II.1 Distribution of Plants Genetic Resources and their conservation

H. I. Oka
Department of Agronomy, National Chung-Hsing University,
Taichung, Taiwan, R.O.C.

1.Conservation of Gene Pools to Meet Genetic Erosion

The importance of conservation of genetic resources has become universally recognized, but it will not be futile to consider why genetic variations are resources. The following points may be considered.

(1) Crop breeding needs an assembly of many useful genes. Even though breeding techniques have made progress, they are not effective without enough genetic materials.

(2) Before the practice of pure-line selection in the early decades of the present century, the land races or native cultivars were diverse and polymorphic. As there were various resistance genes, devastating epidemics of diseases and pests were rare. The variability was maintained in association with varying cropping systems and ethnological traditions of different human tribes.

(3) The recent industrialization of agriculture has rapidly reduced genetic diversity in crop plants, resulting in "genetic erosion". In developed countries, a crop species has become represented by only a few varieties which are exceedlingly pure genetically. For instance, more than half of rice fields in Taiwan was planted with Tainan 5 some years ago, and now with Tainung 67. The native varieties were abandoned and disappeared. In parallel to this, crop species

were also reduced to a small number which were most profitable commercially.

After 1960's, with the establishment of international agricultural improvement centers, the replacement of land races with improved high-yielding varieties has been extended in a large area of the world. Even in Afganistan or Ethiopia where richest accumulation of diversity of crop plants has been known, genetic erosion now threatens the geen pools. The genetic basis of crop plants on which the mankind relies was rapidly narrowed.

(4) Needs for genes controlling resistances to diseases, pests, and other unfavorable environmental conditions are increasing. But the breeders can not easily obtain the genes which they need. Moreover, we do not know what genes and genotypes we will need in the future. For instance, before the release of Taichung native 1 in 1956 (authorized in 1960; Oka 1974) followed by IR varieties, nobody knew the priceless value of the semidwarfness gene found in a land race of rice, I-geo-wu-gen, in Taiwan.

(5) It has become understood that monoculture of a cultivar over a large area is risky in danger of epidemics of diseases and pests, while chemical control destroys natural enemies of the pests, and results in disintegration of agro-ecosystems and also in environmental pollution, as has been discussed elsewhere (Plant Breeding Symposium, September 1981, at the Taiwan Agricultural Research Institute). Breeding for resistances is most important, but resistant varieties are generally vulnerable as the result of evolution of new pathogenic races or biotypes. The breeding of multiline varieties to increase diversity and stability is envisaged. Many more resistance genes are wanted. In rice, those

resistance genes have so far been found in the populations of land races and wild relatives (Chang *et al.* 1975).

It is now urgent to intensify the activities of exploration, collection, preservation, and documentation of germplasms of crop plants and their wild relatives on a systematic basis. This work requires much skilled labor, budget, and international cooperation.

2. Geographical Distribution of Gene Pools and Crop Evolution

As is well known, Vavilov (1951, the first publication in 1926) has demonstrated the presence of eight centers of diversity of crop germplasms in the world (Fig. 1A; Table 1). He considered that the diversity centers were the centers of origin of crop plants. But this view was criticized with the advances of studies on crop evolution (cf. Zohary 1970). In the diversity centers, there would be various conditions accelerating evolution, *viz.*, population subdivision, isolation, dissemination, introgressive hybridization, and disruptive selection, all influenced by man's activity which varied according to ethnological traditions.

In certain crops, *e.g.*, sorghum, as well as rice, diversity was found to be spread over a wide area which could not be called a center. In view of this trend, Harlan (1971) proposed the theory of centers and noncenters; there are three major centers and three noncenters in the world (Fig. 1B). On this and other evidences from studies of the hunting-gathering-fishing way of man's life, he also put forward the concept of diffuse origin of agriculture (Harlan 1975). Most probably, as Harlan (1966) has discussed, crop evolution was forwarded by repeated differentiation-hybridization cycles, particularly by introgression between wild plants and

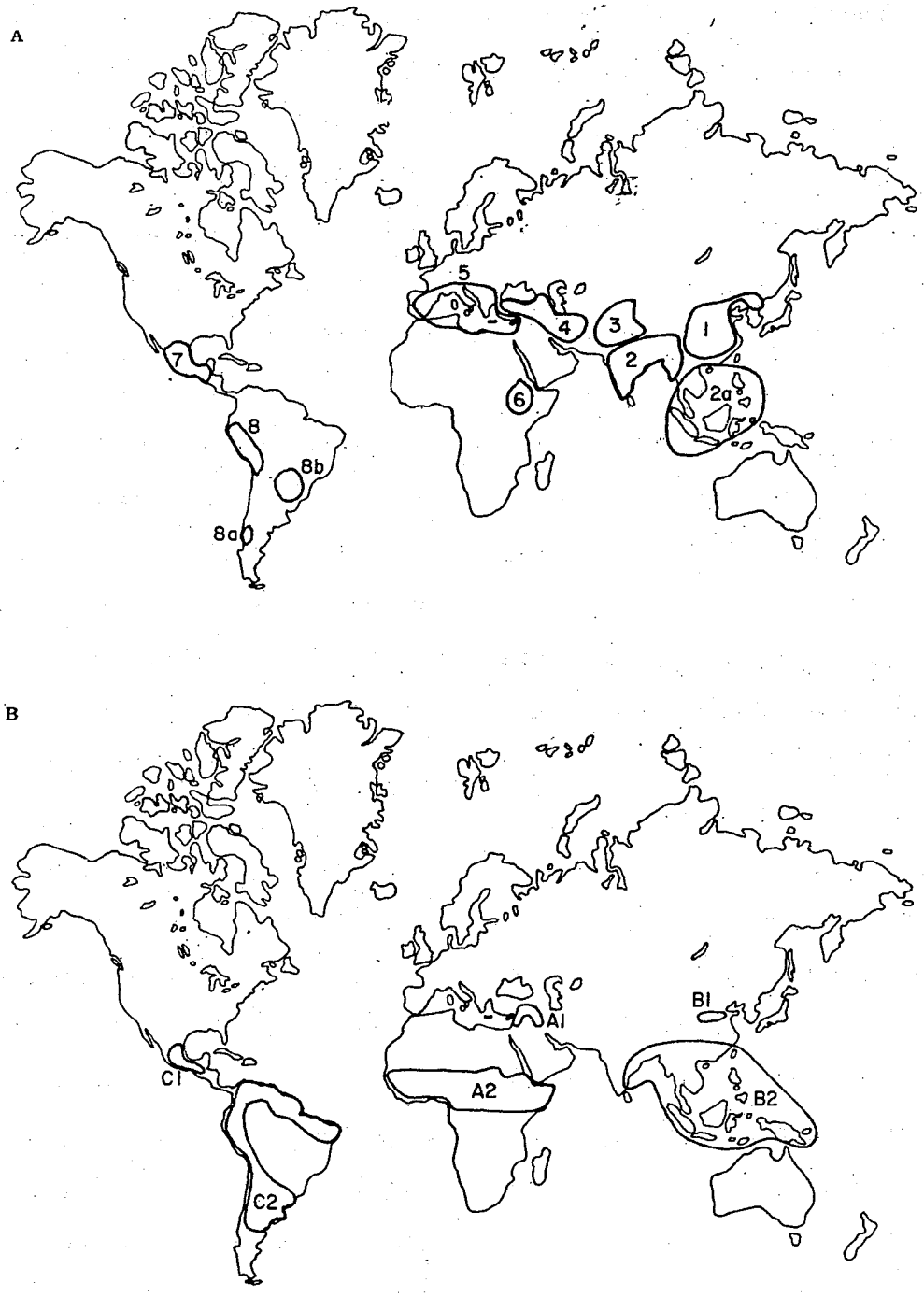


Fig. 1. A: The eight centers of diversity given by Vavilov (1951).

B: Three centers and three noncenters given by Harlan (1971).

(after Harlan 1975, p. 54-55)

Table 1. World centers of diversity of cultivated plants (after Vavilov 1951, abbreviated)

1. The Chinese center: Soybean, Adzuki bean, apricot, peach, orange, tea. 2ndary center: Naked oat, bean leaf mustard (*Brassica juncea*), sesame (dwarf varieties).
2. The Indian center: Rice, millet (*Eleusine coracana*), chick pea, Math bean (*Phaseolus aconitifolius*), rice bean (*Ph. calcaratus*), horse gram (*Dolichos biflorus*), asparagus bean (*Vigna sinensis*), egg plant, radish (*Raphanus caudatus*), taro, cucumber, tree cotton (*Gossypium arboreum*), jute, pepper, indigo (*Indigofera tinctoria*).

2a. The Indo-Malayan center: Yam, pomelo (*Citrus maxima*), banana, coconut.

3. The Central Asiatic center: Bread wheat, club wheat (*Triticum compactum*), shot wheat (*T. sphaerococcum*), pea, lentil, chick pea (*Cicer arietinum*), sesame, flax, safflower (*Carthamus tinctorius*), carrot, radish (*Raphanus sativus*), pear, apple, walnut (*Juglans regia*). 2nd. center: rye.
4. The Near Eastern center: Einkorn wheat, durum wheat (*Triticum durum*), bread wheat (awnless group), two-rowed barleys, rye, red oat (*Avena byzantina*), lentil, alfalfa, sesame, flax, melon, almond, fig (*Ficus*) grape, apricot, pistachio (*Pistacia vera*). 2nd. center: Pea, chick pea.
5. The Mediterranean center: Dullum wheat, hulled oats (*Avena strigosa*), broad bean (*Vicia faba*), cabbage, olive, lettuce.
6. The Abyssinian center: Durum wheat, poulard wheat (*Triticum turgidum*), Emmer wheat, barley, chick pea, lentil, teff (*Eragrostis abyssinica*), millet (*Eleusine coracana*), pea, flax, sesame,

castor bean (*Ricinus communis*), coffee.

7. The south Mexican and central American center: Corn, common bean, red pepper (*Capsicum annum*), upland cotton, sisal hemp, squash-pumpkin-gourd (*Cucurbita spp.*).

8. South American center: Sweet potato, potato, Lima bean (*Phaseolus lunatus*), tomato, sea-island cotton (*Gossypium barbadense*), papaya, tobacco.

8a. The Chile center: Potato (*Solanum tuberosum*).

8b. Brazilian-Paraguayan center: Manioc (*Manihot utilissima*), peanut, rubber tree, pineapple, purple granadilla (*Passiflora edulis*). 2nd. center: Cacao.

domesticates. Some evidence for this was obtained in the studies of rice by the present author and coworkers (Oka and Chang 1961, 1962 ; Chu and Oka 1970)

The pattern of distribution of genetic diversity in a small area is not well known yet. It was suggested that diversity centers are of two kinds, endemic centers which were kept stable for a long time to accumulate variants, and micro-centers resulting from introgression between species in disturbed habitats which are evolutionary active (Harlan 1963). Travelling in Africa and tropical Asia, the present author has recognized that diversity was associated with primitiveness in agriculture. In Nigeria, genetic diversity in rice populations was correlated with species diversity of coexisting wedds (Morishima and Oka 1979).

Many crop species have their respective companion weeds in certain regions. These are weed barley, weed rice, weed sorghum, weed maize (teosinte), weed oats, etc. in cereals, and such are also found in many other crops. Although Vavilov and his followers considered the weedy forms to be the wild progenitor of crops, Harlan (1965) suggested that many of them arose from their wild progenitors with crops side by side. The weedy forms can also occur from hybridization between wild and cultivated plants, as was found in rice (Oka and Chang 1959). The companion weeds sometimes produce hybrid swarms with the crop, and serve as a reservoir of crop germplasms expanding the gene pool substantially.

Studies of crop evolution indicate that plant domestication was a great process of evolution forwarded by natural forces. If compared with this process, our present breeding activities are too small. If the natural evolutionary forces have ceased to

work on account of genetic erosion, how can we survive a long time in the future so far as we feed on crop plants? This is a fundamental question in agriculture in the present.

3. Sample Size and Related Problems in Germplasm Conservation

The object of conservation is apparently the genes. An assembly of many unspecified genes should be considered as we know only a small fraction of them analytically. The geographical distribution of genes is the result of adaptive evolution and non-random, but the distribution within a population may be assumed random.

Plant populations are genetically polymorphic and subjected to natural selection. Such a population can be maintained as it is only when seeds are sampled at random from a number of plants on a single plant basis. How many plants will be necessary? If the number is too small, genes will be lost by random drift. This problem is involved in the sampling from populations in the field and also in the multiplication of stored seeds for renewal.

The present author (Oka 969, 1975) has proposed a probability formula to estimate the size of samples necessary for germplasm conservation, which will be briefly represented below.

As an example, let us consider a case in which 95 percent of genes distributed in a population with a frequency of 5 percent are to be maintained. The number of samples needed (n) will be:

$$1-(1-0.05)^n = 0.95.$$

Then, $n = 58.4$, or about 60.

When it is assumed that:

Total genetic variation in an area 1

Its fraction represented by a population ..... P

Number of populations sampled ..... N

Fraction of genetic variation in a population represented by a plant · · · · · · · · · · · · · · · p

Number of plants sampled per population · · · · · · · · · · · · · n

Fraction of genetic variation represented by $N \times n$ samples · · · · · · · · · · · · · · · · · · · F

Then, it is expected that:

$$F = 1 - [(1-P) + P(1-p)^n]^N.$$

This formula shows that the size of samples needed depends on the genetic structure of populations. The value of P may be estimated as: $P = \sigma^2_{wn} / (\sigma^2_{wn} + \sigma^2_{bt})$, where σ^2_{wn} is the variance within populations and σ^2_{bt} is the variance between populations. The value of p may be estimated as: $p = \sigma^2_o / (\sigma^2_o + \sigma^2_p)$, where σ^2_p is the variance of progeny line means raised from single plants of a population and σ^2_o is the variance within lines. The data on rice species (Morishima and Oka 1970) suggest that the values of both P and p are 0.3 to 0.1 in partly cross-breeding wild rice if relatively small values are taken for the sake of safety. With domestication, plants become self-pollinated. In highly heterogeneous but homozygous populations of land races, it may be assumed that $P = 0.1$ and $p = 0.05$; in relatively pure cultivars, $P < 0.01$ and $p > 0.95$. Thus, a tentative estimation of sample size was made as shown in Table 2.

The major tasks of a conservation program are 1) maintenace of seeds incold storage, 2) record-keeping and documentation, 3) classification and evaluation of the stocks, and 4) seed multiplication for renewal.

The quality of the seed depends on reliability, germinability, and amount of attached information. Reliability is lowered by errors in management (mis-labeling, etc.) and natural hybridization. Empirically, errors occur at a rate of 0.01 to 0.001 per generation, and natural hybridization may also

Table 2. Fraction of total genetic variation represented by N populations each consisting of n plants (in %)

a. Wild species ($P = 0.2$; $p = 0.2$)

N	n: 1	5	10	20	40
1	4	14	18	20	20
5	18	51	63	67	67
10	33	76	86	89	89
20	56	94	98	99	99

b. Land races ($P = 0.1$; $p = 0.05$)

N	n: 1	5	10	20	40
10	5	20	34	49	60
20	9	37	56	73	84
40	18	60	81	93	97

c. Practically pure cultivars ($P = 0.01$; $p = 0.95$)

N	n: 1	2	5
100	62	63	63
200	85	87	87
400	98	98	98

(after Oka 1975)

be of the same order in autogamous crops. Seed multiplication will lower reliability, but ensures germinability by renewal and gives a chance of evaluation of characters in the field. How often multiplication should be made is a problem of operation.

The seeds of most crop plants can be kept for a long time by desiccation and cold storage, although some are "recalcitrant" and do not withstand desiccation. The half-life (p, in week) of dry seed in storage is expected by: $\log_{10} p = K_v - C_1 m - C_2 t$, where m is seed moisture content (%) and t is temperature (C). K_v, C_1 and C_2 are constants characteristic of species, genotype, and initial physiological condition of the seed (Roberts 1960). In rice, it was found that $K_v = 5.3 \sim 5.6$, $C_1 = 0.159$, and $C_2 = 0.069$. Similar values were found in other cereals. It is not difficult to maintain viability over 100 years if moisture is below 7% and temperature is below zero. It is experienced, however, a small fraction of seeds in storage loses viability much earlier than expected. possibly because of poor initial conditions like immaturity.

4. Present Activities in Genetic Resources Conservation

The first scientist who expressed alarm on genetic erosion was Dr. H. V. Harlan (Father of Prof. J. R. Harlan), who wrote in 1936 "Types without number (of barley) have arisen over an enormous area. Many of the surviving types are old. Spikes from Egyptian ruins can often be matched with ones still growing in the basin along the Nile. The progenies of these fields constitute the world's priceless reservoir of germ plasm. Unfortunately, from the breeder's standpoint, it is now being imperiled. When new barleys replace those grown by the farmers of Ethiopia or Tibet, the

world will have lost something irreplaceable". (Harlan, H. V. and M. L. Martini, USDA Year Book 1936).

International efforts to collect and preserve genetic resources began in 1961 when a Technical Meeting on Plant Exploration and Introduction was called by the FAO at Rome. There was much talk but little action. A panel of experts was appointd in 1965 as recommended by the Meeting. In 1967, the FAO and the International Biological Programme (IBP) sponsored a Technical Conference on Exploration, Utilization and Conservation of Plant Genetic Resources. The Conference yie lded a book entitled "Genetic Resources in Plants"(ed. Frankel and Bennett 1970), and attracted considerable attention of workers in plant genetics and breeding.

In 1972, the "Beltsville Meeting" was held and proposed to establish the International Board for Plant Genetic Resources (IBPGR) under the sponsorship of the Consultative Group on International Agricultural Research (CGIAR). This was established in 1973 at Rome, as an organization attached to the Plant Production and Protection Division of the FAO. In 1973, a second technical conference was held at Rome, resulting in a second book entitled "Crop Genetic Resources for Today and Tomorrow" (ed. Frankel and Hawkes 1975).

The major activities of the IBPGR are to build up liaison between national/regional centers conducting germplasm conservation, to promote exploration activities by those centers, and to hold meetings, symposia and training courses for personnels engaged in germplasm conservation, with the general aim of establishing a global network. International agricultural research centers take part in collection and preservation of

germplasms of certain crop plants. They are: International Rice Research Institute (IRRI), International Maize and Wheat Improvement Center (CIMMYT), Centro Internacional de Agricultura Tropical (CIAT, cassava, beans, tropical forages and livestock), Centro Internacional de Papas (CIP, potato), International Institute of Tropical Agriculture (IITA, tropical tubers, vegetables, cowpea, etc.), International Crops Research Institute for Semi-Arid Tropics (ICRISAT, sorghum, millet, chick-pea, peanut, and pigion pea), and Asian Vegetables Research and Development Center (AVRDC, soybean, tomato, Chinese cabbage, etc.).

Of them, the IRRI seems to have done the best job with rice. Other major centers for plant genetic resources are agricultural research institutes at Addis Ababa, Ethiopia; Trialba, Costa Rica; Bogor, Indonesia; Vavilov Institute of Plant Industry, Leningrad; Laboratorio de Germoplasmo, Bari, Italy; International Plant Introduction Center, Ismir, Turkey, National Bureau of Plant Genetic Resources, New Delhi, India; National Institute of Agricultural Sciences, Tsukuba; Japan; National Seed Storage Laboratory, Fort Collins, Colorado, U.S.A., Potato Research Station, Sturgeon Bay, Wisconsin, U.S.A., etc. A course on plant genetic resources is open in Department of Plant Biology, University of Birmingham (Prof. J. G. Hawkes).

It is agreed that collection of germplasms should be made with priority to the area where genetic diversity is rich but is subjected to erosion. The activities to explore crop germplasms are devoloping. For instance, in 1977, a Meeting on African Rice Species was called by the IRAT/ORSTOM in Paris, and the relevant organizations have started to send experts to

different African countries for exploration of rice germplasms. In compliance with this, the present author and his party also visited Nigeria and some other African countries (Oka *et al.* 1978). However, cooperation among different organizations is not an easy task. For instance, in Japan, there are a number of laboratories engaged in germplasm conservation of different plant species, belonging to different governmental sections or private enterprises and having different standpoints. To establish an information system on the national basis is being discussed.

Summary

The evolution of crop plants was forwarded by natural forces for a long period of time and brought about coadapted systems between plants and man on the basis of a great amount of accumulated genetic variations. Recently, however, genetic erosion has resulted from industrialization of agriculture, and the adaptive evolution of crops has become largely dependent on man's breeding efforts. Monoculture increased the danger of epidemics of diseasea and pests, while chemical control produces various undesirable side-effects on agro-ecosystems, environment, and man. For breeding crop plants, a large number of genes, particularly for resistances, are wanted and much more will be needed in the future. In this paper, in addition to these basic concepts, the size of samples to be maintained in a germplasm conservation program and some other technical aspects were discussed, and the recent activities of genetic resources conservation in the world were reviewed.

References

1. Chang, T.T., S.H. Ou, M.D. Pathak, K.C. Ling, and H.E. Kauffman (1975) The search

for disease and insect resistance in rice germplasm. *In* Frankel, O.H. and J.G. Hawkes (ed.) Crop genetic resources for today and tomorrow, p. 183-200. Cambrdige University Press.

2. Chu, Y.E. and H.I. Oka (1970) Introgression across isolating barriers in wild and cultivated *Oryza* species. Evolution 24: 344-355.
3. Frankel, O.H. and E. Bennett (1970) Genetic Resources in Plants-Their Exploration and Conservation. Blackwell, Oxford.
4. Frankel, O.H. and J.G. Hawkes (1975) Crop Genetic Resourees for Today and Tomorrow. Cambridge Univ. Press, Cambridge.
5. Harlan, J.R. (1963) Two kinds of gene centers in Bothriochloininae. Amer. Nat. 97:91-98.
6. Harlan, J.R. (1965) The possible role of weed races in the evolution of cultivated plants. Euphytica 14:173-176.
7. Harlan, J.R. (1966) Plant introduction and biosystematics. *In* Frey, K.J. (ed.) Plant Breeding, p.55-83. Iowa State Univ. Press, Ames.
8. Harlan, J.R. (1971) Agricultural origins: Centers and noncenters. Science 174:468-474.
9. Harlan, J.R. (1975) Crops and Man. Amer. Soc. Agron., Madison.
10. Morishima, H. and H.I. Oka (1970) A survey of genetic variations in the populations of wild *Oryza* species and their cultivated relatives. Japan. J. Genetics 45: 371-385.
11. Morishima, H. and H.I. Oka (1979) Genetic diversity in rice populations of Nigeria: Influence of community structure. Agro-Ecosystems 5: 263-269.
12. Oka, H.I. (1969) A note on the design of germplasm preservation work in grain crops. SABRAO Newsl. 1(2): 127-134.
13. Oka, H.I. (1974) A short

history of the first hybrid semidwarf rice variety, Taichung native 1. SABRAO J. 6: 241-243.

14. Oka, H.I. (1975) Consideration on the population size necessary for conservation of crop germplasms. *In* Matsuo, T. (ed.) JIBP Synthesis 5, p.57-63. Univ. Tokyo Press.
15. Oka, H.I. and W.T. Chang (1959) The impact of cultivation on populations of wild rice, *Oryza sativa* f. *spontanea*. Phyton 13: 105-117.
16. Oka, H.I. and W.T. Chang (1961) Hybrid swarms between wild and cultivated rice species, *Oryza perennis* and *O. sativa*. Evolution 15: 418-430.
17. Oka, H.I. and W.T. Chang (1962) Rice varieties intermediate between wild and cultivated forms and the origin of the Japonica type. Bot. Bull. Acad. Sinica 3: 109-131.
18. Oka, H.I., H. Morishima, Y. Sano and T. Koizumi (1978) Observations of rice species and accompanying savanna plants on the southern fringe of Sahara desert. Report of study-tour in West Africa, 1977. Rep. Nat. Inst. Genetics, Misima, Japan.
19. Roberts, E.H. (1960) The viability of cereal seed in relation to temperature and moisture. Ann. Bot. 24: 12-31.
20. Vavilov, N.I. (1951) Phytogeographic basis of plant breeding. The origin, variation, immunity and breeding of cultivated plants. Chronica Botanica 13: 1-366. Waltham.
21. 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, p.33-42. Blackwell, Oxford.

討　　論

謝順景：請問 genetic variation resources 是指許多純系所構成的 genetic variation ？或指一個族群（population），內之 heterogeneity 而言？

岡彥一：＂遺傳變異性＂本身是＂資源＂，大部份野生集團是異質性（heterogeneous）。

謝順景：種子貯藏之目的是要保持遺傳變異性（genetic variability），在人爲的遺傳損毀（genetic erosion）很大的情形下，有無很好的方法保存野生種（wild species）的遺傳變異性（genetic variability）？

岡彥一：爲了控制 genetic erosion，作物和有關野生種遺傳變異，需要研究保存技術上的各種問題。

何以涼：野生大豆保存每一個系統（accession），應該要多大的族群大小（population size）？每品種 50 株是否可以？

岡彥一：假使野生大豆是自交性，一個或少數個體即可＂代表＂一個系統。若在同一集團由不同系統所組成，則由其他條件來決定所需要的種子量。可能因品種不同所需株數亦不一同。