for grafting and its fruit has medicinal virtues. "Matuun" (*Atalantia*) is used as stocks for grafting.

Therefore, one or two plants are often in the gardens of farmers. Both of these trees reach to 10m or more in height. The skin of the fruit is hard. In some areas, the tree trunks become submerged in water during the rainy season and those used as stocks sometimes wither and die. "Matuun" and "Makwit" are tolerant to submergence, so they would be suitable for the use as stocks.

2) Indonesia

Much fewer citrus cultivars were found in Indonesia than in either Thailand or Malaysia. This may be because the history of citrus cultivation is relatively short since its introduction far from Assam, where citrus originated.

Java is an agriculturally developed island and only an improved mandarin cultivar called "Siam" is cultivated. Sumatra, Madra and Bali are still developing, and the primitive types of cultivars are sometimes seen in the small areas of certain regions.

3) Borneo

The only citrus cultivated in Borneo are the pummelo and mandarin, and the number of cultivar is small. Since cultivation of citrus is new, cultivars planted are improved ones. Numerous closely related species of citrus were seen in the undeveloped forests and in coastal areas. Closely related species are also planted in private gardens for pharmaceutical use.

5. Conclusion

The cultivation of citrus in Southeast Asia is developing rapidly and remaining old genetic resources may become extinct in the very near future. The wild relatives that grow in tropical forests are also at the brink of extinction due to the advances of deforestation. In view of this situation, the present exploration and collection project provided a very important opportunity.

It must be important to quickly explore and collect the citrus genetic resources in the areas of Myanmar and southern China that are closer to the region where citrus originated.

Photo 1　A farmhouse and citrus tree in southern Thailand

Photo 2　Citrus sp. founded at Malay Peninsula

Photo 3 A collection site in Sumatra

Photo 4 A pummelo fruit in Sumatra

Photo 5 Various fruits at a market in Sumatra

Photo 6 A collection at swampy area in Sarawak

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