REF. NO.4 MARCH 1991

EVALUATION AND CLASSIFICATION OF PLANT GENETIC RESOURCES

TECHNICAL ASSISTANCE ACTIVITIES FOR GENETIC RESOURCES PROJECTS

JAPAN INTERNATIONAL COOPERATION AGENCY

REF. NO.4 MARCH 1991

EVALUATION AND CLASSIFICATION OF PLANT GENETIC RESOURCES

TECHNICAL ASSISTANCE ACTIVITIES FOR GENETIC RESOURCES PROJECTS

JAPAN INTERNATIONAL COOPERATION AGENCY

CONTENTS

VII. Ecotypes of Indigenous Wild Grasses, Wild Legumes and Settled Forage Crops in Japan

by Kouichi NAKASHIMA
National Grassland Research Institute, MAFF

VIII. Description for Plant Genetic Resources and a Computer Management System

by Masamichi UMEHARA[1], Hisato TAKEDA[1], Guo-Liang SHOU[2], Shinji HATTORI[1] and Shoji MIYAZAKI[1]
[1] National Institute of Agrobiological Resources, MAFF
[2] Tokyo University

Introduction

The network of international research institutes supported by the Consultative Group on International Agricultural Research (CGIAR) have played a vital role to resolution of hunger problem in the world. As a part of this effort, International Board for Plant Genetic Resources (IBPGR) is constructing an international network for preservation and management of plant genetic resources, which are expected to contribute for resolution of food and environment crisis through prevention of genetic erosion and genetic improvement of crops.

At the request of IBPGR, the Government of Japan has sponsored a special training course on plant genetic resources for trainees from the countries of Asia and Pacific Region since 1982. This special training course is performed at the National Institute of Agrobiological Resources (NIAR) in Tsukuba as one of the many training courses of Japan International Cooperation Agency (JICA).

NIAR is one of the institutes of Ministry of Agriculture, Forestry and Fisheries (MAFF) responsible for researches on plant genetic resources and biotechnology, and has a fully equipped seed storage facility as the center genebank of MAFF. In 1990, the 9th training course of this project was completed. A total of 101 trainees from 23 different countries have participated in the course since its commencement.

The object of this publication series from JICA is to present the activity on plant genetic resources in Japan for those who are engaging in the field of plant genetic resources. Especially, we hope that this series may strengthen the activity of those who already joined or are expected to join the JICA training course.

Following issues have been published in this series:

No.1 : "Preservation of Plant Genetic Resources"
No.2 : "Exploration and Collection of Plant Genetic Resources
– Seed Propagated Crops – "
No.3 : "Exploration and Collection of Plant Genetic Resources
– Vegetative Propagated Crops – "

The present issue of No. 4 covers the area of "Evaluation and Classification of Plant Genetic Resources", and the following seven articles are compiled.

The first article is on classification of rice by Drs. M. KAWASE, T. NAGAMINE and M. NAKAGAHRA, the second is on evaluation of agronomic traits of rice by Dr. H. UCHIYAMADA, the third is on variation of chemical component of soybean by Dr. K. KITAMURA, the fourth is on variation and identification of *Citrus* fruit trees by Dr. M. OMURA, the fifth is on classification of *Brassica* vegetables by Drs. H. YOSHIKAWA and S. YUI, and the sixth is on evaluation of landraces of grasses by Dr. K. NAKASHIMA.

Evaluation, classification and identification of plant genetic resources provide important information necessary for plant breeding, although each crop species has different problems specific in respective research field. Readers are expected to understand the present situation of our approach to resolve these problems.

The last article is on utilization of computer system for evaluation and management of plant genetic resources by Drs. M. UMEHARA, H. TAKEDA, G. L. SHOU, S. HATTORI and S. MIYAZAKI. Since items and methods of evaluation for agronomic traits of plant genetic resources are different from one crop to another and from one location to another, evaluation data are diverse and include numerous information. Therefore, a precise and easy information management system by computer is needed in this research field. This article shows an example of computer management system which is being developed by Dr. UMEHARA's team.

We hope that this publication will be utilized efficiently together with our previous issues of this series.

Kazumi KAWAGUCHI
Genetic Resources Coordinator
National Institute of Agrobiological Resources
Kannondai, Tsukuba, Ibaraki 305, JAPAN

Genetic Variation and Classification of Rice

by

Makoto KAWASE[1], Tsukasa NAGAMINE[1] and Masahiro NAKAGAHRA[2]

[1] National Institute of Agrobiological Resources, MAFF
Kannondai, Tsukuba, Ibaraki 305, JAPAN

[2] Agriculture, Forestry and Fisheries Research Council, MAFF
Kasumigaseki, Chiyoda-ku, Tokyo 100, JAPAN

CONTENTS

1. Introduction

There are two cultivated rice species, *Oryza sativa* L. and *O. glaberrima* STEUD. Asian cultivated rice, *O. sativa* is distributed throughout tropical, subtropical and temperate areas of the world, while African cultivated rice, *O. glaberrima* is endemic to the savanna of West Africa and is now being replaced by *O. sativa*. *O. sativa* is quite important as one of the staple food crops particularly in Asian countries. Since there is a huge variation in *O. sativa*, many researchers have studied its genetic variation, phylogenetic differentiation and intraspecific classification to provide basic information for rice genetics and breeding.

2. Cytogenetical background and taxonomy of *Oryza* species

The basic chromosome number of genus *Oryza* is 12, and diploid and tetraploid species are reported (Table 1). *O. punctata* includes both diploid and tetraploid which differ ecologically (SANO, 1980). Based on the chromosome behavior in the meiosis of interspecific hybrids, genomes A, B, C, D, E and F were classified (MORINAGA, 1940, 1959, 1964; CHU *et al.*, 1969; LI, 1964; KATAYAMA and ONIZUKA, 1979; NAYAR, 1973, etc.). It is suggested that genomes B, C and D have some partial homology.

Some confusion has occurred in the nomenclature of *Oryza* species which sometimes misled researchers. For example, an African wild species, *O. barthii* was usually referred to as *O. brebiligulata* A. CHEV. *et* ROEHR. while the name *O. barthii* was often used for another African wild rice, *O. longistaminata*. Many synonyms described to *Oryza* species were also misleading.

O. rufipogon, *O. meridionalis* and *O. longistaminata*, which are sometimes referred to as *O. perennis* complex (OKA, 1988), are closely related with Asian cultivated rice, *O. sativa*. *O. sativa* is generally thought to have been domesticated from *O. rufipogon* in Asia.

The presumed wild ancestor of African cultivated species, *O. glaberrima* is an African wild rice, *O. barthii*. Both two cultivated species and their closely related wild species are all diploids and have the same genome constitution, AA. High pollen sterility exhibited in the F_1 plants from the crosses between *O. sativa* and *O. glaberrima* suggests that these two cultivated species have probably been domesticated independently.

Table 1 A list of *Oryza* species with their chromosome number, genome formula and geographical distribution based mainly on the classification by TATEOKA (1964) and CLAYTON (1968)

Section and species	2n	Genome	Geographical distribution	Synonym	Growth habit
Sect. Oryzae					
sativa L.	24	AA	Worldwide (cultivated)		perennial
rufipogon GRIFF.[1]	24	AA	Asia[2], America[3]	*perennis* MOENCH	perennial-annual
meridionalis NG.	24	AA	Australia[4]		annual
longistaminata A. CHEV. et ROEHR.	24	AA	Africa[5]	*barthii* in the sense of TATEOKA, 1964	perennial
glaberrima STEUD.	24	AA	Africa (cultivated)		annual
barthii A. CHEV.	24	AA	Africa	*breviligulata* A. CHEV. et ROEHR.	annual
australiensis DOMIN.	24	EE	Australia		perennial
eichingeri A. PETER	24	CC	Africa		perennial
punctata KOTSCHY et STEUD.	24, 48	BB, BBCC	Africa		perennial-annual
officinalis WALL. ex WATT	24	CC	Asia		perennial
minuta J. S. PRESL. ex CHANDL.	48	BBCC	Asia		perennial
latifolia DESV.	48	CCDD	America		perennial
alta SWALLEN	48	CCDD	America		perennial
grandiglumis (DOELL) PROD.	48	CCDD	America		perennial
Sect. Schlechterianae					
schlechteri PILGER	–	–	New Guinea		?
Sect. Granulatae					
meyeriana (ZOLL. et MOR.)BAILL.	24	–	Asia	*granulata* NEES et ARN. ex WATT	perennial
Sect. Ridleyanae					
ridleyi HOOK.f.	48	–	Asia		perennial
longiglumis JANSEN	48	–	New Guinea		perennial
Sect. Angustifoliae					
brachyantha A. CHEV. et ROEHR.	24	FF	Africa		perennial-annual
angustifolia HUBBARD	24	–	Africa		?
perrieri A. CAMUS	24	–	Madagascar		perennial
tisseranti A. CHEV.	24	–	Africa		perennial

1) An annual form of *O. rufipogon* is sometimes called *O. nivara* SHARMA et SHASTRY.
2) Asian form of *O. perennis* complex in the sense of OKA and his coworkers (OKA, 1988).
3) American form of *O. perennis* complex in the sense of OKA and his coworkers (OKA, 1988).
4) Oceanian form of *O. perennis* complex in the sense of OKA and his coworkers (OKA, 1988).
5) African form of *O. perennis* complex in the sense of OKA and his coworkers (OKA, 1988).

The taxonomy of cultivated crops and their wild relatives provide confusion because there is usually a very wide morphological variation.

According to the biological species concept, either *O. sativa* and *O. rufipogon* or *O. glaberrima* and *O. barthii* can be treated as the cultivated and the wild forms (or subspecies), respectively, of a single biological species.

HARLAN and DE WET (1971) proposed three informal categories considering the possibility of gene flow between wild and cultivated species, primary, secondary, and tertiary gene pools, to provide a genetic perspective and genetic focus for cultivated plants. Primary gene pool corresponds to the concept of biological species. Secondary gene pool includes all biological species that can cross with the crop. Therefore, gene transfer is possible from secondary gene pool to the crop but may be difficult, because there is barriers that separate biological species. Tertiary gene pool from which the gene transfer to the crop is not possible or requires special techniques.

The primary gene pool of Asian cultivated rice includes *O. sativa* and *O. rufipogon*. *O. glaberrima* and *O. barthii* forms another primary gene pool. These two gene pools share a secondary gene pool which is composed of rather distantly related Oryza species.

3. Brief history of intraspecific classification of *Oryza sativa*

Since there is quite a large variation in *O. sativa*, many researchers have paid much attention to clarify genetic differentiation and to make intraspecific classification. ISO (1928) and KATO *et al.* (1928) reported hybrid pollen sterility between Japanese and foreign cultivars of *O. sativa*. KATO *et al.* (1928) considered that *O. sativa* can be classified into two subspecies which they called *japonica* and *indica*. TING (1957) stated that ssp. *japonica* and *indica* correspond with "keng" and "hsien" which have been two different types recognized by Chinese people from ancient times. He proposed to designate these subspecies as ssp. *keng* and *hsien*.

TERAO and MIDZUSIMA (1939) revealed continuous and complex variation in hybrid sterility among rice cultivars rather than two distinct groups, and suggested another group distributed in Java Island and vicinity. MATSUO (1952) investigated morphological and agronomical characters and proposed three different groups, which he designated as A, B and C types.

OKA (1953a, b, 1958) systematically surveyed genetic variation in several physiological, biochemical and morphological characters and performed genetic analysis of hybrid sterility. He classified rice cultivars into two major varietal groups, Continental (or Indica) and Insular (or Japonica) Types, subdividing the latter into Tropical and Temperate Insular Types. MORINAGA (1954) and CHANG (1976) recognized three groups, so-called indica, japonica and javanica. CHANG (1976) proposed the name sinica instead of japonica. OKA (1988) discussed again in his book "Origin of Cultivated Rice" that the Indica and Japonica types differ in many characters and genes, but there is no evidence for the entity of so-called Javanica type as the same rank as Indica and Japonica and that Javanica may be regarded as a tropical subgroup of the Japonica type.

Genetic variation at isozyme loci was examined to make a precise classification (NAKAGAHRA 1978, GLASZMANN 1986, etc.). The isozyme or protein variations of cultivated plants are useful for the studies on crop evolution, because such variations are in general thought to be neutral to selection. Isozyme analysis has an advantage to investigate the variations on a lot of loci simultaneously so that the genetic distance between populations is able to be calculated (NEI 1972). Some researchers, however, claimed that they are not always neutral (NEVO 1978).

NAKAGAHRA (1978) proposed to classify rice cultivars into four groups based on esterase isozyme genotypes. In his classification, the Indica group in a large sense can be divided into Indica in a strict sense and Sinica (or Hsien type), while so-called Japonica group into Japonica in a strict sense and Javanica. Then, the name "sinica" was used for different varietal groups; so-called japonica type of a narrow sense by CHANG (1976) and hsien type cultivars in the southern part of China and its vicinity by NAKAGAHRA (1978). For the convenience of the readers, "sinica" is not used in this article.

GLASZMANN (1986, 1987) studied 15 isozyme loci of 8 enzymes in 1,688 native cultivars from various Asian countries. He recognized six groups designated as I, II, III, IV, V and VI. Among them, groups I and VI were major groups, and II and V were minor ones. When compared with the Indica and Japonica types as classified by OKA (1958), groups VI corresponded to the Japonica including both the temperate and tropical types, while most of the varieties classified as Indica belonged to group I, and a few of them to groups II and V (OKA 1988). This indicates that the Indica type can be classified into at least three subgroups.

OGAWA *et al.* (1990) classified rice cultivars into 4 groups and 4 subgroups according to the different responses to four Philippines races of bacterial blight pathogen. The Japonica and the Javanica types fell into a group, while the Indica type cultivars were classified into several groups.

Recently, the studies on gene structure within a certain gene and the analysis of the restriction fragment length polymorphism (RFLP) served as new tools to clarify genetic differentiation in rice. Based on the RFLP variations of chromosomal DNA, Asian rice cultivars were classified into two major groups, which corresponded with the Indica and the Japonica types in a large sense (KAWASE *et al.* 1990) Each group were subdivided into several minor groups as described later.

On the other hand, folk taxonomy of rice cultivars has been developed traditionally in various regions. For example, different groups of landraces named "aus", "aman", "boro", etc. have been recognized in India according to the cultivation seasons, "hsien" and "keng" in China as previously mentioned, and "tjereh" and "bulu" in Indonesia. These groups are sometimes regarded as agro-ecotypes, which have been well adapted to the natural and artificial environment of specific areas.

4. Genetic diversity of *O. sativa*

It is quite important to know where is the geographical area of genetic diversity or how the landrace groups with specific characteristics are distributed for the researchers working on crop genetic resources for future breeding as well as on crop evolution. Several genetic traits of both "invisible" and "visible" characters have been studied in rice. "Invisible" characters, which are difficult to determine morphologically, includes isozymes, gametophyte genes, sterility genes, etc.. Such characters may not serve as a direct means for artificial selection, and useful genetic markers for the studies on phylogenetic differentiation. Some physiological characters, which are detected only in specific conditions of environmental stress, may have been faced by natural selection. On the contrary, "visible" characters may have been affected more or less by artificial selection. There is a wide range of variation in plant shape, dwarfness, plant coloration, grain size and shape, awn, etc. It is necessary to study genetic variations from both "invisible" and "visible" characters to understand genetic differentiation and genetic diversity in *O. sativa*. Several characters which have been recently studied will be discussed in this article.

1) Genetic variation at isozyme loci

NAKAGAHRA (1977) made genetic analysis of esterase isozymes in *O. sativa*. Based on the combination of 2, 3 and 2 alleles on three different loci, *Est-1, Est-2* and *Est-3*, respectively, he recognized 12 possible genotypes designated as genotypes 1 to 12 (NAKAGAHRA 1978, 1984a). The genotypes, especially genotypes 1, 3 and 6, showed interesting patterns of geographical distribution. Genotype 1 was frequently distributed in the Indian subcontinent, genotype 3 was dominated in South China and Vietnam, and genotype 6 was found in North China and Japan with a very high frequency (Fig. 1). The high genetic diversity was found in the area covering Myanmar (Burma), Thailand, Laos and Yunnan Province of China.

2) Low temperature chlorosis and chilling injury

O. sativa have been cultivated in various climatic conditions ranging from the tropics to the temperate zone. Different environmental conditions may demand different physical characteristics of rice. For example, various degrees of chlorosis are observed by low temperature treatments. CHUONG and OMURA (1982) made the genetical analysis of low temperature chlorosis and recognized five different types using temperature treatments of 20, 17 and 15°C. They identified four gene loci involved in this character. The geographical distribution of the five types showed the diversity in the area including the Indian subcontinent, Southeast Asia and the southern part of China (Fig. 2). It is easily understandable that the rice cultivars in North China and Japan which have cooler conditions in spring show tolerance to low temperature stress, because chlorosis genes should have been eliminated by natural selection. It remains uncertain, however, why most of the cultivars in the Philippines and Indonesia showed the tolerance.

Chilling injury is another symptom manifested in much cooler conditions. NAGAMINE and NAKAGAHRA (1990) screened 2,151 cultivars of rice using the treatment of 5°C for 96 hours. They classified the symptom into 7 grades ranging from fully healthy (grade 0) to completely dead (grade 6). He reported a tendency that the tolerant and sensitive cultivars are distributed in the higher and lower latitudinal regions, respectively (Fig. 3). They also suggested the varieties from Southwest China, Myanmar, Thailand, Bhutan and Malaysia showed a remarkable diversity of chilling injury.

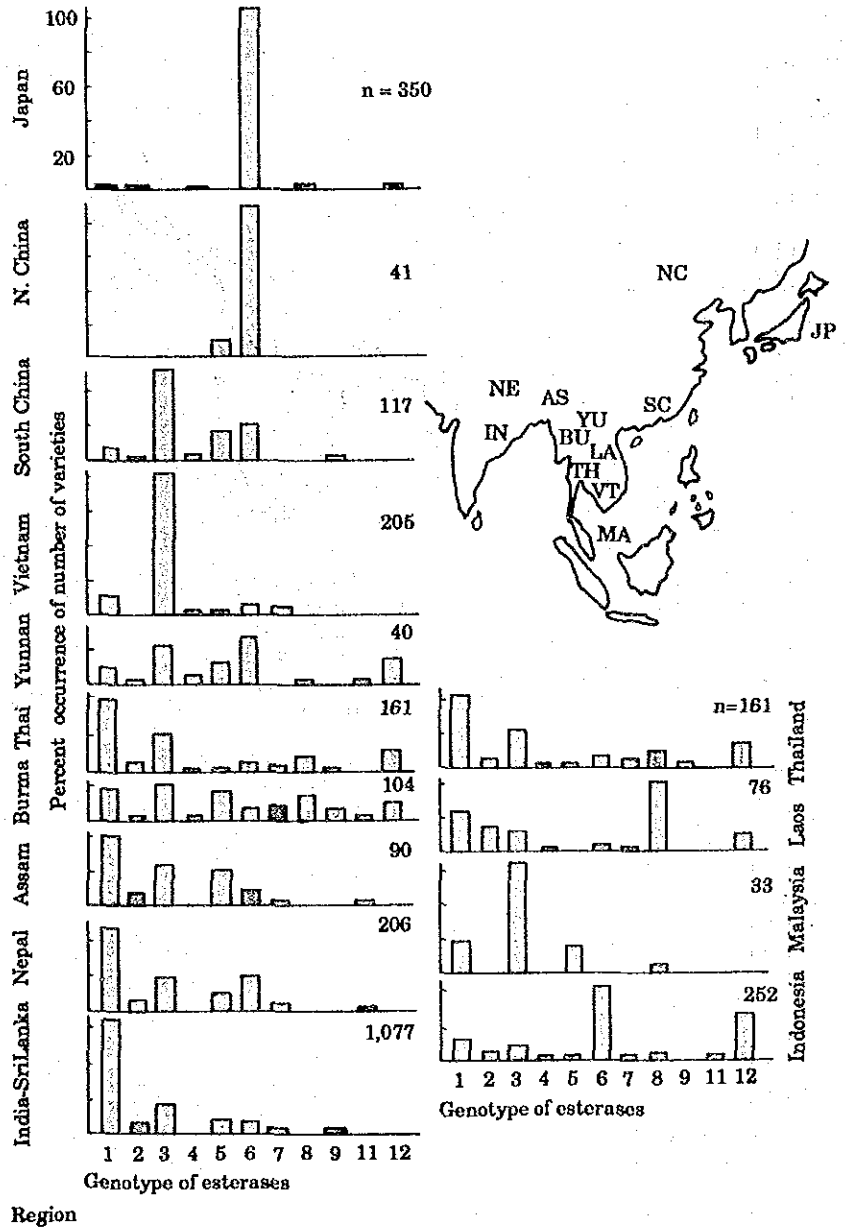


Fig. 1 Geographical distribution of esterase isozyme genotypes of native rice cultivars in Asia (NAKAGAHRA 1984a)

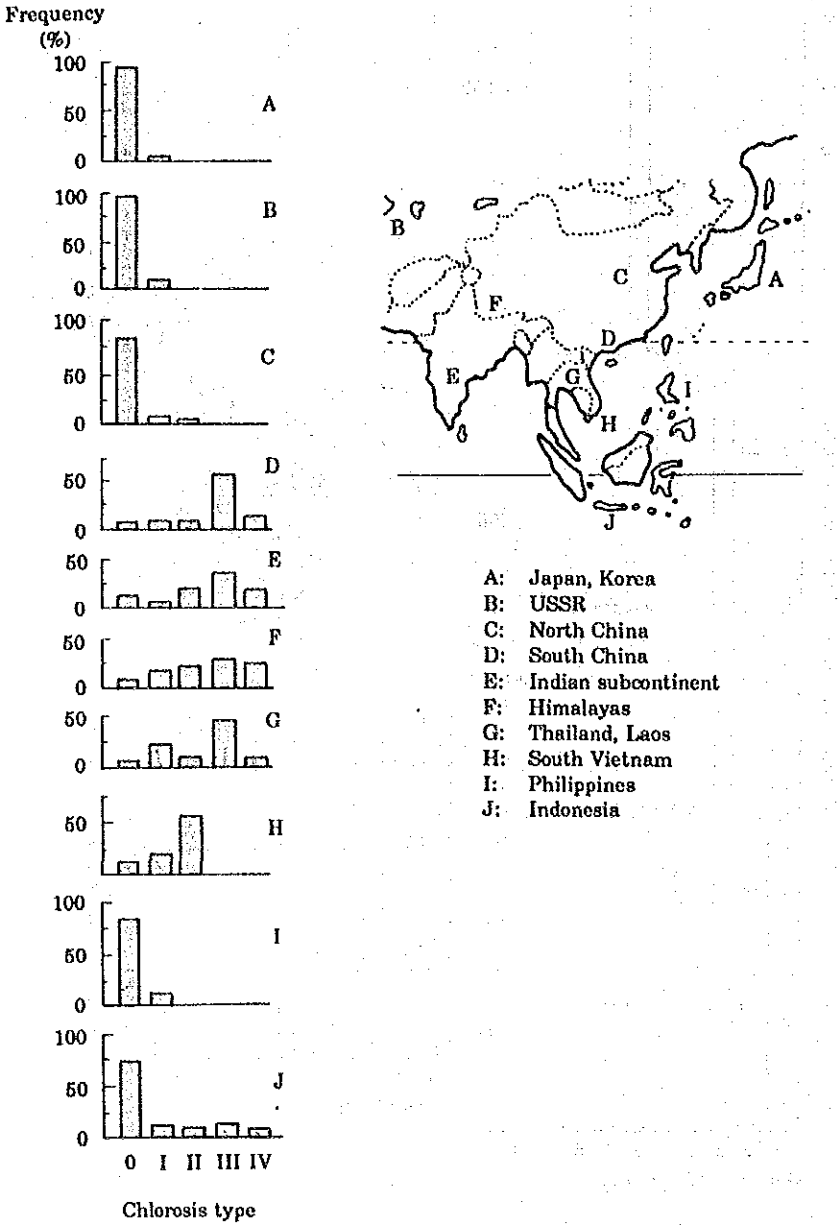


Fig. 2 Geographical distribution of five different types of low temperature chlorosis of rice in Asia (CHUONG and OMURA 1982)

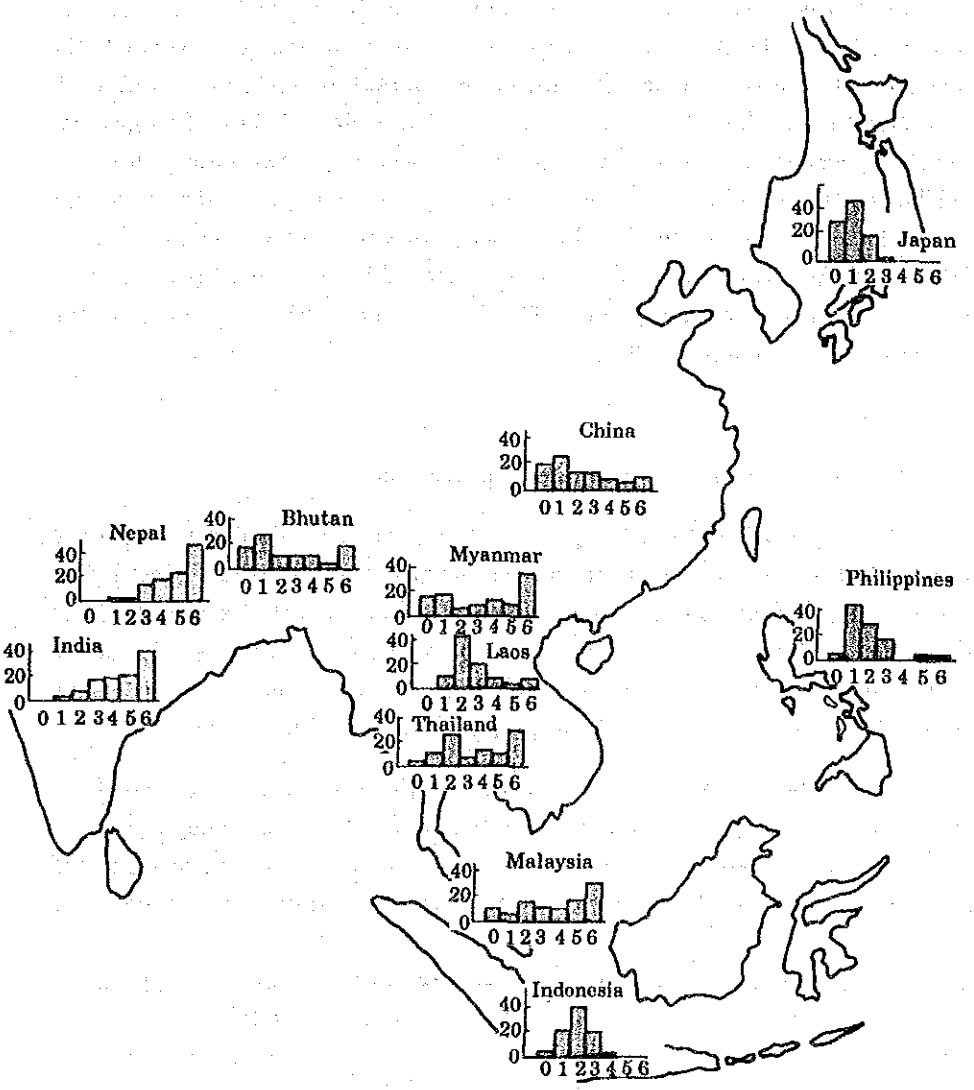


Fig. 3 Geographical distribution of chilling injury in Asian rice (NAGAMINE and NAKAGAHRA 1990)

Note : The vertical axis shows the frequency of occurrence of chilling injury (%). The horizontal axis shows the degree of chilling injury; 0 (healthy) ~ 6 (leaves completely withered).

3) Variation of mesocotyl elongation ability

Another interesting physiological character is the mesocotyl elongation of young seedlings in the dark. KATSUTA (personal communication) investigated this character under the conditions with and without plant hormones among different groups of cultivars (Table 2). The cultivars used were classified into four groups; Indica type, Hsien type, Javanica type and Japonica type by NAKAGAHRA. Without plant hormones, the Indica and the Javanica type cultivars showed remarkable mesocotyl elongation, while the Hsien type cultivars and the Japonica type cultivars did not. When gibberellin A_3 and abscisic acid were added, all four groups increased in mesocotyl elongation. The Hsien type cultivars showed larger increase than the Japonica cultivars.

4) Geographical distribution of gametophyte genes

The segregation of marker genes in the F_2 generations derived from the crosses between rice cultivars sometimes shows deviation from the expected ratio of segregation. This phenomenon, which is called distorted segregation (or segregation distortion), can be explained by the selection of hybrid gametes due to the presence of gametophyte genes closely linked with the marker genes on the chromosomes. It is regarded as another good indicator of genetic divergence.

NAKAGAHRA (1984b) made observations in the F_2 populations derived from 2,700 cross combinations. His findings on the distribution of gametophyte genes are summarized as Table 3. Concerning the gametophyte genes, Japonica type and Javanica type showed to have only a few genotypes, while Indica type (Indian rices

Table 2 Variation of mesocotyl elongation with and without gibberellin A_3 and abscisic acid in the dark (KATSUTA, unpublished)

Cultivar group*	No. of cultivars	Mesocotyl length (mm)		Mean ratio of treated/control
		Control	Treated**	
Indica	36	17.8 ± 1.5	35.8 ± 1.8	2.7 ± 0.1
Hsien	25	3.2 ± 0.7	19.6 ± 3.9	22.9 ± 5.1
Javanica	36	11.7 ± 2.0	23.5 ± 3.9	3.5 ± 0.6
Japonica	37	2.4 ± 0.4	12.0 ± 2.1	14.1 ± 4.6

* Cultivar groups were classified by NAKAGAHRA.

** Germination seed were incubated under the condition of 25ppm GA_3 +0.5ppm ABA in the dark.

Table 3 Distribution of gametophyte genes in Asian rice cultivars (Modified from NAKAGAHRA, 1986)

Varietal groups	Gametophyte gene			
	*ga-2**	*ga-3*	*ga-4*	*ga-8*
Japonica type				
Japonica cultivars (Japan, N.China etc.)	*ga*	+	*ga*	+
Japonica cultivars (Keng from S.China)	*ga*	*ga*	*ga*	+
A part of Javanica cultivars	*ga*	*ga*	*ga*	+
Upland rice cultivars from the Philippines	*ga*	+	*ga*	+
Indica type				
Hsien rice cultivars (subgroup A)	+(A)	+	+	+
Hsien rice cultivars (subgroup B)	+(A)	+	+	*ga*
Indian rice cultivars (subgroup A)	+(B)	+	+	+
Indian rice cultivars (subgroup B)	*ga*	+	+	*ga*
Indian rice cultivars (subgroup C)	*ga*	*ga*	+	*ga*

* *ga-2*+(A) is stronger than *ga-2*+(B) in fertilizing capacity.

and Hsien type cultivars) showed to have many genotypes. This suggests that the indigenous cultivars of India and southern China can be divided into several varietal groups, while so-called Japonica and Javanica types were not differentiated so widely.

5) Variation of amylose content of endosperm

Endosperm characters are very important, because they directly influence the eating quality. It was believed that there were two distinct types of endosperm; non-waxy (non-glutinous) and waxy (glutinous). These two types were easily distinguishable by observation or by KI-I_2 color reaction. They are controlled by the *Wx* allele and the *wx* allele on a single locus. Non-waxy endosperm starch contains both amylose and amylopectin. Almost no amylose is involved in waxy starch. SANO (1984) recognized two dominant alleles, Wx^a (mostly in Indica type) and Wx^b (in Japonica type). Higher amylose content is expressed by Wx^a than Wx^b.

Recent screening of a huge range of rice genetic resources revealed that there is a wide variation of amylose contents in endosperm as shown in Fig. 4 (KATSUTA *et al.* 1989). The indigenous cultivars collected from India and Bangladesh had very high amylose contents showing a narrow variation. The cultivars from Nepal had both very high and high amylose contents, which might reflect Wx^a and Wx^b alleles, respectively. The diversity of this character was found in the area including the

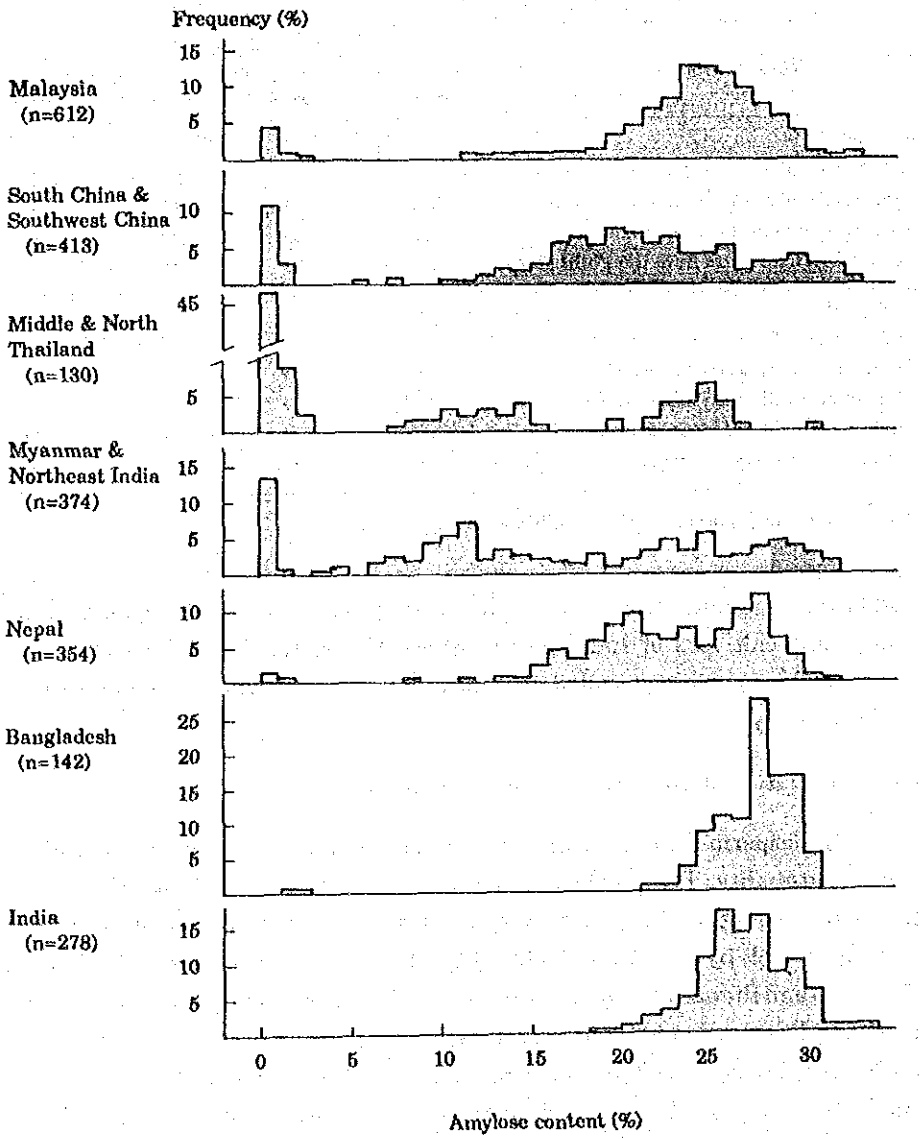


Fig. 4 The variation and geographical distribution of amylose content in rice endosperm starch (KATSUTA *et al.* 1989)

northeastern part of India, Myanmar, Thailand and the southwestern part of China.

6) Restriction fragment length polymorphism

Restriction fragment length polymorphism (RFLP) represents differences in the length of the DNA fragment arising after the treatment with restriction endonuclease. ISHII *et al.* (1987) studied the RFLP of chloroplast DNA in *O. sativa* and related species. The studies on the RFLP of chromosomal DNA in rice have been made mainly for constructing linkage maps (McCOUCH *et al.* 1988, KISHIMOTO *et al.* 1989). Since variation is detected directly at the DNA level, RFLP can provide important information for the studies on genetic diversity and phylogenetic differentiation (TANAKA *et al.* 1989).

KAWASE *et al.* (1990) analyzed RFLP on a total of 135 accessions of rice, *O. sativa* L., of which 123 were local cultivars collected mainly from Asia, using 57 DNA probes. The cluster analysis of unweighted pair group method using arithmetic means and principal component analysis (PCA) were employed for investigating intraspecific variation and classifying rice cultivars (Figs. 5 and 6). The PCA revealed two major groups of landraces, and only a few cultivars were found between them. The two groups clearly corresponded with two large clusters, tentatively designated as clusters I and II in the dendrogram drawn by cluster analysis. The landraces involved in cluster I usually showed a positive phenol color reaction (*Ph* allele) and have $Est3^2$ allele, while most of those in cluster II showed a negative reaction (*ph* allele) and have $Est3^1$. Those two distinct landrace groups are thought to correspond with the conventional varietal groups, the Indica type and the Japonica (including Javanica) type, respectively. The cultivars in cluster I contained those from Malaysia, Vietnam, Bangladesh, India, Yunnan Province of China, Sri Lanka, Myanmar, South China, Thailand, Nepal, etc.. Cluster II included cultivars from Indonesia, Laos, Japan, Nepal, North Pakistan, Yunnan Province of China, the Philippines, etc.. Cluster I comprised small clusters designated as Ia, Ib, Ic, Id and Ie. The landraces involved in clusters Ia, Ib, Ic and Id were distributed with overlapping in the scattered diagram by PCA, while those in cluster Ie were clearly separated from them. Cluster II contained small clusters, designated as IIa, IIb and IIc, of which the landraces were distributed separately in the scattered diagram. The geographical distribution of those two groups are overlapping in Southeast Asia and vicinity.

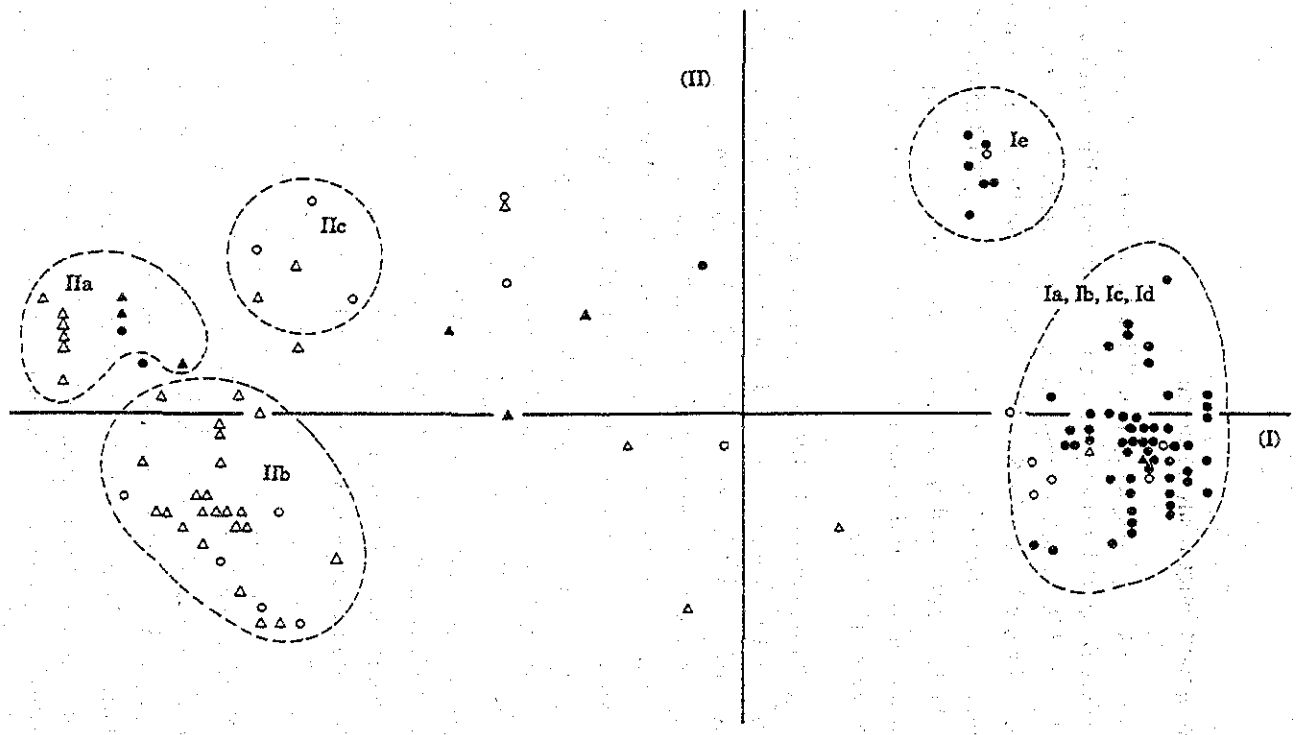


Fig. 5 A scattered diagram obtained from PCA analysis on RFLP of 123 landraces of rice (KAWASE *et al.* 1991)

Note : The horizontal and the vertical axis show the first and second principal component, respectively. A black circle; *Est3*^{a} and *Ph*, an open circle; *Est3*^{a} and *ph*, a black triangle; *Est3*^{b} and *Ph*, and an open triangle; *Est3*^{b} and *ph*.

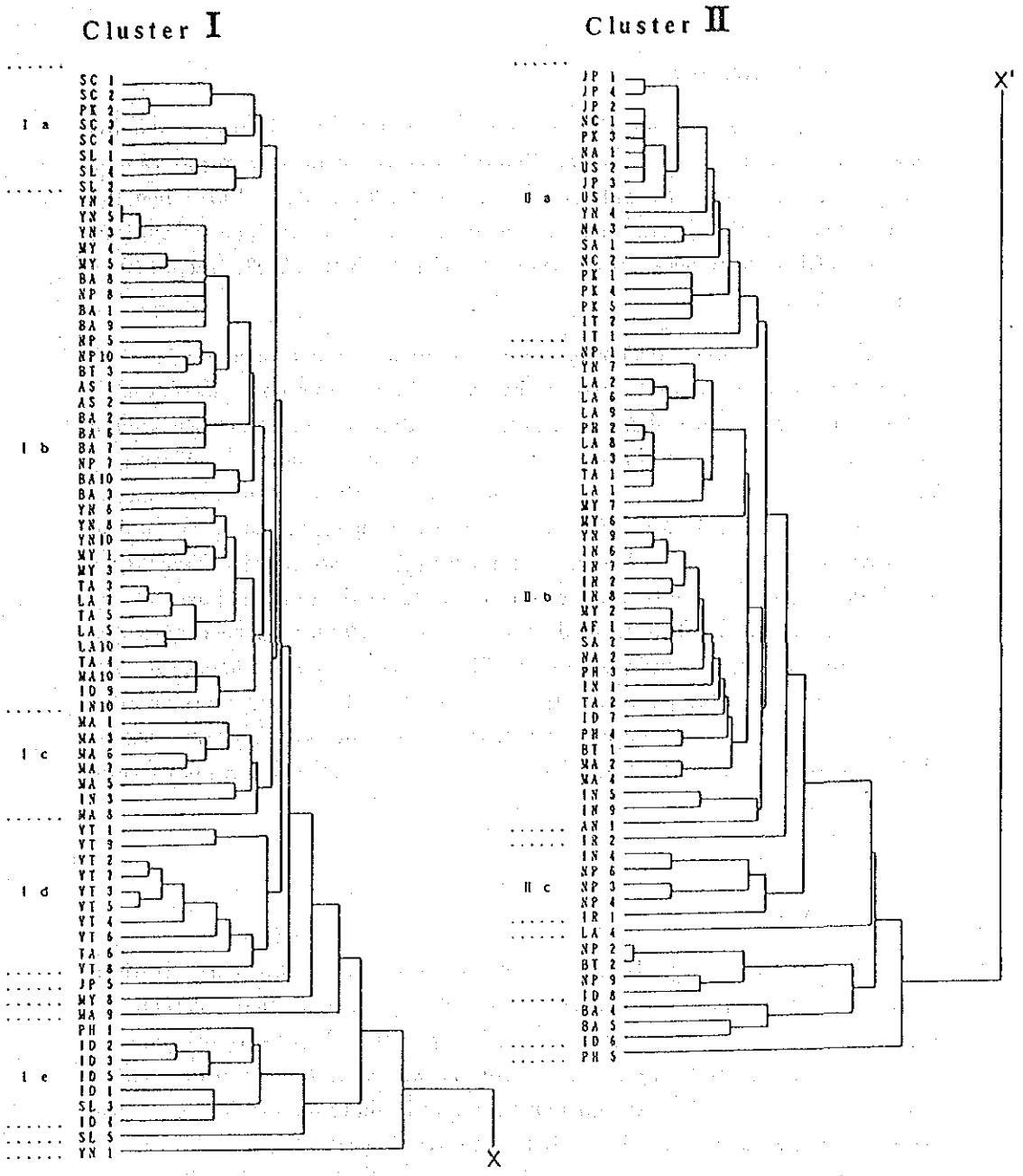


Fig. 6 A dendrogram indicating relationships among 135 rice cultivars based on cluster analysis of RFLP (KAWASE *et al.* 1991)

Note : It is separately shown as clusters, I and II, which are connected between X and X'.

Symbols : JP (Japan), NC (North China), SC (South China), YN (Yunnan Province, China), PH (Philippines), VT (Vietnam), TA (Thailand), MY (Myanmar), LA (Laos), MA (Malaysia), IN (Indonesia), NP (Nepal), BT (Bhutan), AS (Assam State, India), BA (Bangladesh), ID (India), PK (Pakistan), SL (Sri Lanka), IR (Iran), AN (Afganistan), US (USSR), IT (Italy), NA (North America) and SA (South America).

7) Center of diversity

N. I. VAVILOV, a famous Russian geneticist and Agronomist proposed in his book "On the Origin of Cultivated Plants" that the center of origin can be determined by an analysis of pattern of variation (VAVILOV 1926). Since then, the concept of centers of origin has evolved. Some authors, however, pointed out that the center of diversity is not necessary the center of origin (SMITH 1969, HARLAN 1970, ZOHARY 1970).

It is important not only for the crop evolutionists but also for geneticists and/or breeders to know where the genetic diversity of a certain crop is distributed in order to collect and utilize plant genetic resources. Genetic diversity of rice is found in the area including the northeastern part of Indian subcontinent, Myanmar, Thailand and the southwestern part of China (Yunnan Province) in many characters as mentioned above. Most of this area is covered by heterogenous geographical and ecological conditions such as hills and mountains. Ethnological diversity is also found in those who cultivate rice in the area. It is still uncertain how and why the genetic diversity has been formed, although this area have often been suggested as geographical origin of cultivated rice. The discussion of the domestication of Asian rice is not contained in this article, because it needs much more detailed studies on the genetic variations in cultivated rice and wild relatives, on archaeological remains of Neolithic culture as well as on the ethnobiology of wild and cultivated rice.

5. Conclusion

We have two cultivated rice species; Asian cultivated rice, *O. sativa* and African cultivated rice, *O. glaberrima*. Both are diploids having A genome, but there are reproductive barriers between them. The primary gene pool of Asian cultivated rice includes *O. sativa* and *O. rufipogon*. *O. glaberrima* and *O. barthii* forms another primary gene pool. These two primary gene pools share a secondary gene pool which is composed of rather distantly related Oryza species.

Since there is a very wide and complex variation in *O. sativa*, many researchers have tried to classify this crop into cultivar groups. The history, however, of the intraspecific classification was full of trials and errors. It is necessary to study not a single or a few characters but several different genetic traits systematically.

At present, it is generally agreed that there are two distinct cultivar groups of rice; the Japonica type and the Indica type. Both groups can probably be subdivided into several minor groups using morphological characters, isozymes, gametophyte genes, sterile genes, RFLP and so on. There is rather continuous variation within each group compared with between the two groups. Further studies should be needed to integrate our knowledge on rice genetics and to build up a precise classification. For this purpose, studies on DNA level will be quite helpful.

Better classification will be obtained as the result of better understanding of the genetic variation and differentiation in *O. sativa*.

Analyses of different genetic aspects will serve for the evaluation and utilization of the rice genetic resources preserved in the world.

6. References cited

CHANG, T. T., 1976. The origin, evolution, cultivation, dissemination, and diversification of Asian and African rices. Euphytica 25:425-441.

CHU, Y. E., H. MORISHIMA and H. I. OKA, 1969. Reproductive barriers distributed in cultivated rice species and their wild relatives. Japan J. Genet. 44:207-223.

CHUONG, P. V. and T. OMURA, 1982. Studies on the chlorosis expressed under low temperature condition in rice, *Oryza sativa* L. Bull. Inst. Trop. Agr., Kyushu Univ. 5:1-58.

CLAYTON, W. D., 1968. Studies in Gramineae: XVII. West African wild rice. Kew Bulletin 21: 485-488.

GLASZMANN, J. C., 1986. A varietal classification of Asian cultivated rice (*Oryza sativa* L.) based on isozyme polymorphism. *In* IRRI (ed.), Rice Genetics, pp.83-90.

GLASZMANN, J. C., 1987. Isozymes and classification of Asian rice varieties. Theor. Appl. Genet. 74:21-30.

HARLAN, J. R., 1970. Evolution of cultivated plants. *In* FRANKEL, O. H. and E. BENNETT (eds.), Genetic Resources in Plants - Their Exploration and Conservation. IBP Handbook No. 11:19-32. Blackwell Scientific Publications, Oxford.

HARLAN, J. R. and J. M. J. DE WET, 1971. Toward a rational classification of cultivated plants. Taxon 20:509-517.

ISHII, T., T. TERACHI and K. TSUNEWAKI, 1987. Chloroplast genome differentiation in the A genome diploid species of *Oryza*. Japan J. Breed. 37 (Separate vol. 1):98-99 (in Japanese).

ISO, N., 1928. Researches on the Formosan rice plants with special reference to the plant breeding and culture. Rep. Dept. Agr. Govern. Res. Inst. Formosa, Japan 37, pp.315 (in Japanese).

KATAYAMA, T. and W. ONIZUKA, 1979. Intersectional F_1 plants from *Oryza sativa* × *O. ridleyi* and *O. sativa* × *O. meyeriana*. Japan J. Genet. 54:43-46.

KATO, S, H. KOSAKA, S. HARA, 1928. On the affinity of rice varieties shown by the fertility of hybrid plants. Rep. Bul. Fak. Terkult, Kyushu Imper. Univ. 3:132-147 (in Japanese with English summary).

KATSUTA, M., T. NAGAMINE, M. OKA, K. OKUNO, Y. EGAWA, M. KAWASE, M. NAKAGAHRA, 1989. Genetic variations and geographical distribution on amylose content of Asian rice cultivars. Japan J. Genet. 39 (Separate vol. 2):258-259 (in Japanese).

KAWASE, M., N. KISHIMOTO, T. TANAKA, A. YOSHIMURA, S. YOSHIMURA, K. SAITO, A. SAITO, M. YANO, N. TAKEDA, T. NAGAMINE and M. NAKAGAHRA, 1991. Intraspecific variation and genetic differentiation based on restriction fragment length polymorphism in Asian cultivated rice, *Oryza sativa* L. Proc. 2nd Rice Genet. Symp. (in Printing).

KISHIMOTO, N. M. YANO, T. TANAKA, K. SAITO, A. SAITO, S. KUHARA, T. NAGAMINE, M. KAWASE, M. KATSUTA, S. YOSHIMURA, A. YOSHIMURA, M. NAKAGAHRA, N. IWATA, 1989. Linkage mapping of RFLP markers of nuclear DNA, morphological markers and isozyme loci in rice (*Oryza sativa* L.). Proc. 6th Intern. Congr. SABRAO (1989):489-492.

LI, H. W., 1964. Studies on genetic and cytogenetic evidence for species relationships in the Republic of China. *In* IRRI (ed.), Rice Genetics and Cytogenetics, pp.118-131. Elsevier, Amsterdam.

MATSUO, T., 1952. Genecological studies on cultivated rice. Bull. Natl. Inst. Agr. Sci., Japan. D3:1-111 (in Japanese with English summary).

McCOUCH, S. R., G. KOCHERT, Z. H. YU, Z. Y. WANG, G. S. KHUSH, W. R. COFFMANN and S. D. TANKSLEY, 1988. Molecular mapping of rice chromosomes. Theor. Appl. Genet. 76:815-829.

MORINAGA, T., 1940. Cytogenetical studies on *Oryza sativa* L. IV. The cytogenetics of F_1 hybrid of *O. sativa* L. and *O. minuta* PRESL. Japan J. Bot. 11:1-16.

MORINAGA, T., 1954. Classification of rice varieties on the basis of affinity. *In* Studies on Rice Breeding (A separate volume of Japan J. Breed.) 4:1-14.

MORINAGA, T., 1959. Note on genome analysis in *Oryza* species. Int. Rice.Commun. Newsl. 8:10-11.

MORINAGA, T., 1964. Cytogenetical investigations on *Oryza* species. *In* IRRI (ed.), Rice Genetics and Cytogenetics, pp.91-110. Elsevier, Amsterdam.

NAGAMINE, T. and M. NAKAGAHRA, 1990. Genetic variation of chilling injury at seedling stage in rice, *Oryza sativa* L. Japan J. Breed. 40:449-455.

NAKAGAHRA, M., 1977. *Genic analysis for esterase isoenzymes in rice cultivars.* Japan J. Breed. 27:141-148.

NAKAGAHRA, M., 1978. The differentiation, classification and center of genetic diversity of cultivated rice (*Oryza sativa* L.) by isozyme analysis . Trop. Agr. Res. Ser. 11:77-82.

NAKAGAHRA, M., 1984a. Geographical distribution of esterase genotypes of rice in Asia. Rice Genet. Newsl. 1:118-120.

NAKAGAHRA, M., 1984b. Geographic distribution of gametophyte genes in wide crosses of rice cultivars. *In* IRRI (ed.), Rice Genetics, pp.73-82.

NAYAR, N. M., 1973. Origin and cytogenetics of Rice. Advances in Genetics 17:153-292. Academic Press, New York.

NEI, M., 1972. Genetic distance between population. Amer. Natur. 106: 283-292.

NEVO, E., 1978. Genetic variation in natural populations: pattern and theory. Theor. Popul. Biol. 13:121-177.

OGAWA, T., G. A. BUSTO, R. E. TABIEN, G. O. ROMERO and G. S. KHUSH, 1990. Grouping of rice cultivars based on reaction pattern to Philippines races of bacterial blight pathogen. *Japan J. Breed. 40 (Separate vol. 1):282-283.*

OKA, H. I., 1953a. Phylogenetic differentiation of the cultivated rice plant. I. Variation of various characters and character combinations among rice varieties. Japan J. Breed. 3:33-43 (in Japanese with English summary).

OKA, H. I., 1953b. Phylogenetic differentiation of the cultivated rice plant. II. Classification of rice varieties by intervarietal hybrid sterility. Japan J. Breed. 3:1-6 (in Japanese with English summary).

OKA, H. I., 1958. Intervarietal variation and classification of cultivated rice. Ind. J. Genet. Plant Breed. 18:79-89.

OKA, H. I., 1988. Origin of Cultivated Rice. pp. 254. Japan Scientific Societies Press, Tokyo; Elsevier, Amsterdam.

SANO, Y., 1980. Adaptive strategies compared between diploid and tetraploid forms of *Oryza punctata*. Bot. Mag., Tokyo 93:171-180.

SANO, Y., 1984. Differential regulation of waxy gene expression in rice endosperm. Theor. Appl. Genet. 68:467-473.

SMITH, C. E., 1969. From VAVILOV to the present - a review. Econ. Bot.3:2-19.

TATEOKA, T., 1964. Taxonomic studies of the genus *Oryza*. *In* IRRI (ed.), Rice Genetics and Cytogenetics, pp.15-21. Elsevier, Amsterdam.

TANAKA, T, M. KAWASE, A. SAITO, M. YANO, N. KISHIMOTO, K. SAITO, A. YOSHIMURA, N. TAKEDA, T. NAGAMINE, M. NAKAGAHRA, 1989. Intraspecific variation on restriction fragment length polymorphism of nuclear DNA in rice, *Oryza sativa* L. Proc. 6th Intern. Congr. SABRAO (1989): 599-602.

TERAO, H. and U. MIDZUSIMA, 1939. Some consideration on the classification of *Oryza sativa* L. into two subspecies, so-called 'Japonica' and 'Indica'. Japan J. Bot. 10:213-258.

TING, Y., 1957. The origin and evolution of cultivated rice in China. Acta. Agron. Sin. 8:243-260 (in Chinese).

VAVILOV, N. I., 1926. Studies on the Origin of Cultivated Plants. pp.248. Inst. Appl. Bot. Plant Breed., Leningrad.

ZOHARY, D., 1970. Centers of diversity and centers of origin. *In* FRANKEL, O. H. and E. BENNETT (eds.), Genetic Resources in Plants - Their Exploration and Conservation. IBP Handbook No. 11:33-42. Blackwell Scientific Publications, Oxford.

Evaluation of Agronomic Traits of Rice (*Oryza sativa* L.)

by

Hiroshi UCHIYAMADA

National Federation of Agricultural Co-Operative Associations, ZEN-NOH
Higashi yawata, Hiratsuka, Kanagawa 254, JAPAN

CONTENTS

1. Introduction

Japan has about 2 millions ha of paddy fields, where about 180 rice cultivars of non-glutinous and 60 of glutinous are being planted. Of them, two cultivars, "Koshihikari" and "Sasanishiki", occupy about 40% of the whole paddy area, and 76 % of which is covered by 20 high-ranking cultivars. Such predominance by a small number of cultivars is a recent trend not only in Japan but also throughout the world. Whereas, landraces, pure-line selections and outdated improved cultivars developed by cross breeding are about to disappear from farmers' fields.

Plant breeders, who used to search for genetic resources from farmers' fields, have now to find out the resources from gene banks, in which valuable breeding materials are preserved.

Systematic rice breeding at national organizations in Japan started in 1927. As far as the author knows, the number of experimental breeding lines which were developed and given local numbers by the national breeding systems was 2,786. Of them 349 were registered as "Norin"number cultivars by Ministry of Agriculture, Forestry and Fisheries (MAFF). Besides the national breeding system, 3,943 experimental breeding lines were developed by prefectural breeding centers, and of them 466 were registered as cultivars by the prefectural governments. By both national and the prefectural organizations, a total of 6,729 experimental breeding lines were developed, and of them 815 were registered as cultivars so far.

Preservation and evaluation of these experimental breeding lines and registered cultivars are of varietal importance as well as those of landraces for further breeding work. In fact, even improved cultivars disappeared fast from farmers' fields, once they were cancelled from the lists of varietal recommendation. Preservation of such materials, therefore, should be emphasized. As breeding objectives are changed following agricultural situations, those experimental breeding lines and cultivars which were cancelled from recommendation still have a possibility to be utilized as genetic resources when they are evaluated from a viewpoint of new breeding objectives.

2. Information and evaluation of agronomic traits for genetic resources

There are many outstanding breeding materials which were evaluated among preserved genetic resources. Some of them are cited below.

Until the mid of 1960s, farmers in the southern Japan used to plant high-stature cultivars because short-stature cultivars gave low yield in infertile farms which were wide spread in the region. Since the mid of 1960s, rice breeders there have succeeded in developing new short-stature cultivars, such as "Hoyoku", "Reiho", "Nishihomare", and others by utilizing a semi-dwarf gene (*sd-1*) of indigenous variety, "Jukkoku". Since then farmers in this region have stably gained high yield by adopting the semi-dwarf cultivars and by an increased use of fertilizer.

Tolerance to cool temperature is one of the most important breeding objectives in the northern Japan, and indigenous cultivars from the cool region have been collected as genetic resources for breeding cold-tolerant cultivars. Genetic resources for the screening of cold-tolerance have also been collected from central parts of Japan and hilly areas in the tropics. Some cold-tolerant genetic resources, such as "Koshihikari", "Todoroki-wase", "Silewah" and others have been used to strengthen breeding works against cool damage in Tohoku and Hokkaido regions.

The third example is the breeding for stripe-disease-resistant cultivars with progeny lines of a *japonica-indica* cross. As no resistant cultivar was found among Japanese rice varieties, the resistance gene of *indica* varieties had to be introduced in this work. A resistance gene (*Stv-2*[1]) of *indica* variety, "Modan", was introduced into a Japanese variety, "Norin 8", by means of backcrossing, then stripe-disease-resistant varieties, such as "Mineyutaka", "Musashi-kogane", "Tsuki-no-hikari" and others, were developed by the interim lines.

As mentioned above, information on agronomic traits of genetic resources is necessary in utilizing them. Genetic resources conserved without any information on agronomic traits, therefore, are of no value for breeding. The more detailed data exist on agronomic traits, the more valuable become the genetic resources.

Genetic resources information consists of two parts, namely passport data and evaluation data on agronomic traits.

1) Passport data

Passport data consist of histories of accessions and their storage condition. For example, the data include name of crop species, name of cultivar and/or line, history, collection or introduction site, harvested year, quantity, address number in store room, and others. These data are very much important in dispatching accessions safely to plant breeders and researchers.

The MAFF gene bank is routinely working to complete these data by examining storage conditions at the national breeding organizations.

2) Evaluation data on agronomic traits

When new plant genetic resources are introduced from abroad, MAFF conducts three levels of evaluation on agronomic traits by following procedures.

In case of rice, the first level of evaluation is conducted on morphological traits except heading date, such as culm length, panicle length, panicle number, apiculus color, grain length, grain width, glutinous or non-glutinous, and others. The items are chosen from the viewpoint of easiness in measuring, and are those with a high heritability. Data on these agronomic traits are recorded for all of new introductions.

The traits for the second level evaluation are mainly of physiological and ecological ones, such as disease and insect resistance, cool tolerance, and others. Evaluation of these traits are rather laborious and costly, because some special environmental conditions or facilities are required for testing each trait. Therefore, it is not practical to perform the second level evaluation on many traits and for all introductions. The MAFF genebank, at present, conducts the second level evaluation on blast resistance and bacterial leaf blight resistance for nearly all introductions, but brown planthopper resistance and cold-tolerance are evaluated only for some selected introductions.

The third level evaluation are conducted on agronomic traits of practical importance, such as yield, adaptability, grain and cooking quality. The evaluation is usually performed at replicated yield trials or at trials of breeding lines for recommendation which are conducted for more than three years at many locations of respective prefectures.

IBPGR listed 43 items of agronomic traits for evaluation; i.e. leaf characters (7 items), auricle and ligule (5 items), heading and maturing date (2 items), culm (6 items), panicle (7 items), husked grain and glume (17 items). These are mainly morphological traits which are easily measured, and are corresponding to the traits of the first level evaluation of at the MAFF gene bank, though the number of items in the IBPGR list are more than that at the MAFF.

3. Evaluation of rice genetic resources at Hokuriku National Agricultural Experiment Station

In order to find out valuable breeding materials for a significant yield increase in Japanese rice varieties, we planted rice genetic resources which had been preserved in the gene bank of Department of Genetics, National Institute of Agricultural Sciences, and evaluated their agronomic traits from breeder's point of view. The data from this evaluation were utilized efficiently to choose valuable breeding materials in a MAFF special project, "Rice Breeding for Superhigh Yield".

1) Materials and methods

We evaluated 50 items of agronomic traits for about 5,000 accessions in total; of them about 1,700 accessions in 1976, about 1,900 in 1977, about 1,000 in 1978, and about 200 in 1979. Materials were planted in the same way as usual breeding experiments as follows; sowing on protected nursery on early April, removing protecting plastic film from nursery on late April, transplanting seedlings on paddy field on mid May with 1 plant/hill of two rows, and 44 hills/plot without replication. A set of three cultivars, "Reimei", "Koshihikari" and "Nipponbare", were inserted four times for each field (5a) as the standard.

2) Observation methods and levels of significance

The objective traits for the evaluation are corresponding to the first and the second level evaluation of the MAFF gene bank, and some accessions selected from the test were further evaluated at the third level.

The traits evaluated, number of individuals observed, measuring unit, and criteria of classification are shown in Table 1. There are two categories of traits, namely quality and quantity traits. Since the data of quantity traits are easily affected by environmental condition, we calculated standard deviations for the quantity traits with replicated data of three standard cultivars, and used these values, i.e., mean$\pm 2\sigma$ as a measure for the level of significant difference between entries (Table 2).

Table 1 Observation methods and criteria for evaluation (1/2)

Items	Sample size for evaluation	Measuring units and classification criteria
Examination No.		
Entry No.		
Variety name		
Origin		
Heading date	1 plot	Visual observation, month and date ×: non-heading, ××: non-heading, especially late
Culm length	5 ind.	cm
Panicle length	5 ind.	cm (mm as measuring unit)
Panicle number	5 ind.	number/ind.
Plant type	1 plot	Classified into MATSUO's plant type, A, B and C, by visual observation
Grain yield	20 ind.	Percentage for average grain yield of 17 plots/10a of standard variety "Todorokiwase"
Lodging	1 plot	0 (non) to 5 (severe) by visual observation
Bird damage	1 plot	0 (non) to 5 (severe) by visual observation
Panicle blast damage	1 plot	0 (non) to 5 (severe) by visual observation, 6 for badly severe as exceptional case
Stem maggot damage	1 plot	0 (non) to 5 (severe) by visual observation
Rice leaf beetle damage	1 plot	0 (non) to 5 (severe) by visual observation
Herbicide damage	1 plot	0 (non) to 5 (severe) by visual observation
Leaf blast at disease nursery	1 plot	Field resistance : 0 (no lesion) to 9 (extremely weak), "Homarenishiki" (3), "Norin 22" (5), and"Koshihikari" (7) True resistance : R' for *Pi-k*, R for other alien true resistance gene(s)
Bacterial leaf blight by artificial inoculation	2 ind. each for 4 bacterial groups	Differential bacterial group I, II, III and IV, RRSS means that R for I and II group and S for III and IV group
Sheath blight at disease garden	2 ind.	0 (non) to 5 (severe) by visual observation
Germination speed	1 plot	Visual observation after removal of plastic film from protected nursery
Seedling height	1 plot	1 (short) to 5 (long) by visual observation, 6 and 7 for extremly long as exceptional cases
Leaf blade	1 plot	1 (narrow) to 5 (broad) by visual observation
Leaf angle	1 plot	A for vertical, D for horizontal and F for drooping leaf
Discoloration by cool temp.	1 plot	0 (slight) to 5 (severe) by visual observation after removing plastic film at nursery
Killing by cool temp.	1 plot	0 (slight) to 5 (severe) by visual observation after removing plastic film at nursery
Tillering type	1 plot	1 (close) to 5 (open), 3 (medium) for "Koshihikari"
Pubescence of hull	10 grains	0 (non) to 5 (many) by visual observation on hull
Pubescence of leaf	1 leaf	0 (non) to 5 (many) by feeling of finger
Grain number per panicle	2 panicles	Grain number on the longest panicle, 2 ind.

Table 1 Observation methods and criteria for evaluation (2/2)

Items	Sample size for evaluation	Measuring units and classification criteria
Grain number per 10cm	2 panicles	Grain number per panicle / panicle length × 10
Shattering	2 ind.	0 (hard) to 6 (very easy) by hand-gripping
Diameter of culm base	2 stems	0.1mm, mean diameter at 10cm from base
Diameter of panicle base	2 panicles	0.1mm, max. diameter at 1cm under neck of the longest panicle
Sprouting	3 ind.	0 (difficult) to 5 (very easy) by visual observation
Awn	1 plot	0 (non) to 5 (long and many), and 6 for special long
Degree of fixation	1 plot	× means segregation, by visual observation mainly for heading date and plant type
Evaluation at tillering stage	1 plot	◎(good), O (fairly good), △ (medium) and × (poor) by visual observation for tillering type and leaf color except Japanese entries
Evaluation at maturing time	1 plot	◎ (good), O (fairly good), △ (medium) and × (poor) by visual observation for plant type, ripening color and damage except Japanese entries
Husked rice length	5 grains	0.1mm, when ( ) was used, original seed, because these were no heading
Husked rice width	5 grains	0.1mm, when ( ) was used, original seed, because these were no heading
Grain quality	ca. 200 grains	1 (excellent) to 9 (poorest) by visual observation, no notice for grain shape and grain color
white belly	ca. 200 grains	1 (non) to 5 (severe) by visual observation
white core	ca. 200 grains	1 (non) to 5 (severe) by visual observation
Grain color	ca. 200 grains	W (white), W_2 (amber), r_1 (pale red), r_2 (red), r_3 (dark red), r^0 (brown dot), P (purple) and gh (color by golden hull)
Apiculus color	1 plot	I (dark purple), II (purple), III (red), IV (pale red), V (pink), VI (pale pink), b (brown) and g (white)
Glume color	1 plot	Pr (purple), af (redish purple), b (green at heading and black at ripening time), gh (golden), df (stripes), and y (yellow)
Leaf and node color	1 plot	G (green), Pl (purple rice), Pl^m (purple except node), I (inhibited type)
Others	1 plot	special trait and/or malformation if necesary

Table 2 Range of significance for each trait estimated by standard deviation of standard varieties

Traits	LSD by analysis of variance		Range of variation within entries	
	All plot	Range within 4 plots	2σ	3σ
Heading date	0.6	0.3 ~ 1.9	2.3 ~ 3.9	3.4 ~ 5.9 days
Culm length	1.7	3.0 ~ 7.3	4.6 ~ 9.2	7.0 ~ 13.8 cm
Panicle length	0.5	0.6 ~ 1.9	1.4 ~ 2.3	2.0 ~ 3.5 cm
Panicle number	0.7	1.4 ~ 3.1	2.6 ~ 4.2	4.7 ~ 6.3 number per hill
Grain weight ratio	4.8	7.5 ~ 22.6	15.1 ~ 22.1	22.7 ~ 33.2 %
Lodging	0.4	0.8 ~ 1.5	0 ~ 2.4	0 ~ 3.6 index
Sprouting	0.3	0.5 ~ 1.3	0.8 ~ 1.3	1.2 ~ 2.0 index
Tillering type	0.2	0 ~ 0.5	0 ~ 0.7	0 ~ 1.0 index
Grain number per panicle	9.6	19.7 ~ 36.9	31.5 ~ 40.0	47.2 ~ 59.9 grains
Grain number per 10cm length	3.7	8.0 ~ 13.2	12.7 ~ 14.4	19.0 ~ 21.2 grains
Diameter of culm base	2.3	4.0 ~ 12.4	7.5 ~ 10.4	11.2 ~ 15.7 0.1mm
Diameter of panicle base	0.8	1.4 ~ 4.0	2.9 ~ 3.4	4.4 ~ 5.1 0.1mm
Leaf blast resistance			1. 2 ~ 1.4	1.8 ~ 2.2 index

1) The value for analysis of variance was culculated from 3 entries × 4 replications within 5a field.

2) 2σ and 3σ were culculated from data of 24 plots for 3 standard varieties.

3) Standard varieties were C_1 (Reimei), C_2 (Koshihikari) and C_3 (Nipponbare), and those for leaf blast resistance were C_1 (Homarenishiki), C_2 (Norin 22) and C_3 (Koshihikari).

3) Testing methods for three important traits

(1) Resistance to leaf blast disease

Severity of leaf blast damage is recorded in a disease nursery at seedling stage when disease occurrence is observed. A large number of accessions can be simultaneously tested in a small nursery under uniform disease occurrence.

Reaction of each accession to specific races of blast fungus is determined by artificially inoculating specific races before natural outbreak of leaf blast. The earliest leaf blast occurrence at Hokuriku National Agricultural Experiment Station (HNAES) is usually observed around June 20th. In this region, mid June to late July is suitable for leaf blast outbreak, because it is rainy season with a temperature of 20 to 24°C under cloudy weather.

For this evaluation, entries were sown on June 5, and were inoculated with the fungus race 007 (virulence to +, *Pi-a* and *Pi-i* genotype) by means of spreading diseased leaves at early July when seedlings grew at the 5th leaf stage. Five days after inoculation, leaf blast occurred uniformly, and the severity of disease damage was recorded three to four times at mid to late July.

The disease nursery was made in upland condition with rectangular shape of 120cm wide. Susceptible cultivar, "Koshihikari", was sown as a spreader in three rows beside long side (center and both side). One hundred seeds of each entry were sown in a plot with 50cm in length and 10cm in width between plots at a right angle to long side. Three standard varieties, "Homarenishiki" for resistance (R), "Norin 22" for medium resistance (M), and "Koshihikari" for susceptibility (S), were inserted after every 20 to 30 entries for monitoring uniformity of disease severity at the nursery. Differential varieties for specific fungus races were also sown to monitor changes of fungus races. Insecticide and herbicide were treated if necessary, and mulching with wheat straw or hay other than rice straw was effective to get uniform germination.

Disease severity was recorded by the criteria shown in Table 3 with grade of 0 to 9, and an average of three to four times recording was used for determining the degree of resistance for each entry. Irregular disease outbreak was inevitable, though disease occurrence in the disease nursery was nearly uniform. The severity scores of standard varieties which were inserted after every 20 to 30 entries were adjusted to 3 (R) for "Homarenishiki", 5 (M) for "Norin 22", and 7 (S) for Koshihikari", then severity scores of each entry were also adjusted with the standard scale. Entries showing no disease lesion were estimated to have some true resistance

Table 3 Criteria of evaluation for degree of susceptibility to leaf blast

Degree of susceptibility	Circumstances
0	No susceptible (S) lesion
1	Very few S lesion
2	Few S lesion
3	Medium number of S lesion
4	Large number of S lesion
5	Very few killed leaves
6	Few killed leaves
7	Medium number of killed leaves
8	Large number of killed leaves
9	All leaves were killed

gene(s) other than *Pi-a* and *Pi-i*.

(2) Resistance to bacterial leaf blight disease

Bacterial leaf blight (BLB) resistance was evaluated by artificial inoculation with three-point-needle-inoculation method on early July before heading. Central parts of uppermost open leaf was inoculated with BLB bacteria suspension concentration of which is 10^8 to 10^9 cells/ml. Two leaves/hill of two hills were used for each bacterial strain. Bacterial strains in this evaluation were selected from Virulence Type I, II and III, and IV whenever it is necessary. Ten to fourteen days after inoculation, reactions of each entry to each bacterial type were classified into three groups, namely resistance (R), medium resistance (M), and susceptibility (S), on the basis of elongation of disease lesion. Three standard varieties, "Kinmaze", "Kogyoku" and "IR 26", showed the specific reaction of SSS, RSS and RRR to the three differential bacterial groups respectively. The symbols from left to right were reaction patterns to the bacterial type I, II and III.

(3) Cold tolerance

Cold temperature causes three types of cold damage on rice plants. The first type appears as delayed germination and vegetative growth, but this type of cold damage can be recovered when temperature rises to normal level or high. The second type is delayed heading which causes so-called delayed type of cold damage due to high frequency of immatured grain at harvesting time. The third is so-called destructive type of cold damage which is caused by a high sterility ratio resulted from cold temperature during meiosis stage and/or flowering time.

Tolerance to the first type of cold damage was evaluated by observing the degree of germinability and leaf discoloration after removing plastic film covers from the nursery. Rice seedlings at an earlier stage in nursery were protected against low temperature by plastic film covers, but their leaves discolored rapidly when they were exposed low temperature directly after the plastic film cover is removed.

There were big differences in the degree of discoloration among entries, and a number of entries from abroad were found to show a low level of tolerance to discoloration injury. Some entries from low latitude area were observed to be killed or to be inhibited severely in their successive vegetative growth.

Tolerances to the second and the third type of cold damage were not observed in this evaluation, though tolerance to the third type is most important at northern Japan.

Evaluation of tolerance to the third type is usually conducted at breeding stations by following method. At panicle primordia stage plants are kept under water surface in a 20cm depth pool which is irrigated with cool water of 20°C. When water temperature in the pool is kept uniformly, three plants per entry are enough to precisely determine the degree of cold tolerance of the third type by measuring sterility.

4) Outline of evaluation results

The results of evaluation at HNAES for agronomic traits of rice genetic resources were published under the title of "List of Agronomic Traits of Rice Genetic Resource in Japan" from HNAES (UCHIYAMADA *et al.* 1977, 1978, 1980, 1981). The data base on passport data and evaluation data for each entry was built up for reference and statistical analysis by computer system of MAFF.

The outlines of these reports are stated in the following part.

(1) General survey for genetic resources

The first-level evaluation for 225 Japanese paddy rice, 40 Japanese upland rice, and 1,440 alien rice was conducted in 1976.

According to MATSUO's criteria(Matsuo, 1952) for varietal classification, 387 entries were A type, 122 entries were B type, 122 entries were A or B type, and 1,068 entries were C type.

The summary of the evaluation data for several agronomic traits were shown in Fig.1. In the figure, entries from Japan were indicated as A, A and/or B types from abroad were indicated as AB, C types from east Asia were indicated as C1, C types from other Asian area were indicated as C2, and C types from regions other than Asia were indicated as C3.

① Germinability and seedling growth

Entries were sown on the protected nursery on early April in the conventional seeding time in Hokuriku region. Some entries from Nepal and Philippines showed early germination, whereas most of Japanese paddy varieties had a tendency of late germination.

After removing plastic film cover, discoloration of leaves due to low temperature were clearly observed, especially in the entries of C type, whereas it was not so severe in the entries of A and AB. Entries from low latitude area, especially IRRI breeding lines, showed a tendency of stunting, but those from Japan, Taiwan and mainland China did not.

② Culm length

In general, culm length was in the order of A<AB<C1 = C2 = C3. Among Japanese paddy varieties of A, main cultivars of recent showed the shortest stature being followed by old improved cultivars, landraces, then by glutinous cultivars. Culm length of alien entries was generally higher than that of Japanese entries, but high yielding varieties (HYV) from Philippines, Sri Lanka, Indonesia, India, China, and Korea were found to be short as a result of modern rice breeding.

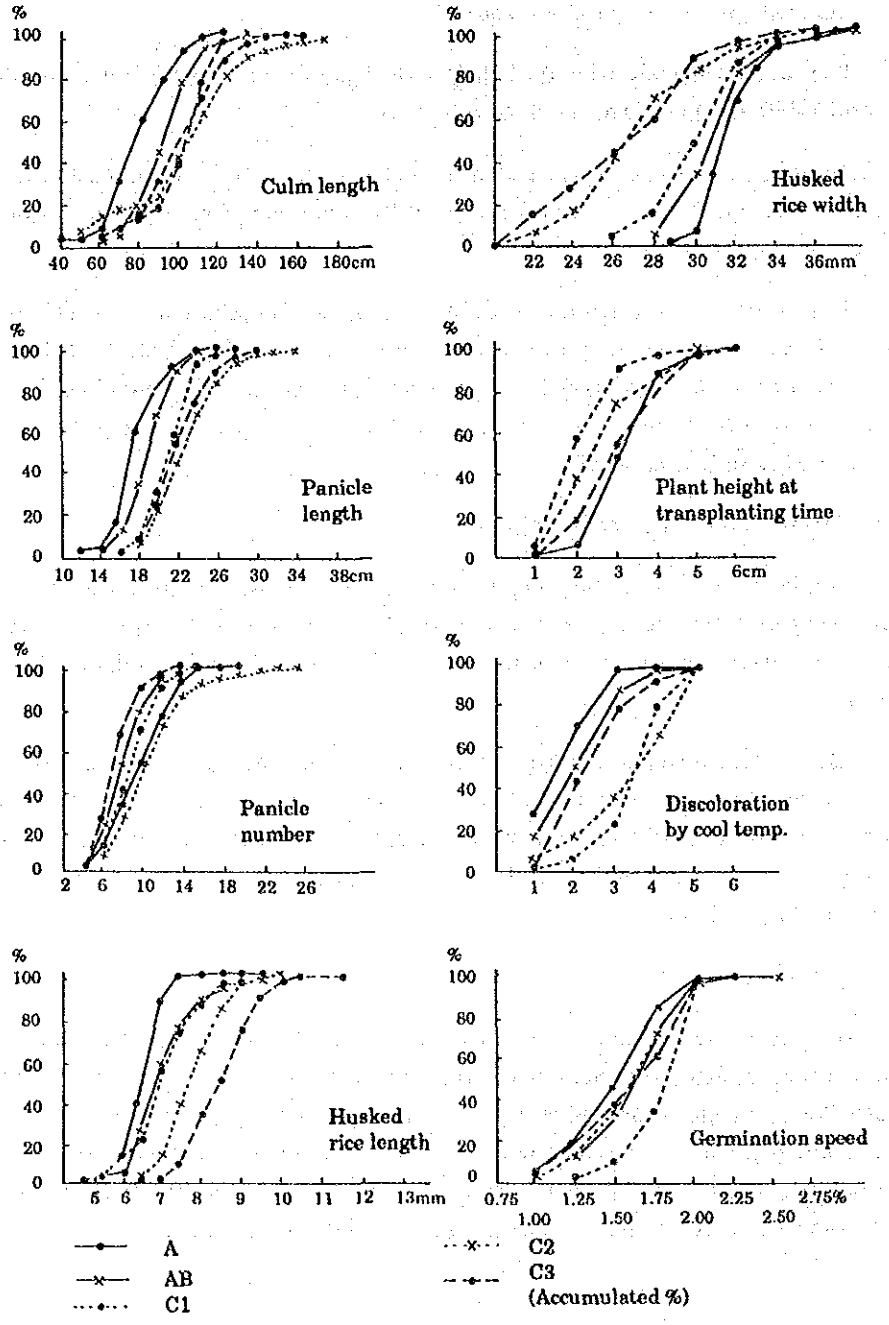


Fig. 1 Frequency distribution of traits among varietal groups

③ Panicle length, panicle number, and grain number per panicle

Panicle length was generally in the order of A≤AB<C1=C2<C3, and entries from Indonesia, Nepal, India, Thailand, and Africa was long in panicle length. The panicle length among Japanese entries was in the order of recent main cultivars<old improved cultivars<landraces<glutinous cultivars. This fact indicated that the rice breeding in Japan changed plant types from long stature, long panicle length, and small panicle number type to short stature, short panicle length and large panicle number type cultivars. Grain number per panicle was in the order of A<AB<C1=C2<C3.

④ Grain characteristics

Grain length was in the order of A<AB=C1<C2<C3, and Japanese entries showed short length with little variation. Grain width was in the order of C3=C2<C1<AB<A. Japanese entries has broad width with little variation. Whereas, entries of C type except C1 had long grain length with wide variation of grain width.

(2) Leaf blast resistance

Seedlings of 4,222 entries were artificially inoculated with the blast fungus race 007 at a disease nursery in 1977, because fungus race 007 is virulent to true resistance gene of *Pi-a* and *Pi-i* which are popular in Japanese entries. Therefore, entries with no disease lesion (R) were estimated to have alien-true resistance gene(s) other than *Pi-a* and *Pi-i*, and a degree of resistance of entries with susceptible lesion (S) showed a degree of field resistance.

Of 785 alien entries, 471 were determined to be R. The R group included 85% of southeastern Asian entries, 100% of African entries, and 11% of AB. Of Japanese entries, 7% were confirmed to be R, indicating the results of modern breeding using alien true resistance gene. Regarding to entries with susceptible lesions, those with high level of field resistance were found more among Japanese entries than among alien entries.

(3) Bacterial leaf blight resistance

Artificial inoculation was performed to 1,702 entries with the differential Bacterial type I, II, and III in 1976. Entries were grouped into SSS, RSS, RRS and RRR type based on their reaction to the above mentioned bacterial types.

SSS ("Kinmaze") type was found throughout the world covering 73% of the entries, especially with a high frequency in *japonica*.

RSS ("Kogyoku") type was also found throughout the world covering 19% of the entries, and this type was mainly found among Japanese upland rice and entries from Taiwan, India, Sri Lanka, and Madagascar. Especially, all 15 entries from Madagascar belonged to this type.

RRS ("Rantai Emas") type was shown by 44 entries with a share of 2.5%, and these were all from Southeast Asia.

RRR ("Wase Aikoku") type was shown by 63 entries with a share of 3.7%, and these were from Southeast Asia and Japanese landraces.

(4) Selection of breeding materials for a special project with the title of "Rice Breeding for Superhigh Yield"

Some entries which were selected from above mentioned evaluation programs at HNAES were further evaluated for their yielding ability in the third level evaluation.

Entries from Taiwan showed good ripening color as well as Japanese rice, but were high in culm length. Entries from USA were also good in ripening color, but low in grain yield.

Short statured entries from IRRI, Korea, and China were found to be high yielding with long panicle length and short culm, but poor in grain quality. Of them, entries from IRRI were susceptible to discoloration of leaves by cool temperature. Entries from China had a tendency to be open-tillering type. Entries from Surinam were short in both culm length and panicle length.

From these materials, IR 2061-214-3, Milyan 23, Milyan 25, Milyan 42, Suwon 258, Gui-zhao 2, Nan-jing 11 and others were selected as promising for superhigh yield breeding. So far, new high yielding cultivars, "Habataki" and "Takanari", were developed from the crosses including these materials in the course of the special project.

4. Evaluation of breeding lines for recommendation

The level of the varietal evaluation for recommendation which is conducted systematically all over Japan corresponds to the third level evaluation for genetic resources. National and prefectural rice breeding stations develop 60 to 120 new experimental breeding lines every year. These new lines are distributed to prefectural agricultural experiment stations for evaluating them for recommendation by each prefecture. About 130 agricultural experiment stations including main stations, sub-stations and branches in 47 prefectures evaluate agronomic traits and adaptability of new experimental breeding lines.

Numbers of entries for the evaluation per station varies 2 to 110 in one year, and new experimental breeding lines are compared with existing recommended varieties. New varieties are recommended usually on the basis of three year data and two year data at farmers' fields (2 to 20 sites).

The number of entries for the local evaluation reaches about 7,000 per year in total of Japan. Items of traits and criteria for evaluation are uniform throughout Japan, and some items may be added at the respective prefectures if necessary. The database of the local evaluation is built up with the computer system of MAFF. Of 55 descriptors in this database, 25 are for experiment methods and materials, and 30 are data for evaluated traits as shown in Table 4.

5. References

MATSUO, T. 1952. Genecological studies on the cultivated rice. Bull.Nat'l Inst. Agr. Sci. D3: 1-111 (in Japanese with English summary).

UCHIYAMADA, H., Y. FUJITA, K. KIMURA and T. YAMADA 1977. Analysis of agronomical characteristics on the japanese and foreign rice cultivar. Hokuriku Agricultural Research Data 3:1-137 (in Japanese).

UCHIYAMADA, H., Y. FUJITA, K. KIMURA, K. TAKAYANAGI and T. YAMADA 1978. Analysis of agronomical characteristics on the Japanese and foreign rice cultivar (2) Hokuriku Agricultural Research Data 4:1-167 (in Japanese).

UCHIYAMADA, H., Y. FUJITA, K. KIMURA, K. TAKAYANAGI and K. MORI 1980. Analysis of agronomical characteristics on the japanese and foreign rice cultivar (3) Hokuriku Agricultural Research Data 7:1-153 (in Japanese).

UCHIYAMADA, H. 1981. Possibility to breed up very high yielding rice cultivar for feed. Recent Progress of Plant Breeding, Japan Soc. Breed. 22:20-52 (in Japanese).

Table 4 Items of traits in evaluation of breeding lines for varietal recommendation

Items of traits	Measuring unit	Reference
Heading date	Month and date	Visual obsrvation
Maturing time	Month and date	Visual observation
Culm lengtrh	cm	20 to 30 individuals per plot
Panicle length	cm	20 to 30 individuals per plot
Panicle number	Number/m^2	20 to 30 individuals per plot
Total weight	kg/a	About $3m^2$
Grain weight	kg/a	About $3m^2$
Grain quality	1 to 9	Classified by visual observation
Degree of damage	0 to 5	Classified by visual observation
Lodging		
Leaf blast		
Bacterial leaf blight		
Cool injury and others		
Promising degree	1 (very promising) to 9 (no promising)	
Advantage traits	Note by code number for 5 traits	
Disadvantage traits	Note by code number for 5 traits	

Two types of trial are necessary, such as standard and heavy fertilizing, or standard and early planting condition with three to four replications.

Genetic Variation and Improvement of Seed Components in Soybean

by

Keisuke KITAMURA

National Agriculture Research Center, MAFF
Kannondai, Tsukuba, Ibaraki 305, JAPAN

CONTENTS

1. Introduction

Around 50 years ago, soybeans was produced only in a limited area of Asia. The people in the area have long used the bean for direct human consumption in various traditional foods. Until the 1950's, the crop had become one of the dominant sources for vegetable oil for human consumption and protein for animal industry in the world. Today, soybean oil and protein are used not only for human foods and animal feeds but also for industrial products in the developed world such as USA, Europe and Japan. In more recent years, soybean production and utilization have gained popularity in the developing world, such as tropical Africa, India and Latin America. Generally, the peoples there do not have modern industries for soybean processing, and do not necessarily accept the traditional oriental soybean-based foods. Hence, they have to develop recipes that suit local taste so that soybeans become a part of the daily diets for them.

Accordingly, there is a worldwide desire or a need to develop soybeans with new or modified seed components for various oil and protein uses as well as for whole soybean consumption.

In Japan, the total consumption of soybean currently amounts to about 500 million tons, though it is not a major crop. The rate of self-sufficiency is only 5%, the rest is imported. All the locally produced soybeans are used only for just direct human consumption in the traditional soy foods such as tofu, nimame and natto. So, special attention has been paid in soybean breeding programs to factors which determine high quality and excellent appearance of the soyfoods such as large or extremely small seed sizes, yellow-white hilum, and high protein contents in addition to agricultural traits such as high yield and disease and pest resistance. Recently, improvement of the bean components has beeen expected to improve food-processing quality for the processed soybean products such as soy milk and various edible ingredients as well as for the traditional soy-foods in Japan.

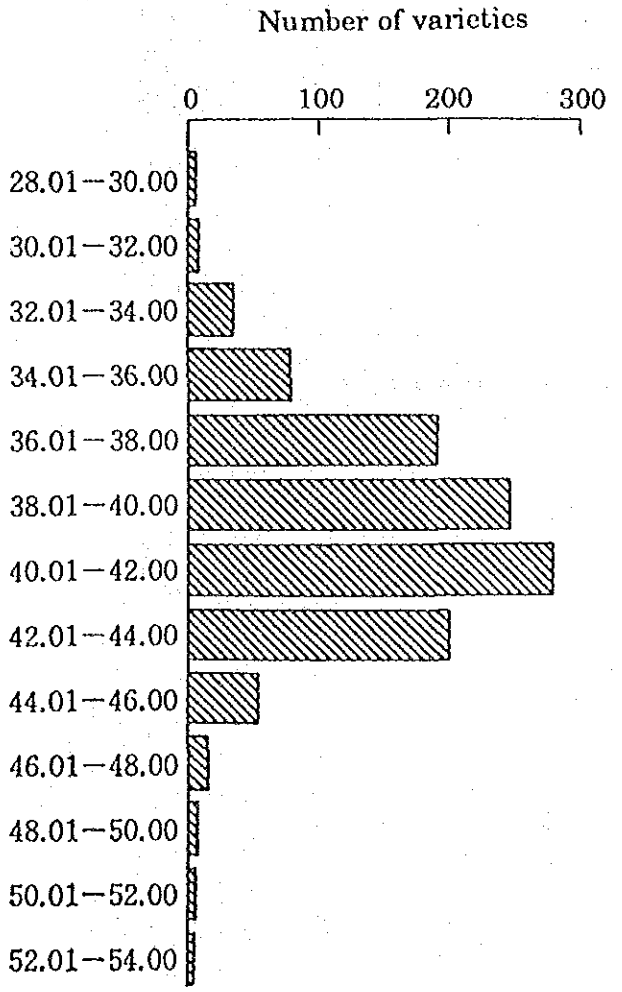
In this paper, breeding trials for improving food-processing quality of soybeans in Japan are introduced. They include increasing protein content, modification of protein composition using mutant genes for the storage protein subunits and eliminating undesirable beany flavor using null genes for the lipoxygenase isozymes. Further, chemical evaluation and genetic variation of other components for better quality of soybeans is to be discussed.

2. Increasing protein content

"Tofu" is the most important soybean product in Japan. More than 60% of the domestic soybeans are consumed in the form of tofu. So, chemical evaluation of the seed components affecting tofu processing is important to develop suitable soybeans for tofu-making. Many studies on the chemical evaluation performed so far revealed that the protein content of the whole beans is the most important factor for the processing of tofu by determining hardness and yield of tofu, though other factors such as the proportion and content of protein, oil and carbohydrate as well as some minor components such as minerals and organic acids influence also the tofu-making quality. In fact, the two leading cultivars, "Enrei" and "Fukuyutaka" having relatively high protein contents (43~45% in dry weight basis of N X 6.25) are presently most popular soybeans for the tofu production.

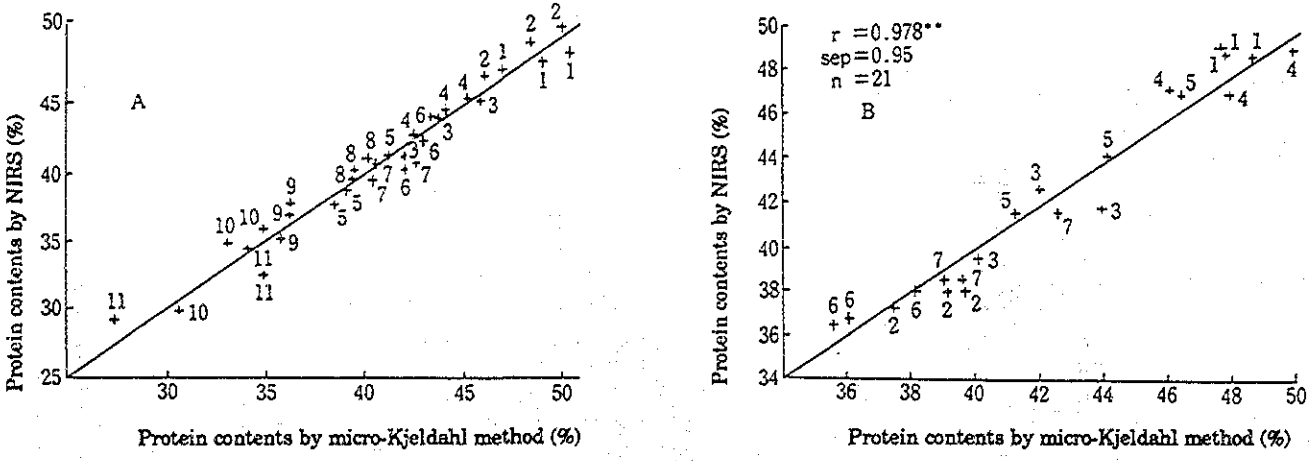
Since soybeans with protein contents higher than 48% exist in the germplasm of soybeans (TAIRA *et al.* 1976, WATANABE *et al.* 1989), it will not be so difficult to develop soybean cultivars with the high protein contents of 50% (Fig.1). In 1960's, a special research project supported by Ministry of Agriculture, Forestry and Fisheries was conducted to develop soybeans with high protein contents in Saga Prefectural Agricultural Experiment Station. The two high protein cultivars, "Saikai 20" and "Higomusume" which are early maturing (summer-type) soybeans having protein content of nearly 50% were released from the station. A similar breeding project has been undertaken at the Tohoku National Agricultural Experiment Station using "Saikai 20" as a donor of high protein to develop high protein varieties for the northern Japan since 1970's. Although some promising high protein lines were developed lately, until now no commercial high protein cultivars have been released. It is easy to develop soybeans with protein contents of higher than 48% being adapted to that area. But it is difficult to increase the protein content of soybeans with a minimum loss of yield while maintaining large seed size which is a key factor determining market price of soybeans.

The protein content has been estimated using crushed soybeans by either Kjeldahl method or a near-infrared reflectance spectroscopic method (NIRS) in ordinary laboratories for soybean breeding. Very recently, a research group of soybean breeding laboratory in Nagano Prefectural Chusin Agricultural Experiment Station succeeded in developing non-destructive NIRS method to estimate the protein contents with satisfactory accuracy using intact single soybean seeds (Fig. 2). Since it is rapid to analyze and seeds analyzed by the non-destructive method can be sown to proceed a generation, it is possible in early generations to



Protein contents (dry weight basis) %, N X 6.25

Fig.1 Distribution of the seed protein contents in the germ plasm varieties of soybeans (TAIRA *et al.* 1976)



Used wavelengths: 1240, 1254, 1290, and 1310 nm
Multiple correlation coefficient: 0.98
Standard error of estimate: 1.09
Calibration varieties: 1 Saikai No. 20, 2 Sin No. 2, 3 Asoaogari, 4 Norin No. 2, 5 Miyagisirome, 6 Enrei, 7 Kirin No. 10, 8 Kokunou No. 10, 9 T201, 10 Tousan No. 90, and 11 Tousan No. 89

Prediction varieties: 1 Tohoku No 74, 2 Tachinagaha, 3 Ooturu, 4 Nattoshoryu, 5 Morse, 6 Lee (nonnodulating), 7 Kokunou No. 1

Fig.2 Calibration (A) and prediction (B) for protein contents of soybean seeds by near infrared reflectance spectroscopy (NIRS) using individual whole seeds (unpublished results in Nagano Chusin Agricultural Experiment Station)

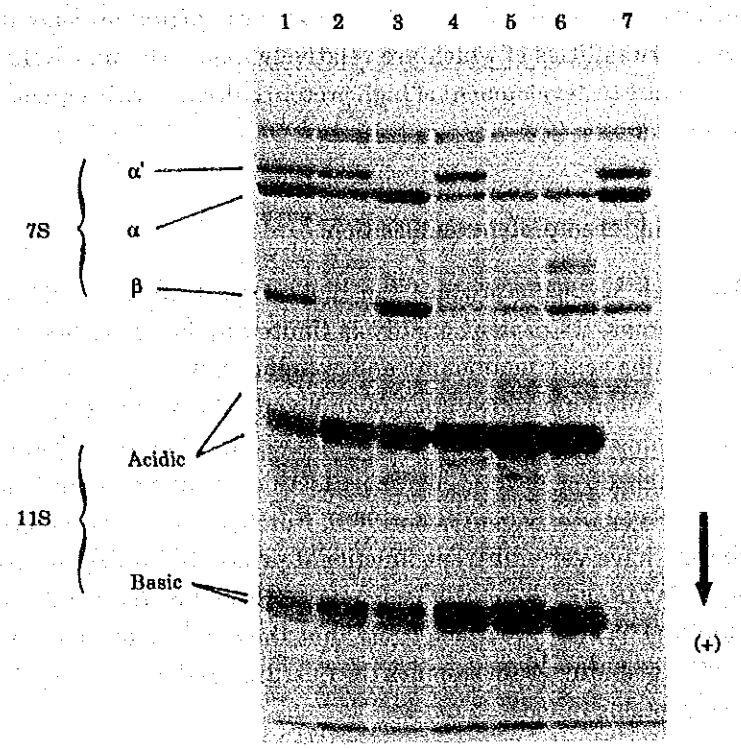
select soybeans with desirable phenotypes such as high protein contents and large seed sizes, heritabilities of which are relatively high. The new NIRS technique would contribute to development of high protein cultivars with a great commercial value of soybeans.

3. Modifying seed protein composition

Although soybeans produce the highest seed protein yield and content among seed crops, its protein quality is limited by low contents of the sulfur-containing amino acids, methionine and cysteine. Seven S and 11S globulins are the two major proteins which amount to about 70% of the total seed proteins. Sulfur-containing amino acid contents of the two globulins are quite different: 11S globulin contains three to four times more methionine and cysteine in unit protein than that of 7S globulin (KOSHIYAMA 1968). Furthermore, it was reported that the two globulins have very different functional properties in the soy-protein foods (SAIO and WATANABE 1978): the isolated 11S globulin fraction formed a much harder tofu-gel. These reports suggest that the increase of 11S globulin at the expense of 7S globulin would enhance the nutrition and improve the functionality of soybean proteins in food.

So far, the two types of mutant varieties (Fig. 3; 3 and 4) were identified by screening using SDS-polyacrylamide gel electrophoresis (PAGE) in the germplasm varieties of soybean: "Keburi" and "Mo-shi-dou (Gong 503)" which were characterized by the absence of α'-subunit and low levels of both α- and β- subunits of 7S globulin, respectively (KITAMURA and KAIZUMA 1981). The absence of the α'-subunit is controlled by a single recessive allele and the reduction of the α- and β-subunits by respective independent single alleles (TSUKADA *et al.* 1986).

By gathering the three variant alleles for the subunits of 7S globulin into one genotype, we obtained two types of 7S-low lines (A and E lines in Fig. 3) which are genetically fixed for the three alleles for the 7S globulin subunits and the allele producing or lacking the intermediate subunit of 11S globulin (OGAWA *et al.* 1989). Contents of 7S and 11S globulins of the 7S-low lines and the ordinary cultivars were estimated by single radial immunodiffusion analyses using anti-7S and anti-11S sera, respectively. Table 1 shows that the 7S-low lines have only a half 7S content of those in the ordinary cultivars, on the contrary, about 15% higher 11S content than those of the ordinary ones.



1 Suzuyutaka

2 Williams

3 Keburi (α'-null)

4 Mo-shi-dou Gong 503 (α-and β-low)

5 A-line (α'-null, α-and β-low, A_5-type)

6 E-line (α'-null, α-and β-low, A_4-type)

7 a mutant line lacking all group I subunits of 11S globulin

Fig.3 SDS-PAGE patterns of the total seed protein in soybeans

Table 1 Total and fractional protein contents of ordinary varieties and 7S-low lines (OGAWA *et al.* 1989)

Group of variety	No. of lines tested	Total protein %	% of total protein	
			7S	11S
7S-low lines:				
A lines (7S-low, A_5-type)	5	43.4 a[1)]	8.7 d[1)]	52.5 a[1)]
E lines (7S-low, A_4-type)	5	44.4 a	11.7 c	44.7 b
Ordinary variety:				
A5-type variety (normal, A_5-type)	20	41.4 b	17.3 b	38.3 c
A4-type variety (normal, A_4-type)	20	41.1 b	19.5 a	31.2 d
Keburi (α'-null, A_5-type)	–	39.4	15.7	41.8
Mo-shi-dou Gong 503 (α-low, β-low, A_5-type)	–	42.9	9.9	45.7

1) Different letters in a column indicate statistical significance of the differences among the mean values within a column at 5% level.

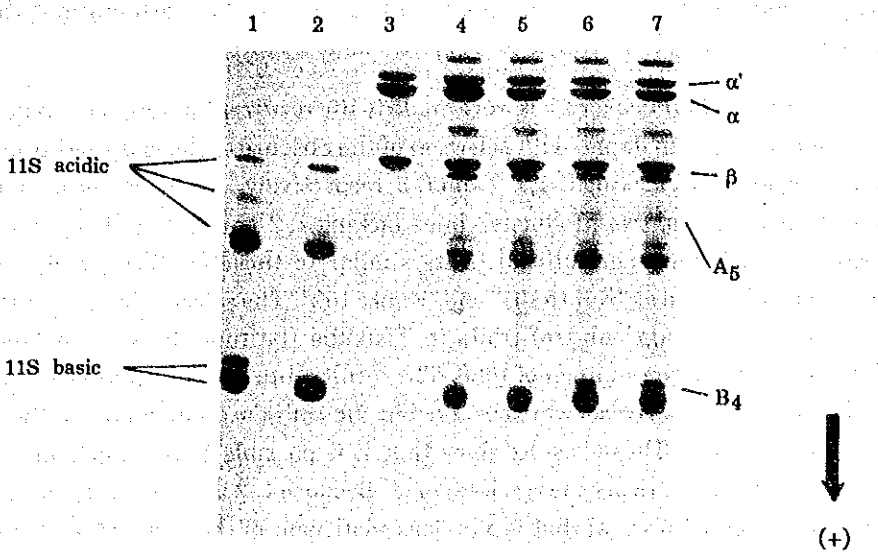
A highly negative correlation was found between the 7S and 11S globulin contents. The results suggest that 11S globulin might be overproduced to compensate for the reduction of 7S globulin keeping normal levels of the total seed protein content in the 7S-low lines. No deleterious effect was observed despite the marked modification of protein composition in the lines. The mean value of sulfur-containing amino acid contents of the 7S-low lines was about 20% higher than that of the ordinary cultivars (OGAWA *et al.* 1989).

On the other hand, there exists genetic polymorphism of 11S globulin independent of that of 7S globulin. Eleven S globulins can be classified into two types, A5 or A4 types, according to the presence or absence of the intermediate subunit which is formed by the A5-subunit and the paired basic B4-subunit of 11S globulin (Fig. 4). The absence of the intermediate subunit is controlled by a single recessive allele (HARADA *et al.* 1983). It was shown that the presence of the subunit is closely related to properties of gel formation of 11S globulin by heating (NAKAMURA *et al.* 1984) and of tofu-gel formation (HARA and NEGISHI 1988).

Very recently, KAIZUMA *et al.* (1990) identified an induced mutant soybean lacking all group I intermediate subunits of 11S globulin with gamma-ray irradiation. The lacking characteristic has been shown to be controlled by a single recessive gene and to extremely decrease 11S globulin contents, on the contrary increasing 7S globulin contents in the seeds (Fig. 3; 7). It was shown that despite the marked reduction of 11S globulin, no deleterious effect was observed on the total protein contents as well as physiological aspects such as seed development and germination. Because 7S globulin has much superior food-functionalities such as water-holding ability, adhesiveness and solubility at high temperature to those of 11S globulin, soybeans with extremely high 7S/11S ratios would be effectively used for special soy-protein ingredients in food industry.

4. Eliminating seed lipoxygenases

Normal soybean seeds contain three lipoxygenase isozymes, called L-1, L-2 and L-3. These enzymes are responsible for the generation of grassy beany flavors and tastes which have limited the wide utilization of whole soybeans and soybean protein in certain food products. The three types of spontaneous mutants lacking L-1, L-2 and L-3, respectively, were detected in the early 1980's. Genetic studies have demonstrated that the absence of L-1 (HILDEBRAND and HYMOWITZ 1981), L-2 (KITAMURA *et al.* 1985, DAVIES and NIELSEN 1986), and L-3 (KITAMURA *et al.*



1 purified A5-type 11S globulin

2 purified A4-type 11S globulin

3 purified 7S globulin

4 Suzuyutaka

5 Raiden

6 Nattoshoryu

7 Bonminori

Fig.4 SDS-PAGE patterns of soybean seed proteins (OGAWA *et al.* with partial changes)

1983) from the seeds is under the control of single recessive alleles, lx_1, lx_2, and lx_3, respectively. By the use of these recessive alleles, we have been attempting to breed new cultivars with low levels of the objectionable flavors.

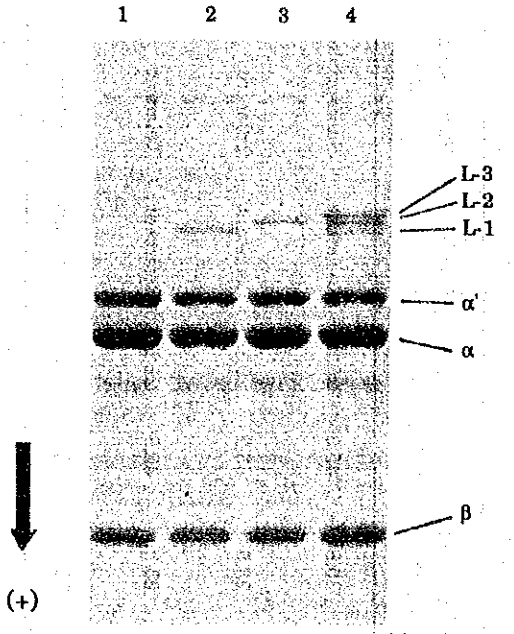
Three to four backcrosses were made to the recurrent parent "Suzuyutaka", a leading cultivar in Japan with selection of the absence of the isozymes to obtain near-isogenic lines lacking L-1, L-2 and L-3, respectively. From crosses among the near-isogenic lines, two productive lines lacking L-2 and L-3, and L-1 and L-3, respectively, having agricultural traits similar to those of "Suzuyutaka" were selected and named as "Kanto 101" and "Kanto 102". The selected lines were tested to compare some agricultural traits in Tsukuba (latitude 36°N), and Morioka (latitude 40°N) in the summer of 1988. The results obtained in Tsukuba are shown in Table 2. No differences were observed in the traits examined among the lines and the cultivar. These results show that it is possible to develop commercial soybean cultivars lacking the two isozymes. Because L-2 is largely responsible for the generation of hexanal that is a major constituent of the flavor (MATOBA *et al.* 1985), soybeans lacking L-2, and L-2 and L-3 would be acceptable by soy-food industries and consumers in the world.

However, neither double mutant seeds lacking both L-1 and L-2 nor triple mutant seeds lacking all the isozymes have been identified yet, presumably due to close linkage between L-1 and L-2. Very recently, however, HAJIKA *et al.* (1990) succeeded in inducing a soybean line lacking all the isozymes by gamma-ray irradiation (Fig. 5). The triple mutant soybeans went through two generations and no physiological and agricultural problems were encountered, so far. In another experiment, it has been shown that there are no significant differences in activity levels of lipoxygenase in leaves, which seem to take important physiological roles (HILDEBRAND 1990) among the triple line, "Kanto 101" and "Kanto 102" lines and "Suzuyutaka". This seems to explain why soybeans lacking the seed lipoxygenase isozymes are physiologically normal. Soybean cultivars lacking all the seed lipoxygenases could be economically valuable on account of their utilization, and the storage stability of soybean would be enhanced since the effect of the enzymes which act as major oxidative factors associated with the deterioration of oil and protein in the seeds during storage would be eliminated.

Table 2 Performance and some characters of two leading varieties, Enrei and Suzuyutaka and the near-isogenic lines lacking L-2 and L-3 (Kanto 101), and L-1 and L-3 (Kanto 102) in Tsukuba in 1988

Variety/line	Flowering time	Maturing time	Stem height cm[a]	Seed yield kg/a[a]	100 seeds weight g	Protein %[b]	Oil %[b]	Soybean mosaic virus	Purple seed stain
Enrei	Aug. 1	Oct.10	43	24.0	30.8	45.3	20.3	Resistant	Susceptible
Suzuyutaka	Aug. 3	Oct.15	43	32.5	23.5	43.2	20.3	Resistant	Tolerant
Kanto 101	Aug. 3	Oct.13	38	31.2	23.2	42.9	21.1	Resistant	Tolerant
Kanto 102	Aug. 4	Oct.17	39	30.8	22.4	42.0	21.1	Resistant	Tolerant

[a] Averages of random 20 plants in two replicates. [b] Estimated by NIRS analyzer on a dry matter basis.



1 a line (M_3) lacking all the isozymes

2 a line (Kanto101) lacking L-2 and L-3

3 a line (Kanto 102) lacking L-1 and L-3

4 Suzuyutaka

Fig.5 Resolution of the lipoxygenase isozymes in soybean seeds by SDS-PAGE

5. Modifying fatty acid composition

Soybean oil is the most dominant vegetable oil used in world food consumption and is considered as nutritionally good because of its high content of essential fatty acid as vitamin F, linoleic acid (C18:2). However, compared with the other vegetable oils like corn, cottonseed and peanut oil it contains relatively high contents (about 9%) of linolenic acid (C18:3) which greatly decreases the oxidative and flavor stability during storage or under high heat like frying. After the development of gas chromatography to analyze fatty acids, breeding trials were initiated to develop soybeans with low levels (3~4%) of linolenic acids in USA around the early 1970's. The trials have been partially successful: genetically stable soybeans having 3~4% linolenic acid have been developed with about 20% lower of standard yield potential (WILSON 1987). On the other hand, it has been recently found that linolenic acid confers important nutritional roles for development of brain and nerve, and for prevention of cancer and heart diseases which are not available from linoleic acid. One might expect nutritionally functional soybeans with high levels of linolenic acid. It seems to be possible to develop such soybeans because there are soybeans in germplasm varieties with the linolenic contents of 13~15% and the wild soybean, *Glycine soja* has higher (16~20%) linolenic acid (Table 3).

Although, soybean oil is generally composed of about 55% linoleic, 20% oleic, 10% linolenic acid, and 15% the others, there exist domestic soybean cultivars having enhanced levels (40~45%) of oleic acid at the expense of linoleic and linolenic acid (Table 4). From the fact that packed tofu products made of soybeans lacking the seed lipoxigenases show very light or plain taste, it is assumed that certain substances derived from unsaturated fatty acids oxidized by the lipoxygenases would be involved in tastes of the tofu. If this assumption is true, the remarkable difference of the fatty acid composition should affect the tofu tastes because both linoleic and linolenic acids can be used as substrates against lipoxygenases but oleic acid can not. In fact, it was recognized by taste panels using the packed tofu products made of soybean cultivars listed in Table 4 that there are significant positive correlations between deliciousness and good body of the tofu tastes and contents of the two acids: linoleic and linolenic acid, on the contrary significant negative correlations between the taste and oleic acid contents (Table 5).

Table 3 Fatty acid composition of selected soybeans and the wild soybeans (*Glycine soja*)

Germplasm	Fatty acids (%)					*
	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)	
Dare (Normal)	12.3	2.9	21.3	56.1	7.4	a)
A6	8.0	28.1	19.8	35.5	6.6	a)
A5	6.3	3.9	39.8	42.9	4.1	a)
PI 171441	12.6	3.1	15.4	55.9	13.0	a)
C 1640	10.6	3.6	24.6	56.4	4.2	b)
Bay (Normal)	10.7	2.7	20.0	57.3	9.4	c)
B 739	7.9	4.3	14.3	55.1	18.4	c)
G. soja (USSR)	13.9	3.3	9.1	57.0	16.8	d)
G. soja (Hidaka)	10.8	3.1	11.5	57.6	17.1	d)
G. soja (Tochigi)	12.4	3.1	10.6	54.0	19.9	d)
G. soja (Wakayama)	12.7	2.9	10.6	55.9	17.9	d)

*: Literature

a) WILSON, R. F., 1987 "Soybeans: improvement, production, and uses" 2nd edition, ed. by J .R .WILCOX, p.637.

b) MOUNT, T. L. *et al.*, 1988 JAOCS: 624-628.

c) TAKAGI, Y. *et al.*, 1989 Japan J. Breed. 39:403-409.

d) KITAMURA, K. *et al.*, 1989 Japan J. Breed. 39 (Suppl. 1):428-429

Table 4 Seed component contents and fatty acid compositions of ten domestic soybean cultivars produced in 1989 (averages of two replicates, %)

Cultivars	Protein[1]	Oil[1]	Others[2]	Fatty acids		
				Oleic (16:0)	Linoleic (18:1)	Linolenic (18:3)
Enrei	44.6	19.4	36.0	38.6	41.6	7.8
Hourei	42.1	21.0	36.9	37.2	39.6	7.7
Tachinagaha	41.7	21.6	36.7	36.7	44.2	7.2
Tamahomare	41.3	20.4	38.3	20.3	59.9	8.4
Nakasennari	41.3	20.3	38.4	22.1	55.1	10.5
Sinanomidori	43.6	19.8	36.6	15.1	59.2	9.5
Fukuyutaka	44.9	18.3	36.8	22.1	56.6	8.3
Akisirome	41.8	20.4	37.8	17.8	58.9	10.3
Higomusume	49.9	16.6	33.5	45.8	34.9	4.4
Koganedaizu	43.8	19.9	36.3	40.1	39.9	5.0

1) Values estimated by NIRS (dry weight basis).
2) Mostly consist of carbohydrates.

Table 5 Correlation relationships between the seed component, and fatty acid contents and tastes of packed tofu from the ten soybean cultivars in Table 4

Tofu taste	Seed components			Fatty acids		
	Protein	Oil	Others	Oleic	Linoleic	Linolenic
Sweetness[1)]	−0.701	0.507	0.798	−0.860*	0.858*	0.767
Sweetness[2)]	−0.911*	0.794	0.901*	−0.545	0.549	0.727
Good body[1)]	−0.623	0.382	0.779	−0.853*	0.879*	0.784
Good body[2)]	−0.736	0.548	0.823*	−0.777	0.826*	0.655
Deliciousness[1)]	−0.810*	0.625	0.882*	−0.788	0.796	0.845*
Deliciousness[2)]	−0.851*	0.665	0.920*	−0.800*	0.809*	0.851*

1),2): Results obtained using the packed tofu prepared on the different days.

* : Significant at 5% level.

It can be judged from fatty acid compositions analyzed so far that soybeans with high contents of oleic acid have low contents of linoleic and linolenic acids without exception, and that the characteristics of high oleic acid is genetically stable. Hence, the soybeans with high linoleic and linolenic acid would be suit for production of tofu products with good body tastes which are said to be popular in the older consumer, on the other hand the soybeans with high oleic acid can be used for making the light or plain tofu products which is probably getting popularity in the younger consumers in this country.

6. Improving aftertaste of soybeans

Although elimination of the seed lipoxygenases will undoubtedly improve the beany flavor of soy products, other sources of undesirable aftertastes referred to as astringency and bitterness may remain. The astringency and bitterness of soybeans are believed to be caused by saponins, isoflavone glucosides and their aglucones (HUANG *et al.* 1981, OKUBO *et al.* 1983). These compounds may be carried to the soyfood products through the processing operations. The astringency due to the saponins could be reduced by mechanical removal of seed hypocotyl because the levels of saponins are much higher in hypocotyl than cotyledon, and acetyl-soyasaponines (Saponin A and the aglycon, Sapogenol A in Fig.6) which have more undesirable tastes occur almost only in hypocotyl (OKUBO *et al.* 1983). So, soybean isoflavones might be responsible for the major aftertaste obstacle of processed soybean products such as soy milk, protein concentrates and textured concentrates.

Genistin and daidzin, the glucoside forms of genistein and daidzein comprise about 95% of the total isoflavones in whole soybeans (Fig. 7).

It has been known that in soy milk and tofu, the proportion of the glucosides decreases significantly, whereas the concentration of the aglucones, genistein and daidzein increases (MURPHY 1982). Recently, MATSUURA *et al.* (1989) revealed that β-glucosidases in soybean seeds are responsible for the hydrolysis of daidzin and genistin to produce daidzein and genistein, respectively during the processing operation of soy milk. As the astringency and bitterness of the aglucones are much more intense than those of the glucosides (OKUBO *et al.* 1983), increase of daidzein and genistein by the action of the β-glucosidases will result in large increase of the objectionable aftertastes of soy milk. The action of the enzymes occurs not only during soaking and homogenizing but also during storage and milling of soybeans, it might be difficult to lower the undesirable action by processing treatments

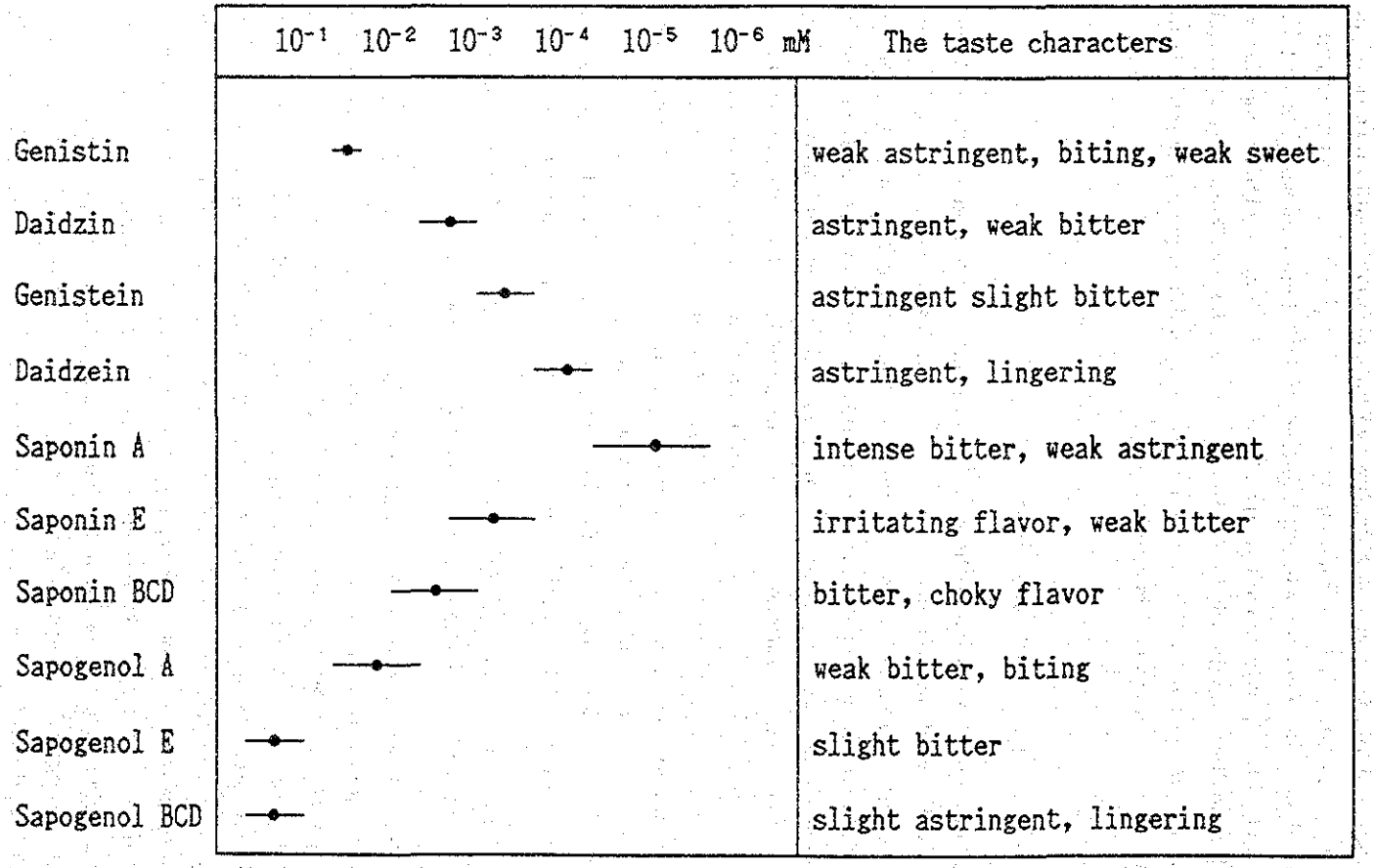
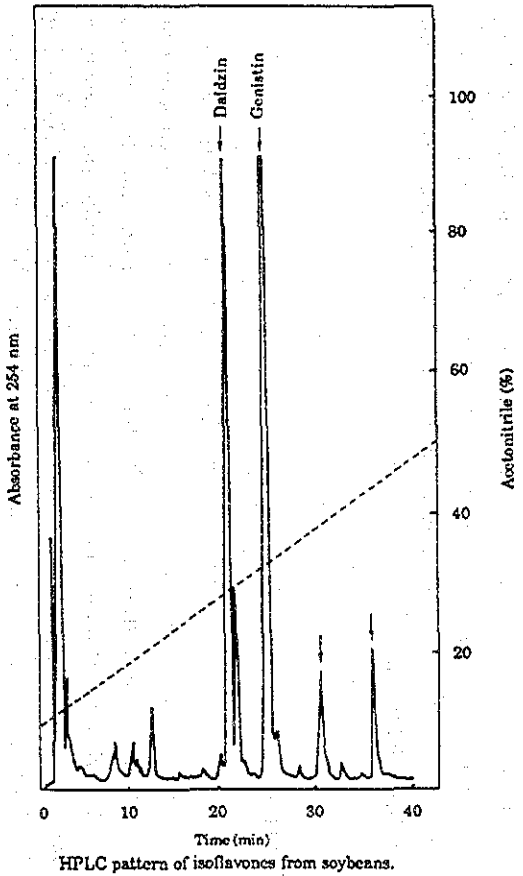
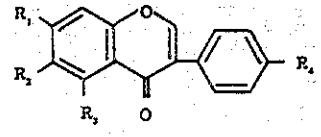


Fig.6 Taste characters and the threshold values of various glycosides and their aglycons (ILJIMA *et al.* 1987)



HPLC pattern of isoflavones from soybeans.



daidzein, R_1=OH; R_2=H; R_3=H; R_4=OH
daidzin, R_1=O-glucosyl; R_2=H; R_3=H; R_4=OH
genistein, R_1=OH; R_2=H; R_3=OH; R_4=OH
genistin, R_1=O-glucosyl; R_2=H; R_3=OH; R_4=OH

Fig.7 Chemical structures and HPLC pattern of isoflavones from soybean seeds (MATSUURA *et al.* 1989)

without quality deterioration of soybean products as in the case of the lipoxygenase action. So, breeding programs to reduce the levels of β-glucosidases should be initiated.

7. Other possible improvement for better quality

Soybean seeds contain 10~12% total soluble sugars of which 4~6% is sucrose, 2% is raffinose, and 3~5% is stachyose. Sucrose can be metabolized to produce energy, but the oligosaccharide: raffinose and stachyose can not. Raffinose and stachyose cause intestinal gas (flatulence) when soybean products are consumed by humans and other monogastric animals. Further, sucrose acts as a preferable factor for the fermented soyfoods such as natto and miso, and for nimame, boiled soybeans with sugar and soy-sauce. So, it is valuable to develop soybeans in which the oligosaccharide content is greatly reduced or eliminated, instead, the sucrose content increased.

Food allergies occur in a lot of food products as a result of immune reactions. The soy product is not an exception of this category. Recently, it has been suggested that the Kunitz trypsin inhibitor and the soybean lectin are major factors of soybean allergens (OGAWA *et al.* 1989). This implies that it may be possible to develop soybeans with low levels of the antigenecity because the respective null-alleles of the trypsin inhibitor (ORF and HYMOWITZ 1979) and the lectin (PULL *et al.* 1978) have been detected.

8. Conclusion

Advances in chemical and biological sciences enabled us to detect various genetic mutants of the seed components in the world germplasm varieties of soybeans. Some of the mutants, e.g., the lipoxygenase null, and the Kunitz inhibitor null soybeans, no doubt had practically important implications because such mutants enabled us to develop newly modified soybean cultivars having potential values for processors and consumers. Further chemical evaluation of soybean germplasm is necessary with the worldwide collaboration in order to improve soybeans for better human uses.

9. References

DAVIES,C.S. and N.C.NIELSEN 1986. Genetic analysis of a null-allele for lipoxygenase-2 in soybean. Crop Sci. 26: 460-462.

HAJIKA, M., K.KITAMURA and K.IGITA 1990. Production of soybeans lacking all lipoxygenase isozymes induced with gamma-ray irradiation. Japan J.Breed. 40 (Suppl. 2):218-219 (in Japanese).

HARA, K. and M. NEGISHI 1987. The tofu-making qualities of Japanese domestic soybeans. Res. Bull. of Kanagawa Agr. Res. Inst. No. 195:58-68 (in Japanese with English summary).

HARADA,K., Y.TOYOKAWA and K.KITAMURA 1983. Genetic analysis of the most acidic 11S globulin subunit and related characters in soybean seeds. Japan J. Breed. 33:23-30.

HILDEBRAND,D.F. and T.HYMOWITZ 1982. Inheritance of lipoxygenase-1 activity in soybean seed. Crop Sci. 22:851-853.

HILDEBRAND, D. F. 1989. Lipoxygenases. Physiol. Plant. 76:249-253.

HUANG,A.S., O.A.L.HSIEH, and S.S.CHANG 1981. Characterization of nonvolatile minor constituents responsible for the objectionable taste of defatted soybean flour. J. Food Sci. 47:19-23.

KAIZUMA, N., H. ODANAKA, H. SATO and H. KOWATA 1990. Mutants on soybean storage proteins induced by gamma-ray irradiation. Japan J. Breed. 40 (Suppl.1): 504-505 (in Japanese).

KITAMURA, K, and N. KAIZUMA 1981. Mutant strains with low levels of subunits of 7S globulin in soybean (*Glycine max* MERR.) seed. Japan J. Breed. 31:353-359.

KITAMURA, K., C.S. DAVIES, N. KAIZUMA and N.C. NIELSEN 1983. Genetic analysis of a null-allele for lipoxygenase-3 in soybean seeds. Crop Sci. 23:924-927.

KITAMURA, K., T. KUMAGAI and A. KIKUCHI 1985. Inheritance of lipoxygenase-2 and genetic relationships among genes for lipoxygenase-1, -2 and -3 isozymes in soybean seeds. Japan J. Breed. 35:413-420.

KOSHIYAMA, I. 1968. Chemical and physical properties in soybean globulins. Cereal Chem. 45:394-404.

MATOBA, T., H. HIDAKA, H. NARITA, K. KITAMURA, N. KAIZUMA and M. KITO 1985.Lipoxygenase-2 isozyme is responsible for generation of n-hexanal in soybean homogenate. J. Agr. Food Chem. 33:852-855.

MATSUURA, M., A. OBATA and D. FUKUSHIMA 1989. Objectionable flavor of soy milk developed during the soaking of soybeans and its control. J. Food Sci. 54:602-605.

MURPHY, P.A. 1982. Phytoestrogen content of processed soybean products. Food Technology 36:60-64.

NAKAMURA, T., S. UTSUMI and T. MORI 1984. Cultivar differences in gelling characteristics of soybean glycinin. J. Agr. Food Chem. 32:647-651.

OGAWA, T., E. TAYAMA, K. KITAMURA and N. KAIZUMA 1989. Genetic improvement of seed storage proteins using three variant alleles of 7S globulin subunits in soybean (*Glycine max* L.) Japan J. Breed. 39:137-147.

OGAWA, T., N. BANDO, H. TSUJI and K. SASAOKA 1989. Characterization of allergenic proteins in soybean. In abstract of the 14th International Congress of Nutrition, Aug. 20. Korea. p.500.

OKUBO, K., Y. KOBAYASHI and K. TAKAHASHI 1983. Improvement of soy milk and tofu process on the behavior of undesirable taste component such as glycosides. Up to Date Food Processing 18:16-22 (in Japanese).

ORF, J.H. and T. HYMOWITZ 1979. Inheritance of the absence of the Kunitz trypsin inhibitor in seed protein of soybeans. Crop Sci. 19:107-109.

PULL, S.P., S.G. PUEPPKE, T. HYMOWITZ and J. H. ORF 1978. Soybean lines lacking the 120,000 dalton seed lectin. Science 200:1277-1279.

SAIO, K.and T. WATANABE 1978. Differences in functional properties of 7S and 11S soybean proteins. J. Texture Studies 9:135-157.

TAIRA, H., H. TAIRA, N. KAIZUMA J. FUKUI and S. MATSUMOTO 1976. Varietal differences of seed weight, protein and sulfur-containing amino acid contents of soybean seeds. Proc.Crop Sci.Soc.Japan 45:381-393 (in Japanese with English summary).

TSUKADA, Y., K. KITAMURA, K. HARADA and N. KAIZUMA 1986. Genetic analysis of subunits of two major storage proteins (α-conglycinin and glycinin) in soybean seeds. Japan J. Breed. 36:390-400.

WATANABE, I., M. SATO, T. NAGASAWA, A. OKABE and S. SAKAI 1989. Chemical composition and appearance of soybean seeds in germplasm collection in Japan. Misc. Publ. Tohoku Nat'l Agr. Exp. Stn. No.9:21-131 (in Japanese).

Classification and Evaluation of *Citrus*

by

Mitsuo OMURA

Okitsu Branch, Fruit Tree Research Station, MAFF
Okitsunaka-cho, Shimizu, Shizuoka 424-02, JAPAN

CONTENTS

1. Introduction

The phylogenetic relationships of *Citrus* species has been remained unclear, and there were many arguments until now (see Proceedings for 6th International Conference of Citriculture, Tel Aviv, Israel, 1989). The propagation system in *Citrus* which includes vegetative propagation, apomictic propagation and hybrid seedlings, makes it difficult. *Citrus* plants are usually classified into several "conventional" groups such as lime, lemon, citron, mandarin, sweet orange, sour orange, pummelo, and grapefruit. This grouping is based mainly on visible and commercially important characteristics. In the practical sense, morphological and physiological characteristics are very important to recognize their worth for commercial use, but recent development in *Citrus* breeding requires more detailed and wider information. Classification techniques in *Citrus* has been developed in various methods such as numerical approach, chemotaxonomy, karyotype analysis, and micromorphology.

Recently, molecular methods including isozyme analysis and DNA fragment analysis are applied to phylogenetic study in *Citrus*. These molecular methods clearly show the genetic variability within/between groups in gene level instead of practical traits in conventional methods. The evaluation of genetic polymorphism would provide the basic information for breeding and germplasm conservation.

2. Isozyme analysis

1) Isozyme diversity in *Citrus*

Since an enzyme is direct product of gene, isozyme analysis can provide direct information of diversity in genetic loci. As a change in coding region of DNA induces somewhat structural modification of protein such as molecular weight and electric charge, the change will be detected as different bands from original position by electrophoresis or electrofocusing.

For example, LAP (leucine aminopeptidase) shows variation among species and clones in *Citrus* (Fig.1). We can see mainly two groups of bands with a slow or fast migrating rate. Within the variation, we have to find stable band(s) among different leaf ages and seasons. In the case of LAP analysis, the band in fast migrating region (*LAP-2*) is unstable, and therefore genetic analysis is rather difficult, whereas the band with slow migrating region (*LAP-1*) is stable. As LAP is monomeric enzyme, *LAP-1* is inherited and expressed codominantly, therefore,

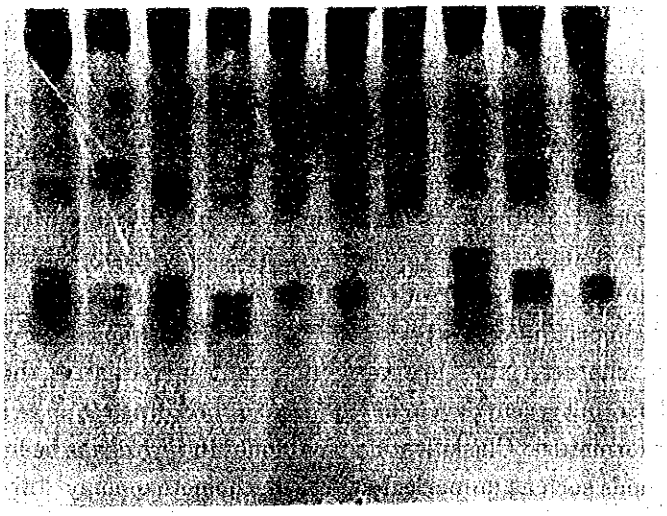


Fig. 1 **LAP zymogram in *Citrus***
(from left lane to right *C. sinensis, C. grandis, C. clementina, C. aurantium, C. limon, C. aurantifolia, C. medica, C. hystrix, C. tachibana, C. junos*)

heterozygosity can be directly detected. Segregation of the stable band (*LAP-1*) in progenies is shown in Fig. 2. Phenotypic patterns are divided into three types among progenies. Plants phenotypically having only one band (S or F) are homozygous in the *LAP-1* locus and progenies having two bands (FS) are heterozygous. On the other hand, dimeric enzymes such as GOT (glutamate-oxaloacetate transaminase), PGI (phosphate glucose isomerase), MDH (malate dehydrogenase) show three bands including heterodimer with two homodimers in heterozygous progenies.

Isozymes of *Citrus* leaf were extensively analysed for genetic and phylogenetic studies by TORRES *et al.* (1978, 1982). They reported similarities in the isozyme genotype within conventional groups. Within each group, isozyme alleles were recognized fairly homogenous. A model genotype of *GOT-1* was identified as FF for lime, pummelo and citron, SS for sour orange, mandarin and sweet orange, and FS for rough lemon, lemon and grapefruit. TORRES *et al.* analyzed totally ten loci of eight enzymes, and decided their model genotypes which were different for each group.

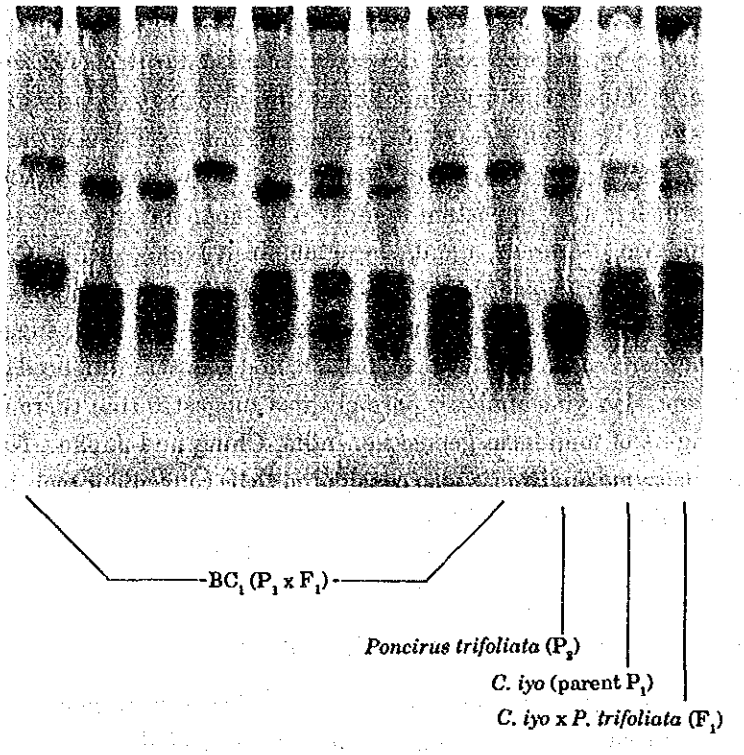


Fig. 2 **Segregation of LAP isozymes in progenies of *C. iyo* and *Poncirus trifoliata***

Based on genetic analyses, the isozyme diversity in *Citrus*, especially in mandarins, was investigated (HIRAI *et al.* 1986, HIRAI and KAJIURA 1987). Mandarins have relatively wider variation than other groups of *Citrus*, and are classified into some separate groups depending on similarities of isozyme genotypes (Table 1); Mandarins originated from China such as *C. kinokuni* Hort. ex TANAKA, *C. sunki* Hort. ex TANAKA, *C. erythrosa* Hort. ex TANAKA, have fundamentally same band patterns in eight isozyme loci, and *C. unshiu* MARC. (Satsuma mandarin) which is very important mandarin in Japan has the same genotype as Chinese mandarin. Whereas, mandarins endemic in Japan, such as *C. tachibana* (MAK.) TANAKA, *C. yatsushiro* Hort. ex TANAKA have different genotypes in *GOT-2*, *MDH-1* and *Px* (peroxidase) loci from Chinese ones. It is strongly suggested that *C. unshiu* was derived from Chinese mandarin, and was not influenced genetically by *C.tachibana*. Isozyme analyses possibly also suggested that there were three different origins of mandarin genes; i.e. India, China and Japan. Not only for mandarins, isozyme analysis is also considered to be convenient tools for study of phylogenetic relationships in *Citrus*.

2) Some techniques for isozyme analysis in *Citrus*

Isozyme analysis technique by polyacrylamide gel electrophoresis (PAGE) is employed in our laboratory by simplified procedures as follow.

Citrus leaves are good material for enzyme extraction, because they are evergreen and have a low content of polyphenolic compounds. Enzymes are extracted from 0.2g fresh leaves with 1.0ml of 0.1M Tris-ascorbate buffer (pH 8.0) supplemented with NaCl and Triton X-100 in ice cold-mortar. Supernatant after centrifugation at 2,000rpm for 10 minutes is used as crude enzyme solution. After adding sucrose to weigh the solution, the enzyme solution is applied to a top of polyacrylamide gel. Twelve sample solutions are electrophoresised at 15mA during 10cm long gel with 1mm thickness. When visible marker (BPB) reaches bottom of gel, usually electrophoresis finishes, and then the gel is stained.

GOT, PGI (phosphate glucose isomerase), SOD (superoxide dismutase), LAP, ShDH (shikimate dehydrogenase), PPO (polyphenol oxidase), and EST (non specific esterase) are easily detectable by respective active staining. When starch is mixed to polyacrylamide gel, amylase and catalase become detectable. For some kinds of enzymes such as MDH, partial purification through Blue sepharose CL-6B is required for good separation of isozymes. After fixation, gel could be preserved as dry specimens on paper.

Table 1 Genotypes of isozymes in mandarin (After HIRAI *et al.* 1986, HIRAI and KAJIURA 1987)

Species	*GOT-1*	*GOT-2*	*GOT-3*	*MDH-1*	*Px*	*PPO*	*SOD-1*	*SOD-2*
1. Originated from China								
Citrus kinokuni Hort. ex TANAKA	SS	MM	E-	DD	DD	AA	AA	AA
C. sunki Hort. ex TANAKA	SS	MM	E-	DD	DD	AA	AA	AA
C. ponki Hort. ex TANAKA	SS	MM	E-	DD	DD	AA	AA	AA
C. erythrosa Hort. ex TANAKA	SS	MM	E-	DD	DD	AA	AA	AA
C. oleocarpa Hort. ex TANAKA	SS	MM	E-	DD	DD	AA	AA	AA
C. succosa Hort. ex TANAKA	SS	MM	E-	DD	DD	AA	AA	AA
C. tankan HAYATA	SS	MM	E-	DD	DD	AA	AA	AA
C. suhuiensis Hort. ex TANAKA	SS	MM	E-	DD	CD	AA	AA	AA
2. Originated from Japan								
C. tachibana (MAK.) TANAKA	SS	MA	E-	BD	CC	AA	AA	AA
C. nippokoreana TANAKA	SS	MA	E-	BB	CC	AA	AA	AA
C. keraji Hort. ex TANAKA	SS	MA	E-	BD	DD	AA	AA	AA
C. oto Hort. ex Y. TANAKA	SS	MA	E-	BD	CD	AA	AA	AA
C. yatsushiro Hort. ex TANAKA	SS	MA	E-	BD	CD	AA	AA	AA
C. unshiu MARC.	SS	MM	E-	DD	DD	AA	AA	AA
C. ujukitu Hort. ex TANAKA	SS	MM	DE	DD	DD	AA	AA	AA
3. Originated from other areas								
C. reticulata BLANCO	SS	MM	E-	DD	CC	AA	AA	AA
C. tangerina Hort. ex TANAKA	SS	MM	E-	DD	CD	AA	AA	AA
C. deliciosa TANAKA	SS	MM	E-	DD	DD	AA	AA	AA
C. nobilis LOUR.	SS	MM	E-	DD	DD	AA		
C. reshini Hort. ex TANAKA	SS	MM	E-	DD	DD	AA	AA	AA

For the classification and phylogenetic studies through isozyme analysis, it is required that many enzymes can be examined in short period as much as possible. PAGE takes about four to five hours for each enzyme in usual methods. As reported by ASHARI *et al.* (1989), isozymes of ShDH, 6-PGD (6-phosphogluconate dehydrogenase), APH (acid phosphatase), F1,6DP (fructose-1, 6-diphosphatase) among mandarin species and hybrids can be also analyzed by somewhat different procedures. Recently, prepared gels can be supplied for micro-electrophoresis, in which electrophoresis or electrofocusing finishes within 30 minutes for about 1μl samples applied on the gel. By usual staining, GOT, POD, SOD and PGI can be detectable on native PAGE and MDH on IFE gels. Through such technical improvement, isozyme analysis will provide new information on phylogeny and breeding on *Citrus*.

3) Application of isozyme analysis in explored *Citrus* germplasm

Many *Citrus* species produce nucellar embryos together with zygotic ones in a seed. Nucellar embryos can be utilized in germplasm collection instead of vegetative scions, because they have genotype identical to maternal plants due to apomictic nature. However, on-typed nucellar seedlings have to be distinguished from off-typed zygotes for germplasm depository. Mean embryo number in each seed varies from 11 to 26 within grapefruit, from 9.6 to 19.7 within sweet orange, from 7.7 to 50.0 within mandarin, from 1.9 to 4.5 within lemon, and from 2.0 to 2.9 within lime, although almost all pummelo have monoembryonic trait (UENO *et al.* 1967). To find hybrid or selfed seedlings (off-type), isozyme analysis has been applied to Citrus breeding (UENO and NISHIURA 1976, TORRES *et al.* 1982). For this purpose, heterozygous isozyme loci have been conveniently investigated. For example, a majority of lemon and lime clones is heterozygous in *GOT-1* (genotype: FS), *-2* (SM), *-3* (AE/AC), *MDH-1* (AD), *Px* (CC/CD), *SOD-2* (AA/AB), *-3* (AE/AC).

Percentage of detectable off-types for each locus among all off-type seedlings is 50%, therefore five loci can detect 97% of off-type, if they are independently inherited. The percentages of heterozygous genotype in each locus are given in Table 2 to apply isozyme screening for various species of *Citrus*. Fig. 3 shows an example of checking GOT isozymes among seedlings collected. Table 3 shows also examples in an application of isozyme assay to off-types in seedlings collected in Nepal. Genotype of on-type seedlings can be decided by discarding off-types. At the same time, genetic diversity in isozyme loci could be recognized.

In the case of evaluation of germplasm collection from Nepal, each accession was discriminated at first with vegetative characteristics such as leaf shape, spine, and color of young leaf. Based on the evaluation data on quantified leaf characteristics, unidentified accessions were classified into respective "conventional" groups by a discriminant function method.

Dendrogram for discriminating the mandarin group by cluster analysis for clones is shown in Fig. 4. It clearly shows that newly rearranged accessions into mandarin from other groups were placed apart from typical mandarin called "Suntala" in Nepal.

Table 2 Variation of genotype in *Citrus* isozymes (After HIRAI *et al.* 1986, HIRAI and KAJIURA 1987)

Group	Isozyme locus							
	GOT-1	*GOT-2*	*GOT-3*	*MDH-1*	*Px*	*PPO*	*SOD-1*	*SOD-2*
Lemon and lime	H(2)	H(2)	H(4)	H(3)	L(2)	–(1)	L(2)	H(3)
Pummelo	L(3)	–(1)	H(5)	–(2)	–(2)	L(2)	L(2)	–(1)
Grapefruit	H(2)	–(1)	H(2)	H(2)	H(2)	–(1)	–(1)	–(1)
Sour orange	H(2)	–(1)	H(2)	H(2)	H(2)	–(1)	H(2)	–(1)
Sweet orange	–(1)	–(1)	H(2)	–(1)	–(1)	–(1)	–(1)	–(1)
Mandarin	–(2)	L(1)	–(2)	L(3)	M(3)	–(1)	L(2)	–(1)
Number of loci	(4)	(4)	(7)	(6)	(5)	(2)	(2)	(4)

( ) shows number of alleles.

–, L, M, and H : mean ratio of heterozygous genotype among clones, <10%, ~ 50%, ~ 90%, and ~ 100% respectively.

Table 3 Off-type screening of Lemon-Lime type seedlings collected from Nepal by isozyme (GOT) analysis

Acc.No.	Genotype		Off-type seedlings			
	GOT-1	*GOT-2*	*GOT-1*	*GOT-2*	*GOT-3*	Off-Total/ On
Lemon						
84338	FS	SM	3	3	0	4/7
84349	FS	MM	1	1	0	2/4
85361	FS	SM	1	0	0	1/6
85409	FS	SM	1	1	0	1/5
85410	FS	SM	2	1	0	2/13
Rough lemon						
85382	FS	SS	0	0	0	0/2
85398	FS	SS	0	0	0	0/9
Lime						
84336	FF	SM	0	0	0	0/13
84339	FF	SM	0	0	0	0/10
84341	FF	SM	0	0	0	0/13
84587	SS	MM	0	0	0	0/6
85390	FF	SM	0	0	0	0/9

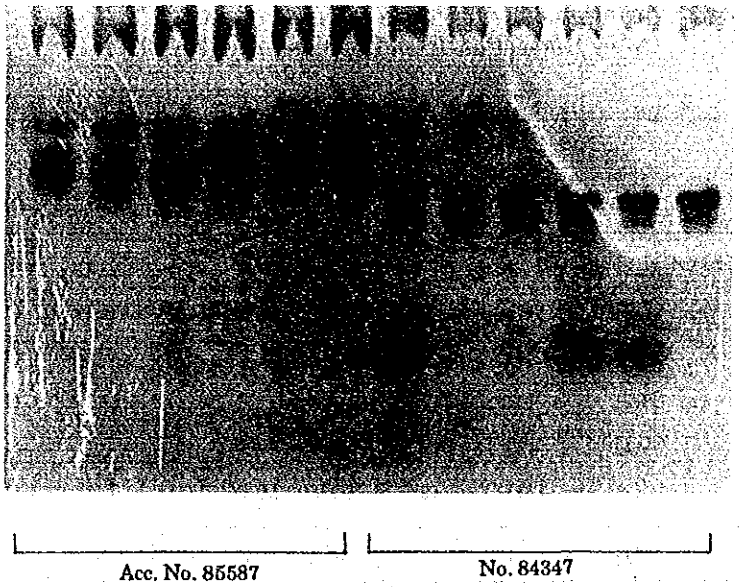


Fig. 3 Detection of Off-type seedlings by GOT isozymes

Isozyme analysis for germplasm collection from Nepal was conducted by four enzymes including seven loci (*GOT-1, -2, -3, PGI, LAP-1, LAP-2,* and *ShDH*), of which genetic analysis of *LAP-2* is not completed. The zymogram of typical mandarin such as Acc. No. 84334 has genotypes of SS, MM, E-, FF, FM, – –, and FM respectively. Among 30 accessions examined, there were 2 off-types in *GOT-1*, 1 in *GOT-2*, 8 in *GOT-3*, 14 in *PGI*, 9 in *LAP-1*, 10 in *LAP-2* and 6 in *ShDH*. Heterogenity was relatively high among clones which were discriminated to mandarin by numerical analysis of leaf characteristics. However, 19 clones which were placed in the upper part on dendrogram (from No. 84332 to No. 85606) showed relatively close relation on leaf characteristics, and exhibited relatively high homogenity in isozyme genotypes. Within these 19 clones, only 16 out of 133 isozymes were off-type, but 11 clones in bottom on the dendrogram showed 34 off-types for 77 examined. Not only the percentage of clones having off-type in each locus, but also number of off-type loci in each clone was extremely high in clones in the bottom groups. Of them, Acc. No.85081 and 85413 had the typical sweet orange genotypes in all seven loci. Therefore, numerical discrimination by leaf characteristics between mandarin and sweet orange was not complete at young seedling stage. The clones relatively apart from the typical mandarin within the group may be hybrids or other species. Application of isozyme analysis would make it possible to develop a more precise classification of *Citrus* germplasm.

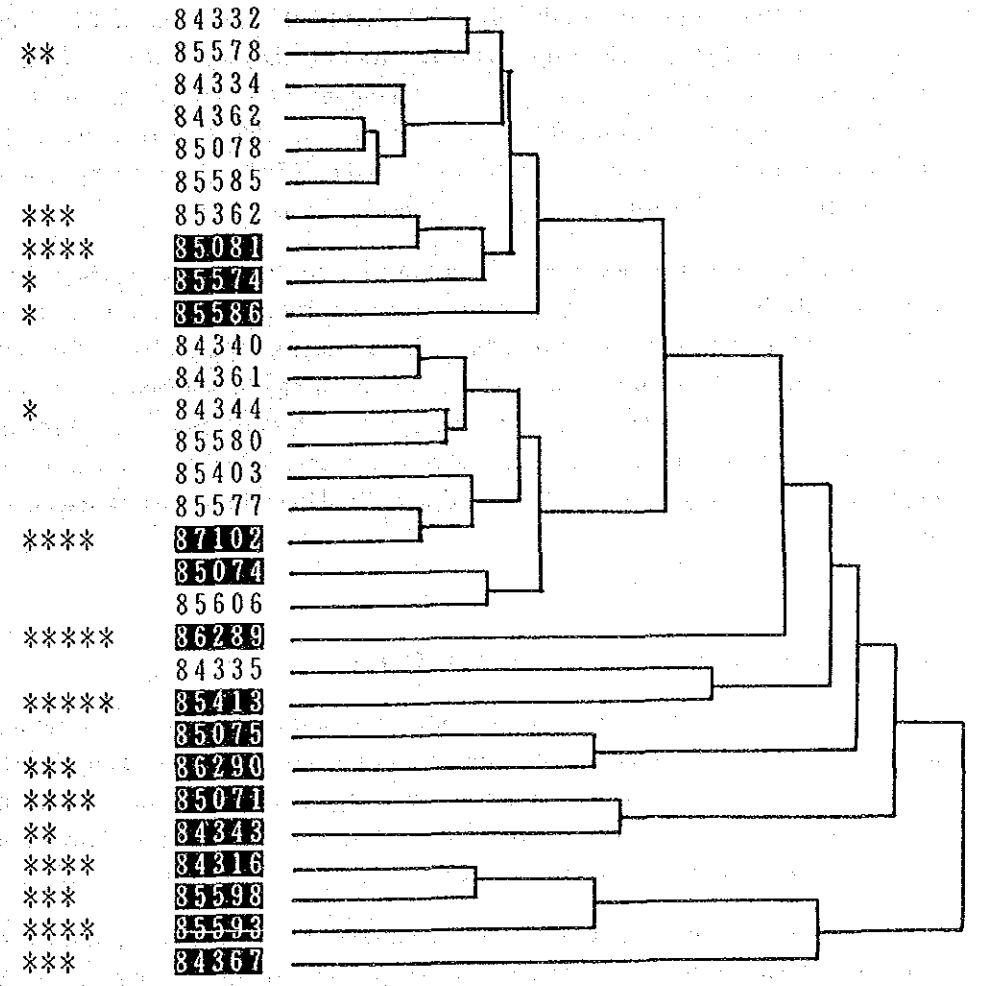


Fig. 4 **Dendrogram by cluster analysis in leaf characteristics of *Citrus* germplasm (mandarin group) collected in Nepal**

Note: Accession numbers written with white figures mean clones which were grouped into mandarin by discrimination analysis of leaf characteristics.

* numbers represented the different genotypes from typical mandarins in 7 isozyme loci.

4) Linkage analysis combined with RFLP markers

Restriction fragment length polymorphisms (RFLPs) of nuclear DNA has been employed to phylogenetic studies in *Citrus*. ROOSE (1988) detected many RFLPs by the probes of cDNA from m-RNA of rough lemon leaf. There was also variation in the spacer region of ribosomal-DNA (UEMATSU *et al.* 1990). It revealed wide variation among mandarin clones than those expected. RFLPs will make it possible to quantify the classification of *Citrus* on molecular basis.

As RFLPs could detect many polymorphic loci in *Citrus* genome, they were mapped on the linkage groups together with isozyme markers (JARRELL and MOORE 1990, LIOU *et al.* 1990, DURHAM *et al.* 1990). Those efforts will be applied to find out loci for commercially important characteristics including quantitative characteristics, disease resistance and so on. Therefore, recent classification techniques on molecular basis could direct to the genomic analysis for closely related variation in important traits. Isozyme analysis can also be utilized in making the linkage maps of *Citrus*.

3. Variation in chemical constituents in *Citrus*

In Rutaceae including *Citrus*, there is a remarkable variation in secondary metabolites such as flavonoids, triterpenoids, and essential oils. Although such chemical constituents are not direct product as isozyme or protein, information of the variation of chemical constituents is also informative in the classification of Rutaceae or Aurantioideae (WATERMAN 1975, 1982).

Acridone is one of plant alkaloids contained in Rutaceae plants, mainly in root bark. Its basic structure, 9(H)-acridone, consists of three hetero-rings in which B ring includes nitrogen and oxygen double bond. Many natural derivatives of acridone were found in Rutaceae. Not only monomeric derivatives, but also dimeric alkaloids even with coumarins were discovered recently.

Simply, acridones are analysed by crude extracts with acetone on TLC, and their special patterns are measured by chromato-scanner. In detail, fractions on TLC are further separated by column chromatography using various solvents such as various composition of hexane, benzene, acetone, ethyl acetate. Purified components are analysed by NMR, IR and mass spectrography, and their molecular structures are decided.

Table 4 shows components of acridones in some *Citrus* species (after WU and FURUKAWA 1983, WU *et al.* 1983a,b). There is a wide variation in composition of derivatives among *Citrus* species.

Besides on taxonomical importance, some acridones have medicinal values. Some structures of acridone, such as acrocynine, were known as a natural occurring antitumor agent. Recently, a kind of acridone, Citrusinine-I, was found to exhibit potent activity against herpes simplex virus (HSV) and cytomegalovirus at low concentration which did not affect cell activity (YAMAMOTO *et al.* 1989). A dimeric acridone, Atalaphillinine, was confirmed to suppress 90% or more of *Plasmodium yoelii,* which caused malaria in rodents (FUJIOKA *et al.* 1989).

Table 4 Variation of acridone alkaloid components in some *Citrus* bark (After WU and FURUKAWA 1983, WU *et al.* 1983 a and b)

Species / Alkaloids	*C. depressa* HAYATA	*C. sinensis* OSBECK	*C. grandis* OSBECK
Bark weight	0.25kg	0.5kg	1.6kg
Citacridone-I	++	+	++
Citacridone-II	+		++
Citrusine-I			
Citrusinine-I		+	+
Citrusinine-II		+	
Citpressine-I	+		+
Citpressine-II	+		+
Prenylcitpressine	+		+
5-hydroxy noracrycine	+		+
Crenulatin		+	
Citbrassine		+	
Glycocitrine-I			+
Glandisinine			+
Grandisine-I			++
Grandisine-II			+
Total	701mg	297mg	14,100mg

++: >0.1% of dry bark, +: ≤0.1% of dry bark, no sign: not detectable.

New derivatives of acridone were reported for phylogenetic and medicinal purpose (ITO *et al.* 1990a), further analysis would provide new keys for classification of *Citrus*.

4. Classification and cultivar identification

Micromorphological studies through scanning electron microscope (SEM) revealed differences in pollen surface among *Citrus* species (KOZAKI and HIRAI 1981).

Cytological researches were also improved to produce clear karyotype and to reveal banding patterns of *Citrus* chromosomes (ITO *et al.* 1990b). Recent studies combined with molecular techniques will present the chromosome mapping for special regions on chromosomes by *in situ* hybridization. Those cytological techniques will present new information on evolutional relationships among species or genera. Classification techniques which include morphological, chemical, cytological, and molecular techniques can be applied to identify cultivars in addition to above described techniques, DNA fingerprinting methods by using hypervariable probes can be investigated for practical identification of cultivars (JEFFREY *et al.* 1985, NYBOM *et al.* 1990).

In fruit species including *Citrus*, cultivars are practically identified or discriminated mainly in fruit characteristics, but it requires for long duration to evaluate fruit characteristics. Early estimation with advanced techniques will become more important for breeding and germplasm management.

5. References

DURHAM, R., G. MOORE and. C. GUY 1990. Linkage of restriction fragment length polymorphisms and isozymes in a backcross of *Citrus* and *Poncirus*. Hort Science 25:1154 (abstract).

GREEN, R. M., A. VARDI and E. GALUN 1986. The plastome of *Citrus*. Physical map, variation among *Citrus* cultivars and species, and comparison with related genera. Theor. Appl. Genet. 72:170-177.

HIRAI, M., I. KOZAKI and I. KAJIURA 1986. Isozyme analysis and phylogenic relationship of *Citrus*. Japan J. Breed. 36:377-389.

HIRAI, M. and I. KAJIURA 1987. Genetic analysis of leaf isozymes in *Citrus*. Japan J. Breed. 37:377-388.

ITO, C., S. TANAHASHI, K. FUJISAWA, M. NAKAGAWA, M. JU-ICHI, Y. FUJITANI, M. INOUE, A. T. McPHAIL, T. S. WU, I. KAJIURA, M. OMURA and H. FURUKAWA 1990a. Isolation and structures of Citropone-A, -B, and -C from *Citrus* plants, the first examples of naturally occurring homoacridone alkaloids containing a seven-membered ring system. Chem. Pharm. Bull. 38:1881-1885.

ITO, Y., M. OMURA, H. NESUMI and T. YOSHIDA 1990b. Application of C-banding methods to Citrus chromosome. Japan J. Breed. 40 (Suppl. 2):350-351.

JARREL, D. C. and M. L. ROOSE 1990. A genetic map of citrus based on isozymes and RFLPs. Hort Science 25:1154 (abstract).

KOZAKI, I. and M. HIRAI 1981. Pollen ultrastructure of Citrus cultivars. Proc. 4th Int'l. Citrus Congress (Tokyo) Vol.1:19-22.

LIOU, P., F. G. GMITTER, Jr. and G. A. MOORE 1990. Molecular characterization and linkage mapping of the citrus genome using isozyme and RFLP markers. Hort Science 25:1154 (abstract).

JEFFREY S, A. J., V. WILSON and S. L. THEIN 1985. Hypervariable 'minisatellite' regions in human DNA. Nature 314:67-73.

NYBOM, H., S. H. ROGSTAD and B. A. SCHAAL 1990. Genetic variation detected by use of the M13 "DNA fingerprint" probe in *Malus, Prunus,* and *Rubus* (Rosaceae). Theor. Appl. Genet. 79:153-156.

ROOSE, M. L. 1988. Isozymes and DNA restriction fragment length polymorphisms in Citrus breeding and systematics. Proc. 6th Int'l. Citrus Congress (Tel Aviv, Israel) 155-165.

TORRES, A. M., R. K. SOOST and U. DIEDENHOFEN 1978. Leaf isozymes as genetic markers in citrus. Amer. J. Bot. 65:869-881.

TORRES, A. M., R. K. SOOST and T. MAU-LASTOVICKA 1982. Citrus isozymes; Genetics and distinguishing nucellar from zygotic seedlings. J. Hered. 73:335-339.

UEMATSU, C., M. OMURA, T. HIDAKA and Y. OGIHARA 1990. Strain diagnosis of *Citrus* species by means of RFLPs analysis. Japan J. Breed.40 (Supple.2): 452-453.

UENO, I., M. IWAMASA and M. NISHIURA 1967. Embryo number of various varieties of *Citrus* and its relatives. Bull. Hort. Res. Stn. B-7:11-21.

UENO, I. and M. NISHIURA 1976. Application of zymography to citrus breeding. I. Identification of hybrid and nucellar seedlings in citrus by peroxidase isozyme electrophoresis. Bull. Fruit Tree Res. Stn. B-3:1-8 (in Japanese).

WATERMAN, P. G. 1982. Phylogenetic implications of the distribution of secondary metabolites within the Rutales. In chemistry and chemical taxonomy of the Rutales (Ed. WATERMAN, P. G. and M. F. GRUNDON) Academic Press 377-400.

WU, T. S. and H. FURUKAWA 1983. Acridone alkaloids. VII. Constituents of *Citrus sinensis* OSBECK var. *brasiliensis* TANAKA. Isolation and characterization of three new acridone alkaloids, and such a new coumarin. Chem. Pharm. Bull. 31:901-906.

WU, T. S., C. S. KUOH and H. FURUKAWA 1983. Acridone alkaloids and a coumarin from *Citrus grandis*. Phytochemistry 22:1493-1497.

WU, T. S., C. S. KUOH and H. FURUKAWA 1983. Acridone alkaloids. VI. The constituents of *Citrus depressa*. Isolation and structure elucidation of new acridone alkaloids from *Citrus* genus. Chem. Pharm. Bull. 31:895-900.

Classification and Identification of Crucifer Vegetables in Japan

by

Hiroaki YOSHIKAWA and Susumu YUI

National Research Institute of Vegetables, Ornamental Plants and Tea, MAFF
Kusawa, Ano, Mie 514-23, JAPAN

CONTENTS

1. Classification of *Brassica oleracea*

Brassica oleracea has B genome with chromosome n=9. Common cabbage, cauliflower and broccoli are important vegetables in this species. There are some other crops of this species in Japan.

1) Cabbage (*B.oleracea* var. *capitata* L.)

(1) Common cabbage

In Japan, varieties sown in very early and early autumn, those sown in early and middle summer and those sown in middle and middle-late autumn were developed by using European and American varieties which were introduced into Japan during Meiji era. Table 1 shows major characteristics for classification of the common cabbage.

Nowadays almost all commercial varieties are F_1 hybrids, and they are classified into following three groups; spring sowing group, summer sowing group, and autumn sowing group.

a. Spring sowing group: In this group, there are three types. The first one which is a varietal group for highland culture is derived from "Copenhagen Market, Just Light, Glory of Enkuizen (early), Succession (middle), Nanbu (late) and Sapporo (very late)", and is characterized by heat tolerance, firm-head, and tolerance to transportation. The second one which is a varietal group for early spring sowing is derived from "Kuroba Succession, Aichi and Atsumi", and is characterized by late bolting and heat tolerance. The last one which is a varietal group for early summer sowing is derived from "Yoshin", which was a local variety in Taiwan. It is characterized by heat tolerance, good texture, soft leaf, low transportability, and early bolting.

b. Summer sowing group: In this group, there are three types. The first one which is a varietal group with early growth habit is derived from "Atsumi and Aichi", and is characterized by heat tolerance, cold tolerance, and stable heading. The second one which is a varietal group with middle growth habit is derived from "Atsumi and Aichi", and is characterized by good head formation under low temperature, cold tolerance, and frost tolerance. The last one which is a varietal group with late growth habit is derived from "Aichi, Danish Ballhead and Flat Dutch", and is characterized by stable and good head formation under low temperature, cold tolerance, and late bolting.

Table 1 Main characteristics to classify common cabbage (*Brassica oleracea* var. *capitata* L.)

Characteristics	Grade	Remarks
Days to mature	Very early	Less than 50 days to harvest after transplanting
	Early	50-60 days to harvest after transplanting
	Semi-early	60-70 days to harvest after transplanting
	Medium	70-80 days to harvest after transplanting
	Semi-late	80-90 days to harvest after transplanting
Head shape	Flat	
	Semi-flat	
	Semi-round	
	Round	
	Pointed	
Plant form	Erect	
	Medium	
	Spread	
Nature of leaves		Hard or soft, hairy or glabrous, pale green or deep green, waved or smooth
Stress resistance		Heat tolerance, cold tolerance, drought tolerance, excess moisture tolerance

c. Autumn sowing group: In this group, there are four types. The first one which is a varietal group with very early growth habit is derived from "Nakano Wase and Jersey Wakefield", and is characterized by long juvenile phase, earliness, small head, and few outer leaves. The second one which is a varietal group with early growth habit is derived from "Nozaki Wase and Kiba Succession", and is characterized by short juvenile phase, yellowish and thin leaf, and semi-flat and semi-round of head shape. The third one which is a varietal group with middle-early growth habit is derived from "Kiba Succession and Succession", and is characterized by short juvenile phase, high yield, and good quality. The last one which is a varietal group with middle-late growth habit is derived from "Kuroba Succession and Aichi", and is characterized by the shortest juvenile phase, the highest heat tolerance, large and dark leaves, flat and large head, and adaptability to a culture of late-spring or early-summer harvest.

As to the head shape of Japanese cabbage, semi-flat or semi-round types are popular, round types are not so common, and pointed types are rare. Recently, small

round types (green ball) are becoming popular because of their good quality, such as tender and juicy tissue and deep green color.

(2) Savoy cabbage

Savoy cabbage which is derived from "Perfection Drumhead, Best of All, Chieftain Savoy etc." is characterized by highly wrinkled foliage, dark green, little bloom foliage, sweet taste, and good for salad, but low heat tolerance, easy to bolt, and not of much commercial importance. Though the cultivation area of this type in Japan is very much limited, this type is adapted to the cultures of spring sowing in highland and cool region, and of summer sowing in lowland.

(3) Red cabbage

Red cabbage which is derived from "Mammoth Rock Red, Red Acre, Nigger Head etc." is distinguished from all others by its deep purplish-red color in both leaf and head. Red cabbage is characterized by large outer leaf, small and firm head, high in storage ability, and cold tolerance, but low in heat tolerance, and easy to bolt. The number of varieties is small.

2) Cauliflower (*B.oleracea* var. *botrytis* L.)

Cauliflower varieties differ from each other in plant size, time or range of maturity, and foliage and curd characteristics. Classification based on the maturing time is common, and the days from transplanting to maturity varies from 55 days in very early varieties to over 180 days in late ones. Almost all Japanese varieties belong to very early to medium types. Moreover, most of them are F_1 hybrids of snowball type. Generally, very early types are dwarf with medium-large leaves, and their inner leaves curl over the head, while outer leaves curl outward at the tip. The curd is of moderate size but tends to be flatter and thinner than those of medium varieties. Medium varieties are generally more erect and sometimes longer in leaf length, and the curds are more rounded, thicker, and heavier than those of early varieties.

3) Broccoli (*B.oleracea* var. *italica* PLEN.)

Green sprouting broccoli is becoming more popular than cauliflower in Japan, and most of broccoli varieties are very early to medium late in maturity.

The days from transplanting to maturity is about 40 days in very early varieties, and about 85 days in medium late ones. These varieties are characterized by dark bluish-green color, and produce a large compact apical head and many small lateral heads after cutting the apical head. Very early and early varieties are bred for harvesting the apical head only, and medium and late medium varieties are bred for continued harvesting by cutting both the apical head and some lateral heads.

4) Brussels sprouts (*B.oleracea* var. *gemmifera* ZENK.)

In Japan, Brussels sprouts are cultivated only in a limited place, because optimum growth period is too short in most regions. Though the number of commercial varieties is small, they are divided into two types. One grown in warm regions is sown in summer, and harvested from autumn to winter. They are characterized by earliness and dwarfness. The other grown in highland is sown in spring, and harvested from summer to autumn. They are medium tall (75-90cm) and early.

5) Other *oleracea* crops

Kohlrabi (*B.oleracea* var. *gongylodes* L.), Chinese kale (*B.oleracea* var. *alboglabra*) and kale (*B.oleracea* var. *acephala* DC.) are also cultivated, but very few in terms of both areas and numbers of varieties. Most of their seeds are imported.

2. Classification of *B.campestris*

B.campestris has A genome with chromosome n=10. Chinese cabbage and turnip are important vegetables which belong to this species. There are some other leaf vegetables or salt greens of this species in Japan.

1) Chinese cabbage (*B.campestris*, pekinensis group)

Chinese cabbage can be classified into three types according to its heading characteristics, i.e. heading, semi-heading and non-heading types. Differences among them are, however, quantitative and continuous.

(1) Heading Chinese cabbage: Table 2 shows main characteristics to classify the heading Chinese cabbage. The days to maturity is recorded in late summer to autumn sowing, which is a major cultivation season of heading Chinese cabbage in the central Japan. Head shape is the most important characteristics for classification of heading Chinese cabbage. Those which originated in the southern China tend to be globular or round, while those which originated in the northern China tend to be long.

Number of leaves in a head determines whether a variety is leaf number type or leaf weight type. Nature of leaves is important from the point of postharvest and consumer's preference. As to their bolting, low-temperature-sensitive varieties are derived mainly from materials of warm southern regions, and less sensitive ones are derived from materials of cool northern regions.

(2) Semi-heading and non-heading Chinese cabbage: Degree of heading, leaf shape, leaf color and degree of incision are applied to classify these Chinese cabbage varieties.

2) Turnip (*B.campestris*, rapifera group)

Turnip is an important root vegetable in Japan. It has been cultivated for more than one thousand years. Several hundreds of local varieties are distributed all over Japan. An area covered by each variety is rather small except one called "Kanamachi" which is said to be originated from European "Milan White". Table 3 shows some important characteristics of turnip. Of them, shape, size and color of root are used for classification of turnip varieties, and leaf shape and days to maturity are also important characteristics.

3) Salt greens (*B.campestris*)

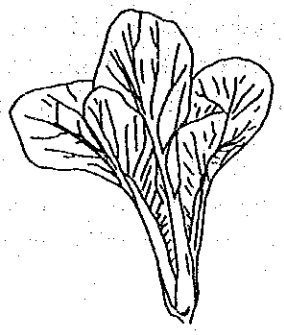
There are many non-heading leafy varieties of *B.campestris* in Japan (Fig. 1). Most of them are harvested in young seedling stage around one month after sowing.

Table 2 Main characteristics to classify heading Chinese cabbage (*Brassica campestris*, pekinensis group)

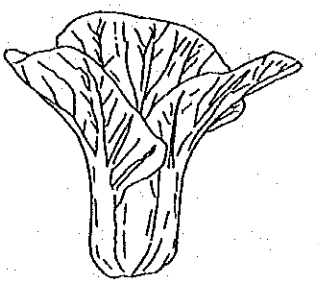
Characteristics	Grade	Remarks
Days to mature	Very early	Less than 50 days to harvest after sowing
	Early	50-60 days to harvest after sowing
	Medium	60-70 days to harvest after sowing
	Late	70-80 days to harvest after sowing
	Very late	More than 80 days to harvest after sowing
Head shape	Globular	
	Conical	
	Elliptical	
	Cannon ball shaped	
	Cylindrical	
Number of leaves in head	Plenty	More than 60 leaves, each leaf is small
	Intermediate	Around 50 leaves
	A few	40-50 leaves, each leaf is big
Nature of leaves		Hard or soft, hairy or glabrous, pale green or deep green
Bolting character	Early	Sensitive to low temperature
	Late	Less sensitive to low temperature

Table 3 Main characteristics to classify turnip (*Brassica campestris*, rapifera group)

Characteristics	Grade
Root shape	Round
	Intermediate
	Long
Root color	White
	Cream
	Red
	Purple
Root size	Small
	Intermediate
	Big
Leaf shape	No incision
	Incision
	Deep incision
Days to mature	Early
	Intermediate
	Late

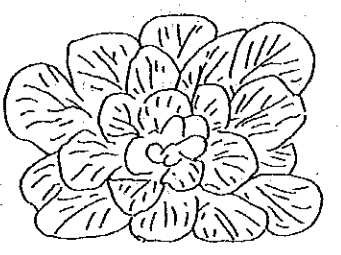


Rapifera

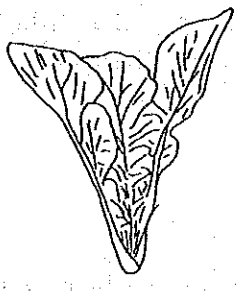


Chinensis

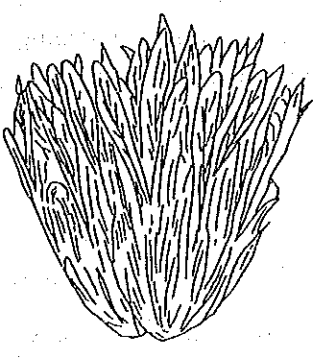
Parachinensis



Narinosa



Campestris



Japonica

Fig. 1 Plant shape of salt greens in *Brassica campestris* (syn. *rapa*)

(1) Rapifera group: Major varieties which have no swelling root are grouped into "Komatsuna". Though their root is slender, it is said to be originated from turnip. They have green and elliptical leaves with slender petiole. "Komatsuna" is produced all year round especially around Tokyo. There are some other turnip varieties which have rather small root. These are cultivated mainly as leaf vegetables.

(2) Chinensis group: This is characterized by leaves with flat and wide petiole. Compared to Chinese cabbage, its petiole is abaxially protruded. Wide diversity of leaf characteristics is observed within this group.

(3) Parachinensis group: This group was introduced as flower stalk vegetable mainly from southern China. Early maturing varieties bolt within 40 days after sowing. They do not require low temperature period to bolt. Medium to late maturing varieties require some degree of low temperature for their bolting. Leaf shape of parachinensis group is similar to that of chinensis group. These two groups seem to overlap.

(4) Narinosa group: This group is characterized by deep green leaf, long flat petiole, and numerous leaves. This group is adapted to cold condition.

(5) Campestris group: This group was cultivated in the past for oil production from seeds in Japan. But now it has no commercial value as oil seed rape. These varieties are not much refined as vegetable. Their petiole is comparatively narrow, and the lower part of leaves is incised.

(6) Japonica group: This group is Japanese origin. From its slender petal, branching character and pungent or hot taste, it might be related to *B. juncea*. Two different leaf shapes are recognized within the group. Those which have long elliptical leaves are called "Mibuna" or "Kyona", and the others with deeply incised leaves are called "Mizuna".

3. Classification of *B. juncea*

Mustard is a popular vegetable or oil seed rape throughout Asian countries. Being the most tolerant species to high temperature in *Brassica*, it is a major crop in the tropics and the subtropics. These are grouped into four types according to KUMAZAWA (1965) and NISHI (1980).

(1) Hakarashina group: Incised or pinnate leaves. It might be closely related to an original variety of *B.juncea*.

(2) Nekarashina group: Swelling root.

(3) Serifon group: Branching, incised and glabrous leaves.

(4) Takana group: Thick and round leaves. Petiole is also well developed and wide.

As shown in Table 4, leaf shape, shape and width of petiole, shape of leaf edge, degree of branching, degree of swelling in root or stem are applied to distinguish these four groups.

Table 4 Main characteristics to classify varieties of mustard (*Brassica juncea*)

Characteristics	Grade
Leaf shape	Round Elliptical Long elliptical
Shape of petiole	Round Intermediate Flat
Width of petiole	Wide or narrow
Shape of leaf edge	No incision Incision Deep incision
Degree of branching	High or low
Degree of swelling in root or stem	No swelling Intermediate Swelling

4. Classification of *Raphanus sativus*

Raphanus sativus includes Japanese radish (daikon group), radish (radicula group) and Chinese radish (lobo group). Of them, Japanese radish is popular, and radish and Chinese radish are very much limited. Cultivated area of radish is 63,200ha in 1988, and it is the largest in Japanese vegetables. Japanese radish is classified into following 15 groups from the root and leaf characteristics. There are wide variation in size, length, color, earliness, root shape, bolting, disease resistance and other characters, especially in weight (a few grams to more than 20kg) and length (a few centimeters to more than 120cm). Root types of each group are shown in Fig. 2.

1) Japanese radish (*R.sativus*, daikon group)

(1) Shiju-nichi group: This group has earliness, heat tolerance and tender leaf. Its root is slender in tip and 20-25cm in length. This group is cultivated in spring to early summer season, but recently its area of cultivation becomes very small.

(2) Minowase group: This group is characterized by earliness, heat tolerance, disease resistance and erect growth. Minowase group is cultivated in a hot season being sown from spring to early summer. Green leaf color type has a late bolting habit and can be sown in spring, while deep green leaf color type is more heat-tolerant than other.

(3) Nerima group: This group may be originated from "Horyo", and differentiated into many types, such as "Nerima, Nerima-shiriboso, Nerima-marujiri, Risou, Akitsumari, Miura". These cultivars can be classified into three subgroups; the first one is "Nerima" type. It has along root of about 80cm, and most part of the root grows underground. Its texture is suitable for salt pickles. The second one is "Akitsumari" type which has soft and juicy flesh suitable for fresh consumption. The last one is "Risou" type which is bred out by the cross of former two types. "Risou" which means "Ideal" in Japanese language is suitable for both pickles and fresh consumption.

(4) Horyo group: This group has soft, juicy, and sweet root, light green and hairy leaf, and curved root shape. Root quality is excellent.

(5) Moriguchi group: This cultivar has a slender and extremely long root of about 3cm in diameter, 120cm in length and 350g in weight. It is cultivated in special farms with deep and light soil.

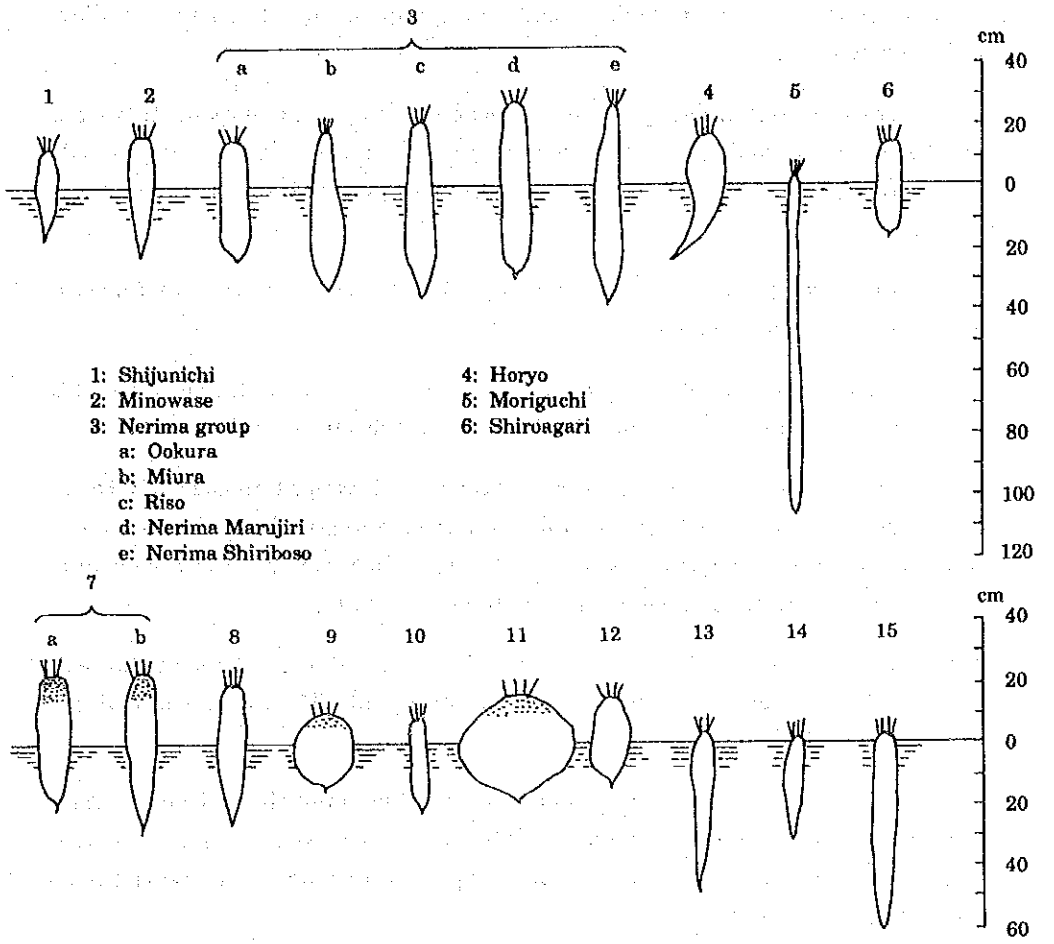


Fig. 2 Root shape, size, exposure from soil surface of typical varieties in Japanese radish

(6) Shiroagari group: This group has a short cylindrical root of about 25cm in length with soft flesh and low development of pithy root. A large portion of the root grows up from the ground level, so that this group can be grown in a shallow cultivated soil.

(7) Miyashige group: This group is the most widely grown in Japan. Its root is cylindrical and green topped with sweet and juicy flesh suitable for fresh and salt pickles. A large portion of the root stand out from the ground level. The plant form is erect.

(8) Awabansei group: Root is about 45cm in length, 6cm in diameter with crisp flesh suitable for salt pickles.

(9) Shogoin group: Root is round-shaped and green topped, 2-3kg in weight, with tender and fine flesh. About 1/3 of its root stands out from the ground level.

(10) Sakurajima group: This is a very late harvested group. It needs 90-150 days to harvest. The leaf is dark green, and has many small stipules. Root is round-shaped and extremely big with a weight of 15-20kg. The root flesh is extremely fine, firm and crisp with no pungency, and the top is fairly tall.

(11) Haruhuku group: This group is late bolting, suitable for spring harvest. Root grows mostly under ground, and is tolerant to cold. The top of leaf blades is larger than that of other groups.

(12) Ninengo group: This group is characterized by late bolting, cold and heat tolerance, and is suitable for sowing in fall for spring harvest. Most part of its root grows under ground. Root is about 40cm in length, white, tender, having crisp flesh with rather strong pungency.

(13) Local varieties in cold regions: These are superior in storage, firmness and earliness, and are short in length of less than 30cm.

(14) Local varieties in warm regions: These are superior in late bolting, having a large root and fine flesh.

(15) Shigatsu daikon group: This group is late variety with dark green leaf-color, and is suitable for sowing in fall for spring harvest in warm regions. Most part of its root grows underground.

2) Radish (*R.sativus*, radicula group)

Most seeds are imported, and its cultivation area is limited. Varieties with glove shape, bright scarlet red root, and white and crisp flesh are much popular in Japan.

3) Chinese radish (*R. sativus*, lobo group)

Japanese radish is mostly originated from southern Chinese radish.

Northern Chinese radish is introduced again, but the cultivation area is limited.

5. Classification of other crucifer vegetables

1) Hakuran (*B.* × *napus* hort.)

This is a new crop, which was artificially developed in Japan by an embryo culture technique for interspecific hybridization between Chinese cabbage (A genome, n=10) and cabbage (C genome, n=9). Hakuran (AC genome, n=19) is a heading vegetable, and is characterized by few fiber, soft, crisp, juicy and sweet flesh and good for salad, cook by boiling and salt pickles. The plant form looks like cabbage in younger stage, and its head looks like Chinese cabbage. The head weight is about 1.5-2.0kg.

2) Wasabi (*Eutrema wasabi* (SIEB) MATSUM. n=14)

This crop originated in Japan is a evergreen perennial, and characterized by hot taste in all parts of the plant, especially in rhizomes. To harvest large-sized rhizomes, it needs 2 or 3 years. This crop is extremely sensitive to high temperature, and cultivated usually in a farm with shallow stream of spring water. The optimum water temperature for growth of wasabi is 12~13°C. The temperature of spring water varies 8~18°C throughout year. Wasabi is consumed specially in Japanese traditional food such as sushi and sashimi. Although quality of wasabi is much superior to that of horseradish (*Armoracia rusticana* ph. GAERTN.), consumption of horseradish becomes popular instead of wasabi due to its low price.

6. References

KUMAZAWA, S. 1965. Vegetable horticulture. Yokendo. Tokyo (in Japanese).

LI, C. 1981. The origin, evolution, taxonomy and hybridization of Chinese cabbage. in Chinese cabbage. AVRDC. Tainan.

NISHI, S. 1980. Differentiation of Brassica crops in Asia and the breeding of "Hakuran", a newly synthesized leafy vegetable. In Brassica crops and wild allies. Japan Scientific Societies Press, Tokyo.

SHIMIZU, S. 1980. Encyclopedia of vegetable horticulture. Yokendo. Tokyo (in Japanese).

The Japanese Society for Horticultural Science 1979. Dictionary of horticultural crops names. Yokendo. Tokyo (in Japanese).