Plant Genetic Resources Newsletter

Bulletin de Ressources Phytogénétiques

Noticiario de Recursos Fitogenéticos

No. 103, 1995

Food and Agriculture Organization of the United Nations and the International Plant Genetic Resources Institute
Organisation des Nations Unies pour l'alimentation et l'agriculture et l'Institut international des ressources phytogénétiques
Organización de las Naciones Unidas para la Agricultura y la Alimentación y el Instituto Internacional de Recursos Fitogenéticos

Plant Genetic Resources Newsletter

Aims and scope

The *Plant Genetic Resources Newsletter* publishes papers in English, French or Spanish, dealing with the genetic resources of useful plants, resulting from new work, historical study, review and criticism in genetic diversity, ethnobotanical and ecogeographical surveying, herbarium studies, collecting, characterization and evaluation, documentation, conservation, and genebank practice.

Management

The *Plant Genetic Resources Newsletter* is published under the joint auspices of the International Plant Genetic Resources Institute (IPGRI) and the Plant Production and Protection Division of the Food and Agriculture Organization of the United Nations (FAO).

Availability

The *Plant Genetic Resources Newsletter* appears as one volume per year, made up of four issues, published in March, June, September and December. *Plant Genetic Resources Newsletter* is available free of charge to interested libraries of genebanks, university and government departments, research institutions, etc. The periodical may also be made available to individuals who can show that they have a need for a personal copy of the publication.

Types of paper

Articles

An article will publish the results of new and original work that makes a significant contribution to the knowledge of the subject area that the article deals with. Articles, which should be of a reasonable length, will be considered by the Editorial Committee for scope and suitability, then assessed by an expert referee for scientific content and validity.

Short communications

A short communication will report results, in an abbreviated form, of work of interest to the plant genetic resources community. Short communications in particular will contain accounts of germplasm acquisition missions.

Other papers

The *Plant Genetic Resources Newsletter* will publish other forms of reports such as discussion papers, critical reviews, and papers discussing current issues within plant genetic resources.

Book reviews will be printed, as well as a News and Notes section. Suggestions for books to review are invited, as are contributions to News and Notes.

Submission

In the first instance papers may be submitted in typescript form or as an Email message. The final version may be submitted as an Email file or as an MS-DOS-readable file on diskette.

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Cover: Dr. W. Plarre took this photograph of an Irian Jayan woman removing the kernels from *Pandanus* fruits with a stone axe that has all the characteristics of a Neolithic culture. See pp. 1-13 for the full review

Bulletin des ressources phytogénétiques

Domaine d'intérêt

Le *Bulletin des ressources phytogénétiques* publie des articles en anglais, en espagnol et en français, sur les ressources génétiques de plantes utiles, fruit de nouvelles recherches, d'études historiques, d'examens et de critiques concernant la diversité génétique, d'études ethnobotaniques et écogéographiques, d'études d'herbiers, d'activités de collecte, de caractérisation et d'évaluation, de documentation, de conservation et les pratiques des banques de gènes.

Parrainage

Le *Bulletin des ressources phytogénétiques* est publié sous les auspices de l'Institut international des ressources phytogénétiques (IPGRI) et de la Division de la production végétale et de la protection des plantes de l'Organisation mondiale de l'alimentation et de l'agriculture (FAO)

Distribution

Le *Bulletin des ressources phytogénétiques* paraît une fois par an en un volume regroupant quatre numéros publiés en mars, juin, septembre et décembre. Il est distribué gratuitement aux bibliothèques des banques de gènes, universités, services gouvernementaux, instituts de recherche, etc. s'intéressant aux ressources phytogénétiques. Il est aussi envoyé sur demande à tous ceux pouvant démontrer qu'ils ont besoin d'un exemplaire personnel de cette publication.

Types de documents publiés

Articles

Un article contient les résultats de travaux nouveaux et originaux qui apportent une contribution importante à la connaissance du sujet dont traite l'article. Les articles, qui doivent être d'une longueur raisonnable, sont d'abord examinés par le Comité de rédaction qui en évalue la portée et la validité, puis par un expert qui en examine le contenu et l'intérêt scientifiques.

Brèves communications

On entend par brève communication un texte contenant, sous une forme abrégée, les résultats de travaux présentant un intêrêt pour tous ceux qui s'occupent de ressources phytogénétiques. Elle contient en particulier des comptes rendus des missions d'acquisition de matériel génétique.

Autres documents

Le *Bulletin des ressources phytogénétiques* publie d'autres types de rapport tels que des documents de synthèse, des études critiques et des articles commentant des problèmes actuels concernant les ressources phytogénétiques.

Le Bulletin publie une revue de livres ainsi qu'une section intitulée Nouvelles et Notes. Les auteurs sont invités à envoyer leurs suggestions pour les livres à passer en revue ainsi que des contributions aux Nouvelles et Notes.

Présentation

En premier lieu, les documents doivent être soumis dactylographiés ou par courrier électronique. La version définitive doit être présentée en fichier de courrier électronique ou sur disquettes compatibles MS-DOS.

Rédaction

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Couverture: Cette photographie, prise par le Dr. W. Plarre, représente une femme de l'Irian Jaya extrayant les amandes de fruits de *Pandanus* avec une hache de pierre qui a toutes les caractéristiques d'une culture néolitique. Voir pages 1-13.

Noticiario de Recursos Fitogenéticos

Objetivos y temas

El *Noticiario de Recursos Fitogenéticos* publica documentos en inglés, francés y español que tratan de los recursos genéticos de plantas útiles para la humanidad, y que son el fruto de nuevos trabajos, estudios históricos, revisiones y análisis críticos relacionados con la diversidad genética, investigaciones etnobotánicas y ecogeográficas, estudios de herbarios, trabajos de colección, caracterización y evaluación, documentación, conservación, y prácticas en bancos de germoplasma.

Dirección

El *Noticiario de Recursos Fitogenéticos* se publica bajo los auspicios conjuntos del Instituto Internacional de Recursos Fitogenéticos y la Dirección de Producción y Protección Vegetal de la Organización de las Naciones Unidas para la Agricultura y la Alimentación.

Distribución

El *Noticiario de Recursos Fitogenéticos* aparece como un tomo anual compuesto por cuatro números, que se publican en marzo, junio, septiembre y diciembre. Se distribuye gratuitamente a las bibliotecas de bancos de germoplasma interesadas, facultades universitarias y ministerios estatales, centros de investigación, etc. También pueden obtener la revista las personas que necesiten una copia de la publicación.

Tipos de documentos

Artículos

Los artículos, que deberán tener una extensión razonable, divulgarán los resultados de un trabajo nuevo y original que contribuya de modo importante al conocimiento del tema tratado. El Comité de redacción examinará la pertinencia e idoneidad de los artículos y posteriormente una comisión de expertos juzgará su contenido y validez científicos.

Comunicaciones breves

Las comunicaciones breves informarán de modo conciso sobre los resultados de un trabajo de interés para las personas que se ocupan de los recursos fitogenéticos. Las comunicaciones breves incluirán, en particular, resúmenes sobre las misiones de adquisición de germoplasma.

Otros documentos

El *Noticiario de Recursos Fitogenéticos* publicará otros tipos de informes, como documentos de trabajo, análisis críticos, y documentos que examinen cuestiones de actualidad relacionadas con los recursos fitogenéticos.

El Noticiario publicará una reseña de libros así como una sección de Noticias y Notas. Las propuestas de libros para reseñar y las contribuciones a la sección de Noticias y Notas serán bien acogidas.

Presentación

Los documentos deben entregarse, incialmente, en forma de texto mecanografiado o de correo electrónico. La versión final debe presentarse como un archivo de correo electrónico o en disquete compatible con el sistema operativo MS-DOS.

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Portada: Fotografía tomada por el Dr. W. Plarre que representa a una mujer de Irian Jaya mientras quita los granos a las frutas de *Pandanus* con un hacha de piedra, lo que tiene todas las características de una cultura neolítica. El artículo se encuentra en las páginas 1-13.

Plant Genetic Resources Newsletter, 1995, No. 103: 1-13

REVIEW
Evolution and variability of special cultivated crops in the highlands of West New Guinea (Irian Jaya) under present Neolithic conditions[1]

W. Plarre
Institute of Applied Genetics, Free University of Berlin, Berlin, Germany

Summary
Samples of all cultivated crops and some wild types utilized for specific purposes (e.g. edible fruits, fibre plants) were collected, described and investigated to assess their nutritional value in the central highlands of West New Guinea (Irian Jaya) in the area of the Mek population (at 4° S, 140° E). Large variability was observed within all the different cultivated crops for morphological features such as shape and colour of leaves, stems and tubers of root crops, as well as for physiological characters such as protein content, cooking quality and taste. From two locations (Kosarek at 1500 m a.s.l., Eipomek at 1800 m a.s.l.) data were collected on all important food crops: sweet potatoes (*Ipomoea batatas*) >90 clones, taro (*Colocasia esculenta*) >60 clones, bananas (*Musa* spp.) >20 clones, sugarcane (*Saccharum* spp.) >30 clones and different kinds of vegetable crops such as *Setaria palmifolia, Amaranthus* spp., *Rungia* spp. and others. The highlands of New Guinea can be assumed to be a gene centre, especially for vegetative crops.

Introduction
In 1974 a joint Indonesian-German research project, financed by the Deutsche Forschungsgemeinschaft (DFG), was started under the title: 'Man, culture and environment in the central highlands of Irian Jaya.' An interdisciplinary programme has been carried out for several years in cooperation with the Cenderavasih University (UNCEN), Jayapur/Manokwari. For that research, scientists of ethnography, under the leadership of Gerd Koch, Berlin (1983), selected an area at 4°S and 140°E at an elevation between 1500 and 2000 m a.s.l. This was terra incognita for ethnobotanists at that time.

All that was known about the people living there was that they belonged to the Mek group, and lived more or less isolated in small settlements in these highlands. These pygmaean folk have a Neolithic culture, with little evidence of outside influences, which was well adapted to their original environment (Schiefenhövel 1978; Michel 1983). However, in the neighbourhood Christian missionary stations had been established with the intention of spreading out more and more into the Central Highlands. In 1978, when I got permission to start my work, the missionaries had already arrived.

The project gave high priority to two main aspects:

- collecting data from all disciplines dealing with the people, their culture and environment, as much as possible without influencing their acculturation,
- finding out the basis of the material culture and, if there were any gaps concerning malnutrition and/or unsatisfactory health conditions, making recommendations to the Indonesian research institutions for any needed improvement.

Methods and materials
Investigations concerning all wild plants and original ecosystems were carried out by Hiepko and Schultze-Motel (1981). I was asked to deal with the utilized and intensively cultivated crops, including cultivation systems.

For good reasons only a few scientists were living in the highlands with the native people. I started my field work in December 1978 and finished in the middle of March 1979. I was accompanied only by my wife (Plarre 1985). During that time I collected, recorded and described

[1] Paper presented by the author in St. Petersburg, on the occasion of the Centennial Anniversary of the Vavilov Institute, on behalf of the oldest German Institute of Genetics founded in 1911 at Berlin. In Berlin Vavilov presented for the first time his "Theory of the centres of origin and diversity of cultivated plants" and in 1928 Erwin Baur visited St. Petersburg to participate in a congress and to present a paper. The breach caused by World War II resulted in no contact at all between the Vavilov Institute and the German Institute of Genetics for nearly 60 years. The author was the first to end that period of no contact, and was honoured to present a report of his research work on the origin and variability of cultivated crops in the highlands of New Guinea. In his work, he attempted to follow the footsteps of Vavilov, and this paper represents his small contribution to Vavilov's gene centre theory.

all kinds of cultivated crops at two different locations: Kosarek 139°32'E, 4°06'S and Eipomek, 140°01'E, 4°27'S (straight line distance about 70 km, see Fig. 1, Table 1).

Close contact with the inhabitants in both of the settlements, where we always met friendly people, made it easy to get all the information we wanted. They understood very quickly that I was interested in their work of shifting cultivation, preparing the garden land, planting, weeding, harvesting and food preparation. In all these occupations I could observe them immediately, and therefore was able to find out the primary behaviour of the people in their environment. I could observe and study what was going on, if the people utilized wild plants and handled them carefully in their original environment. Also, distinct cultivation methods were very interesting

Table 1. Climatic data of some locations in Irian Jaya

	Manokwari†	Sentani† (N coast)	Kosarek‡	Eipomek§
Altitude (m a.s.l.)	3	98	1500	1800-2000
Annual precipitation (mm)	2600	1830	>5000	5900
Average temperature (°C)				
Minimum	18.5	18.5	15.0	12.0
Maximum	30.7	31.5	31.0	24.0

† = 5-year average.
‡ = Calculation from 2 months.
§ = 1-year measurement by G. Hoffman: Forschungsprojekt Klimatologie, Führungsblatt Nr. 5, Ausstellung: Steinzeit heute. Staatl. Mus. Preuß. Kulturbesitz, Berlin 1979.

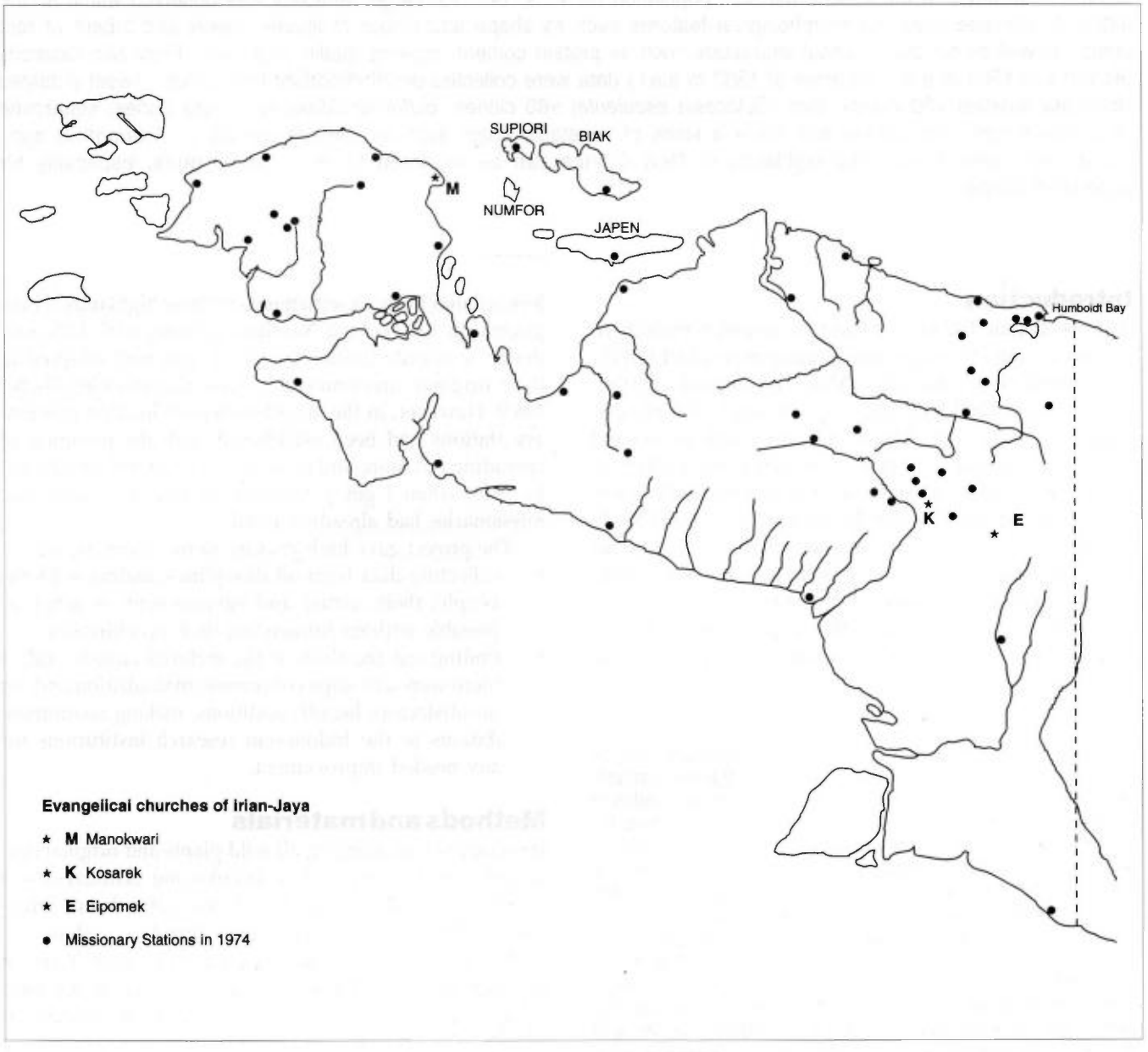

Fig. 1. Map of Irian Jaya marked with the localities of Kosarek and Eipomek within the settlement area of the Mek population

to me in connection with selective processes. When I summarized all these items, it was possible to get facts about the first steps of evolutionary processes, i.e. how domestication occurs under Neolithic cultural conditions when the people are only looking carefully, thinking logically and have only simple tools to improve plant production. Some examples shall be mentioned below.

For nearly all the cultivated food crops, photographs were taken, leaves were collected as herbarium material (sweet potatoes) and roots/tubers prepared for protein and mineral analyses. Many of the different phenotypes—clones of sweet potatoes, taro and sugarcane—were replanted in a breeding garden at Kosarek. However, as I learned from Heeschen, the linguistic scientist of the German team (1980, pers. comm.) those collections were not handled carefully later on. Nevertheless, I assume that most of the clones in both areas of about 25 km^2 each around the settlements of Kosarek and Eipomek can be found again (see Table 2). All of them are named, and some native people are able to identify and to distinguish the different phenotypes or genotypes. My best informants were children of about 12 years of age. As a breeder myself, I have experience identifying clones by specific morphological features, but I was really surprised by a young girl who showed me that two sweet potato clones which seemed to me completely identical were not: one had fine-haired stems and the other had naked stems.

Table 2. Sweet potato collections made in Kosarek and Eipomek, Irian Jaya

Location	Collecting date	Material	Number
Kosarek			
Collection 1	January 1979	named clones	39
Collection 2	March 1979	named clones, correctly recognized	30 (77%)
		not in accordance with Collection 1	4 (10%)
		not found again	5 (13%)
		named new clones	6
		Total	45
Eipomek	February 1979	named clones (a few are identical with those of Kosarek)	50

I think such information is very important for further expeditions into these regions. Acculturation is in progress, and there is the danger of changing food consumption patterns, e.g. by the introduction of rice. Then the original crops will disappear. Gene resources and genetic diversity of such minor crops, well adapted to the specific environments, will become poorer.

Concerning the physiological characters of the collected material the analyses of protein and mineral content were carried out in Berlin on tubers of sweet potatoes, rhizomes of yam and taro corms. Some clones from the sweet potato material were specifically investigated for their reaction to different soil temperatures under greenhouse conditions (Fisher 1985).

Results and discussion

Studies of evolutionary processes

During my field work when I was accompanied by my informants I became familiar with the many questions and problems concerning the provision of daily food, mostly from plant products. I made a number of very interesting observations.

Pandanus *trees*

When new garden land was required, usually an area of secondary forest was cleared, and sometimes primary forest also was cleared. Shifting cultivation took place at intervals of 4-6 years. All the young trees were removed, but there were exceptions. Some mature trees of *Pandanus* spp. (Pandanaceae) standing in a small group were not removed. The reason was that larger fruits could be expected from solitary trees. From the kernels of these fruits

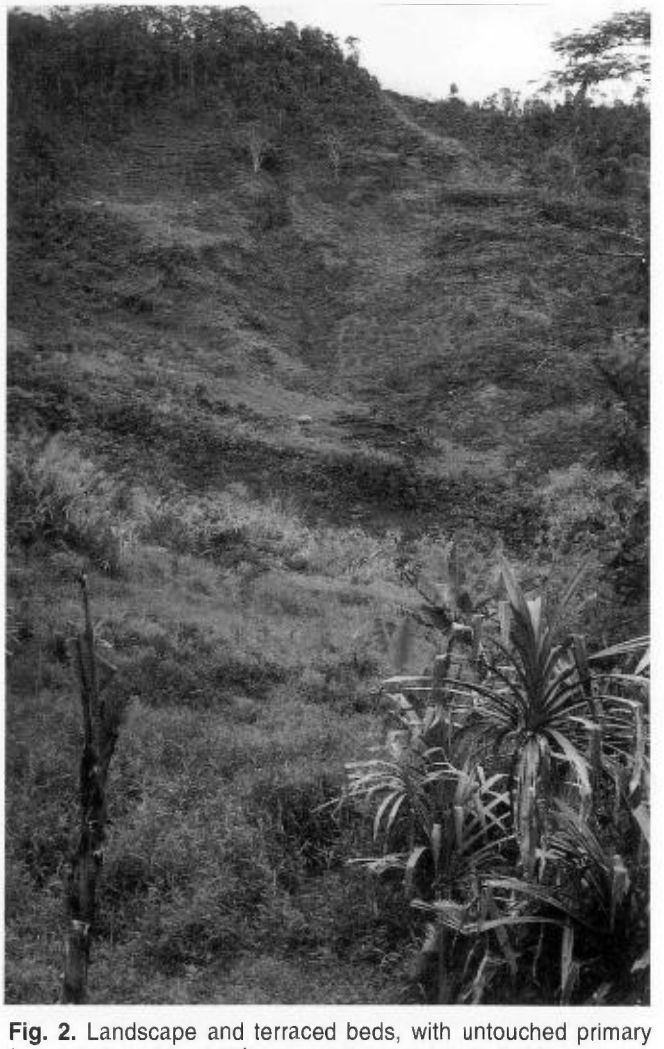

Fig. 2. Landscape and terraced beds, with untouched primary forest on top of the hill for preventing erosion; in the foreground, some *Pandanus* trees have been left for harvesting of fruit

a higher quantity of oil can be pressed for specific diets. A kind of sauce mixed with vegetables was prepared from this oil which is a source of fatty acids and vitamins (Figs. 2-4). The leaves are also used to cover the roofs of the huts. Details of the classification of *Pandanus* spp. are given in Hiepko and Schultze-Motel (1981).

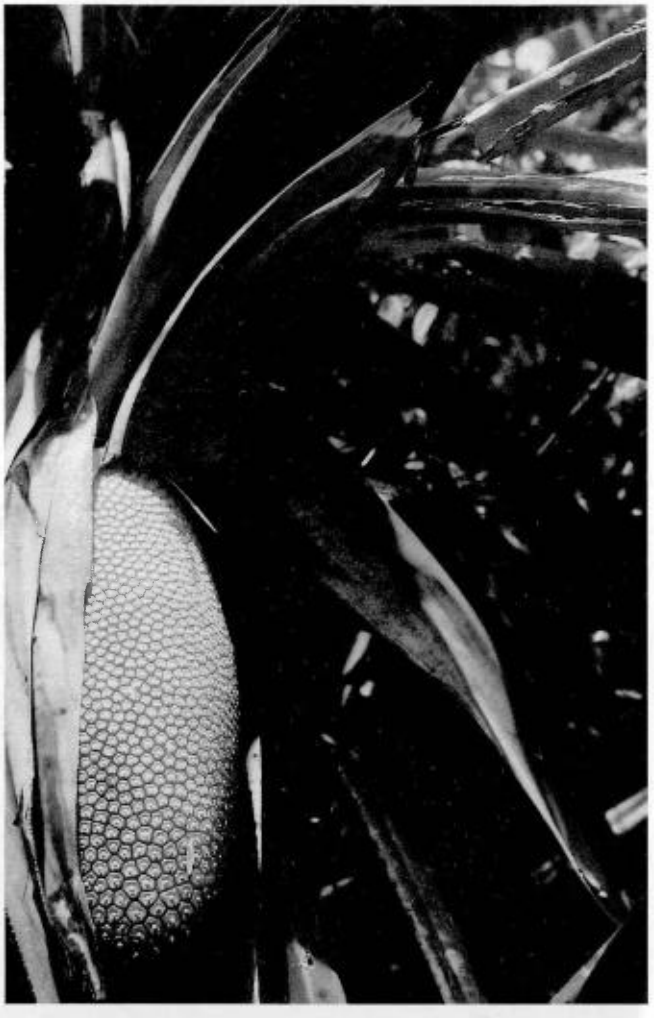

Fig. 3. Fruit of *Pandanus* spp. nearly ripe, length about 50 cm

Fig. 4. Removing the kernels from *Pandanus* fruits with stone axes (Neolithic culture) in the area of Kosarek

Pandanus is a wild plant in that region and is not planted by the people. The fruits are collected in the forest. Leaving some well-formed types means that selection takes place. This is a first step of domestication.

Eleocharis *spp.*

Eleocharis is another example of domestication. The raw material of the grass aprons for the girls and women is collected from the reed grass *Eleocharis dulcis* (Hiepko and Schultze-Motel 1981). One of the first observations I made was that single plants of that wild Cyperaceae grass were attached to small sticks to avoid lodging of smooth and long tillers (Figs. 5, 6). This use of sticks can be considered as a cultivation method to improve production, but in the same way it is a method of selection, a means of singling out genotypes that produce a better quality tiller. Differences in growth habit can be recognized more distinctly. The next step will be that fast-growing types with long tillers will be multiplied artificially by extra plantings. We should not forget that each anthropogenic influence is always a selection method in the direction of domestication.

Fig. 5. Girls watching the development of the raw material of their grass aprons

Fig. 6. Wild plants of *Eleocharis dulcis* are attached to small sticks to hold the tillers in an erect position; this produces better tiller quality for preparing grass aprons

Fig. 7. Garden-cane not stiff enough for erect growing is attached to long poles

Sugarcane

A procedure similar to that mentioned above concerns the cultivation of sugarcane (*Saccarum officinarum*). Stalks attached to long poles allow better development of soft but not stiff genotypes, which have a higher sugar content (Fig. 7). The sweeter types, which would otherwise lodge, can be selected easily. In this way the so-called 'garden-cane' has been derived, which was first detected in New Guinea by Stevenson (1965). This cultivation method is an example of the traditional way of domestication which began in prehistoric times in the highlands of New Guinea. Perhaps 10 000 years ago clones of cross-products between different ecotypes ($2n$=60 and $2n$=80) of *S. robustum* were used for planting and selecting in the gardens of the highlanders. Within these clones a large range of 70 to 148 chromosomes can be observed. From the best-adapted and sweetest types (>7% sugar content) primitive cultivated forms were derived, classified as *S. officinarum*. Nowadays a great genetic diversity in sugarcane exists in the New Guinea highlands, which must be considered as a primary centre of genetic resources, and which should be maintained by *in situ* conservation (Table 3).

Saccarum *edule*

This species is another cultivated relative of the genus *Saccharum*. The edule cane is used as a fine vegetable. It must have been selected in ancient times by the highlanders and probably also in other regions of Oceania.

The whole inflorescence has been changed into finely branched leaflets compressed into a hull of covering leaves, comparable with the inflorescence of cauliflower. *Saccharum edule* is unable to flower (Fig. 8). It can be multiplied only vegetatively. However, I collected a lot of different phenotypes for which each one is a specific genotype. The edule cane can be recognized easily in the field when planted in mixed cropping with sugarcane by its hairy stems (Fig. 9). Its closest relative is probably *S. robustum* and it could be derived from this ancestor directly by macromutation in one character. The hairy stems and the high fibre content are indicators for this suggestion.

The changed inflorescence can be harvested easily as a whole piece after removing the upper leaves near the top of the stems (Fig. 9). The whole thing can be eaten fresh or prepared in an earth-stone oven with other vegetables. It is rich in protein content and has a good flavour.

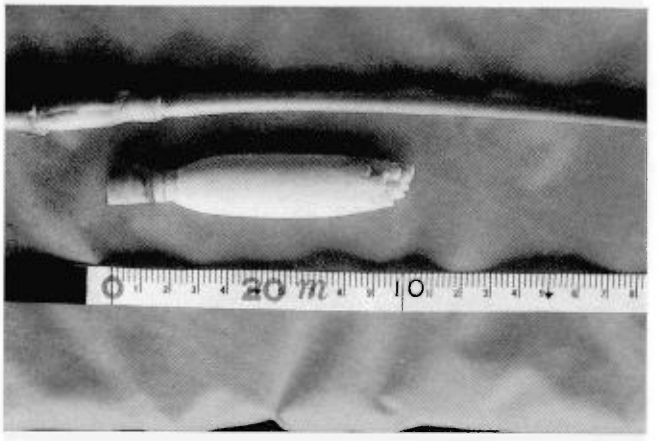

Fig. 8. On the top, the upper part of a *Setaria palmifolia* tiller; instead of an inflorescence, only leaves have developed. On the bottom, the inflorescence of *Saccharum edule*

Fig. 9. *Saccharum edule* can be recognized easily by its hairy stalk

Setaria plicata/S. palmifolia

The grass *S. plicata* (Gramineae) is common in Southeast Asia and people in the highlands sometimes use the tillers to treat stomach ache (Hiepko and Schultze-Motel 1981). It differs from *S. palmifolia* in that the former develops flowers and seeds in an inflorescence of a panicle typical for *Setaria* species and the latter only fine leaflets rolled in the upper part of the tillers. I made longitudinal sections and removed all these fine leaflets. In the centre of a tiller a tip of a vegetation point could be found, but nothing else (Figs. 8; 10).

Nowadays, *S. palmifolia* can be found in Asia as well as in America. However, only in the highlands of New Guinea is it cultivated and planted systematically as a vegetable crop in the gardens of the native people. It produces no flowers and seeds and so can only be propagated vegetatively. I found a large genetic diversity: clones with very distinct morphological characters. All of them are named (Fig. 11, Table 3).

In my opinion *S. palmifolia* has been derived by macromutation in *S. plicata* which can be assumed to be the ancestor because this wild type is the closest relative to it. By

Fig. 10. On the left side *Setaria palmifolia*; on the right side its ancestor *S. plicata*

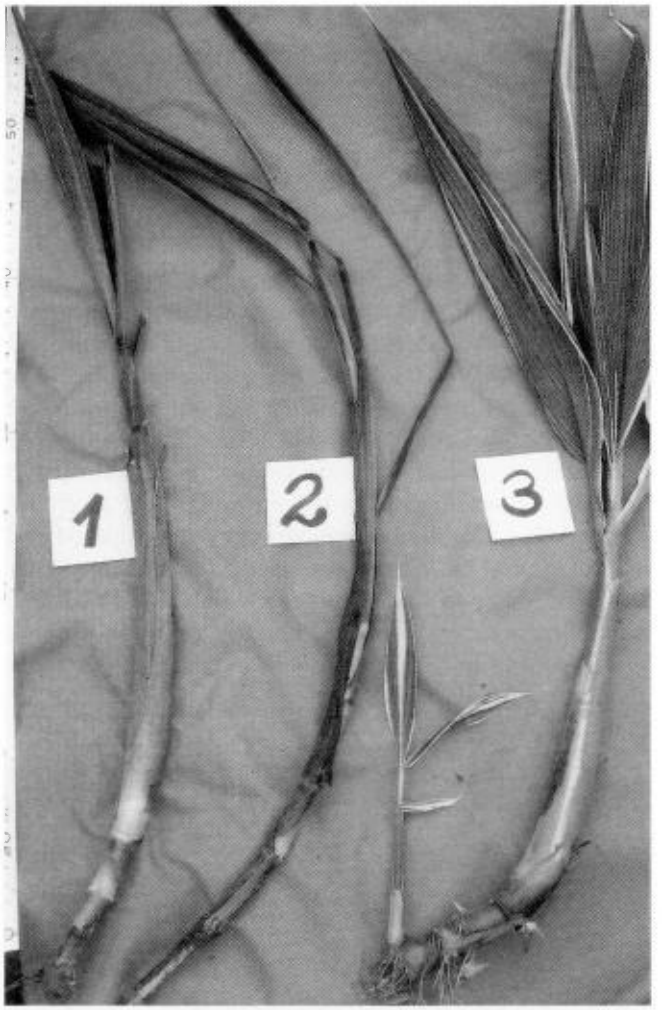

Fig. 11. Three different clones from *S. palmifolia*; on the right a variegated type with young shoot for vegetative propagation

further small mutations the original *S. palmifolia* segregated in some other different clones. An equal evolutionary process happened in the case of *S. edule* described above (Table 3). This characteristic change in related genera of the same family (Gramineae) was named by Vavilov (1928) as a parallelic variation evolved during the domestication process.

The young shoots of *S. palmifolia* are eaten raw or prepared with other vegetables in earth-stone ovens as mentioned above. However, my wife cooked those shoots together with *S. edule* and other leafy greens. Never have I eaten such delicious vegetable soup as I did in Kosarek and Eipomek!

Lagenaria siceraria

This species is distributed worldwide in the tropical and subtropical regions. We find a very large variability in all characters, especially in fruit shape. Native people in some tropical rainforest areas in South America as well as in New Guinea prefer the longer forms, which they use as penis sheaths.

Table 3. Cultivars of the most important food crops within the area of two settlement locations in Irian Jaya

Species	No. of cultivars with given names	
	Kosarek 1500 m	Eipomek 1800 m
Main food crops		
Sweet potato (*Ipomoea batatas*)	45 (11)†	50 (12)
Taro (*Colocasia esculenta*)	36 (?)	34 (20)
Sugarcane (*Saccharum officinarum*)	16 (?)	20 (6)
(*Saccharum edule*)	8	5
Vegetables		
Malvaceae (*Hibiscus manihot*)	4	4
Millet (*Setaria palmifolia*)‡	7/8	5
Amaranthaceae (*Amaranthus* spp.)	3	2
Acanthaceae (*Rungia klossii*)	1	2
Fruits		
Banana (*Musa* spp.)	19 (3?)	8
Drugs		
Tobacco (*Nicotiana tabacum*)	1 (?)	3

† Recently introduced. Of lesser importance, but introduced recently: yam, tannia (cocoyam) *Alocasia*, cucumbers/cucurbits, tomato, Chinese cabbage, garden beet, potato, *Phaseolus* beans, castor bean, maize, *Solanum nigrum*.
‡ Ancestor (wildtype) is *Setaria plicata*.

We met a proud man who wanted to harvest long, evenly shaped fruits (Fig. 12). He had planted the seedlings near poles to which a rack had been attached. By this means the climbing plants would develop long-shaped fruits because they grow downward as long as they are able. The final length depends on the genetic background and on the nutrition available to the plants. There is no other limitation and thus the whole genetic potential in growth length can be utilized. By such technical aid the most suitable phenotype with high heritability can be selected. We should keep in mind that each cultivation method, even the simplest one, is functioning as a selection parameter.

Variability of cultivated food crops

Basis of the food supply

Root and tuber plants are the staple food crops in the highlands of New Guinea. Besides sweet potatoes, a minor crop like taro plays a great role. But, as mentioned above, sugarcane is also an important caloric source providing energy. It can be estimated that about 80% of the calories consumed is provided by sweet potatoes, 10% by taro and 10% by sugarcane.

Different kinds of vegetables and bananas are also cultivated as food crops (Table 3). The number of crops utilized for a complete diet is not very high, but within one species a lot of different variants occur, and they have very distinct physiological and morphological characters.

Fig. 12. *Lagenaria siceraria* planted on a frame where the fruits are developing downward without any obstacles

The people are skilful in preparing specific dishes. For instance, from time to time they collect vegetables—roots, tubers, leafy greens, bananas and others—which they mix together, wrap in envelopes of banana leaves and roast on hot stones in earth-ovens.

Botanical classification of the food crops

Obviously we find a large variability within a specific crop. It was very simple to ask the children to collect cuttings and tubers. As mentioned above, my informants were familiar with the names of the different types of crops.

Nearly all the investigated species are multiplied vegetatively in the tropical highlands environment. We learned from the highlanders that they give names to their cultivars, even though their way of life is still on the level of the Neolithic culture. This is a very important perception to me. As a plant breeder and geneticist I have full respect for their intelligence, their capacity for recognizing small differences between plants, their mentality in their behaviour to nature, and the care they take to produce their daily food!

In most cases it is not clear if the names of the clones have a special meaning. Sometimes there is a connection to a specific locality from which the cultivars have been introduced. Some names can be directly translated, e.g. a sweet potato clone named 'wesen' with greenish-yellowish colour similar to the plumage of the parrot *Charmosyna papou* (Heeschen and Schiefenhövel 1983).

Variability of root and tuber crops

Sweet potatoes (*Ipomoea batatas*) are not an endemic crop in New Guinea in contrast to taro (*Colocasia esculenta*) and sugarcane. Sweet potato was introduced after contact with the Americas. The large variability of 45 to 50 different clones in Kosarek and Eipomek, respectively, is derived from mutations and segregation after seed setting and selection of seedlings, which were multiplied as clones (Table 3, Fig. 13). Similar numbers of cultivars (30, 34 and 44 different clones) have been recorded in three other locations (Hiepko and Schiefenhövel 1987). This same variability occurs with taro: 36 and 34 clones in Kosarek and Eipomek, in comparison with 39, 56, 65 cultivars in the three other locations.

Fig. 13. Sweet potato clone 'tinta' flowering and seed setting

The origin of taro can be assumed to be Southeast Asia, where it was first cultivated about 12 000 years ago. From this centre the cultivated types spread out to New Guinea, Oceania. By way of the highlands many cultivars were derived, especially by polyploidization, with very different numbers of chromosomes. Aneuploids ($2n$=22, 26, 28, 38, 42) can survive by vegetative propagation. They are often specifically adapted to adverse environments. Taro was the most important food crop before sweet potatoes were introduced, and it is still the staple food in some regions of New Guinea, where it is planted as a monoculture (Morren and Hyndmen 1987; Tumana 1987).

The external characters—the skin and the flesh colour of sweet potato tubers—serve to identify the clones. The findings on skin colour are summarized in Table 4 (Fig. 14). As the results show clearly, a correlation exists with environment conditions. At higher altitudes red, skinny tubers are preferred because they seem to have a selection advantage. As we know, anthocyanins have a metabolic function of protection against disease infection and adverse conditions such as cooler soils, but their quality is negatively influenced. However, it can be seen that red, skinny tubers have mostly white flesh, thus there is a free combination of the skin and flesh characters. White fleshy tubers have a better taste, and so they have been selected. We have tested many cultivars because sweet potatoes were part of our daily diet, and we found extreme differences in taste and cooking quality. The native people distinguish the qualitative characters exactly. For taro, the leaves of only 4 clones are eaten as a vegetable at Kosarek.

Special investigations were made on the colour of the taro leaf petioles, which is a suitable mark for clone description and identification. The details are published in Plarre (1981). In Table 5 I have summarized all the clones in 6 colour classes. By this differentiation, the large genetic variability is expressed exactly. Petiole colour is not influenced very much by environmental factors. Therefore this feature confirms a high concentration of different genes incorporated in the highlands taro material (Fig. 15).

Table 4. Colour classes of sweet potatoes collected in the highlands of Irian Jaya

	Manokwari		Kosarek		Eipomek	
	No.	%	No.	%	No.	%
No. of clones	10	100	42	100	45	100
Skin colour						
Red	4	40	16	38	30	66.7
Yellow	2	20	11	26.1	6	13.3
White	4	40	15	35.9	9	20
Flesh colour						
Reddish	2	20	2	4.8	3	6.7
Yellow	4	40	2	4.8	2	4.4
White	4	40	38	90.4	40	88.9

Calculation using χ^2-test shows an equal distribution of the three colour classes at Kosarek (χ^2 = 1.00 no significance) and an unequal distribution at Eipomek (χ^2= 22.8, significance P = 0.1%). In comparison between Kosarek and Eipomek (χ^2 = 42.3, significance P = 0.1%). That means the percentage of red skinny clones is significantly increased up to 2/3 of the total production.

Table 5. Colour classes of taro leaf petioles for clone description: variation and frequency distribution in highland clones from two locations in Irian Jaya, 1979

	Kosarek 1500 m	Eipomek 1800 m
n	31	33
Colour		
Nearly white	1	1
Light yellow to yellow-green	3	5
Light to dark green	10	9
Red to blue violet	10	9
Dark violet	2	2
Variegated[†]	5	7

[†] Striped and mottled in different patterns.

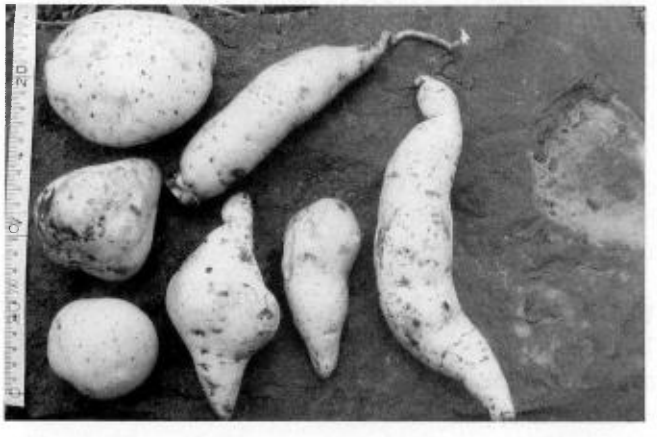

Fig. 14. Five clones of sweet potatoes; on the upper left and lower left are two European potatoes

Fig. 15. Four clones of taro, variegated types as mentioned in Table 5

Of the internal characters, protein content has been analyzed. If we consider the values in Table 6, it can be assumed that genetic differences exist. The coefficients of variation (*s*%) indicate large variability. The frequency distribution follows a normal curve, well known for quantitative features influenced by environmental but also by genetic factors. There is a great chance to achieve an improvement in protein content by selection of positive variants in sweet potatoes as well as in taro.

Table 6. Variation and frequency distribution in protein content in highland clones of sweet potato and taro from Irian Jaya, 1979.

	Sweet potato	Taro
Protein content (*N*x6.25) in g/100 g dry matter		
≥1.6	2	1
1.7-2.3	5	3
2.4-3.0	15	3
3.1-3.7	5	3
3.8-4.4	3	0
≥4.4	0	2
n	30	12
$\bar{X}$	2.65†	3.48†
S	± 0.7	± 1.89
S%	26.4	54.3

† No significant difference.

From my own calculations about protein supply in comparison with findings from other colleagues (Schiefenhövel 1978; Gunawan 1979; Plarre 1979) it can be estimated that the Mek population obtains 75-80% of its daily protein from sweet potatoes, 10% from taro corms and the rest from vegetables and small portions of any animal protein. Sometimes pig meat, small animals (rats, birds, lizards), insect larvae and other animal forms are consumed. The male adults of Mek pygmies have an average body weight of 43.7 kg (Büchi 1981). The protein yield calculated from the yield of the harvested crops analyzed covers the required daily supply. However, the question arises: is the protein quality sufficient, particularly for children—babies are nursed up to 2 years old—and pregnant women? This is still an open question and further investigations have to follow.

Concerning the components of amino acids in sweet potatoes it has been found that the highlands material has a more favourable composition of essential amino acids than lowlands cultivars, but the total amount of protein is reduced (Oomen *et al.* 1961). I think that protein quantity and quality can be improved in sweet potatoes as well as in taro by precise screening. Additional attention should be paid to the root and tuber crops introduced not long ago: yam (*Dioscorea* spp.) — I found three different types — tannia (*Xanthosoma* spp.) and cassava (*Manihot esculenta*). Yam has a great range in protein content (Plarre 1981).

With respect to the variability of the two most important food crops it can be postulated that a secondary gene centre or smaller microcentres exist in the highlands of New Guinea. The environmental conditions which provide the requirements for the concentration of very different genotypes in limited areas are pointed out in the Conclusion.

Variability of vegetable crops

Many of the vegetables cultivated in the highlands were introduced in recent years by missionaries (see Table 3 footnote). Some of them are only growing in the mission gardens, not in those of the native people. But these kinds of vegetables are being increasingly accepted if the native people appreciate the flavour. These include beans, cucumbers, pumpkins, chayote (*Sechium edule*) and peanuts. I detected a well-grown tree tomato, *Cyphomandra betacea*, in the garden of the mission in Eipomek. It could not be determined if there are different cultivars of these vegetables. The less important *Solanum nigrum* is classified as *S. nodiflorum* by Hiepko and Schultze-Motel (1981), but the plants grown from seeds, which were taken to Germany, could be crossed with our *S. nigrum*, and the F_1 was completely fertile. It is remarkable that the alkaloid content of the leaves is high (Ullrich 1985), but the people consume them without getting any digestive complaints. The highlanders in the Andean mountains also eat alkaloid lupines.

The most important endemic vegetables, from which different variants could be identified, are mentioned in Table 3. Only from five genera were specifically named cultivars found. However, we should not forget that wild plants are also collected and sporadically used, especially leafy greens (Hiepko and Schultze-Motel 1981).

The differentiation of *Hibiscus* (now *Abelmoschus manihot*) of *Amaranthus* spp. and of *Rungia klossii* is based first of all on the colour and shape of the leaves. In comparison with the observations of Hiepko and Schiefenhövel (1987) at three other locations, the number of cultivars there was higher: 13, 12, 13 for *A. manihot* and 5, 8, 9 for *Rungia klossii*. There could be different names for the same cultivar and therefore a higher number has been recorded. All these species are widely distributed in New Guinea.

The different cultivars of the more important vegetables (*S. edule* and *S. palmifolia*) can be identified mostly by the colour of their stalks or stems, respectively (Fig. 11). This is also true for sugarcane.

Variability of bananas

The cultivated types of banana (*Musa* × *paradisiaca*) originated in Southeast Asia, but we do not know where and when this happened. There are two diploid wild ancestors of *M. acuminata* (A-genome) and *M. balbisiana* (B-genome). They could not be found by Hiepko and Schultze-

Motel (1981). Therefore, we must assume that the domesticated banana was introduced, but very long ago. From the investigations I made by cross- and longitudinal sections, I found that all of them contained seeds. I assume that they are diploid. Modern cultivars with fruit length up to 30 cm are triploid or tetraploid and seedless.

Concerning the differences between the cultivars, it can be seen clearly from Fig. 16 that fruit shape, length and colour as well as flesh colour vary. There are also distinct differences in the internal characteristics such as odour and taste. The cultivar 'debit', with thick and relatively short fruits (11.2 cm on average), has a hard, dark green skin as a ripe fruit but a very aromatic taste like carrots. It probably has a high vitamin A content. It is remarkable that bananas grow well at altitudes of 1800 and 2000 m.

We can assume that a gene centre for bananas occurs in the highlands, in which very valuable genotypes are maintained. Every clone is specifically adapted to its environment. These genetic resources should and could be utilized for breeding programmes. If we consider the released cultivars of bananas available in our markets, we must say that they are a very poor offer. My wife and I were satisfied and happy to eat instead the delicious bananas of the New Guinea highlands.

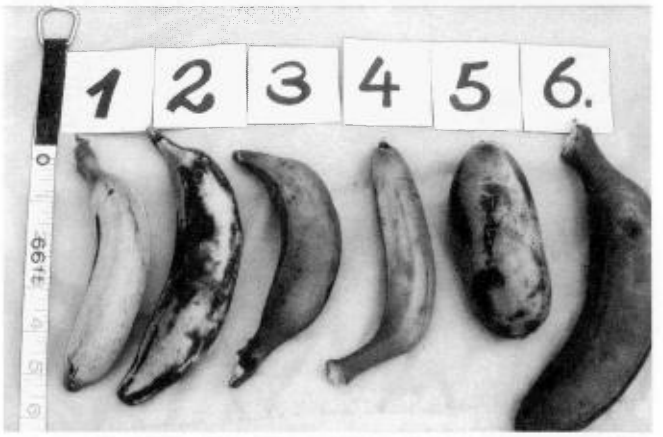

Fig. 16. Six clones of banana, different in fruit colour and in shape, no. 5 is the cultivar 'debit' (see text)

Variability of drugs

When I was looking around in the first days after my arrival in the highlands—by that time especially the children were in company with me—I was really surprised to see that some small boys of about 10 years of age were smoking like adults without any shame. Tobacco, *Nicotiana tabacum*, was introduced by Europeans probably in the 17th century (Hiepko and Schultze-Motel 1981), but leaves from some other plants, which we could not identify, were also used for smoking. Dried, rolled leaves are prepared as small cigars.

Tobacco generally is planted in front of the huts or nearby. The seed beds and the seedlings are handled very carefully, and weeding takes place all the time. Differences in the leaf shape and in colours are distinct, and therefore it is not surprising that the people have selected different variants. In Eipomek I found three: 'bunyefol' with light green, broad leaves, 'bayang' with large narrow leaves and 'butor' with dark green and very narrow leaves. The last named is that of a bush, from which fibres are taken for making stringbags (Heeschen and Schiefenhövel 1983).

From plants which are propagated vegetatively as clones, it is easier to develop new cultivars than in crops, which are seed multiplied. This is the case with tobacco. Selection needs more attention within a seed progeny, where only a few different genotypes derive from a segregating heterozygote mother plant. The changed types have to be selected during the seedling stage, and they have to be multiplied separately to get a more or less uniform new cultivar, which will be named. This requires not only a high knowledge about plant development but also a special sense in recognizing small phenotypical differences controlled by genes. The Mek people are good observers of natural processes, and therefore they are able to improve their plant production by real breeding work.

Only a few plants are used as stimulants. Tobacco is the most important one. Hiepko and Schultze-Motel (1981) mention some others: leaves from *Piper* spp. and rhizomes from *Zingiber* spp. It seemed to me very remarkable that the highlanders do not prepare any drinks containing alcohol or stimulants such as tea. They only drink pure water, collected in bamboo stalks from clear wells.

Conclusion

If we assume that a gene centre or smaller microcentres have developed in the highlands of New Guinea we have to ask: what are the prerequisites? We have to consider all the environmental parameters and also the anthropogenic factors. The findings are as follows:

- the terrain consists of deep valleys with steep slopes at altitudes of 1500-2000 m (Fig. 17),
- annual precipitation is >5000 mm,
- the temperature varies greatly between day and night (Table 1, Fig. 18),
- soil conditions vary considerably (Sieveking 1985),
- the settlers have cultivated the land for gardens on terraces, and have developed a special system of hilly-bed cultivation (Fig. 19),
- crops are always planted in mixtures of different clones and different species, i.e. populations are grown and virtually no monoculture is practised (Fig. 20; Plarre 1988),
- the growing season is continuous throughout the year,
- in such environments a high mutation pressure can be expected.

Under these circumstances vegetatively multiplied crops have a selection advantage. They grow more strongly

Fig. 17. Highlands in the area of the Mek population; in the right corner a settlement and terraced garden-land

Fig. 18. Impression in the highlands: nearly daily rain changes into sunshine very quickly; happy people under the rainbow

and faster by bud sprouting, which is superior to seed germination in small-seed crops. There must be a high somatic mutation rate. In taro, for example, this is confirmed by a high frequency of variegated types in which at least two different genotypes are involved. That means they are genetic chimeras, which can be segregated by sprouting (Fig. 15; Plarre 1981). In sweet potatoes, genetic variability is also enlarged by seed multiplication and segregation in the progeny of a heterozygote mother plant. In taro, I only found one clone flowering, but it was not utilized.

Native people are looking for diversity in all their cultivated crops. This minimizes the risk of damages caused by all adverse factors, e.g. diseases, pests and abiotic factors. By this means the derived different genotypes are maintained, and if they are well adapted the yield will be stabilized.

The valuable genetic resources, particularly in sweet potatoes, taro, sugarcane and bananas, must be maintained. There is a great need to utilize them in modern plant breeding, e.g. for special programmes in Papua New Guinea (Morren and Hyndman 1987; Tuman 1987). I recommend *in situ* conservation in connection with an on-farm project. This could be organized in collaboration with Indonesian institutions.

Fig. 19. General view of a garden-land area, where people are preparing and planting the hilly beds working only with wooden sticks (Neolithic culture). In the foreground one man holds an iron spade. It and the "Berliner Grabegabel", which was brought to Kosarek as a gift, became helpful tools

Fig. 20. Mixed cropping on terraces: in the background taro and sugarcane, in the foreground *Setaria palmifolia*, taro and various clones of sweet potatoes (on the right); in the middle, a pig collecting some feed

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Résumé

Evolution et variabilité de certaines espèces des régions montagneuses de la Nouvelle Guinée occidentale (Irian Jaya), cultivées dans les conditions du néolithique

Des échantillons de toutes les plantes cultivées et de certaines espèces sauvages utilisées à des fins précises (par exemple, fruits comestibles, plantes à fibres) ont été collectés, décrits et étudiés, pour déterminer leur valeur nutritionnelle, dans les régions montagneuses centrales de la Nouvelle-Guinée occidentale (Irian Jaya), dans la région habitée par la population Mek (à 4° S, 140° E). Une grande variabilité a été observée à l'intérieur des différentes espèces cultivées, tant pour des caractéristiques morphologiques telles que la forme et la couleur des feuilles, de la tige et des tubercules chez les espèces tubérifères, que pour des caractéristiques physiologiques telles que la teneur protéique, la qualité à la cuisson et le goût. Des données ont été collectées dans deux sites (Kosarek à 1500 m d'altitude et Elpomek à 1800 m) sur les cultures vivrières les plus importantes: patates douces (*Ipomoea batatas*) >90 clones, taro (*Colocasia esculenta*) >60 clones, bananier (*Musa* spp.) >20 clones, canne à sucre (*Saccharum* spp.) >30 clones et sur différentes sortes de légumes tels que *Setaria palmifolia, Amaranthus* spp., *Rungia* spp. et d'autres. Les régions montagneuses de la Nouvelle Guinée peuvent constituer un centre de diversité génétique, surtout pour les plantes à multiplication végétative.

Resumen

Evolución y variabilidad de cultivos especiales producidos en las tierras altas de Nueva Guinea Occidental (Irian Jaya) en las actuales condiciones neolíticas

En las montañas centrales de Nueva Guinea Occidental (Irian Jaya), en la zona de la población Mek (4 grados S, 140 grados E), se recogieron, describieron y estudiaron muestras de todas las plantas cultivadas y de algunos tipos silvestres utilizados con fines específicos (por ejemplo, frutas comestibles, plantas fibrosas) a fin de evaluar su valor nutricional. En todos los cultivos se observó una gran variabilidad de características morfológicas, como la forma y el color de las hojas, los tallos y los tubérculos de los cultivos de raíces; así como de caracteres fisiológicos, como contenido de proteína, sabor y calidad para la cocción. En dos localidades (Kosarek a 1500 m.s.n.m., y Eipomek a 1800 m.s.n.m.) se recogió información sobre todos los cultivos alimentarios de importancia: batata (*Ipomoea batatas*) >90 clones, colocasia (*Colocasia esculenta*) >60 clones, banana (*Musa* sp) >20 clones, caña de azúcar (*Saccharum* sp) >30 clones y diferentes tipos de cultivos hortícolas como *Setaria palmifolia, Amaranthus* spp., *Rungla* spp. y otros. Las tierras altas de Nueva Guinea pueden considerarse un centro génico, especialmente para cultivos vegetativos.

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Cocoa germplasm conservation in Ghana

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Summary
A major component of successful breeding programmes is the collecting and conservation of germplasm with the view of exploiting the variation that may exist in the populations introduced. At the Cocoa Research Institute of Ghana efforts have been made since 1944 to introduce, conserve and utilize various cocoa populations. These introductions have contributed immensely to the development of improved cocoa hybrid varieties. Several problems, such as losses through diseases and other environmental hazards, have been associated with the conservation of cocoa germplasm in the field. This paper reports on efforts that have been made to introduce and conserve cocoa germplasm in the field, threats to the collection and work done to alleviate the dangers posed by those threats by way of duplication, seed storage and tissue culture methods.

Introduction
There are conflicting reports in the literature of when cocoa (*Theobroma cacao* L.) was first brought to Ghana but it is reasonable to assume that cocoa cultivation commenced in the early 1800s (Dickson 1963). During the last 50 years cocoa breeding has been an active part of the cocoa research programme. It is generally agreed that without serious efforts to collect and conserve cocoa germplasm, current and future breeding programmes for the genetic improvement of cocoa will be adversely affected. Posnette (1943a) made several collections from all the cocoa-growing areas of the then Gold Coast. These were mainly locally adapted Amelonados and local hybrids (often referred to as local Trinitarios). Evaluation of these collections indicated that they were genetically uniform in several attributes. Thus the need for introductions from elsewhere to generate the necessary variations needed for genetic improvement of the crop in Ghana was established.

A large collection of cocoa types has been assembled at Tafo, ranging from those of descendants from early introductions (pre-1910) through the Trinidad introductions (Posnette 1943a) and the Colombian introductions (Mckelvie 1957) to the present-day collection comprising some of the collections of Chalmers (1970) and Allen (1987; Allen and Lass 1983). The history of cocoa introductions and conservation has been well documented by Lockwood and Gyamfi (1979). In all cases the introductions have been conserved in the field.

This paper reports on the conservation of the introductions, dangers associated with conservation in field genebanks of cocoa and efforts being made to conserve the materials using alternative methods.

Conservation in field genebanks
The first major effort to introduce, conserve and utilize cocoa germplasm in Ghana was made in 1944 and 1946 by Posnette (Anon. 1944, 1946). These introductions greatly broadened the genetic base of cocoa in Ghana, and its importance is manifested by the role it has played in major breeding programmes, especially in the development of the Amazon-Amelonado/Trinitario hybrids (series II hybrids) and the inter-Amazon hybrids (Glendinning 1963). Posnette's 1944 introductions were mostly crosses between materials that had been introduced to Trinidad from the Upper Amazon by Pound (1936). Also included were other related cocoa species. These were introduced as pods and after passing through quarantine were planted in the field. Out of a total of 121 (T1 - T121) different pods introduced by Posnette only the progenies of 25 pods are still at Tafo (Table 1), the rest of the progenies having been lost in the field largely through cocoa swollen shoot disease (CSSVD) infection. Most of these did not prove readily relevant to the breeding programme at that time.

The next major introduction of significance was the selections made by the Anglo-Colombian collection expedition in 1952. In all, 14 different clones from this collection were introduced into Ghana and planted in the field (Mckelvie 1957). In 1956, a second consignment of Anglo-Colombian collections was brought in together with 'Lafi 7' and 'Cam 12b' which were not part of the Anglo-Colombian collections. Table 2 shows the types introduced to Tafo and their present situation. A substantial number of these introductions has been lost in the field through CSSVD infection. Some of the Anglo-Colombian introductions have been used in crosses but to date only

Table 1. The surviving progenies of Posnette's 121 (1944) collections

Code at Tafo	Progeny
T9	Open-pollinated from Costa Rica, clone 613
T12	Scavina 12 open-pollinated
T16	IMC 24 open-pollinated
T17	IMC 53 open-pollinated
T60	PA7 x NA 32
T61	NA 33 x NA 32
T62	NA 33 x NA 34
T63	PA 35 x NA 32
T65	PA 7 x IMC 47
T67	*H. mariae*
T72	NA 32 x IMC 60
T76	PA 35 x NA 31
T79	Reciprocal of T60
T81	NA 32 x NA 31
T82	Reciprocal of T63
T85	IMC 60 x NA 34
T86	PA 35 x PA 7
T87	Reciprocal of T85
T90	Reciprocal of T101
T92	Reciprocal of T81
T104	*Theobroma speciosum*
T106	*Theobroma grandiflorum*
T108	*Theobroma microcarpum*
T111	*Theobroma obovatum*
T121	*Theobroma augustifolium*

Table 2. The present conditions of the Anglo-Colombian and other collections introduced in 1952 and 1953

Code	No. of surviving trees
15	3
18	8
41	21
52	5
53	0†
54	0†
111	0†
127	0†
135	0†
136	0†
160	3
186	0†
189	5
194	2
195	4
Lafi 7	2
Cam 12b	3

† Lost through CSSVD.

'Col 41' is actively being used (Adu-Ampomah 1982/83-1984/85) because of its thick and hard pod husk which was erroneously thought to impede the spread of blackpod.

The next introductions of significance were those started in 1969 by the British Research Team (BRT) and which have continued to the present day. A total of 434 different clones have been introduced and planted in the field (Table 3). The clones are brought in as budwood and budded onto Amelonado rootstocks and transplanted to the field after 6 months in quarantine. In the field the clones are planted at random with a minimum of 10 trees per clone. This random planting was adopted largely because of the method of controlling the CSSVD infection which entails the cutting out of infected and all contacted trees. Random planting also minimizes the risk of complete loss of clones as a result of bushfires and falling trees. Maintenance consists of manual weed control, removal of basal chupons to ensure that the rootstock does not overgrow the scion, removal of diseased pods (mainly due to *Phytophthora palmivora*) and regular inspection for CSSVD infection and treatment.

Once a year, a census is carried out in the genebank and clones which are not up to 10 trees are multiplied and planted. In spite of all these efforts, the danger of losing materials in the field is always there. In 1984 alone 36 clones were lost (Table 4). From the table, it is clear that most of the losses were due to CSSVD infection. Losses through old age came as a result of poor regeneration capacity of such trees.

Some of these recent introductions are being used for the development of alternatives to the Amazon hybrids. Others are being used to replace pollen parents in our seed gardens with the objective of developing hybrids with better resistance to the CSSV disease.

A factor which has not been highlighted as a hazard to the germplasm is bushfires. The effects of bushfires have not yet been felt at CRIG, but over the past decade the spate of bushfires during the dry season caused the loss of some of our outstation trials. The need to safeguard our germplasm collection in the field against all these hazards has been a major concern in our conservation programme.

Efforts to safeguard cocoa germplasm in Ghana

Replication of collection

A number of efforts are being made to reduce the risk of losing our germplasm either completely or in part. The Eastern Region where both the Institute and the site for the germplasm collection are located is also the area where CSSVD is endemic. Thus the danger of losing the materials due to the disease is great, especially since the only effective treatment is to cut the infected tree together with those whose branches or leaves are in contact with the infected tree. We are therefore making efforts to replicate the collection at two additional sites, one in the Ashanti Region and the other in the central Region of Ghana.

Table 3. Cocoa germplasm collection assembled in Ghana since 1969 and their origin

Collection code	No. of accessions	Origin
G	1	Getas estate
DR	2	Java
GW	1	A cross from Wageningen
SNK	2	Cameroun
SL	1	Sao Thome'
SM	1	Sao Thome'
PA (Parinari)	45	Pound's selections at Parinari
Pound	19	Pound's selections in the Amazon basin
ICS	17	Trinidad
WA	3	Crosses from Wageningen
NA (Nanay)	146	Pound's Nanay selection
P	8	Mexico
EET	16	Ecuador
EQX	5	Ecuador
GA	1	Haiti
GS	4	Grenada
R	5	Mexico
SIC	1	Brazil
UF	16	Costa Rica
IMC	40	Pound's Iquitos Collection
SCA (Scavina)	10	Pound's Scavina Collection
MXC	1	Mexico
SIAL	6	Brazil
INUS	1	Bougainville
CC	8	Costa Rica
Ma	1	Manaus, Brazil
Rb	7	Brazil
Be	2	Belem, Brazil
C-Sul	1	Brazil
APA	1	Palmira, Colombia
SPA	5	Colombia
SC	8	Colombia
SGU	6	Guatemala
Catongo	2	Balina, Brazil
CAS	2	Brazil
EEG	5	Brazil
GC	1	Jamaica
Matina	3	Costa Rica
Mocorongo	1	Unknown
MOQ	7	Ecuador
SCr	2	Costa Rica
LF	1	Costa Rica
MO	5	Pound's selection from Morona River
AMA(Z)	3	Iquitos
NAPO	2	Ecuador
TJ	1	Guatemala
VIL	1	Chalmers collection from Villano River area
LAFI	1	Samoa
LCT-EEN	3	Reading
Larange	1	Lower Amazon
LA-ESMIDA PENT.	1	KEW
LA-ESMIDA AMARI	1	KEW

Table 4. The list of clones lost by 1984 and the causes for the losses

Clone	Cause of loss		
	CSSVD	Old age	Poor field establishment
SPA 5	x		
Spec 160-9	x		
PA 29	x		
PA 200	x		
PA 219	x		
P2B	x		
P11	x		
P21A	x		
P21B	x		
Pound 11A	x	x	
N8/112	x	x	x
Amazon 3-2	x		
EQX 94B	x		
ICS 16	x		
IMC 36	x	x	
IMC 69	x	x	
P4A	x		
P4	x		
PA 30	x	x	
SIC 5	x		
EQX 3345	x		
EQX 3339	x		x
ICS 1	x	x	x
IMC 3	x	x	
IMC 14	x	x	
IMC 30	x		
IMC 31	x		
IMC 54	x	x	
MO 216	x		
MOQ 258	x		
NA 27	x		
NA 142	x		
R117	x		
RB39	x		
SCA 3	x	x	
VIL I	x		

These areas have very low incidence of CSSVD and they are located so far apart that the probability of both being destroyed by bushfires at the same time is reduced.

CSSVD treatment

The swollen shoot disease of cocoa is caused by a virus which is normally transmitted in the field by mealybugs as vectors. Trees die within 2 years after infection. Attempts to control the mealybug vector by chemicals (Hannah 1954; Bowman and Casida 1958) or by biological control (Donald 1953; Decker 1955) have not been successful. The only effective method of controlling the disease in the field is by removal of infected trees as well as those in contact with them (Posnette 1943b). Thus in a germplasm plot the treatment of an infected tree leads to the destruction of at least four other trees.

A new method being adopted is to limit the number of contacted trees destroyed during treatment for CSSVD. A new approach is to uproot the infected tree but cut (coppice) the contact trees 2 feet (0.6 m) above the ground and allow them to rejuvenate. New leaf flushes from the coppiced trees are assessed for CSSVD symptoms and only trees showing the symptoms are uprooted. This way uninfected contact trees are saved.

Tissue culture

Studies elsewhere (Passey and Jones 1983; Pence *et al.* 1979) have shown the potential for shoot proliferation and rooting *in vitro* of *T. cacao* shoot tips and nodal cuttings. Studies are being conducted at Tafo to identify some of the factors which affect regeneration of plants from shoot tips and nodal cuttings *in vitro* to obtain a reliable technique for plant regeneration from cocoa shoot tips and nodal cuttings (Adu-Ampomah *et al.* 1992). The production of plantlets from cocoa shoot tips and nodal cuttings has been achieved but attempts to obtain plants have been difficult because of excessive callusing of the plantlets which obscures further differentiation. Efforts were made to delimit some of the factors which enhance differentiation and growth but reduce excess callus formation on the plantlets. The indications are that light durations of between 16 and 20 h at 2000 lux coupled with regular subculturing, especially once a week, favour development of plantlets to plants (Adu-Ampomah *et al.* 1991). If the studies prove successful, the technique will serve as a means of quick multiplication of new clones, transferring of germplasm in sterile conditions, as well as collection and storage of germplasm for long periods. It can also be used alone or together with chemotherapy or thermotherapy to rescue CSSVD-infected clones in the field.

We also have been able to produce plantlets via somatic tissues (Adu-Ampomah *et al.* 1988). Attempts are now being made to find the ideal factors that promote efficient induction of somatic embryogenesis and production of plants via somatic embryos in cocoa. Although regeneration from somatic embryonic tissues is not a clonal propagation of maternal material, it could be of use in preserving progenies of germplasm *in vitro*.

Seed conservation

In Tafo, efforts are being made to explore the possibility of storing cocoa seeds to augment our conventional conservation efforts. Preliminary results indicate that cocoa seeds from four varieties stored in PEG 6000 at high osmotic potential had an average of 28% viability at 14 weeks. No significant varietal differences were observed but it is possible that some clones could have better storage potential than others. Further investigations in this area are being carried out. If this method is successful, it will be a great advantage as seeds could be stored during the dry season when protracted drought could cause bushfires. This way many progenies from our original collection could still be preserved.

Conclusions

In the past several germplasm introductions were made into Ghana. These introductions have helped in various breeding programmes, resulting in the provision of genetically superior varieties to the Ghanaian farmer. Future breeding achievements will largely depend on our ability to conserve our present collection and also to introduce and conserve new collections. However, conservation of cocoa collections is mainly done in the field with the attendant danger of losing them through natural causes such as diseases as well as accidents including bushfires. These are not peculiar to Ghana. Other institutes elsewhere in the world which are trying to introduce and conserve germplasm will face similar problems. Thus it is important that, in the first instance, efforts are made to distribute the available cocoa germplasm among institutions involved in cocoa breeding so that the danger of losing certain collections completely is reduced. Secondly, it is necessary that, in view of the dangers associated with conservation in field genebanks, other conservation methods such as seed storage and tissue culture techniques are exploited so that they can be used to augment conservation in field genebanks.

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Résumé

Conservation du matériel génétique de cacaoyer au Ghana

Le succès des programmes de sélection dépend pour une large part de la collecte et de la conservation du matériel génétique dans le but d'exploiter la variation qui peut exister dans les populations introduites. A l'Institut de recherche sur le cacao, du Ghana, des efforts sont déployés depuis 1944 pour introduire, conserver et utiliser différentes populations de cacaoyers. L'introduction de ce matériel a grandement contribué à l'obtention de variétés hybrides améliorées. Plusieurs problèmes, notamment les pertes dues aux maladies et autres risques liés à l'environnement ont pu être mis en relation avec la conservation du matériel génétique sur le terrain. Cet article rend compte des efforts réalisés pour introduire et conserver le matériel génétique de cacaoyer en champ, des menaces pesant sur la collection et du travail qui est effectué pour minimiser les effets de ces nombreux risques, grâce à la multiplication, à la conservation des semences et aux méthodes de culture de tissus.

Resumen

Conservación del germoplasma de cacao en Ghana

Un componente importante de un provechoso programa de mejoramiento es la recolección y conservación de germoplasma con miras a explotar las variaciones que pueden existir en las poblaciones introducidas. Desde 1944, en el Instituto de Investigación del Cacao de Ghana se han realizado esfuerzos por introducir, conservar y utilizar diversas poblaciones de cacao. Esta labor ha contribuido enormemente a la obtención de variedades híbridas de cacao mejoradas. Varios problemas, como las pérdidas causadas por enfermedades y otros peligros ambientales, se han asociado con la conservación del germoplasma en el campo. El presente artículo informa sobre las actividades realizadas para introducir y conservar germoplasma de cacao en el campo, los peligros que amenazan la colección, y la labor realizada para reducir los riesgos que tales amenazas plantean mediante la duplicación, el almacenamiento de semillas y métodos de cultivos de tejidos.

Plant genetic resources in South Italy and Sicily — studies toward *in situ* and on-farm conservation

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Summary

Exploration missions in South Italy and Sicily have been carried out jointly by the Istituto del Germoplasma (Bari, Italy) and the Institute of Plant Genetics and Crop Plant Research (Gatersleben, Germany) since 1980. About 2000 accessions threatened by genetic erosion have been collected and integrated into the participating genebanks for *ex situ* conservation. This activity was supplemented by systematic field studies on cultivated plants and their wild relatives. Results have been obtained on the flora of cultivated plants of the area, including chronoelements and geoelements. The evolutionary importance of the area is evidenced by the striking examples of introgression from the wild into the cultivated genepools and *vice versa*. The progress of genetic erosion could be investigated. However, there is a relatively large number of landraces still present, mostly in gardens but also in field conditions, which should be the basis for *in situ* and on-farm conservation. Our work has led to a considerable increase in public awareness of the value of einkorn and emmer wheats. Today, larger areas of these cereals are grown than at the beginning of the 1980s, when these species were extremely rare relic crops in restricted parts of Italy.

Introduction

South Italy belongs to the Mediterranean gene centre proposed by Vavilov (1927). Therefore, a large amount of variation in crop plants and their wild relatives has to be expected there. Vavilov collected in the area and gave a brief characterization (Vavilov 1962; see also Hammer 1987). His interest was mainly concentrated on the landraces of wheat, particularly in Sicily (Schreiber 1932; De Cillis 1942).

Although there have been several excellent examples of Vavilov's comprehensive approach, e.g. in Turkey (Zukovskij 1933) and Central America (Bukasov 1930), the Mediterranean area was not further evaluated in this direction by the Russian researchers. Italian activities concentrated on cereals, particularly on wheat (Prestianni 1926; De Cillis 1942; Ciferri and Bonvicini 1959, 1959-60; Perrino and Hammer 1983) and on fruit trees (Morettini 1977).

In 1950 the first comprehensive mission in the Mediterranean was carried out by R. Maly, a former coworker of H. Stubbe (Maly *et al.* 1987). Stubbe, founder of the Gatersleben Institute and inspired by the ideas of Vavilov, further elaborated the Vavilovian approach for collecting expeditions (e.g. performing pioneer work in the Balkan area in 1941/42 (Stubbe 1982)) and initiated and supported this mission. New input came in connection with the foundation of the Laboratorio del Germoplasma (later Istituto del Germoplasma) in Bari in 1969 (Scarascia-Mugnozza and Porceddu 1972; Porceddu and Scarascia-Mugnozza 1972). At first the activities concentrated on wheat (Porceddu and Bennett 1971) but soon other crops were included (Polignano and Perrino 1976). The cooperation between the Bari and Gatersleben Genebanks started in 1980 (Perrino *et al.* 1981) and since than an extensive exploration programme has been carried out. In the beginning it was performed along traditional lines but later on it was progressively directed toward studying the preconditions and possibilities for *in situ* and on-farm conservation.

Collecting missions

An extensive exploration programme was carried out with the help of checklists (Hammer *et al.* 1990; Hammer 1991), not only collecting a large number of landraces (see Table 1) but also compiling data on plant genetic resources, both cultivated plants and their wild relatives, including results of ethnobotanical and ecogeographic studies. Meanwhile the collecting missions covered most of the area (see Fig. 4 in Hammer *et al.* 1992).

Flora of cultivated plants

Our results have shown that a flora of cultivated plants can be defined in the same way as a flora of wild plants. The results of Hanelt and Beridze (1991) confirm this. Thus chrono- and geoelements have to be considered but also evolutionary pathways leading to new variation should be indicated. The basis for a flora is the species. Altogether 524 species were found in South Italy, considering also the infraspecific level 542 taxa which have been compiled (Knüpffer 1992).

Chronoelements

Archaeophytes are plants which were introduced from 5000 to 4000 BC (Zohary and Hopf 1993; e.g. einkorn, emmer, free threshing wheats, six-rowed barley, lentil

Table 1. Collections in South Italy and Sicily jointly undertaken by the Bari and Gatersleben genebanks

	1980[1]	1981[2]	1982[3]	1983[4]	1984[5]	1985[6]	1986[7]	1987[8]	1988[9]	1992[10]	Total
Triticum spp.	30	32	39	22	20	9	1	24	8	5	190
Hordeum vulgare	12	4	13	13	4	8	2	8	6	—	70
Avena spp.	14	10	12	8	7	7	1	3	3	—	65
Secale spp.	—	11	2	18	8	—	—	5	—	1	45
Zea mays	12	24	14	21	15	5	4	22	4	17	138
Other Gramineae	9	15	—	8	—	8	6	1	—	1	48
Phaseolus ssp. and *Vigna unguiculata*	32	23	14	31	33	14	12	39	34	23	255
Vicia faba	72	9	9	14	13	18	2	1	5	5	148
Lupinus spp.	13	2	3	13	4	1	—	1	—	3	40
Cicer arietinum	36	8	7	9	5	7	1	7	5	9	94
Pisum sativum	21	2	—	4	1	3	3	2	4	5	45
Other Leguminosae	47	5	8	12	9	18	1	15	7	11	133
Vegetables, oil, medicinal and other plants	163	43	23	104	66	127	57	37	88	62	770
Total	461	188	144	277	185	225	90	165	164	142	2041

Sources: [1]Perrino *et al.* 1981, [2]Perrino *et al.* 1982, [3]Perrino and Hammer 1983, [4]Perrino *et al.* 1984, [5]Perrino and Hammer 1985, [6]Hammer *et al.* 1986, [7]Hammer *et al.* 1987, [8]Perrino *et al.* 1988, [9]Hammer *et al.* 1989, [10]Laghetti *et al.* 1994

and broad bean) to about the 6th century BC. Archaeophytes are often geoelements of the Near East/Eastern Mediterranean area but also include introductions from central and northern parts of Italy. About 25% of the cultivated plants in the area belong to this group.

Palaeophytes were introduced between the 5th century BC and the 15th century AD. Important inputs came from Central/Middle Asia and, later on, from East Asia. About 20% of the flora of cultivated plants are palaeophytes and about the same amount are neophytes which were introduced from the 16th century on. The most important plants from this group come from the Americas. Of course, often no direct introduction occurred but the material came in via other countries. Maize has the Italian name of 'granturco' or 'grano turco' which could indicate introduction via Turkey, whereas the Sicilian 'frumento d'Innia' or the Calabrian 'granudinnia' could indicate a more direct introduction from the New World. Different introductions via different countries are also possible, but this fact is more important for specific interpretations within archaeophytes and palaeophytes. About 35% of the species were first cultivated and/or domesticated in the area. But this fact has no meaning within this classification.

As has been shown, chrono- and geoelements are often closely connected because they are dependent on the migration of peoples, detection of new areas or other political and economic events. A deeper approach would result in showing very interesting historical relations as it has been demonstrated for Cuba (Hammer *et al.* 1992-94).

Geoelements

For 542 taxa, areas of geographical origin could be considered (Hammer *et al.* 1992), i.e. for some taxa a decision was not possible, because there may be different regions for different races (as in *Lagenaria siceraria* or *Vigna unguiculata*), or the region of origin is not known for certain. In this way the geoelements of the cultivated flora of South Italy could be identified (Table 2). The largest part of the species is assigned to the area. Not all of these species derived from local wild plants but all of them underwent more or less evolutionary changes in South Italy mainly because of the presence of their wild progenitors.

Table 2. Geoelements of the South Italian flora of cultivated plants (after Hammer *et al.* 1992)

	No. of taxa	Percentage
South Italy	202	36.8
Near East/Eastern Mediterranean	85	16.2
Europe	40	7.6
Central/Middle Asia	18	3.4
Western Mediterranean	4	0.8
Northern and eastern parts of Africa	20	3.8
South/Southeast Asia	18	3.4
East Asia	33	6.3
South Africa	11	2.1
Middle South America	56	10.7
North America	26	5.0
Australia/New Zealand	11	2.1
Total number of taxa	524	100

Notable inputs came from the Near East and the eastern Mediterranean from where agriculture was introduced. South Italy in this way was a bridge for the radiation of agriculture from east to west as already proposed by Hehn (1887). Real autochthonous elements are rare, as e.g. *Capparis sicula*, and do not belong to the cultivated plants of greater importance. Consequently there are mostly allochthonous elements in the group belonging to South Italy.

Elements from East Asia are partly early introductions, which arrived via the famous 'silk road' and partly later introductions which came mainly by ship.

A great influence has been plants from the Americas which now belong to the most typical food plants of the area (maize, tomatoes, beans, peppers, zucchini).

Mostly the criterion of the presence of wild relatives for the inclusion into the South Italian category leads to the result that 70.3% of the fodder plants, 55.3% of the medicinal plants, 50% of the spices and condiments and 47.3% of the vegetables are from the area; these can be considered as rather high percentages. On the other hand, starch plants, cereals, oil plants, pulses and fruits with percentages from 0-15% in the area (Hammer *et al.* 1992) are for the most part introduced.

Evolutionary importance of the area

The large number of cultivated species evolved in the area characterizes South Italy as an important centre of origin for new crops. Diversity is present in the number of different species as well as in the infraspecific variation. Ongoing evolution could be observed repeatedly, particularly in cases of introgressions of wild and cultivated plants in both directions. Some striking examples are introgressions between:

-wild (*Secale strictum*) and cultivated rye (*S. cereale*) in several regions of southern Italy (Hammer *et al.* 1985)

-wild (e.g. *Brassica rupestris* and *B. incana*) and cultivated cabbages (*B. oleracea*) in Sicily (Perrino and Hammer 1985)

-wild/weedy (*Beta vulgaris* subsp. *maritima*) and cultivated beets (*B. vulgaris*) in Calabria (Hammer *et al.* 1987) -wild (*Pyrus amygdaliformis*) and cultivated pears (*P. communis*) in Calabria and Apulia (Hammer *et al.* 1987) -wild (*Raphanus raphanistrum*) and cultivated radish (*R. sativus*) on the island of Ischia (Hammer, new observation, April 1994).

Introgressions between different wheat species have been described by Hammer and Perrino (1984). The case reports cited stress the evolutionary power of the area. Even within some neophytes, an impressive amount of infraspecific variation evolved, e.g. in beans, pumpkins, capsicum and tomatoes, as evidenced by a large morphological differentiation. The same is true for other characters, e.g. resistance and quality. Recent research has also detected new variation on the molecular level, e.g. in a new phaseolin type for *Phaseolus vulgaris* (Lioi 1989) from the area.

Genetic erosion

The main obstacle against the writing of a real flora of cultivated plants of South Italy has been the loss of a large part of the variation. Loss on the species level proceeds relatively slowly and most of the species mentioned under cultivation by earlier authors could still be found in recent missions (some exceptions for different reasons are *Cannabis sativa, Cucurbita ficifolia* as a fruit vegetable and *Vicia articulata*). But there is a tremendous loss on the infraspecific level where several severe examples can be found.

The whole process of genetic loss is called genetic erosion. It is difficult to provide a specific estimation on an area level whereas in general the process can be demonstrated quite well by the loss of varieties and the increasing uniformity of modern agriculture. It has to be, therefore, considered as a great advantage that there are three levels of information available in the area for comparative studies:

- early approaches by Italian and Russian researchers, exclusively considering cereals and especially wheats
- results of the more general exploration work done by Maly (Maly *et al.* 1987) at the beginning of the 1950s and
- continued general exploration jointly by the Bari and Gatersleben genebanks.

The distance between the levels is about 30 years each. In the case of the two last activities, for some specific areas a time span of 40 years could be determined.

Considering these three levels, there could be found a general genetic erosion for all parts of the area. Generally this process is more advanced in the lowlands than in the mountainous areas. But genetic erosion has progressed further in the central and northern parts of Italy. There is another interesting result for the groups of crops. Whereas genetic erosion is very high in field crops and a tremendous loss can be observed in cereals, pulses and other crops, garden plants are able to persist much longer. Vegetables, fruit trees and condiments can still be found, even in the surroundings of larger cities such as Bari (Hammer *et al.* 1989).

The most important result of our studies is the specific estimation of genetic erosion on the species level, which is presented in a catalogue (Hammer *et al.* 1992).

Proposals for *in situ* and on-farm conservation

As has been shown, many accessions have been collected during our joint missions. This material is well preserved in the genebanks and available for future use. But it is separated from the ongoing evolution in the area which resulted in so much new and useful variation. Therefore, an important aim of our mission has always been to report the status of plant genetic resources. As has been shown, garden crops are widely preserved by a large

community of users. The general situation with this group should be continuously observed, security duplicates taken into the genebank and only in specific cases, such as introgressions of wild material, is protection necessary, mostly in encouraging interested persons to continue growing special types or using special methods.

A stronger approach is necessary for field crops. Our own activities led to the re-detection of einkorn and emmer in parts of South Italy (Perrino *et al.* 1981) as relic crops. Scientific and popular information about these rare wheats led to an astonishing public awareness (D'Antuono 1989) and today many farmers grow these traditional crops, often under supervision of the Bari genebank. Early proposals for projects funded by EC (Hammer and Perrino 1984) failed at that time. But meanwhile the informal sector has developed effective methods for the on-farm conservation of at least einkorn and emmer wheats. In this way the evolutionary dynamics of these species and others from the genus *Triticum* can be maintained (Hammer 1993).

More complicated is the on-farm conservation of less spectacular species, but for them there is also a growing chance in eco-farming. The basis for the new beginning can be provided by the genebanks.

A special case are introgressions as they have been described with rye. The cases of coincidence of the cultivation of landraces of rye and the presence of wild rye (*Secale strictum*) in the neighbourhood require a combination of on-farm and *in situ* preservation. This combination of farm preservation and nature protection is new and should be developed in future. Several problems have to be solved for such an integrated approach, among others the danger for the genetic integrity of the wild populations, as has been discussed already with *Brassica*.

In all the proposed cases the role of the genebanks can be seen as a catalytic one, combining the efforts of farmers and natural protection, but also as critical observers not only of the process of genetic erosion but also of the ongoing evolution; and, last not least, as back-up for safety duplications. This goes far beyond the traditional work of genebanks as *ex situ* conservation, characterization, evaluation and utilization units.

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Résumé

Les ressources phytogénétiques dans l'Italie du sud et en Sicile - études visant la conservation in situ *et* in horto

Des missions d'exploration en Italie méridionale et en Sicile ont été menées conjointement par l'Istituto del Germoplasma (Bari, Italie) et l'Institut des ressources phytogénétiques et de la recherche sur les cultures (Gatersleben, Allemagne) depuis 1980. Près de 2000 accessions menacées d'érosion génétique ont été collectées et intégrées dans les banques de gènes participantes par leur conservation *ex situ*. Cette activité a été complétée par des études de terrain systématiques sur les plantes cultivées et les espèces sauvages apparentées. Des données ont été obtenues sur la floraison des plantes cultivées de la région, notamment des chrono-éléments et des géo-éléments. L'importance de cette région du point de vue de l'évolution est démontrée par les exemples frappants d'introgression de gènes sauvages dans les pools géniques cultivés et *vice versa*, ce qui devrait permettre d'étudier la progression de l'érosion génétique. Cependant, les races locales subsistent encore en nombre relativement élevé, principalement dans les vergers, mais aussi en conditions naturelles, ce qui pourrait servir de base pour la conservation *in situ* et *in horto*. Notre travail est à l'origine d'une prise de conscience considérable de la valeur de l'engrain (*Triticum monococcum*) et de l'amidonnier (*Triticum dicoccum*). Aujourd'hui, les surfaces occupées par ces céréales sont beaucoup plus étendues qu'au début des années 80, époque à laquelle ces espèces ne représentaient que de rares cultures résiduelles dans des zones très circonscrites de l'Italie.

Resumen

Recursos fitogenéticos en Italia meridional y Sicilia - Estudios para la conservación in situ *y en la finca*

Desde 1980 el Instituto del Germoplasma (Bari, Italia) y el Instituto de Investigación Agrícola y Fitogenética (Gatersleben, Alemania) realizan conjuntamente misiones de exploración en Italia meridional y Sicilia. Se han recogido e incorporado en los bancos de genes participantes para su conservación *in situ* unas 2000 accesiones amenazadas por la erosión genética. Esta actividad se ha complementado con estudios regulares de campo sobre plantas cultivadas y sus parientes silvestres. Se ha obtenido información sobre la flora de las plantas cultivadas de la zona, incluidos los cronoelementos y geoelementos. Los sorprendentes ejemplos de introgresión de los acervos génicos silvestres en los cultivados y viceversa, muestran la importancia de la zona desde el punto de vista evolutivo. Se podría examinar el avance de la erosión genética. Sin embargo, todavía hay un número relativamente grande de variedades locales, sobre todo en jardines, pero también en condiciones normales, que deben ser la base para la conservación *in situ* y en la finca. Nuestro trabajo ha contribuido considerablemente a divulgar el valor del trigo almidonero (*Triticum monococcum*) y la escaña menor (*Triticum dicoccum*). Hoy las superficies cultivadas de estos cereales son más extensas que a comienzos del decenio de 1980, cuando estas especies eran cultivos extremadamente raros en algunas zonas circunscritas de Italia.

Plant Genetic Resources Newsletter, 1995, No. 103: 24-26 ARTICLE

Significance of morphological variability in *Solanum insanum* L. (*sensu lato*)

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Summary
Morphological studies made on 56 *Solanum insanum* (*sensu lato*) accessions from India, Sri Lanka and Bangladesh showed wide variability in most of the 20 observed characters. Only about one-third of the accessions matched closely the existing description of *S. insanum* (or *S. melongena* var. *insanum*). The rest differed in having one or a few features which have so far been regarded as being restricted to *S. incanum* and *S. melongena*. Presence of advanced eggplant (*S. melongena*) features like unarmed stem and leaves, large leaves and purple fruits of large size indicate that at least some *S. insanum* accessions may be descendants of *S. melongena*. Close morphological similarity between the two favours the treatment of *S. insanum* as a variety of *S. melongena*. Relatively free gene flow between *S. melongena*, *S. incanum*, its putative progenitor, and *S. insanum* has probably been the basis for the extensive variability existing in the wild and weedy forms.

Introduction
Solanum insanum L., a species closely resembling *S. melongena* L. (eggplant or brinjal), is found in the plains of India and adjoining countries growing in a semiwild state around village habitations. It resembles *S. melongena* in several features: presence of prickles, stellate pubescence, andromonoecious inflorescence, elliptic or lanceolate floral calyx which becomes accrescent in fruit, petals shallowly divided and fleshy fruits (Roxburgh 1832; Prain 1903; Duthie 1911; Gamble 1921). The characters that according to the above workers distinguish *S. insanum* are its wild or semiwild occurrence, high prickliness and small (ca. 2.5 cm diam.) oval or spherical, often white, inedible fruits; *S. melongena*, on the other hand, being cultivated, is often not at all prickly, and has large variously shaped and coloured edible fruits. The interrelationships and the taxonomic status of *S. insanum* are still not fully resolved. Thus, while Bhaduri (1951) regarded it as the closest progenitor of eggplant, others (Prain 1903; Duthie 1911; Gamble 1921; Lester and Hasan 1991) considered *S. melongena* as the original species and *S. insanum* as its feral derivative. Furthermore, whereas Linnaeus (1753) and Roxburgh (1832) gave the taxon a status of distinct species, others (Prain 1903; Duthie 1911; Gamble 1921; Lester and Hasan 1991) treated it as a variety of *S. melongena*. Deb (1989) made a prominent departure from the above treatments by suggesting the merger of *S. insanum* and *S. melongena* under another closely related species, *S. incanum* L. The author reported the existence of overlapping variability among the three taxa which allegedly rendered their distinction quite arbitary. Hepper (1987) in his enumeration of Solanaceae from Sri Lanka also mentioned the polymorphism prevalent in *S. insanum* populations. Neither of the authors, however, provided significant morphological details. We grew 56 accessions of *S. insanum* (*sensu lato*) collected from various locations in India, Bangladesh and Sri Lanka. Preliminary studies on the variability of morphological characters having bearing on its taxonomic treatment and relationships are reported here.

Materials and methods
Seeds of 56 accessions were sown in nursery beds in the experimental field of this institute. Seedlings (1 month old) were transplanted to the field to single rows of 15 plants per accession with interplant distances of 60 cm between rows and 40 cm within rows. Data on 20 morphological characters (Table 1) were recorded from five randomly selected plants of each accession, excluding border plants. Mean values were used for the analysis of quantitative data.

Results and discussion
Wide interaccession variability was recorded in most of the observed characters (Table 1, Fig. 1). Consequently, only about one-third of the accessions matched unambiguously with the rather uniform description of *S. insanum* (or *S. melongena* var. *insanum*) as provided by Roxburgh (1832), Prain (1903), Duthie (1911) and Gamble (1921). The rest differed in having variations that have been hitherto considered as diagnostic of *S. incanum* (as described from India) and *S. melongena* but not known in *S. insanum*. A triangular calyx, a feature characterizing *S. incanum* (Clark 1883, as *S. coagulans* Forsk.) was found in accessions IC-89925, IC-111380 and EC-316277. The lobing of corolla ranged between shallow, a *S. insanum* feature, to as deep as that of *S. incanum* (IC-89925, EC-316235, EC-316278 and EC-316289). Some features such as absence of prickles, relatively large leaves, and large and purple fruits, normally identified with advanced cultivars of *S. melongena*, were also recorded. Accessions EC-316212, EC-316224, EC-316225, EC- 316231, EC-316248, EC-316256,

Table 1. Morphological variation among 56 accessions of *S. insanum* (figures in parentheses represent % accessions having indicated form of a qualitative character)

Character	Variation pattern		CV	Attribute
	Mean	Range		
Habit				erect (44.44), shrubby (48.1), decumbent (7.41)
Prickliness				nonprickly (26.67), prickly (73.33)
Leaf length (cm)	10.62	4.5-14.9	22.542	
Prickles/leaf	8.38	0-25.5	72.970	
Maximum flowers/cluster	4.52	2-8	27.744	
Calyx shape of fertile flowers				triangular (8.93), elliptic (48.21), lanceolate (17.86), oblong-linear (25.00)
Petal lobing				shallow (92.86), deep (7.14)
Fruits/inflorescence	1.34	1-3	42.978	
Fruit pedicel length (cm)	2.7	1.5-4.3	22.885	
Prickles/pedicel	1.2	0-6	116.239	
Fruit length (cm)	3.1	1.9-4.7	34.363	
Fruit breadth (cm)	2.9	2.0-3.9	18.129	
Calyx length (cm)	1.4	0.6-2.3	25.820	
Calyx length as % fruit length	44.4	30.0-63.6	17.671	
Prickles/calyx	2.61	0-12.8	120.170	
Fruit weight (g)	12.88	3.5-35	56.319	
Fruit shape				globose (66.6), ovoid (33.3), oblong (1.1)
Fruit colour				variegated green (69.7), milky white (9.1), variegated green with grey purple patches (12.1), pink to purple (9.1)
Fruit apex				round (87.9), tapering (9.1), slightly depressed (3.0)
Fruit taste				bitter non-edible (100), edible (0)

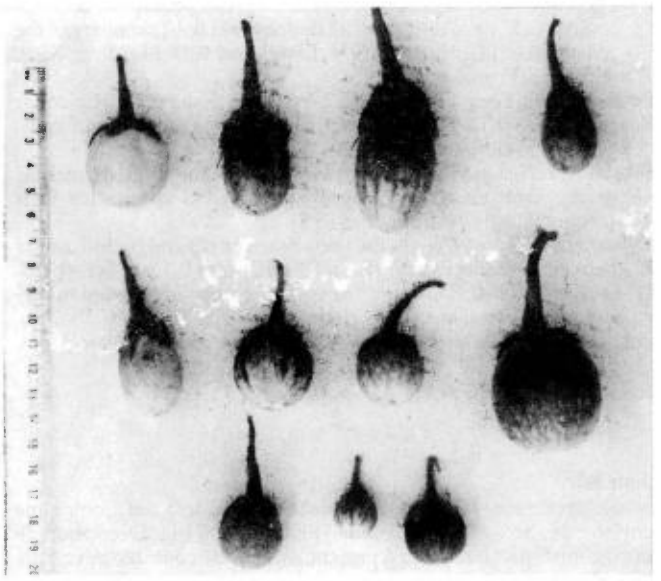

Fig. 1. Fruit variability in *S. insanum*. Each fruit represents a different accession

EC-316258, EC-316264, EC-316277, EC-316278, EC-316289, EC-316295, EC-316297, EC-316303, EC-316304 and NIC-4099 were totally free of prickles. Leaves as long as 13-15 cm were recorded in accessions IC-90061, EC-316223-2, EC- 316235, EC-316274-2, EC-316278 and NIC-4099. Fruit showed wide variation in size, shape and colour. Seven accessions (EC-316224, EC-316227, EC-316235, EC-316253, EC-316275, EC-316302 and NIC- 4262) had immature fruits in different shades of purple, a fruit colour not reported earlier in *S. insanum*. Relatively large fruits (of over 20 g) were recorded in EC-316222, EC-316264, EC- 316302 and NIC-4237. The resemblance of some of the accessions having unarmed stem, moderately large leaves and purple fruits with some of the small-fruited eggplant cultivars was so close that the two could not be distinguished except by the fruit taste, which was bitter in *S. insanum*. Thus on a morphological basis the treatment of *S. insanum* as a variety of *S. melongena* rather than a distinct species is justified.

Several studies have shown that *S. incanum* is the most likely progenitor of eggplant (Pearce and Lester 1979; Lester and Hasan 1991; Sakata *et al.* 1991). Maximum variability of the former is available in East Africa (Whalen 1984) whence some forms are supposed to have migrated to the eastern hemisphere and evolved into the edible *S. melongena* (D'Arcy and Pickett 1991). There exist practically no crossability barriers between *S. melongena*, *S. insanum* and *S. incanum*. *S. insanum* and Southwest Asian as well as Indian forms of *S. incanum* are crossable with *S. melongena*, yielding fertile hybrids (Bhaduri 1951; Narsimha Rao 1979; Pearce and Lester 1979; Zohary 1983). Even natural cross-pollination between *S. melongena* and *S. incanum* from India has been observed that resulted in different types of fruits ranging between those of the two parents (Viswanathan 1975). This more or less free exchange of genes within and between wild *S. incanum* and cultivated *S. melongena* has probably been the cause of the large morphological diversity among the weedy forms existing in South and Southeast Asia. That some of the present *S. insanum* accessions are of recent hybrid origin is revealed by the polymorphism observed in their populations. Both prickly and non-prickly plants

were observed in accessions IC-111312, EC-316217, EC-31622 and EC-316248. EC-316291 had green-fruited as well as purple-fruited plants. Large-fruited and small-fruited plants were observed in IC-111360 and EC-316275. In the latter, with most plants having oval fruits of average 15 g, one plant with as large as 156 g fruits was recorded. But for its bitter fruit, the plant could easily pass for an eggplant cultivar. In EC-316228 having purple-prickled plants, one green-prickled plant was found. Segregation for large and small leaf types was recorded in EC-316228 and EC-316235.

As is evident from the above discussion, the weedy relatives of *S. melongena* grouped under *S. insanum* (*sensu lato*) are probably a complex assemblage of progenitor and derived forms. Detailed systematic studies to resolve the interrelationships should prove extremely interesting. While the present collections along with those of Lester and Hasan (1991) constitute a fairly good representation of weedy forms from South and Southeast Asia, it is important to include accessions from China, West Asia and Southwest Asia in studies on eggplant origin and evolution. These materials should also be of interest to breeders since eggplant wild relatives, including *S. incanum*, are known to possess genes for resistance to several important diseases and pests (Kalloo 1993). Transferring the resistance to cultivars may be fairly straightforward in view of the generally high fertility of hybrids of *S. melongena*, *S. incanum* and *S. insanum*.

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Résumé

Importance de la variabilité morphologique de Solanum insanum *L.* (sensu lato)

Des études morphologiques réalisées sur 56 souches de *Solanum insanum* (*sensu lato*) originaires d'Inde, Sri Lanka et Bangladesh ont montré une grande variabilité de la plupart des 20 caractères observés. Seul un tiers environ des accessions correspondaient de près à la description de *S. insanum* (ou *S. melongena* var. *insanum*). Les autres variaient d'une ou deux caractéristiques qui, jusqu'à présent, avaient été considérées comme n'appartenant qu'à *S. incanum* et *S. melongena*. La présence de caractéristiques plus propres à l'aubergine (*S. melongena*) telles que la tige et les feuilles inermes, la feuille large et les fruits violets de grande taille indiquent que quelques unes, au moins, des accessions de *S. insanum* peuvent être des descendances de *S. melongena*. Une étroite similarité morphologique entre les deux permet de traiter *S. insanum* comme une variété de *S. melongena*. Le flux de gènes relativement libre entre *S. melongena*, *S. incanum*, son géniteur probable, et *S. insanum* est sans doute à l'origine de la grande variabilité constatée entre les formes sauvages et adventices.

Resumen

Significado de la variabilidad morfológica en Solanum insanum *L.* (sensu lato)

Estudios morfológicos sobre 56 accesiones de *Solanum insanum* (*sensu lato*) procedentes de India, Sri Lanka y Bangladesh mostraron una gran variabilidad en la mayoría de los 20 caracteres observados. Sólo alrededor de una tercera parte de las accesiones correspondía casi completamente a la descripción existente de *S. insanum* (o *S. melongena* var. *insanum*). El resto se diferenciaba por tener una o unas cuantas características que hasta la fecha se han considerado propias del *S. incanum* y *S. melongena*. La presencia de características desarrolladas propias de la berenjena (*S. melongena*), como tallos y hojas sin espinas, grandes hojas y frutos de color púrpura de gran tamaño, indican que por lo menos algunas accesiones de *S. insanum* pueden descender de *S. melongena*. Las grandes semejanzas morfológicas entre las dos contribuyen a que se considere al *S. insanum* como una variedad del *S. melongena*. Probablemente un flujo relativamente libre de genes entre *S. melongena*, *S. incanum*, su progenitor putativo, y *S. insanum*, fue el principio de la extensa variabilidad presente actualmente en las formas silvestres y herbáceas.

Factors affecting the cryopreservation of coffee, coconut and oil palm embryos

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Summary

This paper describes the importance of various parameters which can significantly influence the cryopreservability of zygotic and somatic embryos of several recalcitrant and intermediate seed species (coffee, coconut, oil palm). Embryos should be used only when they are in an optimal physiological state as regards notably their maturity and metabolic status. Modifications of recovery conditions can greatly increase the survival rate of zygotic embryos. In the case of oil palm somatic embryos, pregrowth on medium with high sucrose concentration is necessary to induce tolerance to desiccation and cryopreservation.

Introduction

Seeds of a large number of tropical, subtropical and temperate species have been termed recalcitrant (Roberts 1973) since they are sensitive to desiccation and can thus be conserved for short periods only (weeks-months) even in the optimal moisture conditions. Careful adjustment of the storage environment (humidity, temperature) led to improvements in the conservation duration for several of these species such as oil palm and coffee (Ellis *et al.* 1990, 1991) which are now considered intermediate in their seed storage behaviour. Nevertheless, long-term storage of these seeds still remains impossible.

Large-scale multiplication processes by means of somatic embryogenesis in liquid medium are being set up for some species of commercial importance with recalcitrant or intermediate seed storage behaviour, such as oil palm and coffee (Vasil 1991). This leads to the development of an increasing number of strains, which creates laboratory management problems. Moreover, the risks of somaclonal variation increase in line with the duration of *in vitro* culture. Cryopreservation (liquid nitrogen, -196°C) currently represents the only long-term conservation option for zygotic and somatic embryos of these species.

Various cryopreservation protocols have been developed for zygotic embryos of a large number of species, among them many with recalcitrant seed storage behaviour (Table 1). This is not the case with somatic embryos, for which only a limited number of studies have been conducted until now. Research performed on cryopreservation of embryos presently concerns mostly methodological aspects and only a limited amount of work deals with the understanding of biological mechanisms in relation to cryopreservation. Various methods are employed for freezing embryos (Engelmann 1992): classical protocols, comprising pregrowth with cryoprotectants and slow freezing, and encapsulation/dehydration (Dereuddre *et al.* 1991) are used for somatic embryos. Most zygotic embryos are frozen rapidly after partial desiccation. Finally, a new desiccation technique termed flash-drying has been developed with zygotic embryos of *Landolphia kirkii* (Berjak *et al.* 1990). Flash-drying was followed by freezing at an intermediate (Vertucci *et al.* 1991) or ultra-rapid rate (Wesley-Smith *et al.* 1992).

In this article, we present results concerning the importance of various parameters on the cryopreservation of zygotic and somatic embryos of several species with recalcitrant or intermediate seeds and discuss their practical implications. This paper includes results already obtained by the ORSTOM research team either in Montpellier or in collaboration with other institutes.

Cryopreservation of zygotic embryos: coffee, coconut and oil palm

The experiments performed with coffee, coconut and oil palm zygotic embryos aimed at setting up cryopreservation processes for these materials. However, they also allowed the determination of the importance of various parameters such as the maturity and physiological stage of embryos before cryopreservation and the recovery medium.

Embryos of *C. arabica* were frozen at two different maturity stages, as determined by the colour of the fruit: immature embryos extracted from green fruits (i.e. 2

Table 1. Present application of cryopreservation for somatic and zygotic embryos of plant species

Species	Reference
Somatic embryos	
Brassica napus	Uragami *et al.* 1993
Citrus sinensis	Marin and Duran Vila 1988; Marin *et al.* 1993
Coffea arabica	Bertrand-Desbrunais *et al.* 1988
Coffea canephora	Bertrand-Desbrunais 1991; Hatanaka *et al.* 1994; Tessereau *et al.* 1994
Cucumis melo	Shimonishi *et al.* 1991
Daucus carota	Withers 1979; Tessereau *et al.* 1994
Elaeis guineensis	Engelmann *et al.* 1985; Dumet *et al.* 1993a
Juglans	de Boucaud *et al.* 1994
Manihot esculenta	Sudarmonowati and Henshaw 1990
Xanthosoma	Zandvoort 1987
Zygotic embryos	
Aesculus hypocastanea	Pence 1990
Arachis hypogaea	Runthala *et al.* 1993
Araucaria excelsa	Pritchard and Prendergast 1986
Artocarpus heterophyllus	Krishnapillay 1989
Brassica napus	Withers 1982
Carva	Pence 1990
Camellia sinensis	Chaudhury *et al.* 1991
Castanea	Pence 1990
Citrus sinensis	Radhamani and Chandel 1992
Cocos nucifera	Chin *et al.* 1989; Assy-Bah and Engelmann 1992b
Coffea	Normah and Vengadasalam 1992; Abdelnour *et al.* 1992
Corylus avellana	Gonzales-Benito and Perez 1994; Normah *et al.* 1986; Reed *et al.* 1994
Elaeis guineensis	Grout *et al.* 1983
Fagus	Pence 1990
Hevea brasiliensis	Normah *et al.* 1986
Hordeum vulgare	Withers 1982
Howea fosteriana	Chin *et al.* 1988
Juglans	Pence 1990
Landolphia kirkii	Vertucci *et al.* 1991
Manihot esculenta	Marin *et al.* 1990
Musa	Abdelnour *et al.* 1992
Olea europaea	Gonzales-Rio *et al.* 1994
Phaseolus vulgaris	Zavala and Sussex 1986
Pisum	Mycock *et al.* 1989
Poncirus trifoliata	Radhamani and Chandel 1992
Prunus amygdalus	Chaudhury and Chandel 1994
Prunus persica	de Boucaud and Brison 1991
Quercus	Pence 1990
Triticum	Bajaj 1983
Triticale	Bajaj 1983
Theobroma cacao	Pence 1991
Veitchia merrillii	Chin *et al.* 1988
Zea mays	Delvallée *et al.* 1989

months before harvest) and mature embryos (i.e. 1 week before harvest). Even though the desiccation period ensuring the highest survival rates was similar for both categories of embryos (Table 2), 96% of mature embryos withstood cryopreservation but 50% only of immature ones (Abdelnour *et al.* 1992). However, the lower survival of immature embryos could be almost totally overcome by placing them for recovery on a modified medium supplemented with 100 mg/l gibberellic acid (GA_3). In-

Table 2. Effect of maturity stage and desiccation period on the survival of control (-LN) and cryopreserved (+LN) zygotic embryos of *Coffea arabica* (from Abdelnour *et al.* 1992)

Desiccation (hours)	Survival (%)			
	Immature embryos		Mature embryos	
	-LN	+LN	-LN	+LN
0.0	100	0	100	0
0.5	80	50	100	96
1.0	53	34	56	42
1.5	25	14	28	19
2.0	6	0	8	0

deed, in these conditions, survival of cryopreserved immature embryos increased up to 83%. This result demonstrates that the difference in survival noted between the two categories of embryos was not due to a greater sensitivity to desiccation and freezing of immature embryos but to the fact that they were placed for recovery in non-optimal conditions.

Experiments performed with coconut embryos confirmed these observations. High survival rates could be obtained after freezing immature embryos (7-8 months after pollination) but only a limited number of them could develop into plantlets (Assy-Bah and Engelmann 1992a). On the other hand, most mature embryos (11-12 months after pollination) withstood cryopreservation and gave rise to whole plants after freezing (Assy-Bah and Engelmann 1992b). This was due to inadequate recovery conditions for immature embryos (Assy-Bah 1992).

Partial desiccation of zygotic embryos is necessary to obtain survival after cryopreservation. This treatment generally induces a drop in survival in comparison with untreated control embryos. In some cases, modifications in the regrowth pattern of cryopreserved embryos are also observed, such as the non-development of the haustorium of *Veitchia* and *Howea* (Chin *et al.* 1988) and coconut embryos (Assy-Bah and Engelmann 1992b). Experiments performed with oil palm zygotic embryos extracted from hydrated seeds showed that damage could be more severe than those observed with *Veitchia*, *Howea* or coconut embryos (Engelmann *et al.* 1995). Embryos desiccated down to 0.3 g H_2O/g dry weight (DW) and frozen in liquid nitrogen showed high survival rates (90%). However, desiccation induced irreversible damages to some embryos since only 60% of them could develop into plantlets, the others displaying abnormal development (callusing, development of root pole or haustorium only). Another experiment performed with oil palm zygotic embryos showed the importance of their physiological state before cryopreservation. Embryos extracted from rehydrated seeds were desiccated down to the water level of embryos in dry seeds (0.12 g/g DW). In parallel, dry seeds were frozen directly or after partial rehydration

until the water content of embryos reached 0.3 g/g DW, which ensured the highest survival rate with embryos extracted from rehydrated seeds and desiccated before cryopreservation. Embryos were considered surviving when they showed any sign of regrowth, whereas only embryos which developed into a whole plantlet were considered recovered. Survival of embryos was high in all conditions (70-96%). However, recovery of embryos frozen with a water content of 0.12 g/g DW, either extracted from dry seeds or dehydrated to this level, was very low, whereas that of embryos extracted from partially rehydrated seeds was comparable to that of embryos extracted from hydrated seeds and desiccated to 0.3 g/g DW. This increased tolerance may be linked with metabolic changes occurring during imbibition of seeds. Indeed, imbibition very rapidly induces dramatic metabolic changes such as mobilization of stored carbohydrate and lipid reserves and protein synthesis (Bewley and Black 1983). Notably, the degradation of starch which is present in large quantities in oil palm embryos (Vallade 1965) may lead to a rapid increase in the concentration of soluble sugars, which play a crucial role in the acquisition of tolerance to desiccation, by substituting for water in stabilizing membranes in the dry state (Crowe and Crowe 1986) and/or by inducing intracellular vitrification at ambient temperature, thus ensuring subcellular stability in the dry state (Williams and Leopold 1989).

Cryopreservation of somatic embryos: oil palm

Classical cryopreservation protocols including cryoprotective treatment in liquid medium followed by slow cooling are generally employed for somatic embryos (Engelmann 1992). However, an original protocol has been developed recently for oil palm somatic embryos (Dumet *et al.* 1993a). It comprised a 7-day pregrowth treatment of embryos on solid medium followed by partial desiccation (16 hours with silica gel) before freezing. These experiments showed the importance of pretreatment with sucrose for the acquisition of resistance of embryos to desiccation and to cryopreservation (Dumet *et al.* 1993b). Indeed, survival of non-pregrown embryos decreased in line with increasing desiccation periods. Without pregrowth treatment, no survival was obtained after freezing in liquid nitrogen whatever the dehydration duration. With pregrown control embryos, 100% survival was obtained whatever the desiccation period. Survival after freezing in liquid nitrogen was possible at a low rate (40%) without desiccation but it was significantly improved (up to 80-90%) after extended desiccation. Thermal analysis using differential scanning calorimetry revealed that these differences in survival rate could be correlated with differences in the thermal events recorded in embryos during freezing. Non-pregrown embryos displayed crystallization peaks, indicating lethal ice formation, whatever the desiccation period. On the contrary, the increase in survival of pregrown embryos in line with increasing desiccation durations was correlated with the progressive disappearance of crystallization peaks and their replacement by glass transitions. The evolution of sugar concentration in embryos was followed during the pregrowth treatment on medium with high sucrose concentration (Dumet *et al.* 1994a). Sucrose was predominantly accumulated (10-fold increase), whereas glucose and fructose concentration remained constant. Arabinose was the only new sugar detected but its concentration remained very low. Starch accumulation (20-fold increase) was also noted.

Experiments were also performed in order to evaluate the specificity of sucrose in the acquisition of tolerance of oil palm somatic embryos to desiccation and freezing (Dumet *et al.* 1994a). Embryos were pregrown on media containing various sugars or polyols at the same osmolarity. Only sucrose allowed survival after freezing in liquid nitrogen when embryos had not been dehydrated. However, when embryos had been dehydrated, several compounds (galactose, fructose, raffinose) ensured survival rates comparable to that obtained with sucrose. Thus, sucrose seems to have a high specificity in the acquisition of tolerance to freezing of embryos with high water levels, whereas it has a low specificity in the acquisition of tolerance to freezing of embryos with a low water level.

Experiments concerning the effect of various storage temperatures (-12, -80 or -196°C) on the survival of embryos were performed (Dumet *et al.* 1994b). After pregrowth treatment on medium with high sucrose concentration, embryos were desiccated or not before storage. Control embryos were stored at -196°C. Embryos stored at -12 and -80°C were either placed directly at these temperatures or immersed for 5 minutes in liquid nitrogen and then transferred at -12 or -80°C. The evolution of survival was recorded over a 6-month period. Non-desiccated clumps of embryos did not withstand 1 month of storage at -12°C and only 6% of clumps placed at -80°C survived after 3 months if they had been briefly immersed in liquid nitrogen before the storage period. Survival of desiccated clumps stored at -12°C after 6 months decreased progressively down to 27% with and 3% without transitory immersion in liquid nitrogen before storage. Survival of desiccated clumps of embryos stored at -80°C did not vary during the experiment and was comparable to that of clumps cryopreserved in liquid nitrogen (87-100%).

This experiment indicated the importance of dehydration of oil palm somatic embryos before storage, since desiccated clumps could be conserved for 6 months at -80°C without any viability loss in comparison with control embryos stored at -196°C. These results should be due to the fact that all free water has been removed from embryos during desiccation, thus allowing vitrification of intracellular solutes to take place during freezing, as was observed with thermal analysis (Dumet *et al.* 1993b). Moreover, glass transitions were recorded at temperatures between -50 and -60°C. Therefore, embryos stored at -80°C

are in a stable state and may be conserved at this temperature for extended periods without decrease in survival. These results may be of great interest for short-term storage of embryos since freezing in liquid nitrogen appears not to be required for storage periods up to 6 months.

Finally, this cryopreservation protocol was applied to 39 different clones of oil palm somatic embryos (Dumet *et al.* 1993c). This underlined the effect of the physiological state of the cultures on their resistance to cryopreservation. The average survival rate of clones in a good physiological state (i.e. displaying a normal aspect and growth characteristics) was 31% and 12% only for clones in a poor physiological state.

Conclusion

The experiments performed with zygotic and somatic embryos of these various species indicate the importance of several parameters, which should be taken into account when developing cryopreservation protocols for embryos.

Embryos should be used for cryopreservation only when they are in an optimal physiological state since this can greatly influence survival after freezing, as already showed notably in the case of cell suspensions (Withers 1985): zygotic embryos should be selected at a developmental stage at which their *in vitro* culture is fully operational and at which they display a high metabolic activity. Moreover, attention should be paid to the recovery conditions, since slight modifications can significantly enhance recovery rates. The experiments performed with oil palm somatic embryos underlined the important role of sugars, particularly sucrose, for the acquisition of tolerance to desiccation and cryopreservation. Pregrowth treatments on media with high sucrose concentration should be tried with zygotic embryos. These treatments may increase their tolerance to desiccation, thus reducing the extent of damage generally observed.

In conclusion, more fundamental research is needed to understand the mechanisms involved in the acquisition of tolerance to these stresses. In this aim, zygotic and somatic embryos from recalcitrant-seed species should be excellent materials to study desiccation tolerance/sensitivity, since desiccation appears as the key step in most cryopreservation protocols developed for embryos.

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Résumé

Facteurs influant sur la cryoconservation des embryons de caféier, cocotier et palmier à huile

Cet article décrit l'importance de divers paramètres qui peuvent avoir une influence significative sur les possibilités de cryoconservation des embryons zygotiques et somatiques de plusieurs espèces à semences récalcitrantes et intermédiaires (caféier, cocotier, palmier à huile). Les embryons ne doivent être utilisés que si leur état physiologique est optimal, notamment en ce qui concerne leur degré de maturité et leur état métabolique. La modification des conditions de reprise peut grandement améliorer le taux de survie des embryons zygotiques. Dans le cas des embryons somatiques de palmier à huile, une préculture sur un milieu à forte concentration en saccharose est nécessaire pour induire la tolérance à la dessiccation et à la cryoconservation.

Resumen

Factores que afectan la crioconservación de embriones del café, el coco y la palma de aceite

En este artículo se describe la importancia de diferentes parámetros que pueden influir considerablemente en la crioconservabilidad de embriones cigóticos y somáticos de varias especies de semillas recalcitrantes e intermedias (café, coco, palma de aceite). Los embriones deben utilizarse sólo cuando se encuentren en óptimas condiciones fisiológicas, sobre todo por lo que se refiere al grado de madurez y el estado metabólico. Las modificaciones de las condiciones de regeneración pueden aumentar enormemente la tasa de sobrevivencia de los embriones cigóticos. En el caso de los embriones somáticos de palma de aceite, se necesita el precultivo sobre un medio con una elevada concentración de sacarosa para inducir la tolerancia a la desecación y la crioconservación.

Plant Genetic Resources Newsletter, 1995, No. 103: 32-35 ARTICLE

Alginate-coated nodal segments of yam (*Dioscorea* spp.) for germplasm exchange and distribution

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Summary

The nodal segments encapsulation technique using alginate-MS (Murashige and Skoog) medium was used as an alternative method for *in vitro* exchange and distribution of yam germplasm. Nodal segments (3-4 mm) were used as the explants. The explants were encapsulated in MS medium containing 3% (w/v) Na-alginate supplemented with either 0.0, 0.1, 0.3, 0.5 or 1.0M sucrose. The percentage of shoot formation from coated nodal segments was delayed with increasing sucrose contents in the beads. Beads with very high (1.0M) sucrose content caused a high rate of lethality to the coated nodal segments. Results indicated that the alginate-MS solutions supplemented with 0.1-0.3M sucrose are the most suitable media for coating nodal segments of yams for germplasm exchange and distribution. Beads can be distributed in the cryotubes containing semisolid MS medium.

Introduction

Transfer of plant genetic resources in the form of tissue culture is one of the safest and most efficient methods for exchange of vegetative materials (IBPGR 1988; Ng 1991, 1992). In common practice, for distribution of yam germplasm, shoot and nodal cuttings of yam plantlets are inoculated into the tubes containing semisolid medium and cultures for 2-3 weeks to induce rooting and to observe any microbial contamination (Ng 1991). They are then packed and despatched to the recipients by air mail or are hand-carried. However, the plantlets in the culture tubes are in danger of damage due to the unfavourable and prolonged dark conditions during transportation. Hence, the recovery rate of the plantlets after receipt can be reduced significantly to a very low level (IBPGR 1988; Ng 1988).

Microtubers have been used instead of plantlets for distribution of yam germplasm. Microtubers can be kept in the dark for a long period of time and thus can withstand packaging and transportation (Ng 1988). However, this means of transportation is limited to those varieties capable of producing microtubers (Jean and Cappadocia 1991).

This communication describes an alternative method for *in vitro* exchange of yam germplasm by transfer of nodal segments in alginate-coated beads after culturing in cryotubes containing semisolid MS medium. The availability of nodal segments is unlimited as they can easily be obtained from established plantlets (Hasan 1994; Mantel *et al.* 1978). Nodal segments encapsulated in alginate beads are more practical for handling and are protected against the adverse effects of unfavourable conditions during transit.

The alginate-encapsulation technique was originally developed for the production of so-called "artificial seeds", consisting of somatic embryos encapsulated in alginate beads. A similar method was then used to develop an encapsulation-dehydration technique for cryopreservation of the germplasm (Dereuddre *et al.* 1990; 1991b; JICA 1993). In the present study, an attempt was made to adopt such a technique for the purpose of exchange and distribution of yam germplasm.

The presence of sucrose in the alginate-MS solution plays an important role in determining the survival and regrowth of the coated segment. It was found that increasing the sucrose concentration in the bead can delay the regrowth of the encapsulated explants, and thus was useful for germplasm exchange and distribution. However, high levels of sucrose were toxic to the explants (Kitto and Janick 1985; Paulet *et al.* 1993; Uragami 1993). Therefore, it is necessary to test a range of sucrose concentrations in order to determine the most suitable concentration for supplementation in the beads. This study was concerned with the sucrose as the protective agent in the alginate beads. Subsequently, the nodal segments could be transited safely without deterioration or loss of viability.

Materials and methods

Plant materials

In vitro plantlets of yams were grown on Murashige and Skoog's (MS) basal medium supplemented with sucrose at 30 g/L and 0.8% (w/v) agar. Nodal cuttings were micropropagated from the plantlets previously cultured and kept in the incubator room at 25°C under a photoperiod of 16/8 hours light/dark (Hasan 1994). Light was

supplied by daylight fluorescent light tubes producing an intensity of 4000-5000 lux at plant level. Three species were used: *Dioscorea alata* (DA 005), *D. opposita* (DO 002) and *D. rotundata* (DR 002).

Encapsulation

Single nodal cuttings with one axillary bud 3-4 mm long were aseptically obtained from 4 to 5-month-old disease-free plantlets. After dissection, the nodal segments were suspended in the calcium-free liquid MS medium supplemented with 3% (w/v) Na-alginate and combined with either 0.0, 0.1, 0.3, 0.5 or 1.0M sucrose. The alginate solution with the single cutting was sucked through a 10-mm diameter glass pipette and then gently dropped out into 50 ml liquid MS medium consisting of 0.1M $CaCl_2$ and respective sucrose concentration. Cuttings were held for about 30 minutes at 25°C to achieve polymerization of Na-alginate. The alginate beads of about 5-6 mm in diameter containing one segment were collected and each was placed in a 1-ml polypropylene sterile cryotube containing 0.5 ml semisolid MS medium supplemented with 3% (w/v) sucrose and 0.8% agar (Fig. 1). The cryotubes were then closed with a screw cap and the edges sealed with pressure-sensitive laboratory parafilm, and kept under 16 hours of light at 25°C for 1 week.

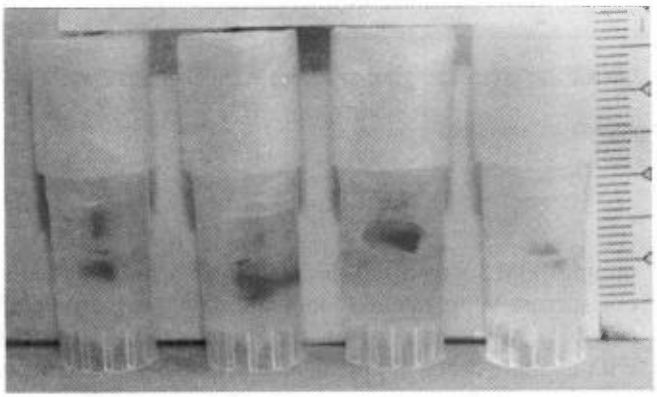

Fig. 1. Alginate beads of 1-ml cryotubes containing semi solid MS medium.

Cryotubes were then transfered into the boxes and kept under 24 hour dark conditions at 25°C for 2 weeks. After that, the beads were recultured onto solidified MS medium supplemented with 3% sucrose and 0.8% agar in Petri dishes and incubated under 16 hours of light at 25°C for about 2 weeks for assessement of recovery growth. At least eight beads were made for each sucrose concentration.

Viability assessment

Assessment of survival of the coated nodal segments was made 3 weeks after treatment and 2 weeks later after transfer onto regrowth medium. The segments were considered alive when the bud had regrown producing new shoots; survival was expressed as a percentage.

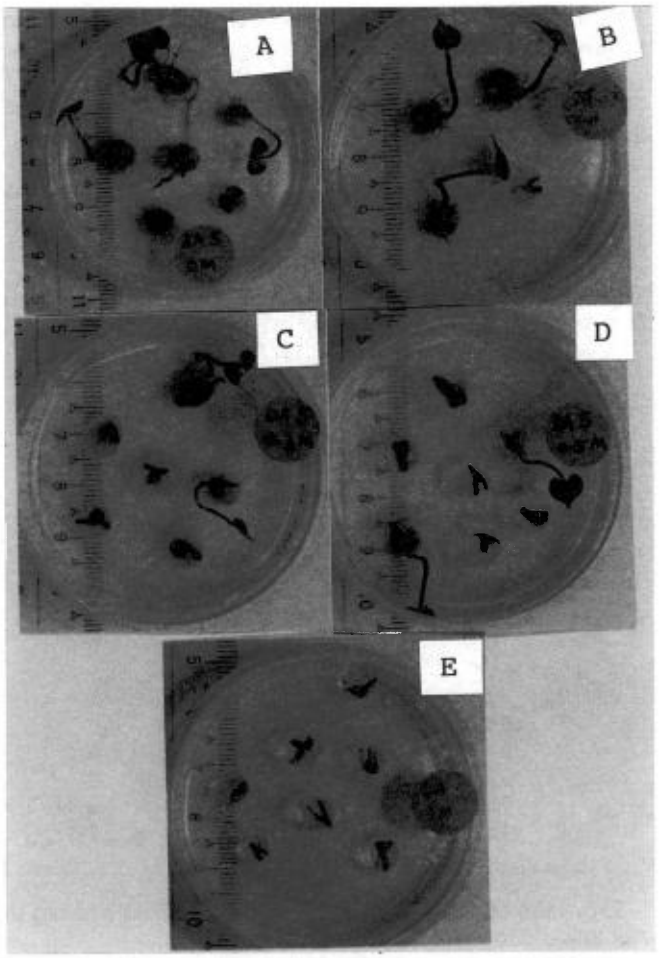

Fig. 2. Coated nodal segments of *Dioscorea alata* (DA 005) after 5 weeks encapsulation (3 weeks in cryotubes + 2 weeks on fresh medium) in alginate MS medium supplemented with various sucrose concentrations: 0.0M (A), 0.1M (B), 0.3M (C), 0.5M (D) and 1.0M (E).

Results

The alginate-encapsulated explants formed whitish, small, shiny beads (Fig. 2). Table 1 lists the effect of sucrose concentration in the beads on the survival of coated nodal segments from *D. alata*, *D. opposita* and *D. rotundata*. The percentage of shoot formation from coated nodal segments was reduced with increasing sucrose content in the beads. Beads containing the lowest sucrose molarity (0.1M) gave the highest proliferation rate, almost equal to those without sucrose supplements. In contrast, beads containing the highest sucrose concentration (1M) had strongly depressed shoot formation and deterioration to the segments.

In *D. alata*, 3 weeks after encapsulating, during which cryotubes were under 24 hour dark conditions for 2 weeks, the alginate beads containing 0.0-0.5M sucrose supported shoot formation from nodal segments (Table 1). The shoot formation had increased 2 weeks later when the beads were transfered onto the fresh medium in the Petri dish (Fig. 2). Beads with 1.0M sucrose supplements failed to initiate growth, even after transfer into the fresh cultures.

Table 1. Survival[†] of the nodal segments of yams (*D. alata*, *D. opposita* and *D. rotundata*) in Na-alginate beads supplemented with different sucrose concentrations

Species	Sucrose molarity in beads	No. encapsulated (beads)	3 weeks after treatments	5 weeks after treatments		
			% with shoots	% with shoots	% green	% deteriorated[‡]
D. alata						
	0.0 M	13	46	62	31	7
	0.1 M	13	38	78	15	7
	0.3 M	13	23	47	38	15
	0.5 M	14	14	29	42	29
	1.0 M	14	0	0	21	79
D. opposita						
	0.0 M	10	30	50	40	10
	0.1 M	12	0	33	50	17
	0.3 M	12	0	33	50	17
	0.5 M	12	0	25	25	50
	1.0 M	13	0	15	38	47
D. rotundata						
	0.0 M	8	0	25	50	25
	0.1 M	9	0	33	33	33
	0.3 M	9	0	0	55	45
	0.5 M	9	0	0	45	55
	1.0 M	9	0	0	22	78

[†] Survival was expressed as percentage of coated segments producing shoots 3 weeks after encapsulating and inoculating in cryotubes and 2 weeks later after transfer onto fresh MS medium in a Petri dish.
[‡] Segments were considered deteriorated when they had changed colour from green to white or pale.

Sucrose at this concentration also caused the highest rate of deterioration (79%) to the coated segments compared with the bead supplemented with 0.0-0.5M sucrose which had between 7 and 29% deterioration rate.

In *D. opposita*, the segments in beads supplemented with 0.1-1.0M sucrose did not produce shoots when inoculated in cryotubes and stored for 2 weeks in boxes (24 hours of dark) (Table 1). Thirty percent of segments in beads without sucrose supplements produced shoots. Two weeks after being returned to the fresh medium, all beads produced shoots ranging from 50% in sucrose-free beads to 15% of 1.0M sucrose-supplemented beads. The deterioration rate of segments was moderate (about 50%) in beads containing 1.0-0.5M sucrose and low (10-17%) in those supplemented with less than 0.3M.

For *D. rotundata*, no shoot formation was observed from nodal segments coated with alginate medium supplemented with any sucrose concentration after 3 weeks (Table 1). On being returned to the fresh medium, only those segments coated with alginate containing 0.0-0.1M sucrose successfully produced shoots. The deterioration rate of nodal segments was high, i.e. 78%, in beads containing 1.0M sucrose and declined to 25% in beads without sucrose supplements. Moderate damage, between 45 and 55%, was observed on the nodal segments coated with alginate-beads supplemented with 0.3-0.5M sucrose.

Discussion

This study showed that the alginate-encapsulated nodal segments can be used as a source of tissue culture for the exchange and distribution of yam germplasm. The high sucrose molarity in the encapsulating medium was responsible for the toxic effect and delayed regrowth of coated segments. There also was variation between species in response to sucrose concentrations in the beads (Table 1).

Nodal segments coated in the beads are not as vulnerable to unfavourable conditions and can be kept in the dark for 2 weeks; this is sufficient time for international germplasm transportation, which is usually by air freight. It is possible to encapsulate the nodal segments of yam into alginate-beads supplemented with 0.1-0.3M sucrose and inoculate into cryotubes containing 0.5 ml semisolid MS medium for international germplasm exchange and distribution. Encapsulation of nodal segments into algi-

nate-medium containing 0.1-0.3M sucrose concentration would delay shoot formation from the explants and thus permit extra time for transportation preparations. However, using alginate-medium with too high, i.e. 0.5M and 1.0M, a sucrose content is not encouraged, since it would cause a high rate of lethality to the coated segments.

Encapsulation of nodal segments into alginate-beads is promising as a practical method for the exchange and distribution of yam germplasm through tissue culture. An additional advantage is that the 1-ml cryotubes used for bead inoculation are smaller and more practical for handling during transportation compared with standard test tubes.

Acknowledgement

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Résumé

Utilisation de segments nodaux d'igname (Dioscorea *spp.) encapsulés dans l'alginate pour l'échange et la distribution de matériel génétique*

La technique consistant à encapsuler des segments nodaux dans du milieu MS (Murashige et Skoog) additionné d'alginate, a été utilisée comme une méthode alternative d'échange et de distribution *in vitro* de matériel génétique d'igname. Des segments nodaux (de 3-4 mm) ont servi d'explants. Les explants ont été encapsulés dans du milieu MS contenant 3% (p/v) d'alginate de sodium avec adjonction de saccharose (0,0; 0,1; 0,3; 0,5 ou 1,0 M). Le pourcentage de formation de pousses à partir des segments nodaux encapsulés est ralenti lorsque la concentration de saccharose ést plus élevée. Une concentration en saccharose très élevée (1,0 M) dans les billes induit un fort taux de mortalité chez les segments nodaux encapsulés. Les résultats indiquent que les solutions d'alginate-MS additionnées de saccharose à 0,1-0,3 M sont les plus appropriées pour enrober les segments nodaux d'igname aux fins d'échange et de distribution de matériel génétique. Les billes peuvent être distribuées dans des cryotubes contenant du milieu MS semi-solide.

Resumen

Segmentos nodales de ñame (Dioscorea *spp.) revestidos en alginato para el intercambio y distribución de germoplasma*

Para la distribución y el intercambio *in vitro* de germoplasma de ñame se utilizó como método alternativo la técnica de encapsulamiento de segmentos nodales usando el medio Murashige y Skoog (MS) con alginato. Como explantes se utilizaron segmentos nodales (3-4 mm). Los explantes se encapsularon en un medio MS que contenía 3% (peso/volumen) de Na-alginato, complementado con 0.0, 0.1, 0.3, 0.5 ó 1.0 M de sacarosa. Al aumentar el contenido de sacarosa en los microportadores, se retrasaba el porcentaje de formación de brotes. Los microportadores con un contenido muy elevado de sacarosa (1.0 M) provocaban un alto índice de letalidad en los segmentos nodales revestidos. Los resultados indicaron que las soluciones de MS-alginato complementadas con 0.1-0.3 M de sacarosa son el medio más adecuado para el revestimiento de segmentos nodales de ñame con miras al intercambio y distribución de germoplasma. Los microportadores pueden distribuirse en los criotubos que contienen el medio MS semisólido.

Plant Genetic Resources Newsletter, 1995, No. 103: 36-38 SHORT COMMUNICATION

From evaluation descriptors of times to flowering to the genetic characterization of flowering responses to photoperiod and temperature

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Introduction

Most organizations concerned with the conservation and utilization of plant genetic resources recognize at least four major categories of information — passport descriptors, management descriptors, characterization descriptors and evaluation descriptors — and some recognize a fifth — environment (site) descriptors.

- Passport descriptors comprise information on systematics and provenance.
- Management descriptors comprise information on where the material is stored, the quantity of material in store, and its condition (e.g. seed viability).
- Characterization descriptors comprise information on strongly heritable characters which are independent of the environment, e.g. taxonomic characters. Some definitions of characterization descriptors include the phrase 'easily visible by eye', but we believe that the inclusion of this term is unnecessarily restrictive.
- Evaluation descriptors comprise mainly those traits of concern in plant breeding programmes which can be thought of as inherited agronomic qualities, the expression of which is often strongly dependent on the environment.
- Environment or site descriptors summarize the conditions under which the characterization and evaluation data were obtained.
- Evaluation descriptors involve those genes which are among the most important to plant breeders, agronomists and farmers. However, these descriptors are also the most problematic because expression depends upon genotype, environment and (in the context of the entire germplasm collection) the interaction between genotype and environment.

Crop duration and flowering time

Descriptor lists for many crops, especially the major cereals and grain legumes, include information on the duration from sowing to flowering. This is because the first step towards maximizing crop yield by agronomic management or plant breeding is to ensure that the phenology of the crop is well matched to the resources and constraints of the production environment (Buddenhagen and Richards 1988; Richards 1989; Shorter *et al.* 1991). In this context, durations from sowing to flowering are of critical importance if cereal and grain legume crops sown on the appropriate date and at the appropriate density are to have the potential to yield well in a given environment (Bunting 1975). In most annual crops, the timing of phenological events is modulated primarily by responsiveness to photoperiod and temperature with strong differences in responsiveness among genotypes (Roberts and Summerfield 1987).

The general photothermal model

A general model of flowering responses to photothermal conditions can be applied in contrasting genotypes of both long-day plants (LDP) and short-day plants (SDP) (Roberts and Summerfield 1987; Summerfield *et al.* 1991a, 1991b). It has been shown that, irrespective of the crop, many advantages accrue from analyses of photothermal responses not in terms of the time from sowing to flowering (f) but in terms of the rate of progress towards flowering ($1/f$).

It is convenient to consider first the case where photoperiod-sensitivity genes are absent (that is, in day-neutral plants) or when for SDP the photoperiod is shorter than the critical photoperiod ($P < P_c$), or for LDP the photoperiod is greater than the critical photoperiod ($P > P_c$). The rate of progress towards flowering is then determined within wide temperature limits according to the equation:

$$1/f = a + bT \qquad (1)$$

where f is the number of days from sowing to the appearance of the first flower, T is the mean pre-flowering temperature (°C) and a and b are genotype-specific constants. Figure 1a shows an example of the response of a photoperiod-insensitive genotype of soyabean (*Glycine max* (L.) Merrill) to photothermal environment in which all the observations can be quantified by a single plane (the thermal plane) defined by Equation (1).

When a plant contains genes which confer photoperiod sensitivity, they are expressed in SDP only when $P > P_c$ (the critical photoperiod) and in LDP only when $P < P_c$. Between P_c and P_{ce} (the ceiling photoperiod) the delay in rate of progress towards flowering is described by the following photothermal regression plane:

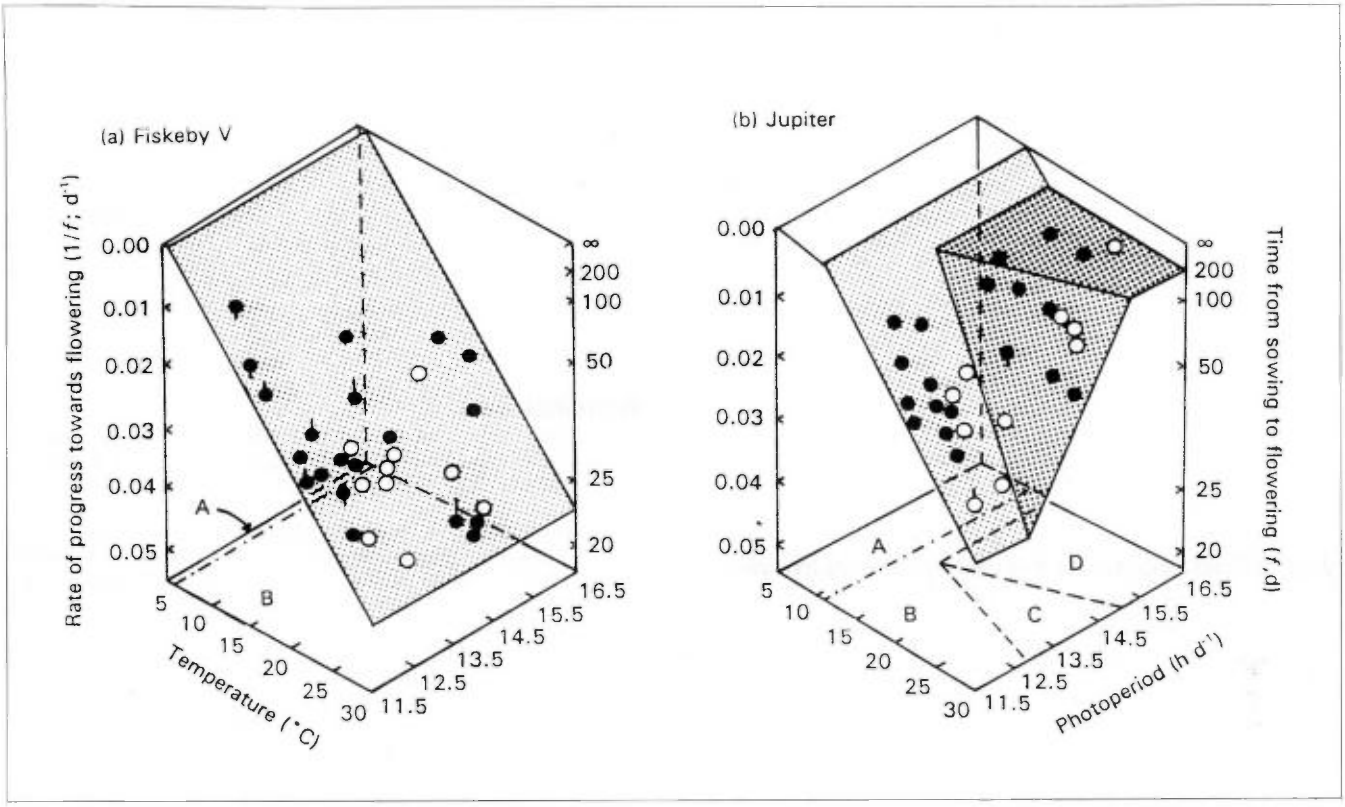

Fig. 1. Photothermal flowering responses of two genotypes (a: Fiskeby V; b: Jupiter) of the SDP soyabean determined from observations from crops sown on various dates at six sites in Australia in 1986-1988 (●) and at one site in Australia and two in Taiwan in 1989-1990 (O). The distances of the experimental points from the fitted response surfaces are shown by vertical lines extending above or below the symbols. The vertical scale ($1/f$) on the left-hand ordinate is converted to f as a non-linear scale on the right of each figure. The base of each graph has been divided into up to four sectors by vertically projecting: (A) the area where flowering cannot occur ($1/f = 0$), because the temperature is cooler than the base temperature; (B) the area projected by the thermal response plane described by Equation 1; (C) the area projected by the photothermal response plane described by Equation 2; and (D) the area projected by the plane of maximum delay described by Equation 3. The line between sectors A and B represents the base temperature; that between B and C represents the critical photoperiod; while that between C and D represents the ceiling photoperiod. From Summerfield *et al.* (1993).

$$1/f = a' + b' T + c' P \quad (2)$$

in which P is the mean pre-flowering photoperiod (h/day) and a', b' and c' are genotype-specific constants. The critical photoperiod occurs as a consequence of the thermal and photothermal planes; that is, it becomes evident under photothermal conditions when Equation 2 predicts a greater delay in flowering than Equation 1, and is therefore at the intersect of these two planes. In LDP the values of c' (sensitivity of rate of progress to flowering to photoperiod) are positive, while in SDP they are negative.

The other photoperiod limit to Equation 2 occurs at the ceiling photoperiod. Here, a plane of maximum delay to flowering is exposed; it is a zone in which in certain SDPs at least (e.g., soyabean) $1/f$ is insensitive to both P and T (Major 1980; Hadley *et al.* 1984; Beech *et al.* 1988), so when $P > P_{ce}$, then:

$$1/f = d' \quad (3)$$

Hence, photothermal flowering responses can be quantified by three intersecting planes which, within wide photothermal limits (defined in detail by Watkinson *et al.* 1994), relate $1/f$ to P and T. These three planes can be characterized for any genotype by six constants (a, b, a', b', c' and d') from which f can be predicted. Fig. 1b shows an example of the three-plane model—and the observations from which it was derived—for a photoperiod-sensitive genotype of soyabean.

Converting evaluation descriptors to characterization descriptors

The three-plane model provided by Equations 1, 2 and 3 was developed from research in controlled environments, but now that the model has been developed it is possible to parameterize it for a particular genotype from observations obtained in natural, and therefore fluctuating, field environments, as Fig. 1 shows. Moreover, while each of the symbols shown in Fig. 1 represents a combination of evaluation descriptors (time to flowering) and site de-

scriptors (mean pre-flowering values of temperature and photoperiod), the fitted planes (or rather the parameters defining these planes) represent characterization descriptors for particular genotypes. This is because the parameter values are not influenced by environment; instead they quantify the response to environment. Indeed, the value of these parameters for genetic characterization has been shown in studies of heritability (Imrie and Lawn 1990) and in studies of the effects of individual genes on progress to flowering in an isogenic background (Upadhyay *et al.* 1994). Moreover, the characterization of accessions by determining the values of the parameters of the model for the world lentil germplasm has identified clear trends in these values with crop domestication and dissemination (Erskine *et al.* 1990, 1994).

RoDMod: Rate of Development Model

Careful examination of Fig. 1b also illustrates one of the difficulties in fitting the three-plane model, however. In photoperiod-sensitive genotypes it is not necessarily obvious whether a particular observation is within the thermal plane defined by Equation 1, the photothermal plane defined by Equation 2, or the plane of maximum delay defined by Equation 3. Similarly, the observations available for a particular genotype may not encompass all three planes; they may encompass only one or two planes because, for example, the range of environments over which observations had been obtained is limited, or the range of photoperiods between P_c and P_{ce} is considerable. In which case it may not be clear how many and which planes the observations span.

In order to solve this bottleneck to the utilization of the three-plane photothermal flowering model, the staff of the Plant Environment Laboratory at The University of Reading, UK, and the Division of Tropical Crops and Pastures, Commonwealth Scientific and Industrial Research Organisation, Australia, have developed the computer program *RoDMod* (*R*ate *o*f *D*evelopment *Mod*el). *RoDMod* is a computer software package that provides an objective procedure for fitting the three planes, defined by Equations 1, 2 and 3, that describe the flowering responses of a genotype to photoperiod and temperature over very wide ranges of photothermal regimes (Watkinson *et al.* 1994).

The *RoDMod* package includes a menu-driven routine and other associated routines along with extensive documentation and tutorials to facilitate its use. The system requirements are an IBM or IBM-compatible Personal Computer, an 8088, 8086, 80286, 80386, or 80486 main processor, 512K RAM, DOS version 2.11 or later, and either a hard disk with 1.2 MB free space or two floppy disk drives at least one of which is a high-density drive (Watkinson *et al.* 1994).

The publication and distribution of this software package represent the outcome of a three-way collaborative research project between the Plant Environment Laboratory of the Department of Agriculture, The University of Reading, UK, the Division of Tropical Crops and Pastures of the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia, and the International Plant Genetic Resources Institute (IPGRI). Copies of the package are now available for distribution directly from the Editorial and Publications Manager, IPGRI, Via delle Sette Chiese 142, 00145 Rome, Italy.

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Seedborne fungi of rice collected from Pakistan

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Exchange of germplasm is an important activity of genebanks. Most germplasm is propagated by seeds. Many pathogens are associated with seeds (Neergard 1979). Storage of seed in genebanks prolongs the longevity of seeds and associated seedborne pathogens (Hewett 1987). Genebanks may serve as important reservoirs of many plant pathogens (Hampton *et al.* 1982). Kaiser *et al.* (1989) reported that seed contamination of lentil with *Ascochyta fabae* reduces the longevity of seeds at different storage temperatures.

Rice ranks second as a staple food crop in Pakistan and is cultivated over an area of 2 million hectares. To preserve the genetic variability in rice, the Plant Genetic Resources Institute (PGRI) of Pakistan Agricultural Research Council (PARC), Islamabad maintains the local germplasm collections which currently contains over 3000 accessions. Ahmad *et al.* (1989), Bajwa and Kausar (1969), Husany *et al.* (1968) and Khanum and Khanzada (1989) studied the seedborne mycoflora of paddy rice mainly collected from Punjab and Sind. Khan *et al.* (1988) evaluated seed health testing techniques for the assessment of seedborne mycoflora of rice. The objective of this study was to clarify the fungal flora contaminating rice seed accessions in the genebank collected from various regions of Pakistan to determine the basis of measures to be taken to minimize the hazards described above.

Seeds of 255 accessions of rice were taken from the genebank of PGRI, Islamabad for this study. These accessions were collected mainly from the provinces of Sind, Baluchistan and North West Frontier Province (NWFP) during 1984 and 1987. The method suggested by International Seed Testing Association (Anon. 1973) was used for the isolation of seedborne fungi after surface sterilization with sodium hypochlorite. Fungi were isolated with the agar plate method and blotter technique. The petri dishes containing seeds were incubated at 20°C for 8 days in a germination chamber with a 12-h photoperiod. After incubation, the seeds were examined for fungal growth under a stereoscopic binocular microscope. Fungi up to genus level were identified.

While investigating the mycoflora of rice germplasm collected from Pakistan, it was found that most of the rice accessions are contaminated with seedborne fungi. Species of 16 fungi genera were isolated from 255 accessions. The number of accessions contaminated with each genera and their percentage of the total is given in Table 1. *Alternaria* spp. and *Helminthosporium* spp. were the main seedborne fungi of rice followed by *Curvularia, Fusarium* and *Aspergillus* spp. All the fungi detected in this study are reported to be seedborne in nature (Richardson 1990). The seed samples included in this study were the collections from southern Pakistan (Sind and Baluchistan Province) and northern Pakistan (NWFP). It was found that material collected from the southern parts was highly contaminated compared with that collected from the northern parts of Pakistan (data not included). The high degree of contamination in material collected from southern parts of the country may be due to the high humidity and high pH of soils in that area. More accessions are being investigated to determine the fungal flora contaminating rice germplasm in the genebank.

Table 1. Seedborne fungi in rice germplasm collected in Pakistan

No.	Genus	Species
1	*Alternaria*	*padwicki, alternata, tenuis*
2	*Aspergillus*	*flavus, niger, terreus*
3	*Botrytis*	*atra*
4	*Candida*	*Candida* spp.
5	*Cephalosporium*	*irregularis*
6	*Cercospora*	*oryzae*
7	*Cladosporium*	*Cladosporium* spp.
8	*Curvularia*	*affinis, geniculata, lunata, oryzae, intermedia*
9	*Fusarium*	*moniliforme, solani, oxysporum, semitectum*
10	*Helminthosporium*	*oryzae*
11	*Helicoma*	*Helicoma* spp.
12	*Monilia*	*sitophyla*
13	*Mucor*	*Mucor* spp.
14	*Nigrospora*	*oryzae*
15	*Penicillium*	*notatum, oxaclium*
16	*Phoma*	*Phoma* spp.
17	*Pyrenochaeta*	*Pyrenochaeta* spp.
18	*Rhizoctonia*	*solani*
19	*Sclerotium*	*oryzae*

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Evaluation, maintenance and utilization of genetic resources of lentil (*Lens culinaris* Medik.) and common bean (*Phaseolus vulgaris* L.) in Slovakia

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Lentil

Evaluation of genetic resources of lentil (*Lens culinaris*) at the Research Institute of Plant Production, Pieštany (RIPP-P) began in 1971 (Gubišová 1975) with evaluation of 39 accessions. In the period 1976-79, 303 accessions of lentil were collected from Bulgaria. Fifty varieties of lentil have been evaluated for agronomic descriptors and 150 for morphological descriptors. From these evaluations, 44 accessions were selected for further research.

Since 1981, 326 samples of cultivated lentil and four samples of two wild taxa (*L. ervoides* and *L. culinaris* ssp. *orientalis*) have been added to the collection of our institute. Of the cultigen, 13 accessions were of domestic origin and three were regional. Each accession is evaluated for chosen morphological, phenological and agronomic descriptors. Prior to storage in the genebank, 103 accessions of lentil were characterized by use of the esterase isoenzyme. The RIPP-P holds the germplasm collection of lentils in Slovakia.

In the years 1988-91, 34 accessions of lentil were evaluated to select accessions for use in breeding programmes. The most important phenological and agronomic descriptors are given in Table 1.

The Slovak 'Lenka' and 'Trebišovská' for many descriptors were below the mean of the accessions tested. The new Slovak breeding line TR-14, released in 1992 under the variety name 'Nelka', gave the best results. It had a medium vegetation period and good resistance to *Fusarium* and lodging. The yield of 'Nelka' (1.54 t/ha) surpassed the total average by 7% and 'Lenka' by 22%. The best exotic genetic resources were Bulgarian varieties which showed the highest degree of adaptation to our conditions. American accessions also performed well. The Bulgarian accessions 'Belogradec', 'Medovina 1', 'Medovina 3', 'Kolos', No. 46 and the accession 'Lo Prenatn' Fonse (France), L.c. 160 586 (USA), V.W. 000 437 (USA), 'Eston' (USA) and 'Ipisor' (Greece) had the highest yields.

Genetic resources with good disease resistance and stable high yield are suitable for utilization in an alternative agriculture. These two criteria have been fulfilled by 'Nelka' from Slovakia, 'Lo Prenatn Fonse' from France, and 'Belogradec', 'Medovina 1', 'Medovina 3', 3/74 and 'Pobeda' from Bulgaria.

Common bean

The common bean (*Phaseolus vulgaris*) collection was started in 1952 at the Institute at Borovce with the goal to breed for high yield and good cooking quality. In 1952 Polerecký (1958) collected initial material from various sites of Slovakia in the Turiec, Žilina, Devínska Nová Ves and Bratislava regions. Also, the collection of domestic and foreign varieties from the Central Control and Testing Institute of Agriculture, Bratislava was evaluated. The 108 varieties and populations, 24 of them bush-type varieties and populations and the others climbing and half-climbing, have been tested.

During 1963-64, Ríman (1965) evaluated the exotic germplasm, introduced varieties of *Phaseolus*. They succeeded in collecting, evaluating and multiplying 1996 common bean genetic resources. This material has been provided for further evaluation and selection to the Research and Breeding Institute of Vegetable Growing, Olomouc.

At the RIPP-P from 1971 to 1988, Pastorek (1975) collected and evaluated 271 accessions of common bean. The material was collected within the former Czechoslovakia as well as from abroad. The Secondary Agricultural and Technical School, Pieštany has collected regional populations in Slovakia and provided them to our institute.

The RIPP-P maintains 352 accessions of common dry bean and common snap bean, including released and restricted domestic varieties, breeding materials and regional populations. Introduced varieties in the collection are from France, Germany, Bulgaria, the USA, Canada and the states of the former Soviet Union. In 1991-93, 19 samples were obtained from the regions of Pieštany, Levice, Kežmarok, Nitra and Myjava. In 1993, the Research and Breeding Institute of Vegetable Growing, Olomouc provided 15 regional populations from east Slovakia. In addition to *P. vulgaris*, *P. coccineus* (3 samples) and *P. lunatus* (2 samples) are maintained in this collection.

In the years 1989-91, 27 varieties (10 varieties of common dry bean and 17 varieties of common green snap bean) were evaluated. The most important economical and biological descriptors are given in Tables 2 and 3. During the 3-year evaluation, the Bulgarian varieties 'Prelom' and 'Trnovo 13' proved to be the best in seed yield of common dry bean. The Slovak variety of common dry bean 'Helia', included as a check, was surpassed by the tested varieties of bean in seed yield and the other

Table 1. Lentil accessions evaluated for important agronomic descriptors

Ser. no.	Variety	Origin†	Descriptor‡						
			1	2	3	4	5	6	7
	Lenka K§	SVK	111	460.8	229.8	25.5	30.7	39.5	1.3
	Trebišovská K§	SVK	110	463.8	246.8	19.6	26.6	37.5	1.3
1	Nelka (TR-14)[2]	SVK	110	471.8	257.6	21.4	25.1	41.9	1.5
2	Belogradec	BGR	110	462.2	232.9	26.3	36.7	30.1	2.0
3	Bistrec	BGR	113	505.5	302.9	27.6	41.3	25.3	1.5
4	Kolos	BGR	112	471.0	257.2	25.4	36.5	40.8	1.7
5	Medovina 1	BGR	111	536.4	305.9	33.1	51.1	25.2	2.0
6	Medovina 3	BGR	112	473.9	274.3	32.7	49.5	25.5	1.8
7	Mizija	BGR	112	562.9	317.8	26.8	38.8	25.9	1.6
8	Naps 2	BGR	111	447.5	234.3	22.7	32.0	38.5	1.4
9	No. 46	BGR	111	493.7	269.7	29.8	44.0	25.6	1.7
10	Pobeda	BGR	112	507.1	294.1	23.4	35.5	26.4	1.6
11	3/74	BGR	113	448.7	261.7	32.9	49.3	24.3	1.6
12	Lo Prenatn Fonse	FRA	112	421.5	232.6	33.6	51.1	26.7	1.9
13	Azer	SUN	111	443.5	239.4	26.1	35.8	31.1	1.6
14	Palouse	NZL	111	447.8	245.9	23.2	26.6	47.8	1.1
15	New Chilean	NZL	110	462.6	267.8	17.8	20.5	46.0	1.2
16	Arachova	GRC	111	360.3	220.2	24.9	29.5	29.5	1.0
17	Arcadia	GRC	110	328.3	190.7	22.1	18.5	36.8	1.0
18	Laird	CAN	112	404.4	220.7	31.1	37.1	37.6	1.5
19	Brewer	USA	111	418.9	222.3	19.7	24.5	52.0	1.6
20	Eston	USA	110	436.9	240.0	31.1	45.3	29.1	1.6
21	Chilean	USA	110	455.8	246.6	25.3	27.2	46.5	1.1
22	L.c. 160 586	USA	110	430.8	209.6	24.4	31.2	47.1	1.7
23	L.c. 810 014	USA	112	437.8	227.2	20.3	21.7	55.0	1.4
24	Red Chief	USA	112	432.2	214.7	23.3	31.9	46.7	1.6
25	V.W. 000 437	USA	111	431.5	212.3	25.1	26.9	45.4	1.6
26	Flip 84-11L	SYR	110	441.7	259.1	25.1	31.6	33.9	1.5
27	Flip 84-14L	SYR	111	343.9	187.9	26.7	34.1	41.0	1.3
28	Flip 84-62L	SYR	111	382.4	196.8	25.6	33.9	34.3	1.6
29	Flip 84-64L	SYR	109	363.7	193.6	22.3	26.4	32.6	1.0
30	Flip 84-67L	SYR	111	293.0	146.2	26.5	37.3	38.1	1.3
31	76 TA 66054	SYR	110	316.7	140.8	19.7	26.7	28.5	0.6
32	78 S 26030	SYR	110	303.9	143.2	22.5	31.3	31.3	1.0
33	78 S 26038	SYR	111	313.3	172.8	18.2	20.4	53.6	0.9
34	ILL-40	SYR	110	307.4	155.9	23.4	32.7	34.0	1.2

† SVK=Slovakia, BGR=Bulgaria, FRA=France, SUN=USSR, NZL=New Zealand, GRC=Greece, CAN=Canada, USA=United States of America, SYR=Syria.
‡ 1. Length of vegetation period (days from sowing to maturity); 2. plant height (mm); 3. height of the lower pod setting (mm); 4. pod number per plant; 5. seed number per plant; 6. 1000-seed weight (g); 7. seed yield (t/ha).
§ Local checks.

studied characters. The control variety 'Salva' was for 2 years best in seed yield. Economical characters of individual varieties important for breeding are: (a) number of pods per plant—'Start' (Hungary) and 'Slowianka' (Poland) varieties had the highest; (b) seed weight per plant—Polish varieties 'Justýnka' and 'Florentýnka', and Bulgarian variety 'Dobrudžanski 7' had the highest; and (c) number of seeds per plant—'Start" and 'Prelom' had the highest. Polish varieties 'Florentýnka' and 'Slowianka' had good yields and seem to be suitable for an alternative agriculture.

In the 3-year evaluation of varieties of common green snap bean, there were no significant differences between varieties in green pod yield (Table 3). The Slovak variety of common green snap bean, 'Hera', included as a standard, was surpassed by the tested varieties for pod yield as well as the other yield descriptors. 'Dita', used as a control, had the highest pod yield. Significant differences were found for pod length. The French variety 'Mangetou Argus' and the control 'Hera' had the longest pods.

Documentation and maintenance

Genetic resources are a valuable resource for breeders in production of improved varieties. Evaluation data for morphological, phytopathological and economical descriptors are used in breeding to select germplasm for use in crop improvement. For this purpose a good documenta-

Table 2. Common dry bean evaluated for important agronomic descriptors

No.	Variety	Origin†	Descriptor‡							
			1a	1b	2	3	4	5	6	7
	K - Helia	SVK	46	101	287.1	136.9	10.9	31.6	220.2	2.0
	K[1] - Salva	SVK	50	101	299.2	126.3	15.4	48.8	241.7	2.3
1	Augustýnka[2]	POL	45	98	286.3	138.6	9.1	25.9	311.7	1.7
2	Aura	POL	45	98	283.3	138.2	8.9	19.3	373.3	1.7
3	Candide	FRA	44	102	366.1	153.3	10.1	26.7	343.3	1.9
4	Dobrudžanski 7	BGR	50	104	578.0	148.6	9.7	33.3	370.0	2.2
5	Florentýnka	POL	46	101	302.0	146.8	9.1	25.7	273.3	1.8
6	Justýnka	POL	45	101	274.4	131.9	7.4	23.5	241.7	1.5
7	Prelom	BGR	53	102	326.3	116.0	10.0	35.7	251.7	2.6
8	Slowianka	POL	47	101	244.3	165.8	10.9	34.6	318.3	1.6
9	Start	HUN	50	102	378.1	114.7	14.7	46.2	183.3	2.1
10	Trnovo 13	BGR	41	101	295.6	121.5	9.5	30.0	328.3	2.0

† SVK=Slovakia, POL=Poland, FRA=France, BGR=Bulgaria, HUN=Hungary.
‡ 1a. Length of vegetation period (days from sowing to beginning of flowering); 1b. (days from sowing to physiological ripeness); 2. plant height (mm); 3. height of the lower pod setting (mm); 4. pod number per plant; 5. seed number per plant; 6. 1000-seed weight (g); 7. seed yield (t/ha).

Table 3. Common green snap bean accessions evaluated for important agronomic descriptors

No.	Variety	Origin†	Descriptor‡							
			1a	1b	1c	2	3	4	5	6
	K - Hera	SVK	48	72	112	172.4	153.5	7.0	4.3	0.9
	K[5] - Dita	SVK	50	77	117	174.0	125.3	9.2	8.1	1.3
1	Almere[6]	NLD	48	75	113	172.0	119.0	10.6	5.2	1.5
2	Amboy	NLD	49	77	109	208.2	133.4	8.9	4.3	1.8
3	Belami	NLD	50	75	110	180.9	103.5	13.8	5.8	1.6
4	Cimbola	NLD	48	76	112	139.8	132.5	8.5	3.1	1.5
5	Demeter	AUT	48	72	105	181.1	120.3	11.6	6.2	2.0
6	Dufrix	BRD	48	73	105	190.0	122.6	9.9	5.4	1.3
7	Lavinia	ROM	47	77	109	187.0	108.9	8.5	6.4	1.5
8	Maestro	NLD	48	75	109	151.2	115.0	11.3	5.8	1.7
9	Mangetou Argus	FRA	48	77	112	202.4	189.0	7.9	4.8	1.5
10	Mirage	NLD	48	75	109	172.7	111.8	10.2	5.0	2.2
11	Mirel	NLD	50	75	113	195.3	114.6	15.2	6.6	2.1
12	Nerina	NLD	48	72	109	279.0	128.0	8.8	5.4	1.6
13	Novostar	NLD	49	76	109	184.2	124.3	13.3	6.3	1.0
14	Ovation	NLD	50	77	113	214.0	123.8	9.8	4.8	1.4
15	Pros Gitana	NLD	50	75	109	170.0	121.3	14.2	6.6	1.3
16	Starnel	FRA	49	75	105	190.1	116.9	13.0	6.1	2.2
17	Tilla	NLD	48	74	109	173.4	117.2	15.1	5.9	1.2

† SVK=Slovakia, NLD=Netherlands, AUT=Austria, BRD= , ROM=Romania, FRA=France.
‡ 1a. Length of vegetation period (days, from sowing to beginning of flowering); 1b. (days from sowing to technological ripeness); 1c. (days from sowing to physiological ripeness); 2. Height of the lower pod setting (mm); 3. Pod length (mm); 4. Pod number per plant; 5. Green pod yield (t/ha); 6. Seed yield (t/ha).

tion system is necessary. At our institute, documentation is done through use of the EVIGEZ system (Dotlačil *et al.* 1992). This is the Czechoslovak information system of genetic resources. This system documents information for all genetic resources maintained and/or evaluated in the territory of the former Czechoslovakia.

As the institute responsible for conservation of the pulse genepool, the Research Institute of Plant Production Pieštany assigned national accession numbers to 200 genetic resources of lentil and 1206 genetic resources of common bean. National accession numbers also have been assigned to collections of genetic resources of common bean maintained in the Czech Republic (Breeding Station Uherský Ostroh and the Research and Breeding Institute of Vegetable Growing, Olomouc). Only seed material of high quality is selected for long-term storage. After desig-

nation with an accession number, samples are dried to the required level of humidity and are placed in hermetically sealed glasses with a desiccant. The base collection is maintained at -10 to -20°C, with seed humidity at 5%. Thirteen Czechoslovakian accessions of lentil and six accessions of common bean have been stored at the Research Institute of Plant Production Praha-Ruzyn . In the active collection at a temperature of 2°C with a seed humidity of 5-10%, 66 accessions of lentil and 22 of common bean are maintained. In the active collection at the Research Institute of Plant Production Pieštany, there are 108 samples of lentil (150 g) and 273 samples of bean (200 g).

Protection of old domestic regional varieties and populations, which represent an important cultural wealth of our nation, is one of the most important objectives for our genebanks. In 1992, the national programme for plant genetic resources began with the following priorities:

- increase of activities in Slovakia of the highest standard,
- full and effective participation in the ECP/GR (European Cooperative Programme for Crop Genetic Resources Networks),
- production of basic technical facilities, especially construction of a Slovak genebank.

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News and Notes

IPGRI's revised collecting form: ethnobotanical information in plant genetic resource collecting and documentation

In July 1995, IPGRI revised its collecting form to include ethnobotanical information in the list of fields on the form. This is part of IPGRI's continuing efforts to increase the use and accessibility of genetic resources held in genebanks by improving the information and documentation systems. It also is designed to help collectors work in partnership with local people to identify useful genetic diversity which would otherwise be lost or ignored. The collecting of plant genetic resources and use of indigenous knowledge is embedded within a code of conduct and ethical concerns to support the traditional resource rights of local peoples. Many of these codes and procedures will need to be developed through the informed consent and partnership of local communities who are the custodians and developers of local knowledge about plant genetic resources.

What is ethnobotanical information?

Ethnobotanical information is what local people know about the plants in their environment. The overarching unit of analysis in ethnobotany is the rural community where the plant genetic resource collecting is to be done. The types of information that local communities possess are mainly about how plants are used, how plants are distributed across the ecosystems they manage and use, and the relationships between plants, people and animals in their ecosystem.

Ethnobotany is a multidisciplinary field with botanists, anthropologists, and ecologists being the main practitioners. Some knowledge of microeconomics or household economics is also useful in order to understand local marketing and exchange of plants. Linguistics is also useful for understanding the indigenous plant classification systems. For those of us concerned with the conservation of biodiversity, understanding how and why people use the plants in their environment is the main reason we include ethnobotany as a core field within the science of plant genetic resources conservation and use.

Why include ethnobotanical information in plant genetic resources collecting and documentation?

When collecting plant genetic resources of cultivated species, ethnobotanical information has always been the starting point for collectors. After all, cultivated plants depend on a local community for their survival, and the practices, preferences and decisions of the local community determine to a large extent their distribution. Sociocultural differences are also important in defining the different distributions of cultivars within essentially homologous environments. Again, experienced collectors have noticed this countless times and pay attention to these sociocultural differences when targeting collecting sites and sampling plant populations.

Ethnobotanical information promotes the use and accessibility of genetic resources stored in a genebank. Experience has shown that the more extensively a genebank is used, the greater its success and the support for its maintenance. As collections get bigger, it becomes harder for users of genetic resources (be they breeders, biotechnologists or conservationists) to know which germplasm contains the genes and attributes they are selecting for. Information provided by the local community where the germplasm was collected can give scientists leads on what useful traits the germplasm contains. There is an urgent need to make what has been implicitly understood in the minds and recorded in the notebooks of plant genetic resources collectors available to users of plant genetic resources. This means that the minimum and essential ethnobotanical information should be included in the passport data and collection forms.

Using ethnobotanical information to target collecting of plant genetic resources

Ethnobotanical information is essential for assessing diversity and adaptation of crops. In eco-geographical terms, "much still remains to be learned about socio-eco-edaphic diversity of crops, and to understand crop adaptation to micro-niches and micro-environments" (Okuno *et al.* 1995).

We can conclude that when collecting genetic resources of cultivated and economically useful species, ethnobotanical information including cultural differences, the socioeconomic systems, and the institutional environment including land tenure regimes, is important in targeting the areas where collecting can capture significant variation within and across species. Ethnobotanical information is essential for identifying microenvironments and niches (spatial and temporal) within the farming system and its surrounding nonagricultural environments that contain diversity within and across species.

Finally, ethnobotanical data provide information about the adaptation of plants to their environment; they give indications of a plant's competitive, complementary and symbiotic relationships with other species, and its resistance to pests and diseases. This type of ethnobotanical information, when included in a collection form and descriptor lists, promotes and facilitates the use of genetic resources stored in a genebank.

Revising IPGRI's collecting forms to include ethnobotanical information

In revising the IPGRI collecting forms we have regrouped and reordered some of the information categories. Our intent is to keep the form simple and avoid adding more sections or making it significantly longer or more time-consuming to fill in.

Other changes are made to include more information on the effects of human and other uses and disturbances on the adaptation, survival and conservation status of the species. This explains the inclusion of a category on the passport data sheet which indicates the parts of the plant used by the community where it was collected. This is crucial and basic information that affects the regeneration, distribution and conservation status of a species. Clearly, whether and which reproductive sections or vegetative sections of a plant are used is crucial information for conservationists as well as plant breeders who are increasingly concerned with the multiple uses of crops in their crop improvement programmes.

Some of the changes are made to allow for greater identification of microenvironments. This includes the edaphic microenvironment as well as the niches within the farm environment such as boundaries, water courses, fallows, etc. One important change refers to the fundamental distinction between wild and cultivated species. There are useful wild species within areas used primarily for cultivation. Cultivated species may also be found in wild habitats. The revised collecting forms allow collectors to record these situations. The ability of wild species to survive in cultivated areas and for cultivated species to survive wild habitats is an important indicator for potential users of germplasm. Finally, a brief section on the local uses of the species in the passport data allows for more rapid screening of conserved germplasm by a wider range of users other than plant breeders.

This exercise in revising the collecting forms is part of IPGRI's continuing work to promote the increased use and accessibility of genetic resources. Passport and collecting data are important aspects of plant genetic resources documentation that allow a broad range of users to screen and gain access to the relevant germplasm. They also allow national plant genetic resources managers to have a better idea of how their germplasm collections are being used and by whom. Including information on gender differences in the access and management of genetic resources is important for identifying plant genetic resources users and partners in conservation that might otherwise be ignored. Finally, the inclusion of ethnobotanical information in passport data is one way that communities which provided the genetic resources can identify and gain access to those genetic resources when needed to restore local genetic diversity that has been lost, or to exchange useful germplasm with other communities.

While we recognize that ethnobotanical information has been an implicit tool that plant genetic resources collectors have used in targeting their collecting sites and in selecting plant samples, explicit recognition of ethnobotanical information in the collecting forms is essential. It is part of the process of validating and supporting the conservation of indigenous knowledge on plant genetic resources by the rural communities that are custodians of the genetic diversity of our economically useful plants.

Further reading

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The European Union Biotechnology RTD Programme (1994-1998)

Introduction

Biotechnology is a domain where the technology trajectory is crucially dependent on basic science and on new applications at the cross-roads of biological disciplines, industrial developments, health and environmental promotion, and social expectations. This programme is therefore trying to mobilize and coordinate the science base in Europe in a way which would be innovative and responsive to problems. Its objective forms an integral part of a more comprehensive effort initiated under Framework Programme IV to promote the applications of the Life Sciences and Technologies. This is to be implemented via three associated specific programmes: Biotechnology, Biomedicine and Health, and Agriculture and Fisheries. The latter two programmes will aim to promote the streamlining of biotechnology applications within their respective sectoral activities linked to the provision of products and services. The Biotechnology programme itself will look for every new opportunity to improve the understanding of living processes in the light of possible future uses.

Earlier Biotechnology programmes, and in particular the programme covering the period of 1990-1994 under Framework Programme III, were schematically driven by a technology push leading to an accumulation of results. The present programme should now initiate a new phase in European Community biotechnology research to stimulate the use of these results. This programme will ensure that advances in sophisti-

cated biological knowledge are constantly taken into account, that is to say in a timely manner and by the appropriate groups of technologists, investors, educators, consumers, and regulators upon which improved practices may be incorporated at a societal level. Such an objective will dictate a different implementation strategy, the definition of which has emerged from extensive consultations of expert advisers in the academic and industrial fields.

This programme has been selective in choosing three approaches, each one with a specific goal and restricted to identified scientific areas.

1. With a view to harvesting the highest potential returns on R&D in the medium term, four areas have been listed which will benefit from **concentrated means**. Task-oriented projects should aim to have measurable impacts, and significantly change the state of the art. A multidisciplinary integrated approach is recommended.
2. Four areas, as described below, will be the focus of coordination efforts where national research programmes will be of primary interest. The aims will be to maintain the high potential for innovative breakthroughs in key areas of research which tend to be developed in relative isolation within Member States, and to increase the value-added efforts from the interaction and harmonization of ongoing activities across borders.
3. With a view to linking academic institutions, research laboratories and industry, thus further enhancing public understanding and clarifying value-laden issues in relation to applications of biotechnology, four horizontal activities will be supported in areas essential to the exploitation of the life sciences. These activities may require special attention in respect of other factors such as socioeconomic or ethical issues. A wider range of participation modalities will ensure the balanced involvement of key players and users.

Following the ratification of the Treaty on the European Union (EU) all Community activities in the field of research, technological development and demonstration are covered by the European Community (EC) Framework Programme for Research and Technological Development (RTD).

On 26 April 1994, Framework Programme IV was adopted. It has a duration of 5 years (1994-1998) and a budget of 12.3 billion (thousand million) ECU (to which in 1996 an amount of 0.7 billion might be added if certain conditions are fulfilled).

Framework Programme IV contains four activities and a number of specified RTD programmes which cover the following areas:

Field	Funding in million ECU (MECU)
Activity 1	
RTD and Demonstration Programmes	10 686
1. Information and communication technologies	3 405
2. Industrial technologies	1 995
3. Environment	1 080
4. Life sciences and technologies	1 572
5. Energy	2 256
6. Transport	240
7. Targeted socioeconomic research	138
Total	10 686
Activity 2	
Cooperation with third countries and international organizations	540
Activity 3	
Dissemination and exploitation of results	330
Activity 4	
Stimulation of the training and mobility of researchers	744
Total	12 300

Shared-cost integrated projects

In order to focus activities, four areas (1 to 4) were chosen on the basis of their wide-ranging significance for the competitiveness of European science and of their proximity to potential interests of industry. An integrated project therefore results from negotiations conducted by the Commission with all selected proposals offering to cover some aspects of the requested tasks. Each contributing laboratory would normally not be expected to maintain more than two man-years/year for its own share of the research effort, and should claim an EC support at the indicative level of 100 000 ECU/year or less.

Shared-cost RTD projects

These projects represent the minimal organization of transnational shared-cost research in multipartner structures, commonly known as 'European Laboratories Without Walls'. They normally involve 2 to 15 participating laboratories (exceptionally more) addressing gaps in basic knowledge or knowledge acquisition systems in areas where industrial or social interest is high.

Concerted actions

Concerted actions will be implemented to create Community networks for competencies for some tasks as well as for certain horizontal activities.

Demonstration activities

Demonstration activities can be linked to any scientific and technological research area included in the work programme. Two types of action are envisaged:

1. *Demonstration project preparatory awards*
   These grants are designed to facilitating the preparation of proposals for technology demonstration projects.
2. *Shared-cost demonstration projects*
   In these projects the resources and interdisciplinary skills of technology producers will be combined with those of technology users to show, on a meaningful scale of operations, the techno-economic advantages offered by state-of-the-art technology concepts with respect to existing practices.

The main objective of demonstration activities in Biotechnology is to prove the technical viability of the new technology, together with, as appropriate, its possible economic advantages. Demonstration activities are expected to speed up the adoption of new biotechnologies by reducing the techno-economic uncertainties and risks associated with innovation and, to enhance the attractiveness of new biotechnological approaches offering environmental advantages, high socioeconomic validity and a large economic potential in industries and services.

Biotechnology and society: Ethical, social and legal aspects

To analyze the ethical, social and legal issues raised by specific applications of biotechnology in view of their being taken into account in public policy deliberations.

To promote a rational and balanced dialogue between the key players (including experts from natural sciences, medicine, philosophy, theology, legislation, economics and social sciences); and to involve the general public in this debate (through, e.g. consumer and patients' groups, industry and trade unions).

To collect scientific data from Commission programmes, national programmes and other expert sources and to provide these data for input into regulatory policies and procedures.

Biotechnology and society: Public perception

Timely information about research objectives, scientific breakthroughs, benefits obtained and obstacles are the key elements for the public perception of biotechnology which must be reviewed in an open discussion.

Socioeconomic impacts

The opportunities offered by biotechnology will be promoted for the competitiveness of European industry and employment.

Efforts will be made to assess the indirect efforts of the biotechnology research programme, to the benefit of industrial branches already in place.

The Commission has presented in its White Paper on growth, competitivity and employment, an analysis of the potential of biotechnology to present certain promises based on the omnipresence of the bioprocesses and the competitiveness of sectors of application.

It will be the responsibility of the Community to promote under this programme further research work where society would expect the highest returns. This points to privileged areas for the exploitation of new knowledge, all of which experience in common an acute need for cross-linking connected topics and/or integrating large groups of experts on an international scale.

With a view to harvesting the highest potential returns on R&D in the medium term, four areas have been listed which will benefit from concentrated means.

Area 1 - Cell Factories
Area 2 - Genome Analysis
Area 3 - Plant and Animal Biotechnology
Area 4 - Cell Communication in Neurosciences

Four horizontal activities will be supported in areas essential to the exploitation of the life sciences.

- Demonstration activities
- Biotechnology and society: Ethical, social and legal aspects
- Biotechnology and society: Public perception
- Socioeconomic impacts

The two areas of interest to Newsletter readers are:

Area 2: Genome analysis
Sequencing: *Arabidopsis* genome. *B. subtilis* genome, Yeast genome, other small genomes.

Area 3: Plant and animal biotechnology
Plants: A typical project will attempt to achieve the appropriate level of integration of plant science with end-user technology, and of target-oriented research with those areas of eukaryotic biology from which key knowledge is stemming (genome analysis, structural analysis of macromolecules and enzymes, signalling pathways, gene expression and stability of expression, bioinformatics, etc.).

Identifying, characterizing and exploiting useful biological traits of agricultural and industrial relevance, in terms of quality improvement and greater environmental acceptability, and their corresponding genes would be the main target for such activity.

Rice germplasm benefits Myanmar

Myanmar is now the world's fourth largest rice exporter and, according to its Minister of Agriculture, one of the factors in this success is the country's active involvement with INGER, the International Network for Genetic Evaluation of Rice.

"Paddy production in Myanmar has jumped from 13 million tonnes in 1991/92 to 17 million tonnes in 1993/94. The production target for 1994/95 is set for 19.5 million tonnes," said the Minister at a recent meeting of the INGER advisory committee in Myanmar. He added that his country had released 26 rice varieties supplied through INGER, which are now extensively cultivated in Myanmar farmers' field.

INGER is based at the International Rice Research Institute (IRRI) in Los Baños, Philippines. With 95 countries and 1000 scientists participating in its activities, INGER is one of the most successful international networks involved in plant germplasm evaluation and utilization. The INGER advisory committee guides the rice germplasm exchange, evaluation and utilization, and provides policy guidelines on safe and unrestricted exchange of germplasm globally.

As a global partnership among scientists of national programmes, IRRI, and other international centres, INGER increases the efficiency of national breeding programmes, shortens the time needed to develop new varieties, and accelerates the transfer of adapted varieties to farmers.

Through INGER, IRRI has made available 15 000 promising lines and cultivars of rice to Myanmar. In return, Myanmar has contributed 1900 indigenous varieties, including 150 wild rices, to the IRRI genepool.

A BASIC program for computing genetic statistics and building frequency tables

Genetic resources workers have for some time been concerned with the need to determine differences within and among genetic resources samples that have been grouped in various ways. A number of computer programs currently exist, and these differ in the statistics that can be computed and/or the usability of the output matrices.

Gene diversity indices can be computed by scoring genetic resources accessions for multiple loci and determining useful populations for within and among comparisons. Allelic frequency tables are then built and subjected to appropriate statistical procedures. Many computer programs that can be used to determine allelic frequencies from raw data, various measures of genetic distance, gene diversity statistics and genetic diversity within and between the populations have been written (NTSYS, BIOSYS, GENESTAT). However, there is no single program that allows the user to build frequency tables from raw data. This process is time-consuming and labourious, especially when the data involve a large number of individuals characterized for many loci. In addition, most of the available computer programs require the construction of multiple matrices for the genetic analysis.

GSTAT has been written to overcome some of these problems. GSTAT will calculate the allelic frequencies from unprocessed datafiles; compute Nei's gene diversity indices and compute various types of genetic distances among the populations based on the distribution of allelic frequencies. GSTAT is written and compiled in standard BASIC to run on any DOS-based personal computer. GSTAT can handle any number of loci, populations and number of samples in each population and is limited only by the computer's RAM. However, the number of alleles for any individual locus should not exceed 50. The output data from all options can either be a fixed format ASCII or a comma-delimited dataset that can then be used with other spreadsheet or database software. Missing values will cause the program to exclude populations to allow for parameters to be calculated based on identical numbers of loci.

Allelic frequencies can initially be computed from a raw dataset. The input data set must consist of allelic scores or designations at each locus for all populations. The output dataset will be a table of allelic frequencies (locus x allele) over all the populations and for each population (locus x allele x population). This option also generates a file (matrix of allelic frequencies with header information) which can then be used by other program options.

Gene diversity statistics can be computed for each locus from allelic frequency data. Genic diversity at each locus and its standard error (*HE*), average gene diversity over all loci and its standard error (*H*), the proportion of polymorphic loci (*P*), effective number of alleles per locus (*NE*) and the average number of alleles per locus (*A*) are computed. Genetic diversity within and between populations is also computed as a separate option. The parameters computed are the gene diversity in the total population (*HT*), in each subpopulation (*HS*), the gene diversity between each pair of subpopulations (*Dst*) and the gene differentiation relative to the total population (*Gst*) and have been computed as given in Whitkus. Monomorphic loci are indicated by zero values.

Several genetic distance measures can be computed in separate options. Included are: Nei's genetic distance, Rogers as modified by Wright, Chord's genetic distance and Balakrishnan and Sanghvi genetic distance. The former includes minimum and maximum and standard genetic distances.

The program is available free of charge from the author:

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Wanted

Viable seeds of *Solanum ferox* and relatives (*S. cyanocarphium, S. involucratum, S. lasiocarpum* and *S. trongum*). This species extends from China to India south to Indonesia and New Guinea and is found both as a wild plant or weed as well as a cultivated plant. The fruit (berry) is used for food, spice and medicine and is often sold in the open-air markets. An excellent treatment, including many of the common names, by Sayed Mohd Zain Hasan and P.C.M. Jansen appears in *Prosea*—Plant Resources of South-east Asia, 8, Vegetables, p. 249-252 (1994).

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Book Reviews

The opinions of IPGRI's book reviewers are personal and do not necessarily represent the positions of the reviewers' employers.

Centres of Plant Diversity. A Guide and Strategy for their Conservation, Volume 1. Europe, Africa, South West Asia and the Middle East

by S.D. Davis, V.H. Heywood and A.C. Hamilton, editors *1994. Hardback. ISBN 2-8317-0197-X. World Wide Fund for Nature.*

Biodiversity is not distributed evenly around the world. Everyone knows, for example, about the downward gradient in the diversity of many taxonomic groups from the tropics to the poles. However, even *within* the tropics and temperate regions there are areas where the numbers of species in different groups are high relative to surrounding areas, and endemism is particularly strong. Identification of such 'hot-spots' of diversity is receiving considerable attention as a tool for priority-setting in conservation.

A particularly important example is the *Centres of Plant Diversity* (CPD) project. This is an attempt:

"1. to identify which areas around the world, if conserved, would safeguard the greatest number of plant species;
2. to document the many benefits, economic and scientific, that conservation to those areas would bring to society and to outline the potential value of each for sustainable development;
3. to outline a strategy for the conservation of the areas selected."

The quote is from the book under review, the first product of the CPD project. There will be two further volumes, on Asia, Australia and the Pacific and on the Americas. The project really got off the ground in 1989, when WWF International provided a grant and arranged for matching funds from the UK Overseas Development Agency, although the antecendents, described in the book, go back to 1982. The European Commission has since also contributed. The project is being implemented by IUCN.

The core of the CPD project is 234 priority sites worldwide selected for Data Sheet treatment. These are considered sites of global botanical importance: it is stressed that if the selection had been done from a national perspective the result would have been quite different. The main selection criteria were species richness (>1000 species for mainland sites) and high endemism (>10% for mainland sites, >50 species or >10% for islands). However, diversity of habitat types, high proportion of species adapted to special edaphic conditions, high proportion of species useful to humans and imminent threat were also considered. Sites were selected for treatment in consultation with local experts and institutions, often in regional workshops. Some 400 individual and 125 institutions are listed as contributors to the CPD project. To give an impression, the nine sites selected for Data Sheet treatment in Europe are: the Baetic Mountains and the Gudar Massif of Spain, the Pyrenees, the Alps, the Balkan Massif of Bulgaria, the mountains of southern and central Greece, Crete, the Troodos Mountains of Cyprus and the mountains of southern Crimea and Novorussia.

Each Data Sheet contains sections on geography, vegetation, flora, useful plants, social and environmental value, economic assessment, threats and conservation. Summary geographical and floristic information is provided in a box at the head of each Data Sheet. The Data Sheet sites range in geographical size from St Helena, 122 km^2, to Madagascar and the mountains of central Asia, both over 500 000 km^2, and in size of flora from the 35 endemic taxa of the subantarctic islands to the near-9000 species of the Cape Floristic Region. This volume treats 9 sites in Europe, 30 sites in Africa, 11 sites in southwest Asia and the Middle East and 7 Indian and Atlantic Ocean islands.

Each of these regions is also described in a regional overview prepared by regional coordinators. The regional overviews give summary botanical and other statistics in a box and have sections on geology, climate, vegetation, flora (including an assessment of how well the flora is known), useful plants, factors causing loss of biodiversity and conservation. In addition to the Data Sheet sites, many more sites are treated in summary paragraphs in these regional overviews. Thus the Africa section of this volume deals with some 84 sites, of which as already mentioned only 30 are given full Data Sheet treatment. The Europe section has another 15 sites in addition to the 9 Data Sheet sites listed above, ranging from the Burren of Ireland to the delta of the Danube. The site descriptions are arranged in the regional overview according to phytochoria (from White's *Vegetation of Africa*, for example) and cross-referenced by country in summary tables. There are reference lists both for the regional overviews and for each Data Sheet.

Here I must declare an interest. Together with Tony

Miller of the Royal Botanic Garden, Edinburgh, I contributed the Data Sheet on the Indian Ocean Island of Socotra. We gathered the information during the course of three botanical expeditions to the island between 1989 and 1992, supported by IBPGR (as it was then), WWF and Edinburgh. I do not think I am being biased, however, when I say that this volume and its forthcoming companions represent a tremendous achievement by the conservation community, and a tribute to international scientific collaboration. They provide not only a programme for the future, but a yardstick against which to measure the success of that programme. If I have criticisms, they are of the petty kind, for example that some Data Sheets have no maps, that the regional overview maps show the location of the Data Sheet sites only, and that the index restricts itself to geographical names, where a species index would have been very useful.

Text and map data from the CPD project are available in electronic format from the World Conservation Monitoring Centre, where they form part of the Biodiversity Information Service.

Luigi Guarino, IPGRI, Nairobi, Kenya

The Life of Isaac Newton

by R. Westfall

1993. Paperback. ISBN 0-521-47737-9. US$11.95, £7.95. 328 pages. Cambridge University Press, UK.

At a time when modern philosophers of science are appraising previous interpretations of Newton's mechanistic world view and exciting new links are being forged between the natural and social sciences in face of boundless global environmental problems, Westfall's **The Life of Isaac Newton** comes as an opportune invitation to get better acquainted with the life and work of this intellectual giant who has shaped much of Western scientific thinking.

From his birth on Christmas day of 1642, to his development as a 'Sober, Silent Thinking Lad' and his fateful enrollment in the renowned Trinity College at Cambridge at the age of 19, the author guides us through the first chapters of Newton's youth.

As a 'Solitary Scholar' at Trinity College the young Isaac Newton devoted much of his time to extensive readings on "...the nature of matter, place, time and motion..." rather than on the assignments required by the limited scholastic curriculum. His structured notebook — the archetypical *Quaestiones quaedam philosophicae* — in which he recorded the fruits of his readings of Descartes, Aristotle, Galileo and others, reveal his incessantly questioning mind and broad interests, as well as his special ability for organizing his thoughts and questions in systematic detail.

In his persuasive account of the *Anni Mirabiles* of 1664-1666, Westfall demonstrates that, contrary to what has been espoused by Newtonian 'myth', these two years marked the period in which Newton laid down only the solid foundations rather than the actual structures of the three colossal pillars for which his name was to live on eternally: mathematics, mechanics and optics.

With his promotion to Lucasian Professor, Newton was granted the privilege of staying on at Cambridge to indulge in his relentless questioning. It was shortly thereafter that the success of his first creation, the telescope, propelled him into the community of natural scientists from where he was never to return.

In the 'Publication and Crisis' chapter Westfall engages the reader in the tumultuous episode of Newton's life as a prominent international figure in the scientific arena. The reader is familiarized with a man who, for the most part, viewed the debates and pressures of the scientific community as disruptive to his studies.

And then follows the era of 'Rebellion' succeeded by the introspective 'Years of Silence' - an unconventional depiction of Newton as a theologian who through his careful studies of the scripture developed strong religious views that were fundamentally against the Undivided Trinity - the very beliefs upon which the College was founded. That he chose not to go public with these views against the Establishment, shows that whereas he enjoyed considerable freedom of expression, he was in many ways bound to the conservative societal order of his times.

In the next chapter, Westfall tells the story of the development of *Principia*, Newton's greatest accomplishment which indeed caused nothing short of the revolution which he goes on to describe in the ensuing pages.

With his account of Newton's assumption of a surprisingly 'non-scientific' yet prestigious occupation as warden of the Royal Mint and then as President of the Royal Society, Westfall expands on his illustration of the protagonist's persona. In the penultimate chapter entitled 'Priority Dispute' the author relates the capturing and seemingly endless episode of Newton's life in which more of his personal qualities surface through accounts of his various personal relationships. The reader is left with impressions of a solitary man who despised criticism; one who, nevertheless, exercised considerable discretion in relating with his counterparts betraying an appreciation of the social status he enjoyed. Newton was not entirely unaware of the legacy he was to be leaving behind through his great scientific achievements.

By the time the 'Years of Decline' come around, the reader will have become well-acquainted with Isaac Newton's complex character; packed with more anecdotes about a man coming to terms with his ending career, this last chapter adds the final touches to this intricate portrait.

Westfall has succeeded in achieving what he set out to do in this enjoyable biography - to make the principles of Newtonian science digestible for a broader group of consumers - certainly broader than the limited circle who actually followed those obscure physics lectures readers are likely to recall.

Hareya Fassil, IPGRI, Rome, Italy

Green Globe Yearbook 1995. Main focus: climate change

1995. Hardbound. ISBN 0-19-823325-6. US$45. 318 pages. *Fridtjof Nansen Institute, Lysaker, Norway*

The Green Globe Yearbook is an independent annual produced by the Fridtjof Nansen Institute in Norway, which specializes in studies of international resource management. The 1995 volume focuses on climate change with a series of articles addressing different aspects of the topic. In addition, the volume contains a number of other articles and a reference section that lists systematically linked data on international agreements on environment and development, inter- and nongovernmental organizations.

The signing of the UN Framework Convention on Climate Change at UNCED and its entry into force less than two years later, on 21 March 1994, raised several basic questions:

- How serious is the potential threat of climate change, for the globe as a whole and its different parts?
- Who bears the responsibility for taking the first steps to counteract the assumed risk, and what are the appropriate measures to be taken in the 1990s?
- How can national action by individual governments be coordinated in a way that makes sense, confronted with a global problem?

This volume of the *Green Globe Yearbook* does not answer these complicated and controversial questions, but seeks to focus attention on the major problems of international collaboration in this area. One paper emphasizes the crucial interface between science and politics, in particular the role of the Intergovernmental Panel on Climate Change (IPCC). Another points to the European Union as a small-scale test case for regional solutions of a kind that could, if successful, show the way towards innovative global schemes of a similar type.

Three articles are concerned with the initial phases of establishing international norms or rules for environmental conduct—the NAFTA negotiations, the Biodiversity Convention, and the discussions on business and sustainable development.

Of particular interest to Newsletter readers is G. Kristin Rosendal's essay on the Convention on Biodiversity. He discusses the "essential controversy [that] revolves around wildlife and habitat preservation versus utilization of biological diversity. This is inherently linked to the dispute over property rights to genetic resources." In the article he gives a useful review of the debate on property rights, especially in the Food and Agriculture Organization and the General Agreement on Tariffs and Trade. Then he goes on to look at how the "Bio Convention" might benefit developing countries, and how the Global Environment Facility could operate to compensate developing countries for external use of genetic resources. Basically a compromise mechanism, GEF has been active for four years. Rosendal has some critical things to say about its apparent bias towards biodiversity projects, and its need to downscale some of its operations.

The reference section is a very useful compilation that lists key data such as objectives, scope and finance of over 100 international bodies. We can learn the Email number of the Framework Convention on Climate Control, the fact that Finland contributes more to UNEP than Switzerland, and that Greenpeace's 1995 budget of $30 million came purely from voluntary donations from the public, and from the sale of merchandise.

The volume is well produced by Oxford University Press on a very unprepossessing, dirty looking 'genuine waste fibre' paper, encased in an expensive hard cover complete with gold blocking on the spine. Overall a very worthwhile addition to any library, both for the interesting essays and the concentrated reference data.

Paul Stapleton, IPGRI, Rome, Italy

Methods for Risk Assessment of Transgenic Plants I. Competition, establishment and ecosystem effects

by Gösta Kjellson and Vibeke Simonsen

1994. Hardbound. ISBN 3-7643-5065-2. Birkhäuser Verlag, Basel, Switzerland

Genetically modified plants (GMPs) will provide society with more plentiful and improved plant food with an additional reduction in the use of fertilizers and pesticides. On the other hand, there is an increasing awareness about potential negative impact on the natural flora and fauna (biodiversity). These two statements, taken from the introduction to the book, summarize concisely its raison-d'être. The catalogue by Gösta Kjellson and Vibeke Simonsen addresses the need to guide researchers and administrators through the process of risk assessment when dealing with field releases of GMPs. It is the result of cooperation among three Danish authorities: the National Forest and Nature Agency, the Danish EPA, and the National Environmental Research Institute (Dept. of Terrestrial Ecology). The EU directive on the deliberate release of genetically modified organisms (GMOs) into the environment provides the basis. It presents us with a compilation of test methods useful in assessing the risk that GMPs may pose with the intention of aiding the environmental researcher to find and compare relevant methods quickly and easily. The target (user) groups are mainly scientists and students, working in plant ecology and risk assessment, and administrators responsible for legislation of GMPs. Furthermore, the catalogue is a general reference work for experimental research in general plant population ecology and genetics, as the methods described originate in these fields. The book is a timely reaction to the increasing number of field release applications for GMPs—over 200 requests in EU countries by March 1994 and more than 1000 experimental field re-

leases already carried out in other countries, mainly the USA. Several companies are ready to apply for the commercial release of different, genetically modified crop species with a number of novel traits—derived from genes inserted through nonconventional gene transfer. This includes characters such as herbicide, insect and pathogen resistances, drought and frost tolerance, improved nitrogen fixation, and increased protein and starch content.

The major processes affecting the fate of plants are covered with emphasis on invasion, competition and establishment, e.g. seed dispersal, density-dependent competition and plant growth. Ecosystem effects and genetic structure are also covered. For each process, a number of relevant test methods have been selected (84 for field, greenhouse or laboratory research) that fall into the following categories, according to the way they apply to plant life stages and population dynamics: general methods (36), specialized methods (30) and data analysis (18). Each method is briefly described and evaluated. Comments on assumptions, restrictions, advantages and applications have been included with each description. An extensive bibliography provides entry to the scientific background, and cross references ease quick finding of all relevant sources. The need for the development of new test procedures for risk assessment of GMPs is also indicated.

Methods to study pollination and gene transfer will be considered in a future volume (in preparation). The present volume deals with the dispersal of inserted genes by seed or vegetative organs and their subsequent survival and competitive interactions with the local flora. Volume 2 will deal with transmission of inserted genes by pollination to nonmodified individuals of the same or related species by hybridization, a highly relevant issue when handling field releases of GMPs.

Regulations for the handling and release of GMPs have been adopted in developed countries and some developing countries. They have all been elaborated on a similar basis, but some are stricter than others. Worldwide homologizing of regulations concerning GMOs in general is urgently needed, to block loopholes for ill-designed experiments to obtain field-release permits. Conversely, overregulation could be deleterious to new developments. Regulations should be reinforced by appropriate experimental designs that allow scientists and administrators, especially those who have to decide on, and monitor, field releases of GMPs, to make the right decisions.

As with regulations, experimental design in risk assessment should also be standardized. The present catalogue presents us with an excellent coverage of experimental design and evaluation methodology from which the most appropriate combination can be chosen. It could be recommended as a standard reference (together with volume 2) in the processing of applications for field releases of GMPs. In such a case, regular updating of the catalogue would become essential.

Few experiments have been performed with real GMPs in natural habitats. In upcoming years we will have to watch carefully as experience accumulates with the first, real-life GMPs in the field. At present we assume a worst-scenario vision, trying to work out the most intricate tests that might detect potential dangers, to nature and to man, in the earliest stages. This vision will soon give way to more factual decision-making.

At first glance, the reader is startled by the plethora of processes and methods, but from the conclusions we learn that, despite the large number of methods presented, we can finally follow a reductionistic approach. We already know a great deal about the tendency of well-known crop plants to invade different habitats. As these plants will be the most common candidates for genetic manipulation, we will be able to draw from this wealth of existing information on the crop, adding only the missing aspects concerning the added trait. Prior to field release, several data on the whole genetic manipulation process are gathered, such as the origin of the gene construct, nucleotide sequence, function, product and general physiological effects on the GMP. Thus, with accumulated experience, additional effort for the introduction of new GMPs will be reduced.

The concluding chapter constitutes an important part of the lecture, for it summarizes the main types of test methods relevant to risk assessment concerning establishment and survival of GMPs in natural habitats. No definitive hierarchy of test procedures is presented, but some suggestions are included. Some general principles are also given to guide the test procedure, as well as comments on invasion and colonization of ecosystems in relation to disturbance.

The methods description is very well organized, allowing the interested person to quickly decide for which level (life stage, genome, population, ecosystem, test system) and processes (related to the specific level or the processes influencing them) the method is relevant when designing a risk assessment strategy. This is followed by a short description of the methodology—supported by citations—and the underlying assumptions and restrictions, including advantages and possible applications. The test system briefly describes where the method may be used. The method is further evaluated in terms of sensitivity, requirements, time and cost.

One reality facing us is that there are very few natural habitats left. Most ecosystems have been manipulated, in one way or another, by human intervention. In most cases, the cardinal issues for the release of GMPs will be the comparative advantage of the new trait within agroecosystems vs. natural ecosystems, and the danger of producing new weeds that demand stronger measures of weed control in cultivated fields. This book (and the following volume) will guide us through the process of finding suitable answers and the taking of appropriate decisions.

Jorge Mayer, CIAT, Colombia

Conseils aux auteurs

Les textes dactylographiés seront préparés en anglais, en espagnol ou en français et envoyés en deux exemplaires au directeur de rédaction. Ils seront présentés en double interligne, avec de grandes marges (3 à 5 cm). Toutes les pages (y compris les tableaux, figures, légendes et références) seront numérotées à la suite.

Titre

Le titre sera le plus court possible et devra contenir le nom commun de toutes les espèces dont il est question dans le document, et le nom des principaux pays visités durant, par exemple, un voyage de collecte de matériel.

Nom et adresse des auteurs

Mentionnez le nom complet de tous les auteurs du document, ainsi que leur adresse à l'époque de l'étude. Indiquez leur adresse actuelle et leur adresse de contact en bas de page sur la première page du document; indiquez également l'auteur auquel doivent être adressées la correspondance et les épreuves.

Résumés

Articles et études doivent être accompagnés d'un résumé en anglais, en espagnol et en français. Envoyez un résumé de 200 à 250 mots au maximum dans la même langue que le texte dactylographié, ainsi que la traduction (y compris le titre) dans les deux autres langues, si possible. Placez-les à la fin de votre texte, après les références et avant les tableaux. Les résumés d'articles devraient mentionner l'objectif de l'étude (hypothèse et buts), le matériel et/ou les méthodes utilisés pour l'expérience, un résumé des résultats et les conclusions tirées de ces résultats.

Mots-clés

Indiquez au maximum six mots-clés qui serviront pour un index, par ordre alphabétique, au-dessous du résumé dans la langue de rédaction au début du texte dactylographié.

Texte principal

Faites bien ressortir les titres et les sous-titres, mais évitez les titres de plus de trois lignes. Ne mumérotez-pas les titres ou les paragraphes, qui devraient être mis en retrait.

Utilisez un langage simple. Il serait préférable qu'avant d'être soumis, le document soit mis en forme par une personne dont la langue maternelle est celle de la langue de rédaction.

Remerciements

Ceux-ci (de même que les dons ou l'aide, etc. éventuellement reçus) devraient figurer à la fin du texte et avant les références.

Références

Les références seront présentées par ordre alphabétique, dactylographiées en double interligne et indiqueront: auteur et année de la publication, par exemple (Dawsib, 1987). On évitera les citations de communications personnelles ou de données inédites. Ces citations ne devraient figurer que dans le texte, comme (E.D. Smith, communication personnelle) et non dans la liste des références. Abrégez les titres des périodiques comme il est indiqué dans le Bibliographic Guide for Editors and Authors (Biosis, Chemical Abstract Service and Engineering Index, Inc., 1974). Adoptez la présentation ci-dessous:

Périodiques

Molina-Cano, J.L., P. Fra-Mon, G. Salcedo, C. Aragonicillo, F. Roca de Togores et F. Gardia-Olmedo. 1987. Morocco as a possible domestication center for barley: biochemical and agromorphological evidence. Theor. Appl. Genet. 73:531-536.

Livres (édités par quelqu'un d'autre que l'auteur de l'article)

Hanelt, P. 1986. Cruciferae (Brassicaceae). Pp. 272-332 *in* Rudolf Mansfelds Verzeichnis landwirtschaftlicher und gärtnerischer Kulturpflanzen (ohne Zierpflanzen), Vol. 2. (H. Schultze-Motel, ed.) Akademie-Verlag, Berlin, Allemagne.

Livres (même auteur et même éditeur)

Chapman, C. 1985. Genetic Resources of Wheat. A Survey and Strategy for Collecting, IBPGR, Rome, Italie.

Nomenclature

Taxinomique: suivre l'*Index Kewensis*. *Génétique:* les applications des termes phénotype et génotype devraient être conformes à Demerec *et al.* (Genetics 54:61-74, 1966); pour des résumés des abréviations génétiques, consultez le *Journal of Bacteriology* qui contient des conseils aux auteurs.

Unités: exprimez toutes les quantités en unités du système international. Si une unité traditionnelle ou locale est utilisée, ou une unité qui pourrait être connue dans un pays seulement, indiquez toujours l'équivalent en unités du système international afin que d'autres chercheurs puissent comprendre les quantités indiquées.

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Figures et tableaux servent à étayer le texte et doivent être organisés logiquement, apparaissant là où ils sont mentionnés. S'il y a une grande quantité d'informations dans un tableau, il vaudrait mieux l'inclure dans une annexe à la fin de l'article. Les figures et les tableaux doivent être clairs et simples. Il s'agit de présenter un matériel complexe sous une forme facile à comprendre. Présentez les données dans le texte, dans une figure ou dans un tableau mais jamais dans les trois à la fois.

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Los textos deben redactarse en inglés, francés o español y entregarse por duplicado al director de redacción. Deben presentarse mecanografiados a doble espacio, con amplios márgenes (3-5 cm). Todas las páginas (incluidos los cuadros, figuras, leyendas y obras consultadas) se deben enumerar consecutivamente.

Título

El título ha de ser lo más corto posible y debe incluir los nombres común y genérico completos de las especies descritas en el documento, así como los principales países visitados, por ejemplo, durante el viaje de colección.

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Incluir los nombre completos de los autores del documento, junto con las direcciones de los autores en el momento de la realización del trabajo presentado. Indicar las direcciones actuales o postales como nota al pie de la primera página del documento. Indicar también el autor designado para recibir la correspondencia y las pruebas.

Resúmenes

Los artículos y recensiones se publicarán acompañados de resúmenes en inglés, francés y español. Entregar un resumen que no exceda las 200-250 palabras en el mismo idioma empleado en el texto mecanografiado, así como, de ser posible, las traducciones (incluido el título) a los otros dos idiomas. Incluir estas traducciones al final del documento, después de la bibliografía y antes de los cuadros. En los resúmenes de los artículos se debe mencionar el propósito de la investigación (hipótesis y objetivos), el material y/o los métodos experimentales, un resumen de los resultados y las conclusiones.

Palabras claves

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Texto principal

La importancia relativa de los títulos y subtítulos debe distinguirse claramente, pero hay que evitar el empleo de más de tres niveles de encabezamiento. No enumere títulos o párrafos que se han de sangrar.

Utilizar un lenguaje sencillo y claro en el texto. Se aconseja que una persona cuyo lenguaje materno sea el empleado en el documento, revise el trabajo ante de presentarlo.

Reconocimientos

Los reconocimientos (al igual que las subvenciones, ayudas etc.) deberán incluirse después del texto y antes de la bibliografía.

Bibliografía

Las referencias bibliográficas deben presentarse en orden alfabético, mecanografiadas a doble espacio, e indicar autor y año de publicación, por ejemplo (Dawsib, 1987). Se deben evitar las citas de comunicaciones personales y datos no publicados. Estas citaciones han de aparecer sólo en el texto (E.D. Smith, comunicación personal), y no en la bibliografía. Abreviar los títulos de revistas de conformidad con el estilo de la Bibliographic Guide for Editors and Authors (Biosis, Chemical Abstract Service and Engineering Index, Inc., 1974). Seguir el modelo siguiente:

Revistas y periódicos

Molina-Cano, J.L., P. Fra-Mon, G. Salcedo, C. Aragonicillo, F. Roca de Togores y F. Gardia-Olmedo. 1987. Morocco as a possible domestication center for barley: biochemical and agromorphological evidence. Theor. Appl. Genet. 73:531-536.

Libros (editados por alguien que no es el autor del artículo)

Hanelt, P. 1986. Cruciferae (Brassicaceae). Pág. 272-332 *en* Rudolf Mansfelds Verzeichnis landwirtsschaftlicher und gärtnerischer Kulturpflanzen (ohne Zierpflanzen), Vol. 2. (H. Schultze-Motel, ed.). Akademie-Verlag, Berlin, Germany.

Libros (del mismo autor y editor)

Chapman, C 1985. Genetic Resources of Wheat. A survey and Strategy for Collecting. IBPGR, Rome, Italy.

Nomenclatura

Taxonómica: de conformidad con el *Index Kewensis*. *Genética*: los términos fenotipo y genotipo se deben aplicar de acuerdo con Demerce *et al.* (Genetics 54:61-74, 1966); para los resúmenes de las abreviaturas genéticas, consultar las Instrucciones para los Autores contenidas en el *Journal of Bacteriology*.

Unidades: expresar todas las cantidades con arreglo al sistema internacional. Si se emplea una unidad tradicional o local, o una unidad que tal vez se conozca sólo en un país, incluir siempre el equivalente en el sistema internacional para que los demás puedan entender perfectamente las cantidades.

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Los cuadros y las figuras complementan el texto y deben presentarse de modo lógico, apareciendo cuando se mencionan. Si un cuadro contiene una gran cantidad de información, tal vez sea mejor incluirlo como apéndice al final del documento. Las figuras y los cuadros deben ser sencillos y claros. Su propósito principal es presentar información compleja en un modo fácilmente comprensible. Presentar los datos ya sea en el texto, en un cuadro o en una figura, pero nunca en las tres formas a la vez.

Instructions to authors

Typescripts should be prepared in English, French or Spanish and submitted in duplicate to the Managing Editor. Typescripts should be double-spaced throughout, with generous (3-5 cm) margins. All pages (including tables, figures, legends and references) should be numbered consecutively.

Title

The title should be as short as possible and should contain the common and full generic name of any species featured in the paper, as well as the main countries visited during, for example, collecting trips.

Authors/addresses

Include the full names of all authors of the paper, together with the addresses of the authors at the time of the work reported in the paper. Indicate current or postal addresses as a footnote on the first page of the paper; indicate also the author nominated to receive correspondence and proofs.

Abstracts

Articles and reviews will be published with abstracts in English, French and Spanish. Supply an abstract not exceeding 200-250 words in the same language as the typescript, as well as translations (including the title) into the other two languages, if this is possible. Include these at the end of the paper, after the references and before the tables. The abstracts of articles should mention the objective of the investigation (hypothesis and aims), the experimental material and/or methods, a summary of the results and the conclusions drawn from the results.

Key words

Provide a maximum of six key words for use in indexing purposes, in alphabetical order, below the native-language abstract at the start of the typescript.

Main text

The relative importance of headings and subheadings should be clear, but avoid using more than three levels of headings. Do not number headings or paragraphs, which should be indented.

Use simple clear language in the text. A native speaker of the language should preferably edit the paper before submission.

Acknowledgements

These (also grants, support, etc. if any) should follow the text and precede the references.

References

The references to the literature should be arranged alphabetically, typed double-spaced and in text referred to as: author and year of publication, e.g. (Dawsib, 1987). Citations of personal communications and unpublished data should be avoided. Such citations should in text appear in the text only, as (E.D. Smith, personal communication), and not in the reference list. Abbreviate titles of periodicals according to the style of the Bibliographic Guide for Editors and Authors (Biosis, Chemical Abstract Service and Engineering Index, Inc., 1974). Follow the style shown below:

Periodicals

Molina-Cano, J.L., P. Fra-Mon, G. Salcedo, C. Aragonicillo, F. Roca de Togores and F. Gardia-Olmedo. 1987. Morocco as a possible domestication center for barley: biochemical and agromorphological evidence. Theor. Appl. Genet. 73:531-536.

Books (edited by someone other than the author of the article)

Hanelt, P. 1986. Cruciferae (Brassicaceae). Pp. 272-332 *in* Rudolf Mansfelds Verzeichnis landwirtschaftlicher und gärtnerischer Kulturpflanzen (ohne Zierpflanzen), Vol. 2. (H. Schultze-Motel, ed.). Akademie-Verlag, Berlin, Germany.

Books (identical author and editor)

Chapman, C. 1985. Genetic Resources of Wheat. A Survey and Strategy for Collecting. IBPGR, Rome, Italy.

Nomenclature

Taxonomical: in line with *Index Kewensis*. *Genetic*: applications of the terms phenotype and genotype should be in accordance with Demerec *et al.* (Genetics 54:61-74, 1966); for summaries of genetic abbreviations, consult the *Journal of Bacteriology* Instructions to Authors.

Units: express all quantities in terms of SI. If a traditional or local unit is used, or a unit that may be well known in one country only, always include an SI equivalent so that other workers can fully understand the amounts.

Preparing figures and tables

Tables and figures support the text and must be organized logically, appearing where they are mentioned. If there is a large amount of information in a table, it may be better to include it as an appendix at the end of the paper. Figures and tables should be clear and simple. Their major purpose is to present complex material in a form that is easily understood. Present data in the text, or as a figure, or a table, but never in more than one of these ways.

La version française des Conseils aux auteurs se trouve à la page 54.

La versión española de las Instrucciones para los autores se encuentra en la página 55.

Plant Genetic Resources Newsletter

No. 103, September 1995

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