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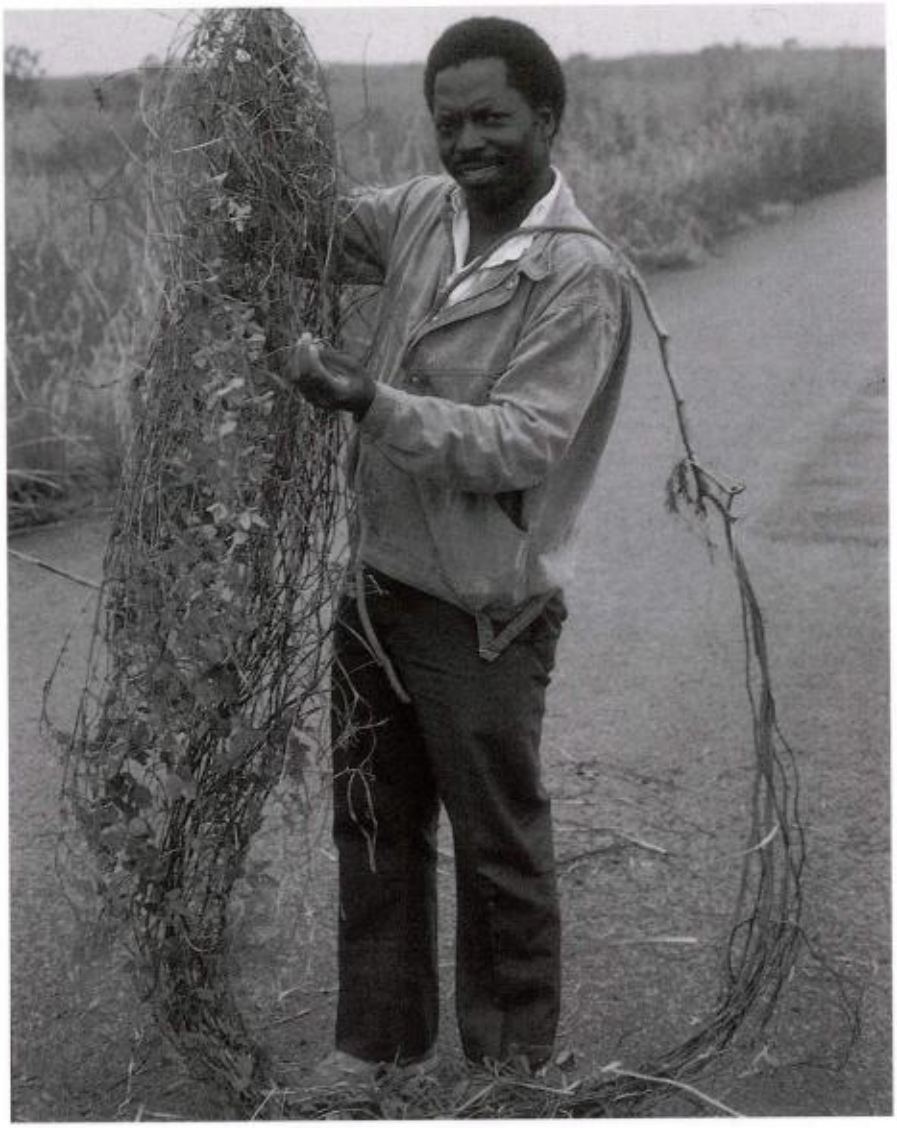

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Cover: A.E. Goli with a single plant of *Vigna radicans* - Zambia.

Couverture: A.E. Goli avec une plante de *Vigna radicans* - Zambie.

Portada: A.E. Goli con una planta de *Vigna radicans* - Zambia.

Germplasm-collecting missions in Africa in the 1980s

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Summary
A series of germplasm-collecting missions took place from 1985 to 1989 in six African countries including Cameroon, Central African Republic, Congo, Guinea, Malawi and Zambia. Exploration routes and strategies in each country are provided here with mention of occasional difficulties encountered. Cowpea, rice, and their wild relatives were the major target species but important African crops such as Bambara groundnut, yam, cassava, fonio, okra and groundnut were also collected. To a lesser extent, minor grain legumes including jack bean, sword bean, pigeonpea, wing bean and Kerstin groundnut were collected when encountered. A total of 2266 accessions was gathered during the six collecting expeditions. Samples within the target crops exhibited impressive variabilities with regard to the plant as well as the seed characteristics. In general, the materials collected were sent to IITA for processing and conservation at the Genetic Resource Unit. A duplicate set of the material collected in each country was left with the collaborating institution for conservation and use by national partners.

Introduction
The science of plant genetic resources is relatively recent in the world and particularly in Africa. The main collecting missions on the continent started in the mid-1970s and reports on them were not always accessible. From 1985 to 1989, the author undertook a series of germplasm-collecting missions in Cameroon, Central African Republic, Congo, Guinea, Malawi and Zambia, mainly during his stay at IITA (International Institute of Tropical Agriculture). Target crops were essentially cowpea, rice and their wild relatives, but other important African crops such as bambara groundnut, yam, cassava and okra were also collected. Interesting reports were written but not published for a wider audience. Today, genetic resources activities are becoming of prime importance in most African countries. In order to plan and organize national activities including germplasm collections, all information on individual countries must be readily available. It is therefore desirable and useful, despite the time elapsed, to publish this information for any germplasm researcher interested in the countries concerned.

Collecting in Central African Republic
The exploration was carried out from 27 October to 16 December 1985, primarily to collect germplasm of cowpea, rice and bambara groundnut and the wild species of these crops. It was the result of a successful collaboration between IITA and the Ministry of Rural Development in Central African Republic. The three-member team was composed of the author, Mr Zaromo-Badaoro Alphonse as technician and guide, and Mr Balebondjo Victor, the driver. It is worth noting the very pleasant attitude of the farmers. They always came to help whenever the team stopped in a village and were generally willing to lead us to their fields and give samples of their seeds. Often, young men even joined the collectors in the search for wild cowpea in neighbouring fields. A few farmers, however, were initially hesitant. They would give their seeds only after they understood the purpose of the mission and realized the size of the sample needed.

A remarkable advantage in that country was the unity of language. Unlike in many African countries, the entire people of Central African Republic spoke the language called 'sango' despite the existence of several ethnic groups with their respective native tongues.

The exploration area was confined to the western half of the country where the vegetation ranges from tropical rainforest in the southwest to guinea savanna in the north with all the gradations in between. This zone is located between 4°10'N and 7°20'N and 15°E to 21°E. Elevation varies from 340 m along the Oubangui river in the south to 1300 m in the northwest. A regrettable practice observed in all the savanna area was bush fire. Villagers set fire to grasses during the dry season for hunting wild animals as well as clearing the land for their crops. The accidental burning of entire villages during the process was not a rare occurrence.

The collecting team was not able to cover all the areas where the target crops were grown. Some interesting zones in the southeast remained to be explored. A summary of the 421 samples collected during the mission is presented in Table 1. The exploration itinerary and sites where wild species were collected are illustrated in Figure 1.

Table 1. Germplasm collected in Central African Republic, 1985

Crop	Species	No. of samples
Cowpea	*Vigna unguiculata*	191
Wild cowpea	*Vigna unguiculata* subsp. *dekindtiana*	19
Wild *Vigna*	*Vigna* spp.	58
Bambara groundnut	*Vigna subterranea*	59
Groundnut	*Arachis hypogea*	3
Lima bean	*Phaseolus lunatus*	4
Winged bean	*Psophocarpus tetragonolobus*	1
Asian rice	*Oryza sativa*	54
African rice	*Oryza glaberrima*	1
Wild rice	*O. punctata*	7
	O. longistaminata	4
	O. barthii	3
Maize	*Zea mays*	17
Total		421

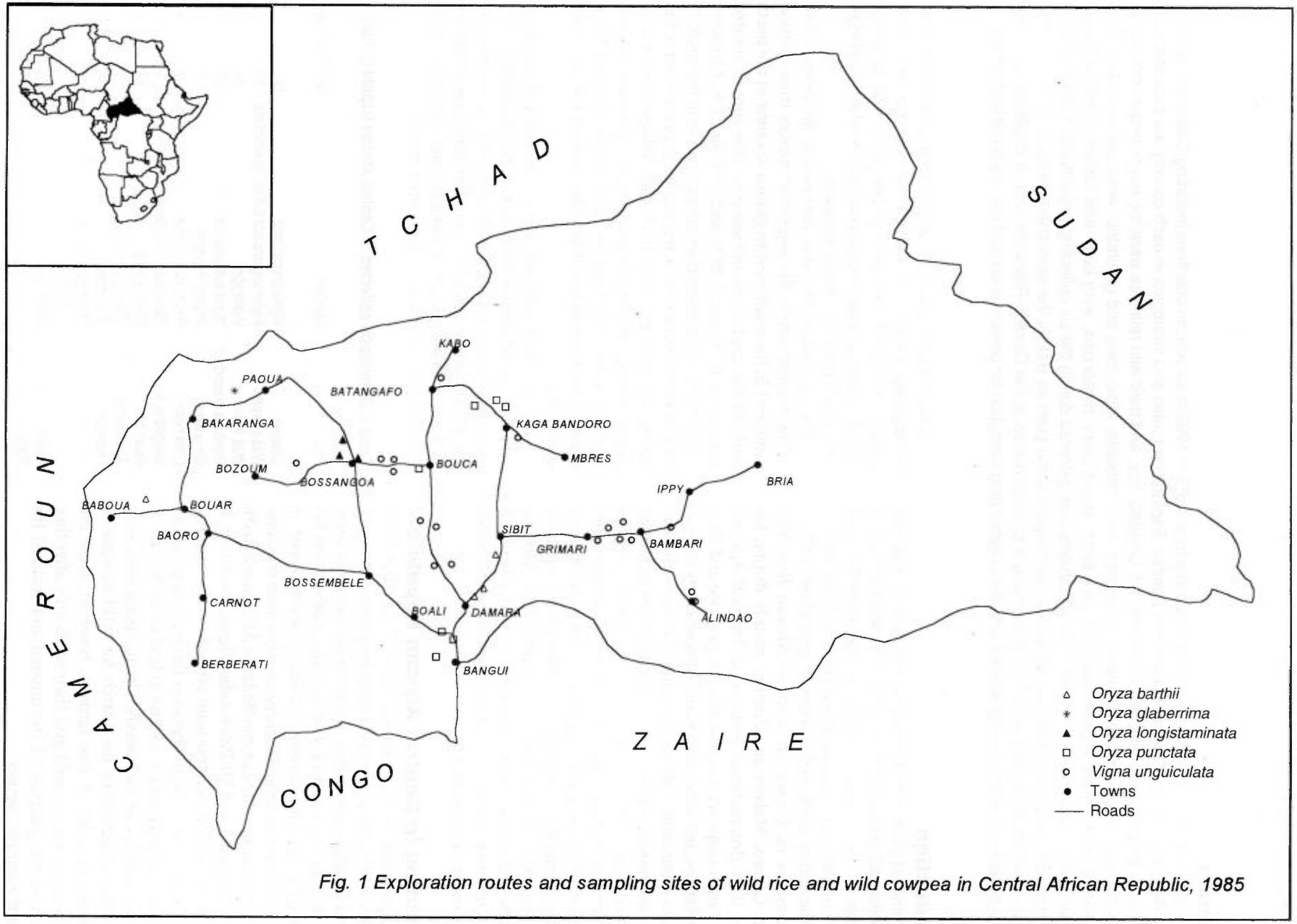


Fig. 1 Exploration routes and sampling sites of wild rice and wild cowpea in Central African Republic, 1985

Fig. 1. Exploration routes and sampling sites of wild rice and wild cowpea in Central African Republic, 1985.

Crop variability

Cowpea, rice and bambara groundnut were grown to various degrees throughout the regions explored during the mission. Variability was greater among samples of cowpea and bambara groundnut than those of rice.

Cowpea

Cowpea was widely cultivated, especially in the south-central area. It was always grown in association with other crops such as cassava, sorghum and particularly cotton. Cowpea in a pure stand was not found. Farmers enjoyed planting cowpea in cotton fields because they took advantage of chemical treatments (fertilizers and pesticides) supplied by agricultural authorities for cotton production. As a result, cowpeas from cotton fields gave higher yields. A danger, however, was that farmers could not help eating the cowpea leaves which had been sprayed with pesticides.

Cowpea samples collected presented great variability with respect to features such as size, shape and colour for both pod and seed. Some pods were very straight, others were spiral-shaped and the majority exhibited various degrees of curvature. Seed coat colour ranged from white or cream, through light and dark brown to red or black.

Wild cowpea (*Vigna unguiculata* subsp. *dekindtiana*) and various wild *Vigna* species were relatively abundant in the south-central part of the country. They became rare in the northwest as the elevation became higher. Wild cowpea was more frequently found in areas where the cultivated type was more abundantly grown.

Rice

Rice was not a staple food in Central African Republic. Many ethnic groups would consume rice only when a shortage of cassava occurred. Nevertheless, a number of families claimed to have been growing rice since the days of their grandparents. When asked about the origin of their seeds, they invariably said that they saved seeds from the previous year. Occasionally, they would buy seeds from a market. Rice samples collected in most areas look similar and are relatively improved. Rice was grown either in mixture with cassava or maize or in pure stand on a piece of land usually not exceeding 0.5 ha.

Only one sample of the African traditional rice (*Oryza glaberrima*) was collected in the northwestern part of the country. The crop was not cultivated but rather growing wild in a stream. It could have escaped from cultivation.

Occasionally, wild rice was collected in ponds or swampy areas. Species found included *Oryza barthii*, *Oryza punctata* and *Oryza longistaminata* (Fig. 1).

Bambara groundnut

This legume was not an intensive crop although it was found almost everywhere. Like cowpea, it was always grown in mixture with other crops. Often the same farmer grew both cowpea and bambara groundnut. The variability among the samples of bambara groundnut collected was almost comparable to that of cowpea. Seeds have an array of size, shape and colour.

The same patterns of seed coat colour described for cowpea were present in bambara groundnut but with much more mottling. Most pods contained single seeds but pods with two seeds also were frequent.

Other crops

Other crop samples collected included maize, groundnut, lima bean and winged bean. Although not widely grown, maize exhibits a notable variability in seed colour and ear size. Groundnut seed size and colour also were variable. Another food crop widely grown but not collected during the exploration was okra.

Collecting in Zambia

The principal aim of the mission was to collect samples of wild species of the genus *Vigna*. Not only did these plant species needed to be safeguarded but they could contribute to the improvement of cultivated *Vigna* species through appropriate interspecific cross-breeding. It was also an opportunity to collect samples of the cultivated cowpea (*Vigna unguiculata*) as well as rice (*Oryza* spp.) and bambara groundnut (*Vigna subterranea*). The mission took place from 16 March to 20 April 1987 with a vehicle supplied by the Ministry of Agriculture and Water Development of Zambia. IITA bore fuel costs and provided travel allowances for the local counterpart, Mr Charles Nkoma, and the driver, Mr K. Simwanza. It was an excellent collaboration between IITA and the Government of Zambia. The collecting team consisted of the author, then scientist at IITA, the national counterpart and the driver.

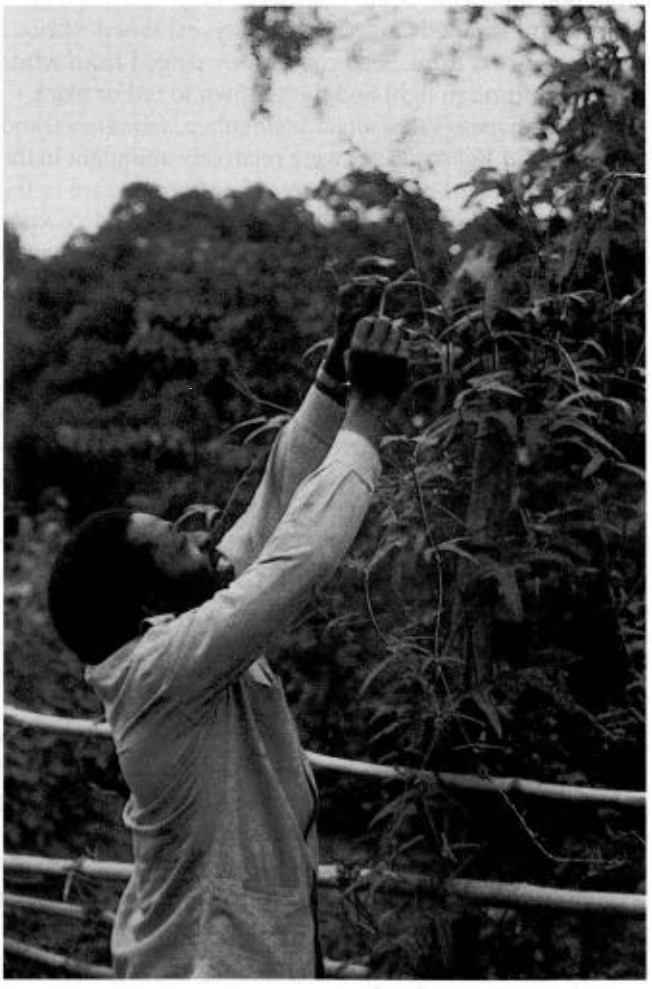
Zambia is a fairly large country covering some 752 600 km^2. It was not possible to cover thoroughly the entire country during the 5-week mission. Much of the northwestern region was not explored. A previous plant exploration mission organized by IBPGR/IITA and led by Dr R.K. Arora took place in Zambia in 1980. The team explored only the central and northeastern provinces of the country. It was a multicrop collecting mission concentrated on cultivated plant species with sampling sources including primarily farmers' fields, threshing grounds, backyards, and village markets. In the present mission, the exploring team stopped about every 10 km or whenever it was appropriate to search for wild *Vigna* species in the natural vegetation. Ponds and rivers or streamsides also were explored for wild rice species. Farmers' fields, farm stores and village markets were visited for collecting the cultivated crop species. It should be pointed out that the mission was scheduled a bit too early. Most crops were still green and immature in farmers' fields in late March and early April, especially in the northeastern province. The period from early May to mid-June would have been more appropriate. The other setback was the flooding of the Zambezi river valley during early April in the western region, which made many roads in the area unfit for motor traffic. In the southern provinces, on the other hand, farmers were not able to plant some crops because of a serious drought.

Travelling difficulties in Congo. This is the national road linking Brazzaville to Pointe Noire.

A.E. Goli is showing a strange cassava cultivar to farmers for further information.

Greeting a yam farmer in his field before requesting samples - Zambia

Using a dug-out canoe to collect deep water wild rice in the Zambezi river - Zambia



Reaching high for a wild cowpea - Congo

Collecting native creeping cowpea on a roof - Guinea

Collecting a wild *Vigna* species - Zambia

Bush fire devastates vegetation out of sight - Guinea

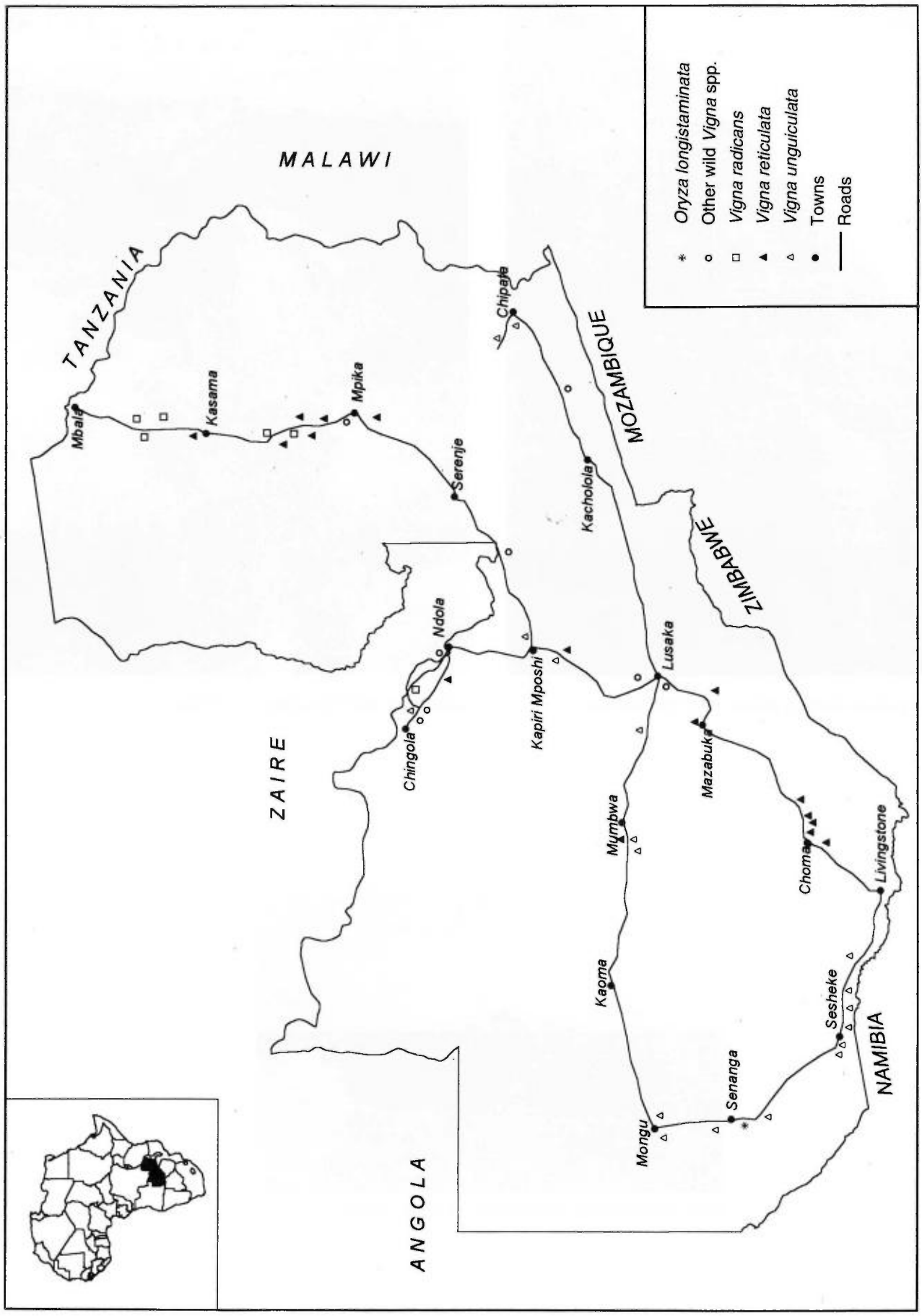


Fig. 2. Exploration routes and sampling sites of wild *Vigna* and wild *Oryza* in Zambia.

A summary of the 168 germplasm samples collected during the mission is presented in Table 2. The exploration route along with the sampling sites of the wild species are shown in Figure 2.

Table 2. Germplasm collected in Zambia, 1987

Crop	Species	No. of samples
Cowpea	*Vigna unguiculata*	52
Wild cowpea	*Vigna unguiculata* subsp. *dekindtiana*	21
Wild *Vigna*	*Vigna reticulata*	18
Wild *Vigna*	*Vigna radicans*	8
Wild *Vigna*	*Vigna oblongifolia* subsp. *parviflora*	1
Wild *Vigna*	*Vigna* spp.	10
Bambara groundnut	*Vigna subterranea*	52
Green gram	*Vigna aureus*	1
Asian rice	*Oryza sativa*	4
Wild rice	*Oryza longistaminata*	1
Total		168

Germplasm variability and distribution

The flora of Zambia is known to contain a wide range of *Vigna* species. The herbarium maintained at the Mount Makulu research station gave evidence of the diversity and variability. During the mission, however, the team did not have the opportunity to discover the full range of these species. They were not easily found, especially during the eastern expedition in late March. In addition to the scarcity, it was very frustrating when no pods could be found on the occasional species encountered. Wild cowpea (*Vigna unguiculata* subsp. *dekindtiana*) occurred frequently in the Chipata area, in the East, and in the western region from Mongu to Sesheke. *Vigna reticulata* was particularly abundant from Choma to Lusaka in the south and from Kapiri-Mposhi to Ndola and Mufulira in the Copper Belt. This species exhibited substantial variabilities in leaflet morphology, hairiness and growth habit. *Vigna radicans* was always present in large colonies. It was found between Kaoma and Mongu in the western region, a little more in the Copper Belt, and abundantly in the northern region from Mpika to Mbala. The normally small plant produced at the base of the lower leaves a stolon-like structure that could stretch over 3 m on the ground. A larger form of the species was found about 85 km north of Kasama. It was a very large creeping plant with the stolon-like structure reaching 7 m.

Cowpea

No cowpea sample was collected in the eastern province. The crop was not as popular as French bean in that area. Farmers in Central and Western Zambia were more familiar with cowpea, where they grew late-maturing cultivars. Planting to harvesting was said to take about 5 months. Several morphotypes occurred with variable pod or seed size, shape and colour. Many farmers in the south did not grow the crop that season because of drought.

Bambara groundnut

This crop followed much the same trend as cowpea. Often they were mixed in the same field. Bambara groundnut was also found in pure stand in fields as large as 1 ha. Among the varieties found in the western province was a type with a prominent red eye in a V shape. Another frequent type had a prominent butterfly-like black eye on a creamy seed coat. Bambara groundnut was not a totally forgotten crop in Zambia. A team of workers at the University of Zambia received funds from the Southern African Center for Cooperation in Agriculture (SACCA) to promote research on the crop. The team launched collecting missions in the eastern, southern and western provinces and gathered some 300 accessions. It was proposed to evaluate and screen the germplasm for nutritional quality and to make crosses among selected accessions.

Rice

Very few rice samples were collected during the mission. Most of the farmers questioned did not grow it or had it in the field still immature. The crop was mostly grown in the regions of Chipata and Chama in the eastern province. The zones of cultivation in the northern province included the region of Kasama and the area around Lake Tanganyika.

Wild rice was equally scarce. The only sample of *O. longistaminata* collected was found in the flooded valley of the Zambezi river, a few kilometers south of Senanga in the western province. The collecting team had to hire a canoe in order to reach the target plants.

Collecting in Malawi

The expedition to Malawi, from 21 April to 16 May, was a continuation of the plant exploration mission in Zambia. It was equally a collaboration between IITA and the Government of Malawi. Another national counterpart, Dr P. Sibale, and another driver, Mr J. Kankono, joined the author to make up the collecting team. Again, the main objectives were to collect species of cowpea and rice with special emphasis on the wild types. Bambara groundnut as well as other minor legumes such as pigeon pea, green gram, black gram and wild winged bean also were collected.

The collecting strategy was the same as in Zambia. Owing to the small size of the country the team was able to cover the entire territory as shown in the exploration route (Fig. 3). Table 3 presents a summary of the 258 germplasm samples collected throughout the exploration.

Crop variability and distribution

Wild *Vigna* species

Wild cowpea and other species of the genus *Vigna* were common throughout the Malawian territory. In general, they were found more readily in areas of high altitude. Malawi is a very mountainous country in most parts. Altitude ranges from practically 0 m in the Shire Valley at the southern tip to 3000 m in the Mulanje vicinity just some 50 km away. Although very few samples were collected because of a lack of

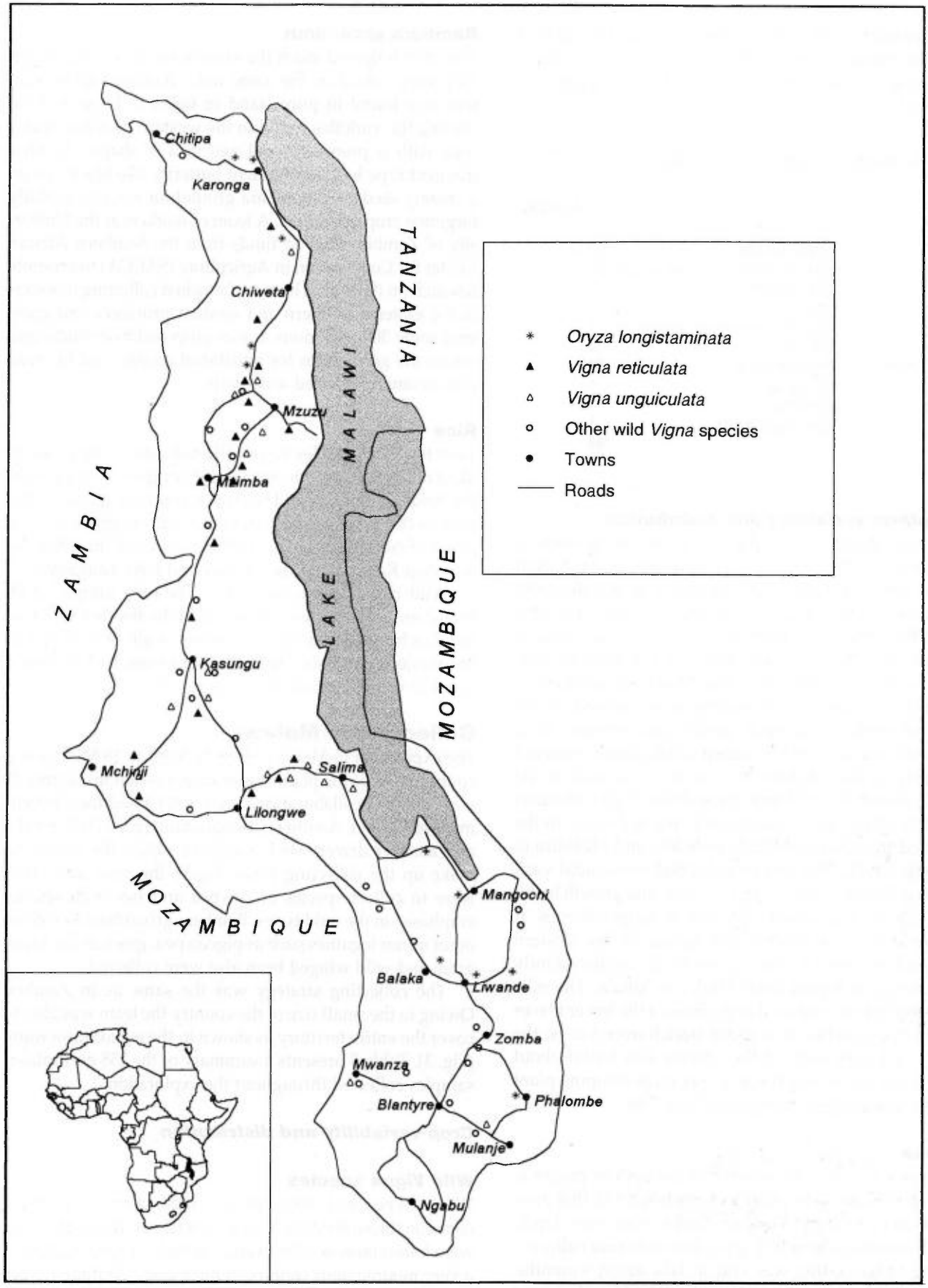


Fig. 3. Exploration routes and sampling sites of wild *Vigna* and wild *Oryza* in Malawi, 1987.

Table 3. Germplasm collected in Malawi, 1987

Crop	Species	No. of samples
Cowpea	*Vigna unguiculata*	89
Wild cowpea	*Vigna unguiculata* subsp. *dekindtiana*	19
Wild *Vigna*	*Vigna reticulata*	18
Wild *Vigna*	*Vigna* spp.	26
Bambara groundnut	*Vigna subterranea*	59
Green gram	*Vigna aureus*	1
Black gram	*Vigna mungo*	1
Pigeon pea	*Cajanus cajan*	2
Wild pigeon pea	*Cajanus* spp.	2
Wild winged bean	*Psophocarpus* spp.	1
Asian rice	*Oryza sativa*	26
Wild rice	*Oryza longistaminata*	14
Total		258

mature pods, the ecology prevailing between Mulanje and Phalombe was quite conducive to the establishment of wild cowpea. The other area with a special abundance in wild *Vigna* species was the zone in the Viphya mountain stretching over 30 km north of Chikangawa town. Samples collected or observed in that area showed remarkable variability in leaf and pod size, hairiness, peduncle length and flower colour. Many plants, however, were just in the flowering stage. The month of June would have been more appropriate for picking most mature pods. *Vigna reticulata* was found frequently along the Lilongwe-Salima axis in the central region and from Mzuzu to Chiweta in the northern province. An unidentified species was found near Chitipa in the extreme north. The plant had a hard reddish stem with small leaflets. Pods were flat when immature and contained small round seeds of deep brown colour at maturity. Other species collected in the Kasangu area had thick leaves resembling blotting paper to the touch.

No wild *Vigna* species was encountered in the southern lowland of the Shire valley or along Malawi lake. These zones generally offered poor vegetation of shrubs and sparse grasses.

Cowpea

This crop was more popular in Malawi and was found everywhere although large fields were not encountered. Some people met in the southern area were even in the business of cowpea commercialization. They were buying tonnes of the grain legume from farmers and selling them to an international agency for feeding refugees from nearby Mozambique. Cowpea was usually grown in association with maize, sorghum, millet, cassava or other grain legumes. The samples collected showed significant variability, especially in relation to the size and colour for both pods and seeds. However, the majority of the cultivars grown had small whitish seeds.

Rice species

Cultivated rice samples were essentially collected in the lowland areas bordering Malawi and Malombe lakes. Specifically, these areas included the Karonga and Chiweta regions in the north and the lakeshore linking Salima to Liwonde via Mangochi in the eastern and central provinces. National rice schemes were implanted around Salima and within the Shire river valley. Farmers associated with these schemes were supervised by extension service agents and grew exclusively improved varieties such as 'Blue Bonnet'. Other independent rice growers made more use of their traditional varieties which exhibited some impressive variability. Awn length, stipule size, glume colour, grain shape and scent were all variable. Some morphotypes were presumably semi-wild as they shared common features with *O. barthii* or *O. longistaminata*.

Among the typical wild rice, only *O. longistaminata* was collected. Very large colonies of the species were found in the Mangochi to Salima area and more strikingly in the northern region around Chiweta. Colonies of smaller size also occurred in the rice schemes and around the townships of Zomba and Karonga.

Bambara goundnut

Like cowpea, bambara groundnut was grown in small patches in most parts of the country. The cultivation was more concentrated in the western and central regions, between Kasunga and Mchinji where the crop was often found in a pure stand. Two types were noted: the small, smooth and thin-shelled pods and the much larger types with a thicker and grooved shell. Variability was also observed in seed coat colour as well as eye colour and pattern.

Other crops

Farmers in Malawi grew a bit of everything, from grain legumes to cereals, through cassava, coffee, tea, tobacco and sugarcane. A visit to a town market enabled us to realize the amazing range of grain legumes available. In addition to cowpea and bambara groundnut, the list included French bean, chickpea, pigeon pea and groundnut. Most of these crops offered incontestable variabilities as a result of the diverse ecologies related to the contrasting elevations.

Collecting in Cameroon

This plant exploration mission was undertaken from 27 October to 6 December 1987 and was confined to the central and southern part of the country, precisely between 2°N and 6°30'N latitude and from 9°15'E to 14°30'E longitude. In fact, it was a complement of a previous mission carried out in Northern Cameroon in 1979 by Dr A.T. Perez, then a plant explorer at IITA. The much-welcomed mission illustrated the good collaboration between IITA and IRA (Institut de Recherche Agronomique) in Cameroon. The collecting team consisted of the author, a national counterpart, Mr Philippe Edimengo, and a driver, Mr Guillaume Assené.

The main target crops during the exploration were cowpea, rice and their wild relatives, yam and bambara groundnut. The exploration route is illustrated in Figure 4. A total of 285 germplasm samples was collected (Table 4).

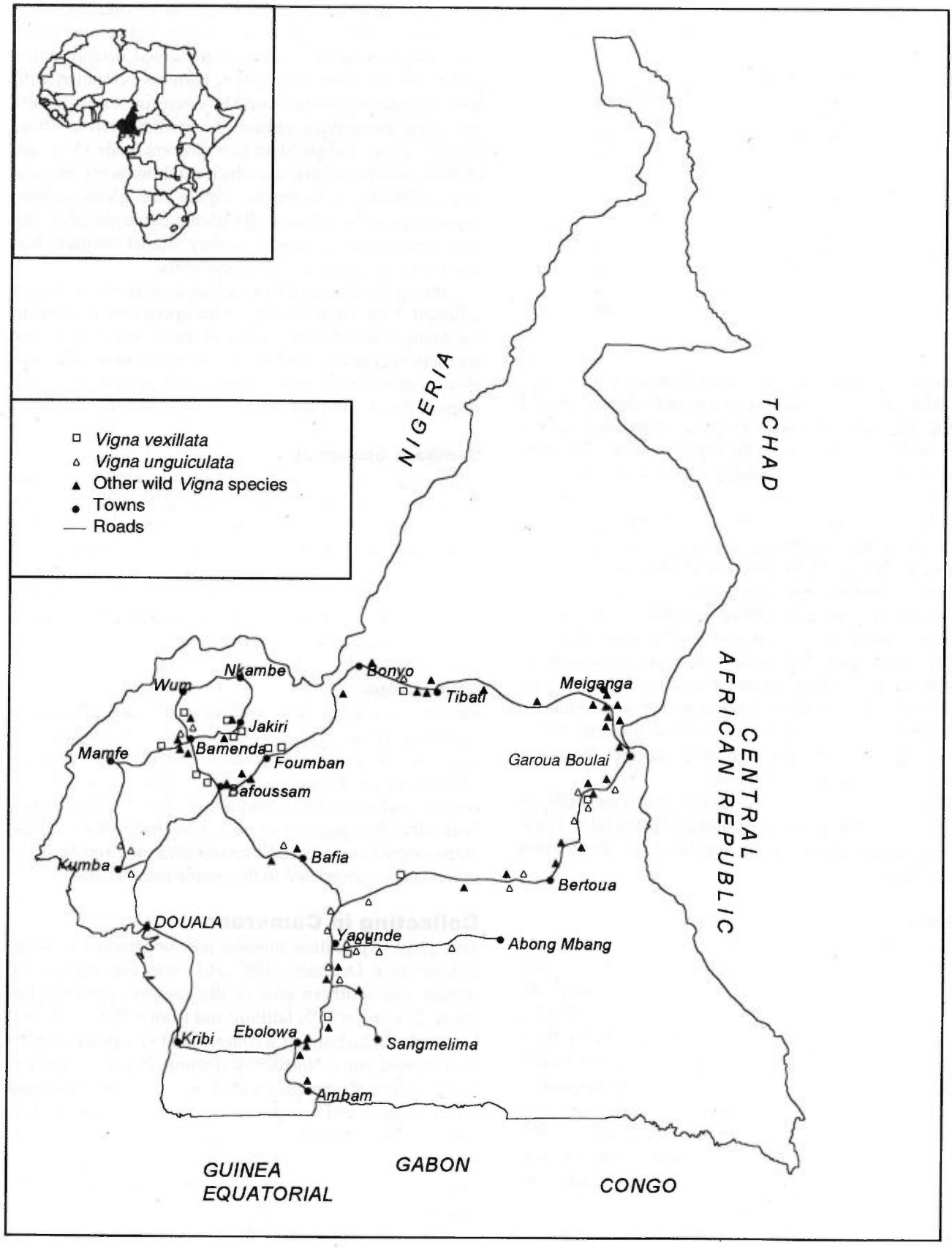


Fig. 4. Exploration routes and sampling sites of wild *Vigna* species in Cameroon, 1987.

Table 4. Summary of germplasm collected in Cameroon, 1987

Crop	Species	No. of samples
Cowpea	*Vigna unguiculata*	64
Wild cowpea	*V. unguiculata* subsp. *dekindtiana*	26
Wild Vigna	*Vigna* spp.	71
Rice	*Oryza sativa*	4
Yam	*Dioscorea* spp.	17
Bambara groundnut	*Vigna subterranea*	32
Common bean	*Phaseolus vulgare*	32
Lima bean	*Phaseolus lunatus*	5
Okra	*Abelmochus esculentus*	23
Cucumber	*Cucumis* spp.	6
Maize	*Zea mays*	5
Cassava	*Manihot esculenta*	2
Jack bean	*Canavalia ensiformis*	1
Sword bean	*Canavalia gladiata*	1
Wild pigeon pea	*Cajanus* spp.	2
Total		291

Crop importance, distribution and variability

Cowpea

This grain legume was important and popular in Cameroon. The major area of cultivation was confined to the northern part of the country with a maximum diversity said to exist in Maroua region. Within the area covered during the present mission, the crop was more frequently found around Foumban township, near Nkambe in the northwestern province, and towards Mamfe and Kumba in the west. Ecotypes collected in the latter region had large pods while the varieties in the northwest all had small, light-brown seeds. Cowpea was scarce in the eastern region, unpopular in the vicinity of Yaoundé, and totally unknown in Kribi district in the southwest. Many villagers in the latter area were inquisitive and often asked to keep the sample shown to them for a trial. This was an indication that the crop could be more widely accepted with proper introduction through the extension service.

Wild *Vigna* species

Wild *Vigna* species were among the main targets during this plant exploration. The team stopped at about every 12 km to search for these species in the wild vegetation. Wild cowpea (*V. unguiculata* subsp. *dekindtiana*) was common in many parts of the explored area. A hairy form of this species was found frequently over 60 km from Yaoundé to Ayos in the lower central province. The species *V. vexillata* with broad whitish or purple flowers was widespread in most areas. Exceptionally, large colonies were encountered in the northwestern province, near Wum and Jakiri townships and within a 40-km radius around Bafoussam. Other species such as *V. reticulata* and *V. luteola* also were frequent, especially from Meiganga to Bertoua in the east. Surprisingly enough, I learned from villagers in Nkambe region (Northwestern province) that they ate the fresh juicy pods of a type of wild *Vigna* identified as *V. ambascencis*. They said the pods were eaten either raw, being a bit sugary, or cooked and added to rice. However, the legume was not cultivated. Wild *Vigna* species were not found in the dense forest in the south or southeast. Collecting sites of these species are depicted in Figure 4.

Rice and wild rice

Traditional rice was reported to be mainly produced in the northern and northwestern provinces with over 60% of the production originating from the latter region. Average yield was estimated to about 1500 kg/ha.

The itinerary of the present mission encompassed the northwestern province but very few rice samples were collected in the area. Most farmers did not grow the crop and the rare fields encountered were planted with improved varieties under the guidance of the extension service agents. The African rice *O. glaberrima* was not found. Wild rice was very scarce throughout the exploration despite the careful search at every pond, swamp and riverbank.

Only one large colony of *O. longistaminata* was found in the Niong river outside Ayos township in the south-central province. The entire field was at the flowering stage and the team was not able to collect a seed sample.

Bambara groundnut

It was a popular food legume in most savanna areas, especially in the district of Foumban and Bafoussam in the northwest and the region of Garoua Boulai in the far east. Fresh pods of bambara groundnut were seen sold on market, not in the usual small quantities, but in large wash-basins of 20 kg or more. Samples collected were characterized by their large pods generally containing two or more seeds. The variability, in terms of seed test pattern, was not very impressive. Most samples had a uniform milky or brown seed coat. Bambara groundnut was not common in the forest zone. No sample was found in the district of Ayos or Sangmelima and the crop was hardly known in the regions of Kribi. Often, only one woman cultivated it in a whole set of villages.

Yam

Yam cultivation is confined to the central and particularly western regions. Total production amounted to nearly 110 000 tonnes annually at an average yield of about 5 t/ha. Yam was grown either as a sole crop or in association with cassava, beans, cocoyam or maize. Most fields covered less than 0.1 ha. Species commonly cultivated included *Dioscorea rotundata*, *D. alata* and *D. bulbifera*. Differences in tuber shape and hairness, peel colour or flesh colouration were observed. Most cultivars on sale were more or less cylindrical with slight or no hair, brown peel and white flesh. An unusual sample collected in the dense south-western forest was a huge, coarse, shapeless tuber hardly believed to be cultivated. Another one, locally called 'Andia' in the deep south near Ambam town, had profuse, strong, long hairs at the apical end. All the yam samples were left with IRA for an initial safe multiplication.

Other crops

Other food crops commonly found throughout the exploration included primarily cassava, common bean (*Phaseolus vulgare*), cocoyam (*Xanthosoma sagittifolium*) and maize. Common bean was much more popular than cowpea in the areas visited. Other crops such as groundnut, sesame, millet and sorghum in Adamaoua province as well as cucumber and okra were readily cultivated. Major cash crops included tea in Ndop district in the northwestern province, coffee, and particularly cocoa in the entire forest zone.

Collecting in Congo

Congo covers 342 000 km^2 and lies between 4°N and 5°S latitude, and ranges from 11°E to 18°40'E longitude. The exploration was confined to the southern half of the country where the vegetation was a mixture of woody savannah and tropical forest. The northern region was covered with a dense equatorial forest where timber, oil palm and cocoa constitute the major resources. The mission took place from 11 April to 28 May 1988 and was a fruitful collaboration between IITA and DGRST (Direction Générale de la Recherche Scientifique et Technologique) of Congo. It was led by the author assisted by a national counterpart, Mr Ndilou Ndilou, and a driver, Mr Albert Benabio. The paths followed during the exploration are shown in Figure 5. Some parts of the roads were not easily passable, especially that linking Loubomo to Pointe-Noire through the Mayombe mountains forest. Nevertheless, the team completed the mission successfully and collected a total of 422 germplasm samples (Table 5). In addition to cowpea, rice, yam, bambara groundnut and cassava, the team devoted a good deal of time to searching for and collecting wild species of *Vigna* and *Oryza*. Figure 5 depicts the collecting sites of these wild species. The mission also was an opportunity for the national counterpart to complete his groundnut collection started a few years earlier but suspended for lack of logistic support.

Table 5. Summary of germplasm collected in Congo, 1988

Crop	Species	No. of samples
Cowpea	*Vigna unguiculata*	76
Wild cowpea	*V. unguiculata* subsp. *dekindtiana*	35
Wild *Vigna*	*V. luteola*	12
Wild *Vigna*	*V. reticulata*	11
Wild *Vigna*	*V. radicans*	11
Wild *Vigna*	*V. vexillata*	6
Wild *Vigna*	*Vigna* spp.	21
Asian rice	*Oryza sativa*	8
Wild rice	*O. longitaminata*	1
Cassava	*Manihot esculenta*	155
Yam	*Dioscorea* spp.	20
Bambara groundnut	*Vigna subterranea*	42
Groundnut	*Arachis hypogea*	17
Common bean	*Phaseolus vulgare*	4
Okra	*Abelmochus esculentus*	2
Jack bean	*Canavalia ensiformis*	1
Total		422

Crop variability and distribution

Cowpea

Cowpea was definitely not an important food crop in Congo although it was cultivated in the Bouenza region comprising the districts of Loudima, Sibiti, Mouyondzi and in the vicinity of Pointe-Noire. In these areas, cowpea was normally sown in the first season and harvested by March. Varieties grown were typically traditional and offered variabilities in pod length, seed colour and size. Strains cultivated in the southeast were extremely susceptible to insects (the pod-sucking bug in particular). Yet, the women kept on trying. This endeavour suggested that the crop could be more widely accepted with the introduction of varieties resistant to pest insects.

Cowpea as a food crop was not known in the central region of the country, from Odziba to Oyo townships. The plant was grown not in the field but around houses, only for its medicinal value. According to villagers, the plant was used traditionally to treat various diseases such as chest pain, skin disorders and other ailments with newborn babies. They were amazed to learn that the seeds were edible. Presumably because of the particular use, only one variety was grown in the entire region. The strain had small pods with small pinkish seeds. Cowpea was totally unknown in the districts of Okoyo, Ewo and Owando.

Wild *Vigna* species

Wild cowpea and wild *Vigna* in general were encountered in most parts of the areas explored. Species collected included *V. unguiculata* subsp. *dekindtiana*, *V. vexillata*, *V. radiacans*, *V. luteola* and *V. reticulata*. Wild cowpea with small leaflets was abundant around Pointe-Noire township. Another ecotype with a long, erect peduncle was encountered all the way from Odziba to Owando in the central region. The plant had a very thick stem and grew along the road, often on the pavement, over 5-6 m. Flowers were conspicuously large and purple. According to villagers met in the region north of Pointe-Noire, wild cowpea was used to cure furuncle or smallpox. Samples of *V. vexillata* collected were often different in appearance. A variant found in the Bouenza region had the typical large, coiled flower but the leaflets were very narrow and the pods resembled those of *V. unguiculata* subsp. *dekindtiana*. The plant was generally large and creeping. *Vigna reticulata* plants encountered in that same region were not bearing pods. No wild *Vigna* species was found in the dense Mayombe forest between Mvouti and Pointe-Noire.

Rice and wild rice

In general, rice was not a staple food in Congo and its already meagre production was continuously diminishing chiefly because of the lack of market. Farmers had no means to carry their products to the cities nor could they sell them on the local market. The few producers were desperately waiting for government agencies to come with help. In some areas, villagers claimed that rice cultivation

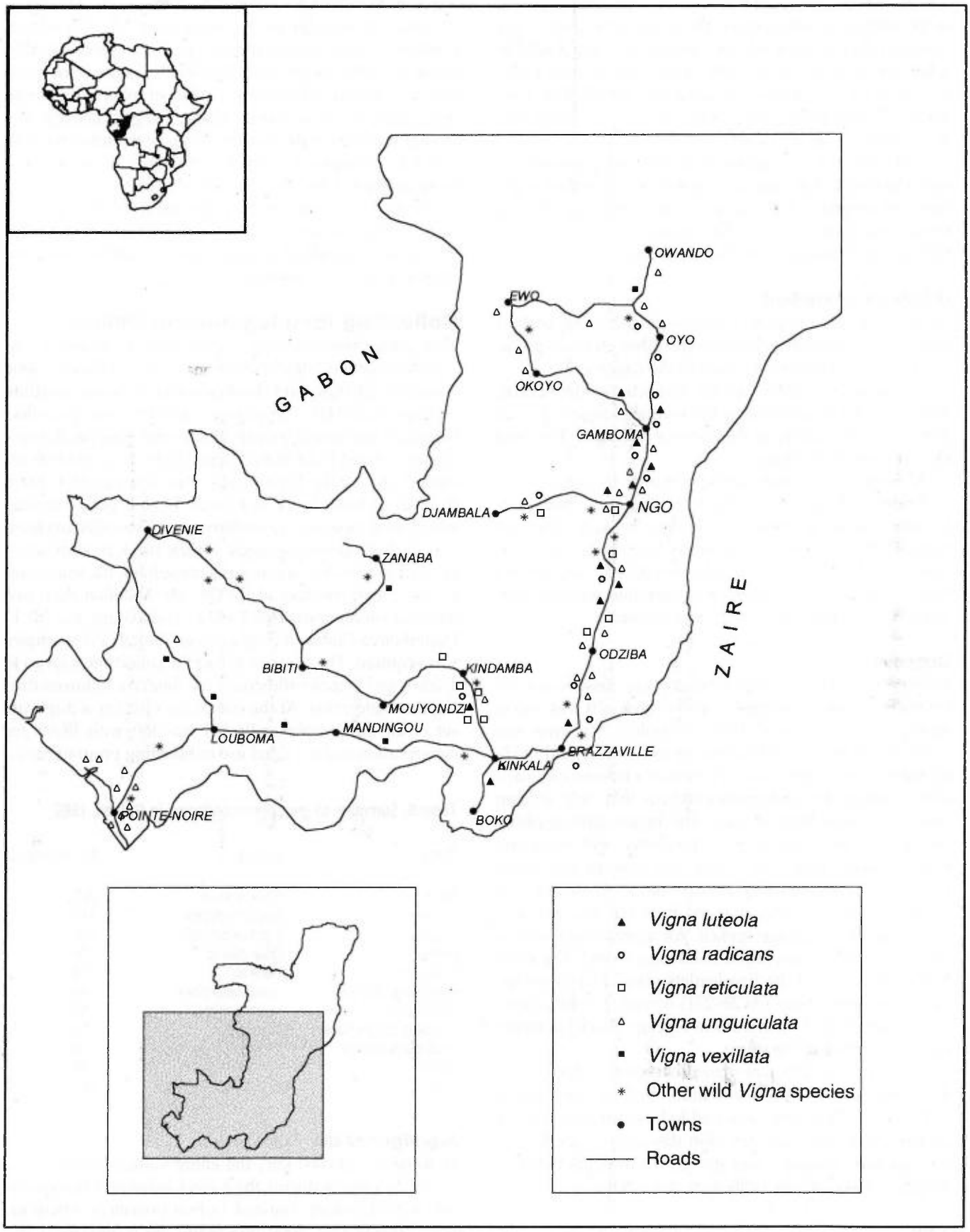


Fig. 5. Exploration routes and sampling sites of wild *Vigna* species in Congo, 1988.

was abandoned because there were no more young people in the villages to take care of the fields or because of the adverse effect of some noxious weeds. Rice was found in relatively large quantities only in the Ewo district. Cultivars grown were mostly improved and supplied by FAO agents. Traditional varieties were scarce where they had not completely disappeared.

Wild rice was not common in the area explored, despite the frequent occurrence of ponds, rivers and swamps. Only one sample of *O. longistaminata* was collected in Loémé river south of Pointe-Noire. The plants, mostly at the grain-filling stage, were growing in deep water.

Bambara groundnut

As in many other African countries, bambara groundnut was not grown intensively in Congo although it was popular and cultivated in many parts of the country.

It was found mainly in the districts of Mouyondzi, Zanaga and Pointe-Noire in the southern region. It was grown equally widely in the districts of Okoyo, Ewo and Oyo in the central region.

Bambara groundnut cultivars grown in Congo were generally characterized by the large size of the pods containing more than one seed. One sample (AG88-234) was particularly striking in its unusually large pods. The seeds were said to be eaten boiled, added to oil, and served with maize, yam or cassava. Bambara groundnut was not cultivated in the north-central region of Owando.

Cassava

In terms of production and consumption, cassava was by far the most important food crop in Congo as it was also in nearby Zaire or Central African Republic. The crop was cultivated in every single corner of the country and made up the base of people's diet. There was a tremendous variability among the numerous cultivars that only women were able to identify readily. The major distinguishing characters were plant height, branching habit, stem and petiole colour, root peel colour and taste of root flesh. Every cultigen had its vernacular name. Naturally, the name of a given variety changed from one region to another with the language spoken. An unusual and strange strain (AG88-255) was found in Zanaga district. The plant had no petiole and the five leaflets grew directly on the stem. Another variety (AG88-251) named 'kikata', meaning 'disabled', had internodes in zigzags which conferred a peculiar shape to the stem.

Although cassava was grown everywhere for food, a particular cultivar (AG88-405) found in the central region and locally called 'Kua' was said to be cultivated only for its medicinal value (to cure skin diseases). According to the villagers, the plant was not grown in fields but only around houses and the roots were not eaten.

Yam

This tuber crop also was popular and widely grown throughout the country. Major production was concentrated in the districts of Bouansa in the south, Kibangou, Divenié, Mossendjo in the southwest, and Djambala, Gamboma in the central region. There was more variability in the cultivars grown in the south. Tubers were cylindrical, roundish or shapeless. Colour of tuber flesh varied from plain white to deep yellow. Length, density and strength of hairs at the proximal ends of the tuber were all variable. Varieties cultivated in the central region were more uniform with long, dark-peeled tubers.

In most areas visited, the period of April-May was too early for harvesting yam. The crop was still in the field, immature. The period of July-August would be more appropriate for yam sampling.

Collecting food legumes in Guinea

This germplasm-collecting mission was a successful collaboration between the International Board of Plant Genetic Resources (IBPGR) and the Agronomic Research Institute of Guinea (IRAG). The primary objective was to collect local food and forage legume species and varieties. Upon a special request from IRAG, fonio (*Digitaria exilis*) was included among the target crops. The mission took place from 25 October to 11 December 1989, a period during which most food crops were harvested. The collecting team was fairly large for the single vehicle used. In addition to the author who was serving as a consultant, the team comprised a staff member of IBPGR, Mr Abdallah Bari, two national counterparts, Mr Fodé L. Guilavogui and Mr L. Doumbouya-Dialakoro, and a driver. A total of 706 samples was acquired. The composition of the collection is given in Table 6 and Figure 6 illustrates the itinerary followed during the exploration. At the end of the mission, a duplicate set of all the samples collected was left with IRAG for conservation and eventual use in breeding programmes.

Table 6. Summary of germplasm collected in Guinea, 1989

Crops	Species	No. of samples
Fonio	*Digitaria exilis*	202
Groundnut	*Arachis hypogea*	148
Cowpea	*Vigna unguiculata*	136
Beans	*Phaseolus* sp.	74
Wild *Vigna*	*Vigna* sp.	56
Bambara groundnut	*Vigna subterranea*	43
Pigeon pea	*Cajanus cajan*	12
Jackbean and swordbean	*Canavalia* sp.	10
Kesting's groundnut	*Kerstingiella geocarpa*	8
Others		17
Total		706

Highlight of the exploration

With nearly 246 000 km^2, the entire Guinea could not be covered carefully during the 6-week mission. The exploration was therefore confined to two provinces where genetic erosion was more important. These provinces, administratively called Moyenne Guinée and Haute Guinée, occupy about 60% of the territory and lie between 9°N and

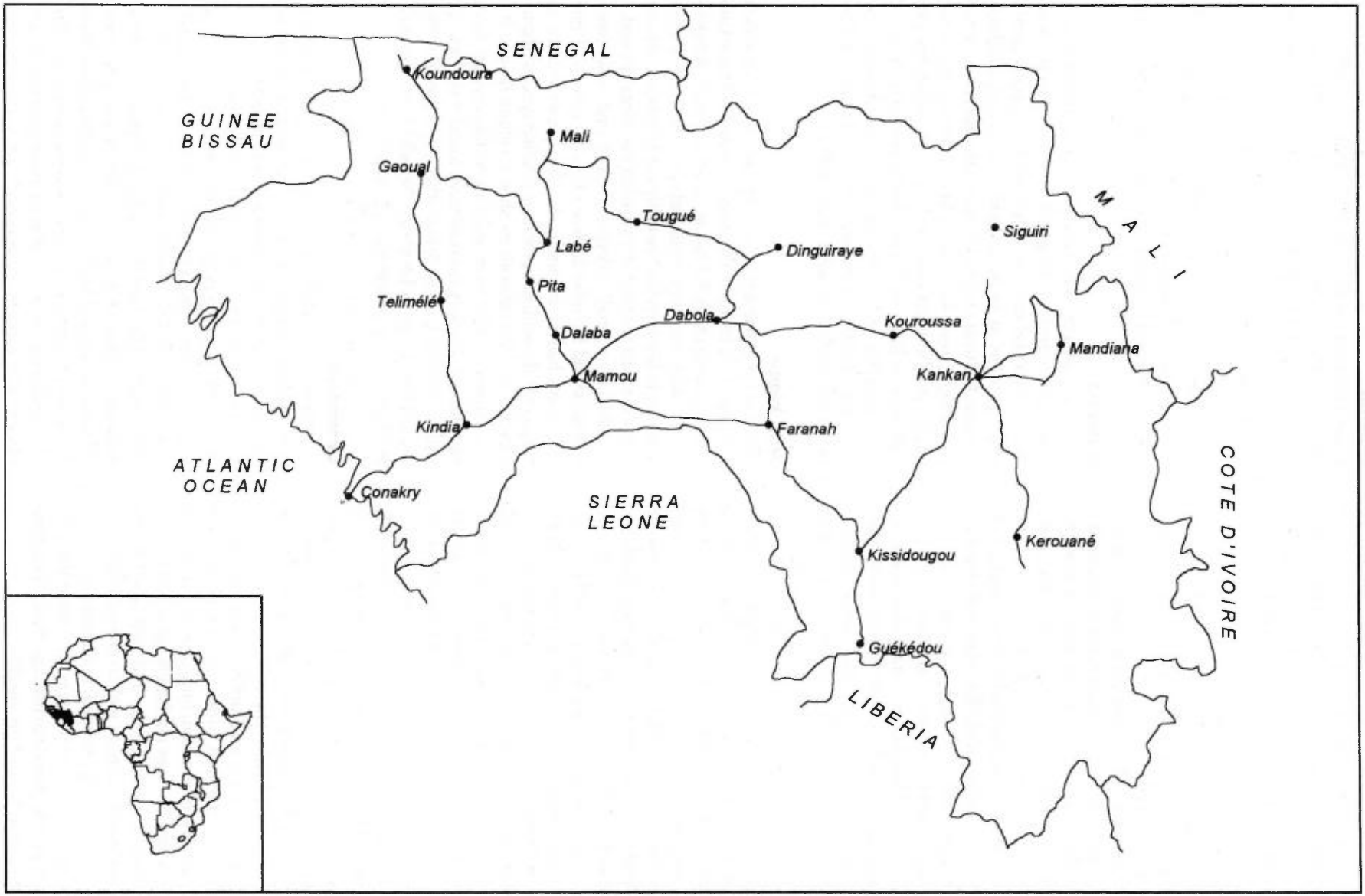


Fig. 6. Exploration in Guinea, 1989.

12°30'N latitude, and from 8°W to 13°30'W longitude. The vegetation was essentially a woody savanna dominated by tall grasses such as *Andropogon* and *Panicum*.

Pockets of luxuriant forest were found occasionally in river valleys or mountains. The exploration zone offered a wide range of altitude, from 70 m in the region of Koundara to 1500 m in the Fouta Djalon mountains. The road infrastructure was not quite adequate, especially in the rocky mountains where average driving speed could be reduced to 12 km/h. Elsewhere, bridges often were broken or ferryboats were not functional.

Despite the occasional difficulties, the mission was generally smooth and pleasant thanks to the admirable cooperation of farmers. At most villages where the team stopped for inquiry, the administrative representative, called chief of district, summoned a number of colleagues around him. After explanation, each of them returned to his house and brought samples of the target crops. With this procedure, the team was able to collect as many as 10 samples at a single site.

Guinea is also a country where animal husbandry is an important activity. Unfortunately, no forage crops were cultivated to feed the cattle which were left to graze in the open nature. This system created a perpetual conflict between crop and animal producers. In fact, the cattle were often unattended and devastated cultivated food crops. As a protective solution, farms in the vicinity of villages were usually surrounded by wooden fences. An important consequence was the reduction in the farm size since erecting a fence was itself an irksome task. Such farms looked more like gardens, rarely exceeding 0.5 ha. So far, there was enough natural pasture for cattle except during the dry season when most of the herbacious vegetation was burned. Bush fire was a widespread practice in the entire savanna area. According to farmers, there was a better regrowth of the grasses after burning. In the Fouta Djalon region, for example, burnt fields often were visible for as far as the eye could see. Bush fire was definitely a sad practice which contributed to the progressive desertification of the region and to the genetic erosion of plant species. In fact, all the plants did not regrow each year after the fire. The importance of germplasm collecting for conservation therefore became apparent.

Guinea has understood the importance and the necessity of a genebank. This was exemplified by the presence of an impressive laboratory with five cold rooms and two drying rooms at the major Agronomic Research Center in Foulaya (CRAF). The laboratory was built in 1984 with the financial assistance of USAID. The cold rooms were well designed and equipped for medium-term seed conservation. Unfortunately, the unit broke down within a few years and by the time of the visit, only one cold room was said to be functional, but was not functioning. Power supply was very erratic at the Center and that could be responsible for the breakdown, as well as the probable mismanagement. All the Center needed, in terms of assistance to pursue the genebank activity, was to restore the cold rooms and have people trained to manage them. A sustainable source of electricity must be sought.

Crop distribution, variability and genetic erosion

Guinea enjoyed an appreciable diversity of ecologies which allowed the cultivation of a large number of crops. Soil type, rainfall and temperature were variable from one region to another. As a result, some crops were restricted to specific areas.

The environmental diversity often led to some degree of variability for crops widely grown. On the other hand, introduction of cash crops or early maturing varieties tended to drive some old traditional cultivars out of the community.

Cowpea

This grain legume was more popular in the south-central part of the country. Very few or no samples were collected in the Fouta Djalon region. The crop was usually grown in small gardens or in association with maize, sorghum or cassava. Cultivars found showed variabilities in maturity, pod length, seed size and colour. Some ecotypes in the southern region had very small, whitish pods while others had pods as large as 30 cm. Most seeds were creamy but some varieties had brown, red or black seed coats. A variety found between Faranah and Mamou was said to be very early, with a growing cycle of 40 days.

Wild *Vigna*

The most popular wild *Vigna* species was *V. reticulata*. It was especially found in the entire province of Haute Guinée but the plants often did not bear mature pods. Samples of wild cowpea (*V. unguiculata* subsp. *dekindtiana)* were collected only between the townships of Télimelé and Gaoual. *Vigna vexillata* was not abundant in the area explored.

Only two small colonies were found, one towards Kerouane and the other between Kissidougou and Faranah. The plants had no mature pods. The species may be more frequent in the southern province. Another species frequently found was *V. luteola* with its showy yellow flower. It was particularly visible from Kankan to Gueckedou through Kissidougou. The species occasionally found was *V. oblongifolia* with its narrow leaflets. A few other *Vigna* species collected needed to be properly identified. No wild *Vigna* was encountered in the rocky mountains of Fouta Djalon.

Groundnut

Groundnut was widely grown in most parts of the provinces explored except the region stretching from Gueckedou to Faranah. About four varieties were available. They differed essentially in maturity, pod length, and seed size and colour. The growing cycle ranged from 3 to 5 months while seedcoat colour varied from pale pink to deep red. Only one variety initially found between Kankan and Siguiri had a red/white mottled seed coat. From the district of Telimelé to that of Koundara, almost every farmer was growing only a particular variety locally called 'Maressi'. According to farmers, it was a productive early maturing (3 months) cultivar introduced from Senegal. It has replaced other ecotypes traditionally grown.

Phaseolus species

Both common bean (*Phaseolus vulgare*) and lima bean (*Phaseolus lunatus*) were readily found during the exploration. They were more popular than cowpea in the Fouta Djalon region. The greatest variability with *P. vulgare* was found in the district of Pita where all kinds of grain colour, size and shape were available. Variability among samples of *P. lunatus* also was impressive. Differences were more pronounced in seed coat colour and seed size. All the cultivated types of lima bean climbed along poles or trees sparsely distributed in other crop fields. Besides the cultivated varieties, many weedy forms were found climbing on fences around houses. Their seeds were smaller, generally pink to red in colour and most people did not eat them. Pods were dehiscent when dry. Leaflet shape among those weedy forms was variable, ranging from lanceolate to roundish.

Bambara groundnut

This grain legume was known but not very popular in the country except in some districts of Haute Guinée province. For most farmers, it was a secondary crop which was sown after priority crops such as groundnut or fonio. As a result, it came to maturity much later. There was little diversity within the samples collected, and basically two varieties were available. Most farmers grew the 4-month cultivar with a reddish seed coat. The other variety had a creamy seed coat but it was less preferred because it had a longer growing cycle (5 months).

Kersting's groundnut

This is an indigenous grain legume which was definitely on its way to extinction. Many young people were not even aware of its existence. The crop was grown very sporadically in some districts of Haute Guinée province, and called 'Duro sosso' in the Malinke language. According to some farmers, they have abandoned cultivating the crop because harvesting its small pods buried in the ground was too tedious and time consuming. They preferred devoting their time to easier and more profitable crops. The few samples collected exhibited some variability in seed size, shape and colour.

Other grain legume species

Pigeon pea (*Cajanus cajan*) was one of the minor food legumes collected. Generally, the crop was used to make a hedgerow around fields. It was not common in the mountain region. Samples collected were much alike with some slight differences in pod length and seed colour. The other grain legumes encountered everywhere were jackbean (*Canavalia ensiformis*) and swordbean (*canavalia gladiata*). The former was more common than the latter. The species were either grown as ornamental plants to provide shade around houses or rarely cultivated for food. In the latter case, the seeds were edible only after extensive boiling with one or two changes of the cooking water and peeling of the seed coat. Without scientific studies, farmers were aware of the toxic compounds, namely the proteins canavalin and concanavalin, contained in the seed. The species also were abundant in the wild state. A particular type with much smaller pods was very common between the townships of Mamou and Pita. Most samples collected had white seeds, but red-seeded types also were found.

Fonio

This non-legume crop was so popular in the country that IRAG made a special request for its collection. The tiny-seeded grass was an old crop in the area but no effort has been made to include it in agricultural research programmes. However, it was widely grown in the entire savanna explored, as indicated by the large number of samples collected. The plant thrived relatively well in harsh environments and was therefore the major food crop in the rocky mountains, especially in the district of Mali. Farmers distinguished about five different varieties according to the growing cycle, plant height, seed size and grain colour. Planting to maturity took 2-5 months, depending on the variety. Differences in seed size were hardly noticeable while grain colour ranged from light to dark brown. Overall, there was no immediate threat of genetic erosion, considering the total absence of improved varieties.

Conclusion and fate of the accessions collected

Despite the absence of national PGR programmes in most African countries in the 1980s, the collecting missions were appreciated and welcome in the various countries. Farmers were instrumental in maintaining genetic diversity within local crop landraces. Women were particularly helpful in the identification of different traditional cultivars within a given crop species.

Wild cowpea and wild *Vigna* in general were abundant in all the countries explored. Wild rice species also were frequently encountered. These wild crop relatives could be useful in breeding programmes for the improvement of existing varieties. Other traditional crops such as bambara groundnut, Kersting groundnut, okra, yam, fonio, etc. needed to be preserved for posterity.

Except for the fonio germplasm from Guinea, all the materials collected were sent to IITA for processing and conservation at the Genetic Resources Unit. A duplicate set of the materials collected in each country was left with the collaborating institution in that country for conservation and use by national partners. Unfortunately, most countries did not have adequate facilities for proper and sustained conservation of the germplasm left with them. That was particularly the case in Cameroon, Central African Republic, Congo and Guinea. As a result, most of the materials either disappeared or lost their viability. On the other hand, the samples taken to IITA were properly managed. All were processed and conserved in cold rooms for medium- or long-term storage. Many accessions had been fully characterized with computerized data. As the accessions entered the genebank, they were distributed around the world upon requests by scientists and other users in various countries.

Résumé

Missions de collecte de matériel génétique en Afrique au cours des années 80.

Entre 1985 et 1989, une série de missions de collecte de matériel génétique ont eu lieu dans six pays africains : Cameroun, Congo, Guinée, Malawi, République centrafricaine et Zambie. Les itinéraires et les stratégies d'exploration utilisés dans chaque pays sont indiqués avec mention des difficultés occasionnellement rencontrées. Le niébé, le riz et les espèces sauvages qui leur sont apparentées étaient les principales espèces recherchées, mais d'importantes plantes cultivées africaines comme le pois Bambara, l'igname, le manioc, le fonio, le gombo et l'arachide ont également été collectées. Dans une moindre mesure, des légumineuses à graines mineures telles que le haricot-sabre, le pois d'Angole, *Psophocarpus tetragonolobia* et diverses autres ont été récoltées au hasard de leur rencontre. Au total, 2 266 accessions ont été recueillies au cours de ces six expéditions de collecte. Des échantillons des plantes ciblées font apparaître une variabilité impressionnante des caractères à la fois de la plante et de la graine. D'une manière générale, le matériel collecté a été envoyé à l'IITA pour y être traité et conservé dans son Unité des ressources génétiques. Un double du matériel recueilli dans chaque pays a été remis à l'institution collaboratrice à des fins de conservation et d'utilisation par les partenaires nationaux.

Resumen

Misiones de recolección de germoplasma en Africa en los años ochenta.

Entre 1985 y 1989 se llevaron a cabo una serie de misiones de recolección de germoplasma en seis países africanos: el Camerún, la República Centroafricana, el Congo, Guinea, Malawi y Zambia. Se indican aquí las vías de exploración y las estrategias aplicadas en cada país junto con las dificultades ocasionales que surgieron. Las principales especies que se recogieron fueron el caupí, el arroz y las especies afines silvestres, pero también se recolectaron importantes cultivos africanos como el maní Bambara, el ñame, la yuca, la digitaria, el gombo y el maní. También se recogieron cuando se encontraron, aunque en menor medida, leguminosas de grano secundarias como el frijol de espada, el frijol de machete, el guandú, el frijol de alas y el maní Kerstin. En las seis expediciones de recolección se reunieron 2266 accesiones. Las muestras de los cultivos mostraron una gran variabilidad con respecto a las características de la planta y de la semilla. En general, los materiales recogidos se enviaron al IITA para su tratamiento y conservación en la Dependencia de Recursos Genéticos. Se entregó a la institución colaboradora un duplicado del material recolectado en cada país para su conservación y utilización por los asociados nacionales.

Long-term storage of old potato varieties by cryopreservation of shoot-tips in liquid nitrogen

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Summary

Collections of plant genetic resources require secure storage systems and simple conservation techniques. A simple freezing method has been developed for the cryopreservation of old potato varieties kept as an *in vitro* collection. To date 219 varieties and genotypes have been frozen and stored, 63 852 shoot-tips in total. Calculated on the basis of the regrowth experiments, a survival rate of approximately 80% has been obtained. More important for a collection of plant genetic resources, however, is the rate of plant regeneration. Using the same regeneration medium for all genotypes approximately 40% of the frozen shoot-tips developed into plants after thawing. Also important is the fact that plants have been obtained from all varieties frozen so far. The genetic stability of regrown plants has been confirmed by comparing morphological parameters, flow cytometric measurements and Restriction fragment length polymorphism (RFLP) analysis.

Introduction

Collections of plant genetic resources require conditions that guarantee genetic integrity. Germplasm of vegetatively propagated crops like cultivars of potato is difficult to maintain. They must be grown continuously or replanted frequently or kept as an *in vitro* collection. For long-term storage, an *in vitro* collection bears the risk of losing material. In addition, it is uneconomical to maintain a large number of cultures not required for immediate use. For long term storage in particular, storage in liquid nitrogen would be advantageous, because once the material is frozen only a regular fill-up with liquid nitrogen would be required. All other risks such as secondary infections of cultures or failure of culture rooms would be eliminated.

There have been many promising attempts to freeze meristems or shoot-tips of a few selected genotypes of *Solanum tuberosum* and *Solanum* species or to improve a method for either variety (Bajaj 1978, 1981; Benson *et al.* 1989; Fabre and Dereuddre 1990; Grout and Henshaw 1978; Harding *et al.* 1991; Henshaw *et al.* 1985; Schnabel-Preikstas *et al.* 1992; Towill 1981a, 1983, 1990). A broader spectrum was published by Towill (1981b, 1984). Although published methods were partially successful, they have never been refined further to allow routine application in a collection of plant genetic resources.

In a joint project of the DSMZ and the FAL, a simple freezing protocol has been developed on the basis of existing methods (Schäfer-Menuhr *et al.* 1994, 1996a; 1996b). This method considers the needs and the limitations of a germplasm collection. This paper describes the application of this technique to 219 varieties and genotypes.

Material and methods

The *in vitro* collection of old potato varieties and the freezing protocol have been fully described elsewhere (Mix 1983, 1984, 1985; Schäfer-Menuhr *et al.* 1994) and will be described only briefly here.

Plants from the *in vitro* collection were propagated further via nodal segments. For the freezing experiments they were grown in a density of 10-15 *in vitro* plantlets in 12 cm high twist-off jars under good aeration. When the plantlets were 10 cm tall, the shoot-tips were cut off and trimmed to 2-3 mm long and 0.5-1 mm thick, depending on the variety. They were incubated overnight (Fig. 1) at 23°C in MSTo medium which contained the macro- and micromineral salts and vitamins of the Murashige and Skoog medium (Murashige and Skoog 1962), sucrose 30 g/L and the hormones described by Towill (1983): zeatinriboside 0.5 mg/L, gibberellic acid 3 (GA3) 0.2 mg/L and indole-3-acetic acid (IAA) 0.5 mg/L. Shoot-tips (100-200) were prepared for each experiment. On the following day the shoot-tips were transferred into the cryoprotective solution (MSTo medium containing 10% dimethylsulfoxide). After incubation for 2 hr at room temperature, 2.5 µl droplets of cryoprotectant were pipetted onto heat-sterilized pieces (0.7 x 2 cm) of 0.03 mm thick aluminium foil at 6 drops per foil. One shoot-tip was transferred into each drop (Fig. 2) and the foil packets were dropped directly into liquid

Fig. 1. Incubation of shoot-tips.

nitrogen. For storage, the foils were transferred to 2-ml precooled cryovials at 2 foils per vial. Each variety or genotype was frozen in three sets of experiments.

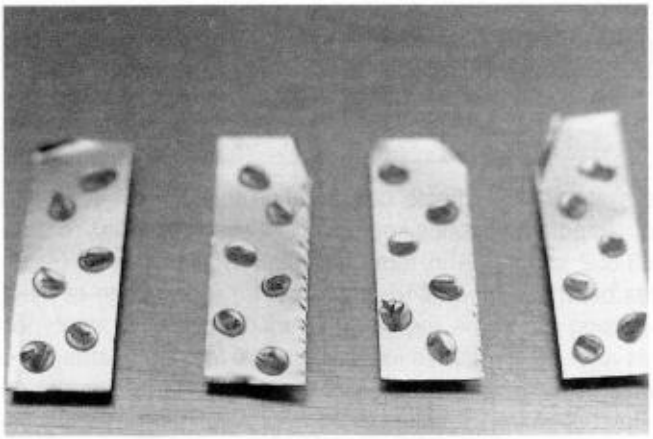

Fig. 2. Shoot-tips in 2.5 µl droplets of cryoprotective solution.

For the control of regrowth, one cryovial of each freezing experiment was thawed and cultured in MSTo medium. For the evaluation of the phenotype, regrown shoots were cultured on hormone-free medium, propagated further and planted out in pots. *In vitro* plants of the respective varieties were planted out as control plants. Photographs of the plants were taken during growth and of the tubers after harvest. The flow cytometric measurements and RFLP analysis were performed as described (Schäfer-Menuhr *et al.* 1996b).

Results and discussion

Freezing methods to be used in a collection of plant genetic resources must meet the following requirements:

- frozen explants must be able to regenerate plants after thawing
- the genetic characteristics of the varieties or genotypes must be maintained
- the method must be simple
- the method must allow a high throughput of samples
- the logistic must allow that vials of a certain variety are found even after a prolonged period of storage time
- the cost must be reasonable.

The methods published for the cryopreservation of *Solanum* species were tested for this purpose in several experiments with different varieties (Schäfer-Menuhr *et al.* 1994). The most important criteria for deciding which basic method to choose were plant regeneration after thawing, high throughput of samples and susceptibility to errors. The requirements were met using the 'droplet' method. With this technique, trimmed shoot-tips in small drops of cryoprotectant supported on aluminium foils were frozen directly in liquid nitrogen.

To date, 219 varieties and genotypes have been frozen and stored. In the liquid nitrogen storage containers are now 63 852 shoot-tips in 5321 cryovials. Of each variety up to about 30 cryovials (= 300-400 shoot-tips) were stored which were the result of three independent freezing experiments. Of every batch frozen, 10-12 shoot-tips have been thawed to check the ability for plant regeneration after freezing.

These regrowth controls were used to calculate the survival and plant regeneration rates. Since only 10-12 shoot-tips were thawed for the regrowth controls, for the first varieties frozen only 5-6, the calculations are more approximations than statistically secured data. As an example, the mean values of the three independent growth controls of the first 150 varieties and genotypes frozen are given in Fig. 3. The remaining 69 varieties frozen follow the same regeneration pattern.

The survival rates of most varieties are high, approximately 80% on the average. More important for a genebank, however, are the plant regeneration rates. They are on the average 40% (40% means in practice 4 plantlets per 10 frozen shoot-tips) and depend on the genotype. This genotypic effect is well known to anyone working with *in vitro* cultures of potato. Although 12 varieties have a low plant regeneration rate, plants are obtained when two or three vials are thawed. The fact that plants have been obtained from all 219 varieties frozen which have not been especially selected makes this freezing method attractive for collections of plant genetic resources.

It is also obvious from Fig. 3 that the ratios of survival rate and plant production within the varieties are very heterogeneous. To understand this fact it is necessary to consider that cryopreservation consists of two processes. One part is the freezing procedure itself, which is independent from the regeneration process. The cryogenic factors seem to be optimized which is shown by high survival rates with the only exception being the variety 'Judika'. The second step, the regeneration, is much more influenced by the variety.

In the freezing routine the same medium was used for the different cultivars and genotypes and was never changed or refilled when it dried out. Therefore, the plant regeneration rate of several varieties could be increased by a more careful treatment of the cultures, for example by refilling the medium in drying-out Petri dishes. For the practical application, such as distribution of stocks to users, the first plants that develop are the important ones. From our experience these plants grew vigorously, were easily propagated and never showed signs of abnormality.

An important question is what rates of plant regeneration are necessary and acceptable. This, of course, will mainly depend on the material. The rates have to be very high, e.g. 80-100%, if heterogenic material has to be stored. With vegetative propagated material like the collection of old potato varieties, the situation is different. The stock material is very homogeneous, being derived from a few selected tubers or *in vitro* plants that had been cloned many times. For this type of material a lower rate of plant regeneration can be accepted as long as some of the shoot-tips of every vial stored regenerated plants and as long as these plants are genetically identical.

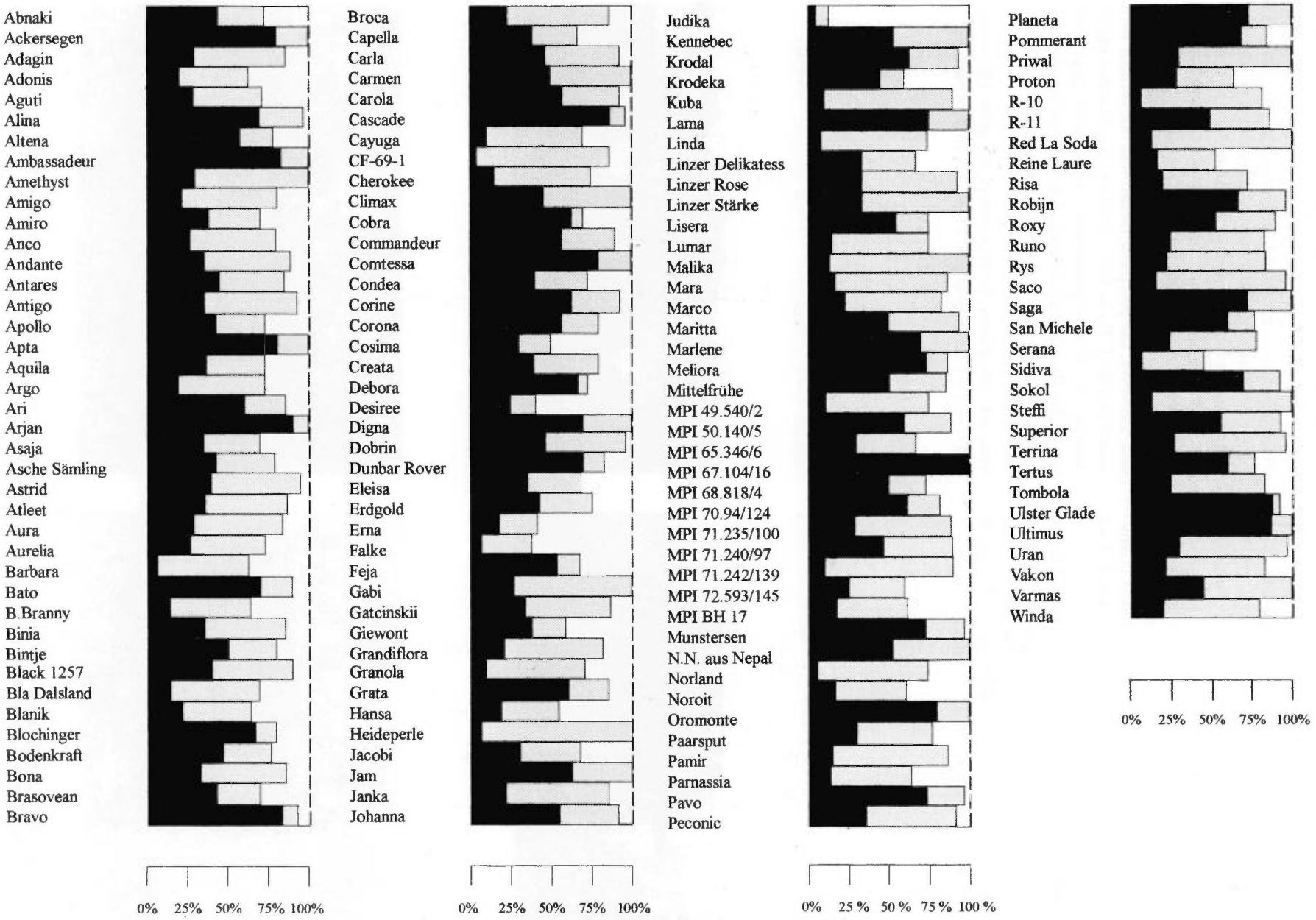

Fig. 3. Comparison of survival and plant regeneration rates of 150 potato varieties after exposure to liquid nitrogen (survival rates light, plant regeneration rates dark).

For those varieties with a low rate of plant regeneration on the MSTo medium, more plants could possibly be obtained when a medium with different combination and/or concentration of phytohormones is used. Sometimes it can be advantageous to exchange the medium after 2 weeks of culture against a medium with a lower concentration of auxins (Schäfer-Menuhr *et al.* 1994).

An important criterion to judge whether or not this freezing method is useful for the long-term storage of potato varieties is the question of whether the genetic characteristics of the varieties or genotypes are maintained. It is a matter of definition how narrow or how wide the term 'genetic identity' is defined. A 100% congruence is difficult to prove and is also not reasonable. For a practical application it should be enough to show that the characteristic attributes of a variety are conserved for a potential use in future breeding programmes.

The tests to check the genetic identity of regrown plants have been described in detail (Schäfer-Menuhr *et al.* 1994, 1996a). The most effective method to show that the freezing and regeneration process did not change the properties of a variety was the evaluation of the phenotype of regrown plants. As an example, Fig. 4 shows control and regenerated plants after freezing in different stages of development.

Among the regrown plants of 98 varieties only one abnormal plant was found which was most likely polyploid. However, the polyploid nature was not verified with another method. There were also a few weak or tiny plants with poor or no tuber production. The reduced vitality was not higher than among control plants. A similar phenomenon has recently been described by Lommen (1996a, 1996b) for minitubers and *in vitro* plants of early varieties. Besides these few exceptions the regrown plants grew vigorously and could not be distinguished from the control plants.

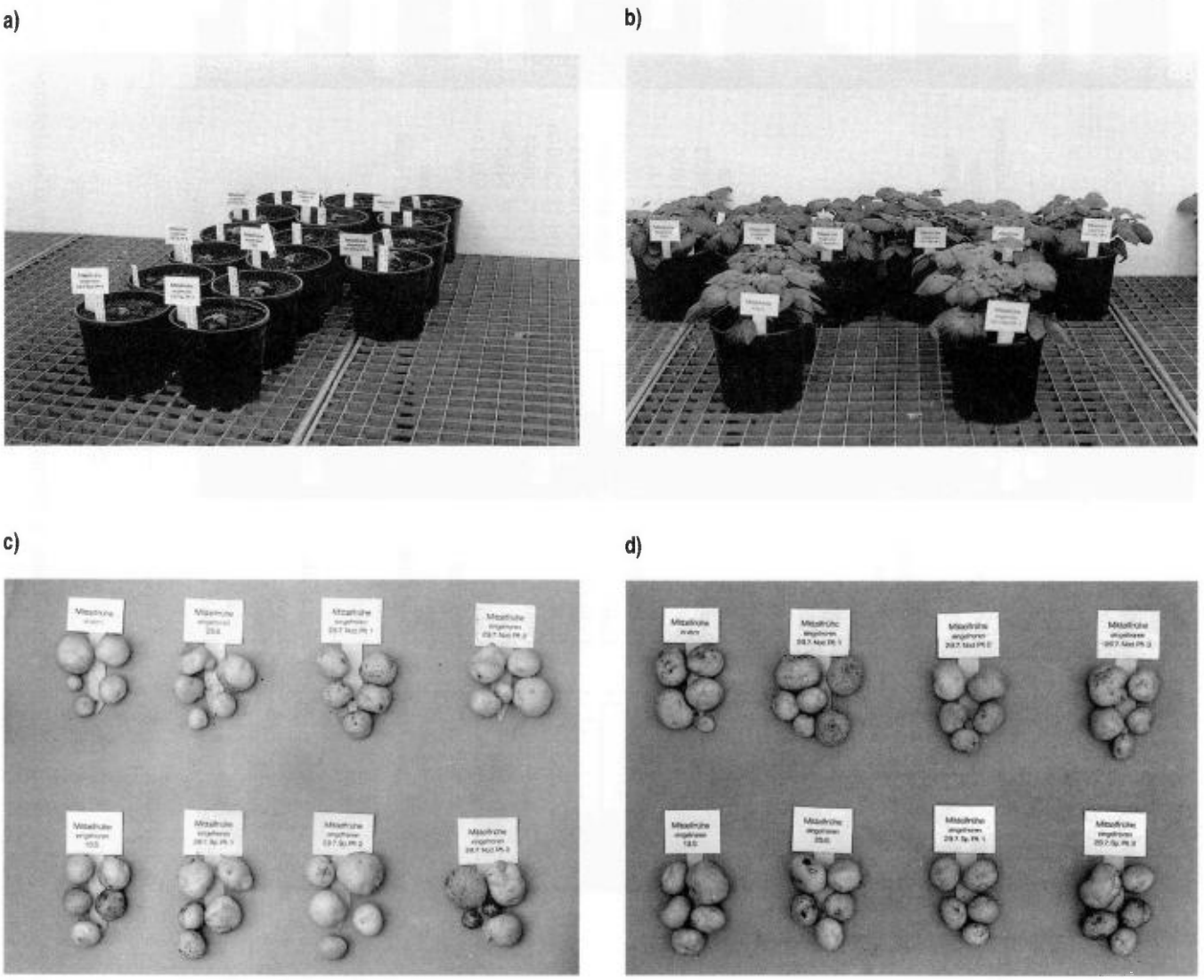

Fig. 4. Comparison of the phenotypes of the frozen material with unfrozen *in vitro* plants of the variety 'Mittelfrühe': a – directly after planting out; b – adult plants; c – tubers of the first harvest; d – tubers of the second harvest.

'Somaclonal variants' which have been described for potato plants regenerated from protoplasts have not been observed (Kumar 1994). This result was expected because the regenerated plants were derived from meristems. Meristem and shoot-tip culture are generally accepted *in vitro* techniques for the propagation and virus elimination in potato cultivars (Hu and Wang 1983).

In addition to the evaluation of the phenotype, the genetic stability was checked by flow cytometric measurements and RFLP analysis (Schäfer-Menuhr *et al.* 1996a). Of 161 samples of regrown plants tested, neither polyploid nor abnormal banding pattern were found. It therefore seems to be safe to conclude that using the cryopreservation technique does not induce somaclonal variation.

Almost 5 years of using this simple freezing technique in the routine of a collection of plant genetic resources has shown that this method allows the secure storage of potato varieties and genotypes.

Compared with all other methods published for the cryopreservation of potato, the procedure is relatively low in cost. The highest costs are for labour. Once the samples are frozen the costs for storage are low compared with a field genebank or *in vitro* storage.

The time necessary to freeze an entire *in vitro* collection depends on the number of accessions and technical assistants. The decision on how long both types of storage are carried out parallel depends on the confidence in the new method and the frequency with which varieties are requested. Cryopreserved varieties are not as readily available as *in vitro* plants and have to be regrown first before they can be sent away. It is therefore advisable to keep frequently requested varieties also in the *in vitro* collection.

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Notice

A fully described protocol of the freezing procedure can be sent on request (contact: G. Mix-Wagner, Institute of Crop Science (FAL), Bundesallee 50, D-38116 Braunschweig, Germany).

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Résumé

Cryoconservation à long terme dans l'azote liquide d'apex de variétés anciennes de pommes de terre

Les collections de ressources phytogénétiques réclament des systèmes d'entreposage sûrs et des techniques de conservation simples. Une méthode simple de congélation a été mise au point pour la cryoconservation de variétés anciennes de pommes de terre sous forme de collection in vitro. A ce jour, 219 variétés et génotypes ont été congelés et entreposés, soit au total 63 852 apex. Des essais ont donné un taux de reprise de 80%. Toutefois, pour les collections de ressources phytogénétiques, le taux de régénération des plantes importe davantage. En utilisant le même milieu de régénération pour tous les génotypes, 40% approximativement des apex congelés ont donné naissance à des plantes après avoir été décongelés. Le fait que jusqu'à présent des plantes ont été obtenues à partir de toutes les variétés congelées est aussi important. On a confirmé la stabilité génétique des plantes régénérées par la comparaison des paramètres morphologiques, par des mesures de cytométrie en flux et par la méthode RFLP.

Resumen

Almacenamiento a largo plazo de variedades antiguas de papa mediante la crioconservación de yemas terminales en nitrógeno líquido

Las colecciones de recursos fitogenéticos requieren sistemas de almacenamiento seguros y técnicas sencillas de conservación. Se ha desarrollado un método sencillo de congelación para la crioconservación de variedades antiguas de papa que se mantienen como una colección *in vitro*. Hasta la fecha se han congelado y almacenado 219 variedades y genotipos, en total, 63 852 yemas terminales. Calculada sobre la base de los experimentos de regeneración, se ha obtenido una tasa de supervivencia de alrededor del 80 %. Sin embargo, más importante para una colección de recursos fitogenéticos es la tasa de regeneración de las plantas. Utilizando el mismo medio de regeneración para todos los genotipos, aproximadamente el 40 % de las yemas congeladas se desarrollaron como plantas después de ser congeladas. Es importante también el hecho de que se han obtenido plantas de todas las variedades que se han congelado hasta la fecha. Se ha confirmado la estabilidad genética de las plantas congeladas comparando parámetros morfológicos, así como mediante mediciones citométricas del flujo y análisis del polimorfismo de la longitud de los fragmentos de restricción.

Analysis of genetic relationships among accessions of *Arachis stenosperma* and *A. duranensis* using DNA markers

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Summary

A DNA amplification fingerprinting (DAF) approach using sequence-specific primers was employed to identify polymorphic DNA markers among eight accessions each of *Arachis stenosperma* and *A. duranensis*, the diploid species within the section *Arachis* to which cultivated peanut belongs. There was considerable polymorphism among the accessions in both species and every accession exhibited a distinct fingerprint profile. A phenogram was constructed based on genetic similarity, and genetic distances were calculated using Jaccard's coefficient. The accessions belonging to two species formed distinct clusters. The DNA marker approach has applications in *Arachis* germplasm research in delineating the genetic relationships of accessions and in fingerprinting of germplasm lines.

Introduction

The wild relatives of peanut (*Arachis hypogaea* L.) are reservoirs of important traits such as disease and pest resistance (Abdou *et al.* 1974; Subrahmanyam *et al.* 1985; Stalker and Moss 1987; Mehan *et al.* 1992). Consequently, there has been considerable interest in studies on such species (Singh and Moss 1984; Kochert *et al.* 1991; Singh *et al.* 1991). The two *Arachis* species *A. duranensis* and *A. stenosperma* are diploid and may have played a role in the evolution of cultivated peanut, the tetraploid *A. hypogaea*. Both these diploid species have distinct distribution patterns in their native South America but may have had a sympatric origin. *Arachis duranensis* is distributed over a narrow strip of the western Chaco region, extending from Villamontes in southern Bolivia to El tunnel (near Joaquin Gonzalez) in northern Argentina without much climatic variation, whereas *A. stenosperma* is limited to two distant locations, the coastal southeast and central plateau of Brazil with distinct ecogeographic environments (Table 1). In an earlier study, the use of seed protein profiles enabled us to discern very little differences among accessions of *A. duranensis* (Singh *et al.* 1996). Similarly, accessions of *A. stenosperma* did not exhibit any variation for seed protein profiles although these accessions were collected from broadly distinct and distant geographic areas (Singh *et al.* unpublished).

Table 1. Identity and collection location of *A. duranensis* and *A. stenosperma* accessions studied by DNA amplification fingerprinting

	Identity			Country of origin†	Province	Lat.	Long.	Altitude
	ICRISAT (ICG)	Other						
		Collector	No.					
A. duranensis								
1	8202	GKBSPSc	30070	Bol	Tarija	2153S	6338W	600
2	8201	GkBSPSc	30069	Bol	Tarija	2148S	6333W	575
3	13183	KSSc	38903	Arg	Salta	2212S	6341W	400
4	8205	GKBSPSc	30075	Bol	Tarija	2118S	6327W	450
5	13243	KSSc	38902	Bol	Tarija	2119S	6327W	450
6	13249	KSBScC	36003	Arg	Salta	UN‡	UN‡	600
7	11552	KSBScC	36002	Arg	Salta	UN‡.	UN‡	600
8	13216	KSBScC	36004	Arg	Jujuy	2416S	6512W	1000
A. stenosperma								
9		V	12488	Bra	Mato Grosso	1534S	5213W	350
10	13233	CIAT	9660	Bra	Mato Grosso	1532S	5231W	350
11	8125	HLK	408	Bra	Parana	2524S	4844W	3
12		V	13260	Bra	Sao Paulo	2419S	4659W	5
13	8126	HLK	410	Bra	Parana	2531S	4831W	3
14		V	10309	Bra	Mato Grosso	1628S	5439W	215
15		V	12646	Bra	Mato Grosso	1543S	5542W	170
16		V	12575	Bra	Mato Grosso	1541S	5246W	360

† Bol = Bolivia; Arg = Argentina; Bra = Brazil. ‡ UN = unknown.

Little or no variation has been observed in the cultivated peanut germplasm using random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) techniques (Kochert *et al.* 1991; Halward *et al.* 1991, 1992; Paik-Rao *et al.* 1992). The use of seed-protein profile (Singh *et al.* 1991, 1994) and isozyme (Lu and Pickersgill 1993; Stalker *et al.* 1994) techniques have found small variation among peanut cultivars. However, the above studies have detected considerable variation among wild *Arachis* species, suggesting that cultivated peanut has a narrow genetic base. As the related wild species have considerable diversity, they hold a larger potential for transfer of desired traits and thus to broaden the genetic base of the cultivated peanut. Our laboratory at Tuskegee University has employed DNA amplification fingerprinting (DAF) and amplified fragment length polymorphism (AFLP) approaches (Caetano-Anolles *et al.* 1991; Vos *et al.* 1995) and has recently successfully identified many polymorphic DNA markers for the first time in cultivated peanut (He and Prakash 1997).

This paper reports on a study with the DAF approach to identify polymorphic DNA markers and to elucidate the extent of genetic relationships among accessions of *A. stenosperma* and *A. duranensis*.

Materials and methods

Plant material

Information on identity and collection site of different accessions of *A. duranensis* and *A. stenosperma* used is summarized in Table 1. Seed samples were obtained from the peanut germplasm world collection of ICRISAT, India.

DNA amplification

Total genomic DNA was isolated from germinated seedlings as described by Wilson *et al.* (1992). Many primers of arbitrary sequences were initially screened individually on many *Arachis* species, and five primers (Table 2) were selected for use in this study based on their ability to detect high levels of polymorphism. The polymerase chain reaction (PCR) mixture (25 µl) contained template DNA (2.6 ng/µL), primer (7.7 µM), $MgCl_2$ (5 mM), 5 units of Stoffel fragment *Taq* polymerase (Perkin Elmer, Norwalk, CT), dNTPs (200 µM each) and buffer (10 mM KCl and 10 mM Tris-HCl). Mixtures were overlaid with two drops of mineral oil and amplified in a Hybaid thermal cycler (National Labnet, Woodbridge, NJ) for 35 cycles after an initial denaturation at 96°C for 10 minutes. Each amplification cycle consisted of 5 sec at 96°C, 20 sec at 45°C and 30 sec at 72°C, with a final extension step of 5 min at 72°C.

Table 2. Sequences of PCR primers used in studying genetic variation among accessions of *A. duranensis* and *A. stenosperma*

Primer code	Sequence (5'-3')										
OPL-2	T	G	G	G	C	G	T	C	A	A	
OPL-3	C	C	A	G	C	A	G	C	T	T	
Hp†-10	G	C	G	A	A	G	C–	C	T	G	
Hp-14	G	C	G	A	A	G	C–	C	G	C	C
8S	A	C	G	G	G	T	G	C			

† Hp refers to mini-hairpin.

DNA electrophoresis and visualization

Amplification products were separated on a polyacrylamide-based vinyl polymer (PCR Purity Plus; AT Biochem, Malvern, PA) gel (0.7 mm thick) prepared in 1X TBE. Electrophoresis was performed in a vertical gel apparatus (Mini-Protean, Bio Rad) at 25 V/cm (He *et al.* 1994). Gels were silver-stained using the procedure of Bassam *et al.* (1991), which was modified to include an oxidization step using an oxidizer solution (Bio-Rad #161-0444) for 5 min at room temperature. Gels were backed with a GelBond plastic film (FMC BioProducts, Rockland, ME) and air-dried at room temperature.

Data analysis

Only intensely staining, clear bands within the size range of 300-1500 bp were scored. Bands in this size range were selected because of their high degree of reproducibility and minimal artifacts as determined in our preliminary studies. Gels were scored visually for the presence (1) or absence (0) of bands using photographs enlarged through an image analyzer. NTSYS-pc software (version 1.7) was utilized to compute Jaccard's similarity coefficients to construct phenograms using the unweighted pair group method with arithmetic averages (UPGMA).

Results and discussion

Genomic DNA isolated from eight accessions each of *A. duranensis* and *A. stenosperma* was amplified under the optimum conditions described above using several primers. The five primers employed detected a total of 131 polymorphic bands in both *A. duranensis* and *A. stenosperma*. Of five primers used, the primers 8S and Hp-10 produced most of the polymorphic bands (8S shown in Fig. 1). The phenogram based on percentage of similarity in bands grouped the accessions of two species separately (Fig. 2). All accessions of *A. duranensis* originating from Bolivia clustered close with each other, while accessions originating from Argentina (except for ICG 13183) formed another close cluster. However, no strict geographic-based grouping was observed among the accessions of *A. stenosperma*, and they were overlapped despite their clear geographical distinction.

Both *A. duranensis* and *A. stenosperma* are known to be resistant to several diseases of peanut (Subrahmanyam *et al.* 1985; Mehan *et al.* 1992) and have been successfully utilized as sources of genes conferring resistance to late leaf spot and rust on the cultivated peanut (Singh and Gibbons 1986; Stalker and Moss 1987). However, there have been conflicting reports on the reaction of *A. duranensis* to certain diseases such as *Aspergillus flavus* (Ghewande *et al.* 1989; Mehan *et al.* 1992). Techniques that can help differ-

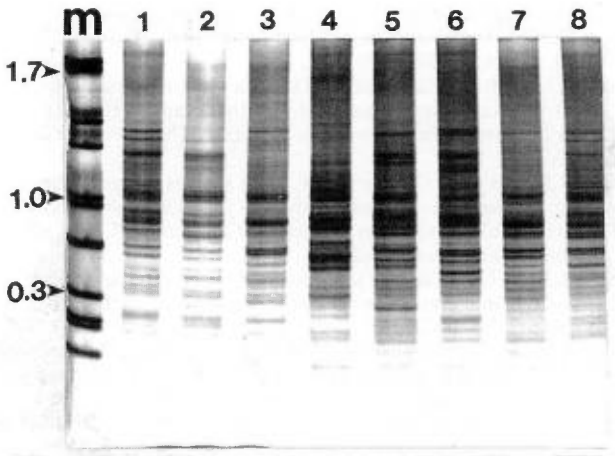

Fig. 1. DNA fingerprint profiles of accessions of *A. duranensis* using primer 8S. Left lane is DNA marker, lanes 1-8 are 8202, 8201, 13183, 8205, 13243, 13249, 11552 and 13216, respectively.

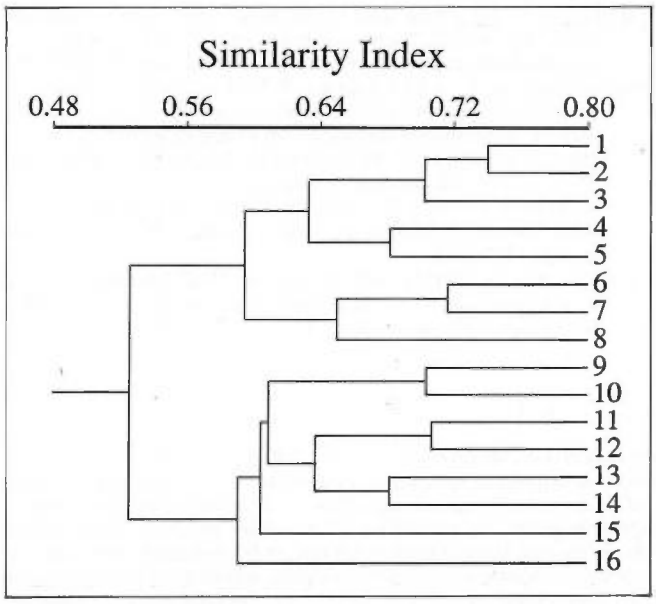

Fig. 2. A phenogram based on percentage of similarity in DNA bands of accessions of *A. duranensis* and *A. stenosperma*. Numbers refer to accessions listed in Table 1.

entiate between accessions of the same species and develop specific markers can be used in the identification of the uniqueness of useful accessions. Additionally, the two species studied also present a unique situation in their distribution pattern: *A. duranensis* is distributed in an area with a narrow range of climatic variation while *A. stenosperma* is found in two distinct ecogeographical regions of Brazil. Therefore techniques in tracing the phylogenetic relationships and the evolution of certain traits in association with geographical distribution should be useful.

Previous studies using protein profiles (Singh *et al.* 1996) and RFLP markers (Stalker *et al.* 1995) with *A. duranensis* could differentiate accessions to different levels, thus emphasizing the potential of molecular techniques in peanut germplasm studies. Protein profiles identified differences among accessions of *A. duranensis* despite being distributed over the same physical environment, but they could not discern the differences between accessions of *A. stenosperma* collected from distinct geographical areas either because of lesser genetic variation or the limited resolution capability of protein profiles. The present study employs a PCR-based technique, which is relatively more sensitive, rapid and simpler than the protein or RFLP approach, to identify micro-evolutionary differences among closely related accessions within *Arachis*.

The five primers employed in the present study produced accession-specific fingerprint profiles in all the accessions of the two *Arachis* species studied. There were also many bands that were identical among accessions of both species, suggesting close relationships among the two species and their accessions. Nevertheless, the presence of many polymorphic bands enabled us to distinguish accessions between and within a species. The variation between the accessions belonging to two species was greater than between the accessions of the same species, resulting in clustering of accessions representing the two species into distinct groups. Although the sample size in this study was small, the results suggest that the DAF technique can complement traditional morphological markers in assigning new collections to either of the two species based on DNA fingerprint profiles (Fig. 1). Further, most accessions of *A. duranensis* from Bolivia clustered together and the accessions from Argentina grouped together. This reflects the potential of the DAF technique in tracing evolutionary relationships of specific sets of loci to a specific region or a location. However, such differentiation was not observed with *A. stenosperma* where the accessions originated from two distinct regions, central plateau and coastal areas of southeast Brazil.

The present results showed the power of the DAF technique in resolving polymorphism that has occurred at micro-evolutionary levels among accessions of a species. This can help in establishing the uniqueness of accessions and relationships to others. Therefore, using specific primers it would be possible to develop fingerprints for selected accessions in relation to specific loci, which can be used in tracing genetic affinity and identity of new collections.

Conclusion

Our study shows that polymorphic DNA markers have potential in the germplasm studies of *Arachis* including studies on the genetic diversity and understanding the nature of genetic relationships among accessions in the germplasm.

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Résumé

*Analyse des relations génétiques entre les accessions d'*Arachis stenosperma *et d'*A. duranensis *à l'aide de marqueurs d'ADN*

Une approche DAF (DNA amplification fingerprinting) utilisant des amorces de séquences spécifiques pour identifier des marqueurs d'ADN polymorphiques parmi huit accessions d'*Arachis stenosperma* ainsi que d'*A. duranensis*, espèces diploïdes de la section Arachis à laquelle appartient l'arachide cultivée. On a observé un polymorphisme considérable parmi les accessions de ces deux espèces, chaque espèce présentant un profil d'empreinte distinct. Un phénogramme a été établi sur la base de la similitude génétique, et les distances génétiques ont été calculées en utilisant le coefficient de Jaccard. Les accessions appartenant aux deux espèces formaient des groupes distincts. La méthode des marqueurs d'ADN trouve des applications dans la recherche sur le matériel génétique d'*Arachis* pour déterminer les relations génétiques des accessions, ainsi que les empreintes des lignées de matériel génétique.

Resumen

Análisis de las relaciones genéticas entre muestras de Arachis stenosperma *y* A. duranensis *utilizando marcadores del ADN*

Se utilizó un sistema de caracterización por amplificación del ADN, empleando iniciadores específicos de cada secuencia para identificar marcadores polimórficos del ADN entre ocho muestras de *Arachis stenosperama y A. duranensis*, las especies diploides de la sección *Arachis* a la que pertenece el maní cultivado. Entre las muestras de ambas especies se manifestó un importante polimorfismo y todas las muestras mostraron un perfil de caracterización específico. Se elaboró un fenograma sobre la base de la analogía genética y se calcularon distancias genéticas utilizando el coeficiente de Jaccard. Las muestras pertenecientes a las dos especies formaron agrupamientos separados. El sistema de marcador del ADN tiene aplicaciones en la investigación sobre el germoplasma de *Arachis* para trazar las relaciones genéticas de las muestras y caracterizar las líneas de germoplasma.

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Creation and composition of the *Brassica oleracea* core collection

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Summary

A core collection of the *Brassica oleracea* genepool (BOCC) was created for an EU (European Union) project entitled: 'The location and exploitation of genes for pest and disease resistance in European genebank collections of horticultural brassicas'. For the development of this core collection the European *Brassica* database (Bras-EDB) proved to be of great value. The BOCC was partly based on pragmatic considerations because of the needs and time constraints of the project, and can be seen as the basis for the development of a more widely coordinated *B. oleracea* core collection. In the process of creating the BOCC a new tool was introduced: the path indicator. The path indicator allows a good overview of the content of the core collection. It points to groups of material instead of individual accessions and can therefore contribute to a better interpretation of evaluation results. It also allows for a better selection of material, in answering requests from users.

Introduction

An EU (European Union) project entitled: 'The location and exploitation of genes for pest and disease resistance in European genebank collections of horticultural brassicas' (Leckie *et al.* 1996) started in 1992. In this project three research teams are screening the *Brassica oleracea* genepool for resistance to cabbage aphid (*Brevicoryne brassicae*), downy mildew (*Peronospora parasitica*) and white blister (*Albugo candida*).

To make this genepool accessible it was decided to select a core collection consisting of *B. oleracea* accessions in European germplasm collections. Frankel (1984) introduced the concept of a core collection which represents the genetic diversity of a crop species and its relatives with a minimum of repetitiveness. Brown (1989) clarified the terminology and expanded the idea by providing genetic and pragmatic arguments supporting the concept.

The Centre for Genetic Resources the Netherlands (CGN) and the Genetic Resources Unit of the Horticulture Research International (HRIGRU) identified a preliminary core collection of *B. oleracea* in their collections representing the available range of crop types and ecogeographical locations. The core collection was expanded and refined by the CGN using the information available in the European *Brassica* database (Bras-EDB, Boukema *et al.* 1995).

Material and methods

To allow a good choice of entries for the *Brassica oleracea* core collection (BOCC), the genepool of *B. oleracea* needed to be structured. This was achieved by stepwise division of the genepool into parts which represented distinguishable groups of accessions. The first division distinguished *B. oleracea* from other closely related *Brassica* species. The second division in the *B. oleracea* group was into a group with cultivated material and a group with wild *B. oleracea* material. The cultivated material was divided into crop types such as cabbages, kales, cauliflowers, etc. Some of these could be further divided, e.g. the cabbages which could be divided into white, red and Savoy cabbages. The last division in this 'line' was according to the geographical origin of the material, such as Northwestern Europe, Southern Europe or the Americas. These regions were further divided into countries.

To document this process of division and subdivision, and to allow for an efficient analysis of the data on the BOCC, a new descriptor called the 'path indicator' was used. This path indicator indicates the 'branch on the *B. oleracea* diversity tree' and consists of a series of ciphers. The first cipher indicates a first division of the genepool into subgroups, the second indicating the division of the subgroup indicated by the first cipher, etc. For example the BOCC path indicator '114121' should be read as: an accession belonging to group 1: '*Brassica oleracea*', to group 11: 'cultivated', which is a subgroup of group 1. It also belongs to group 114: 'cabbage', and to group 1141: 'white cabbage', group 11412: 'pointed headed white cabbage'. Finally it is part of subsubsubsub...group 114121: 'pointed headed white cabbage, originating in Denmark'.

After dividing the genepool into subgroups it was possible to allocate the total number of entries to be included in the BOCC over the subgroups. Because the screening capacity of the research teams in the project was maximal around 400 accessions, assembly of a core collection of about this size was targeted. The number of entries allocated to a subgroup depended on the relative importance of that group, but also on the availability of the material. Some additional groups of material with unknown identity or origin were included since this material was considered as potential sources of resistance.

Since the screening programmes in the project had to start immediately and these programmes required a high quality and quantity of seed, a first selection of 293 accessions was made from the HRIGRU and CGN collections. This selection was heavily based on pragmatic considerations such as immediate availability of the material. Subsequently, using the

information in the European *Brassica* database (Boukema *et al.* 1995), this first selection could be refined with additional material selected from several sources completing the current 411 entries of the BOCC. Parts of this material needed multiplication to meet the standards for quality and quantity of the screening programmes. These multiplications were carried out by CGN and HRIGRU.

Results

The selection of accessions for the core collection resulted in 411 entries representing *B. oleracea* crop types, wild taxa and closely related species. The material included in the core collection was received from six genebanks. Besides 208 accessions from HRIGRU and 86 accessions from CGN, 21 accessions, representing landraces of kale and cauliflower from Brittany, were received from Station d'Amélioration de Plantes, Institut National de Recherche Agronomique (INRA, Le Rheu, France). After regeneration, 40 accessions received from the Institute für Pflanzengenetik und Kulturpflanzenforschung (IPK, Gatersleben, Germany) and five Turkish landraces received from the Plant Genetic Resources Department of the Aegean Agricultural Research Institute (AARI, Menemen, Izmir, Turkey), were included in the core collection. Instituto Superior de Agronomia (ISA, Lisbon, Portugal) provided 51 additional accessions representative of Portuguese brassicas.

The process of dividing the genepool represented in the Bras-EDB resulted in 114 groups. In some cases the final subdivision was achieved after up to eight divisions, resulting in a path indicator with eight digits. The branching of the core collection on the *B. oleracea* diversity tree as far as the main crop types is shown in Figure 1.

In Table 1 the number of accessions per taxon and crop type represented in the core collection is compared with that in the Bras-EDB as it was in March 1996. Of the 6839 cultivated *B. oleracea* accessions included in the Bras-EDB, 405 (6%) are included in the core collection. The kales are represented by 8%, Chinese kales by 20%, cauliflower by 6%, broccoli by 12%, cabbage by 4%, Brussels sprouts by 8% and kohlrabi also by 8%. The tronchudas are represented by 60%, but only a part of the existing collection is included in the Bras-EDB. The group of which the type is unknown is represented by 1%. Of the eight known wild related species comprising 89 accessions in the Bras-EDB, four species are represented in the core collection with six accessions. One probably wild accession of which the species is unknown was also added. The 1578 *B. oleracea* landraces in the Bras-EDB are represented by 119 accessions (8%) in the core collection, the 2989 cultivars by 241 (8%) and the 119 accessions of research material by one (1%). Of the 2662 accessions of unknown population type, 43 (2%) are represented in the core collection.

Table 1. Comparison of the number of accessions of *B. oleracea* and wild related species per group in the Bras-EDB and in the core collection, with path indicators

Path	Group	Common name	No. of accessions Bras-EDB	Core
111	*B. ol. acephala*	kale	891	71
112	*B. ol. alboglabra*	Chinese kale	51	10
1130	*B. ol. botrytis*	cauliflower/broccoli	81	0
1131	*B. ol. botrytis botrytis*	cauliflower	1203	67
1132	*B. ol. botrytis italica*	broccoli	392	46
114	*B. ol. capitata*	cabbage	2700	100
115	*B. ol. gemmifera*	Brussels sprouts	594	45
116	*B. ol. gongylodes*	kohlrabi	236	19
117	*B. ol. tronchuda*	tronchuda	73	42
110	*B. ol.* unknown		618	4
12	*B. oleracea*	wild *Brassica*	12	1
2	*B. villosa*	wild *Brassica*	9	2
3	*B. incana*	wild *Brassica*	3	2
4	*B. montana*	wild *Brassica*	3	1
	Other *B.* species (4)	wild *Brassica*	62	0
0	*B.* species unknown	wild *Brassica*?	368	1
	Total		7296	411

The 411 accessions originate from 39 countries. Table 2 shows the number of accessions per region. Northwest Europe is represented with 179 accessions of which the majority originates from the Netherlands (65), United Kingdom (46), France (31) and Denmark (18). South Europe is represented by 131 accessions, largely from Portugal (68) and Italy (57).

The complete list of the core collection with passport data and an explanation of the paths can be obtained from the CGN.

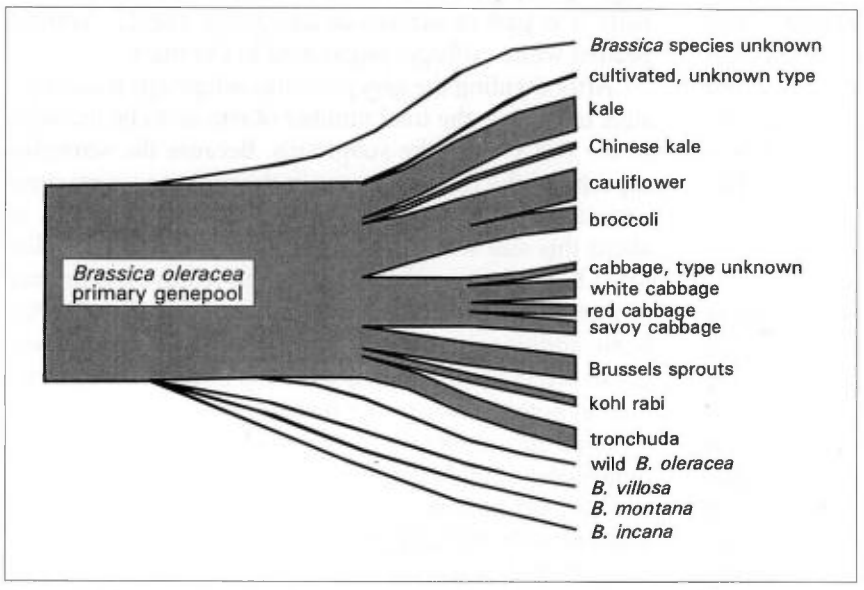

Fig. 1. First part of the diversity tree of the *Brassica oleracea* core collection.

Table 2. Number of accessions of the core collection per region

Region	No. of accessions
Northwest Europe	179
Central Europe	14
East Europe	16
South Europe	131
Middle East	20
Far East	28
Oceania	5
Americas	10
Africa	2
Unknown	6
Total	411

Discussion

The development of the BOCC demonstrates the value of a central database for a crop or group of crops such as the Bras-EDB, promoted by the European Cooperative Programme for Crop Genetic Resources Networks (ECP/GR).

Basic to the construction of a core collection is the knowledge of the genetic diversity of the crop. This includes the knowledge about variation available in the different crop groups, existing morphological types within these groups and regions/countries where high variation in landraces or cultivars of a special type is available. This resulted in deviating representations of the available accessions in the Bras-EDB of the different crop types in the core collection (Table 1). For example broccoli was represented in a much higher percentage than cauliflower. Also accessions from some regions, e.g. Northwest and Southern Europe, are represented in a much higher number than those from other regions (Table 2). Northwest Europe is known for its breeding activities in *B. oleracea* and in Southern Europe the diversity in cauliflower and broccoli (Italy) and in landraces of tronchuda and Portuguese kale (Portugal) is high.

The BOCC is not a core collection by Brown's (1989) definition since it is not a selection of an existing collection, but a selection from the combination of a number of collections. Moreover it is largely based on pragmatic considerations. The reason was the need for continuity between screening programmes and the time constraints of the project. A stated objective of the project was for the three research teams to screen the same accessions. Therefore, no accession was removed from the core collection once used by one group within the project team. For this reason the tronchudas are over-represented in the core collection (Table 1). Only seven accessions of four related wild species were included in the core collection, because only very small amounts of seed were available and seed multiplication of most wild species is difficult and takes several years.

In the process of creating the BOCC a new tool was introduced: the path indicator. The path indicator allows a good overview of the content of the core collection, by its hierarchical subdivision. It points to groups of material instead of individual accessions. Therefore it can contribute to a better interpretation of the evaluation results as is demonstrated by the results of the screening for aphid resistance (P.R. Ellis, pers. comm.). It further allows for a better selection of material, in answering requests from users. As more information becomes available the path indicators of some accessions can be updated, especially those indicating categories of which type or origin is not known.

In a broader context this core collection can be seen as the basis for the development of a more widely coordinated *B. oleracea* core collection under the auspices of the ECP/GR *Brassica* Working Group (Gass *et al.* 1995). Such a core collection would be a fluid entity to be updated as additional accessions representing different parts of the genepool are identified, and where necessary regenerated. Because the core collection acts as a 'window' on the combined European collections it can be the starting point for the majority of requests. Therefore the accessions in the core collection will require large-scale regeneration in order to meet this potential demand. To meet these requests it will be more efficient to store the core accessions in a restricted number of genebanks with long-term seed storage facilities.

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Résumé

Création et composition d'une collection de référence de Brassica oleracea

Une collection de référence du pool génique de Brassica oleracea (BOCC) a été créée pour un projet de l'EU (Union européenne) intitulé "Localisation et exploitation des gènes de résistance aux insectes et aux maladies, dans les collections de brassicas horticoles des banques de gènes européennes. La base de données européenne des brassicas (Bras-EDB) s'est révélée d'une grande utilité pour le développement de cette collection de référence. En raison des besoins et des contraintes de temps du projet, la BOCC repose en partie sur des considérations pragmatiques et peut être envisagée comme base de développement d'une collection de référence de B. oleracea plus largement coordonnée. A l'occasion de la création de la BOCC, un nouvel outil a été mis en oeuvre : l'indicateur de chemin. Cet indicateur permet d'obtenir une bonne vue d'ensemble du contenu d'une collection de référence. Il indique des groupes de matériel au lieu d'accessions individuelles et peut ainsi contribuer à une meilleure interprétation des résultats d'évaluation. Il permet aussi de mieux sélectionner le matériel pour répondre aux demandes des utilisateurs.

Resumen

Creación y composición de la colección básica de Brassica oleracea

Se constituyó una colección básica de genes de *Brassica oleracea* (BOCC) para un proyecto de la Unión europea (UE) titulado: 'La ubicación y explotación de genes para la resistencia a las plagas y enfermedades en las colecciones de bancos de genes europeos de brassicas hortícolas'. Para la creación de esta colección básica resultó de enorme valor la base de datos europea de *Brassica* (Bras-EDB). La BOCC se basó en parte en consideraciones pragmáticas debido a las necesidades y a la premura del tiempo del proyecto y puede considerarse como el punto de partida para la creación de una colección básica de *B. oleracea* con una coordinación más amplia. En el proceso de creación de la BOCC se introdujo un nuevo instrumento: el indicador de la ruta, que permite obtener un panorama general preciso del contenido de la colección básica. No se refiere a muestras individuales sino a grupos de ellas y, por tanto, puede contribuir a interpretar mejor los resultados de la evaluación. Además, permite seleccionar mejor el material, en respuesta a las peticiones de los usuarios.

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Weeds as genetic resources

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Summary

Weeds are often important as genetic resources. Weeds are also useful for evolutionary studies in connection with domestication, as indicators for autochthonous landraces and as basic material for weed control research. Several of the convergent weeds (crop plant mimicry, e.g. *Agrostemma githago*) with high similiarity to crop plants are threatened by extinction. This process particularly affects the infraspecific variation (e.g. adaptations to different crops). A special approach for the *ex situ* conservation of weeds has been developed at Gatersleben genebank within the concept of the integrated genebank. Examples for the conservation of weeds in the Gatersleben genebank are discussed (e.g. *Agrostemma githago, Papaver rhoeas, Bromus secalinus, Lolium temulentum*). *In situ* conservation of crop/weed complexes is a new challenge.

Introduction

A weed is a plant which, by common judgement, is an unwelcome guest in a cultivated environment. Usually weeds are only regarded as competitors for natural resources with cultivars. They handicap land use or they just offend our sense of orderliness, which is often mistakenly confused with a sense of beauty. Sometimes even cultivated plants are declared to be weeds. This, for example, is the case with rapeseed, which emerges in summer barley from shattered seeds from previous growing seasons. Thus a plant that in one year is pushed to maximum yield by all available means may sometimes be considered a weed in following years.

Agricultural sciences usually only deal with weeds in order to find effective methods for removing or eradicating them. In contrast, crop plant research takes a broader interest in weeds. In many languages the term for weeds bears negative attributes, and therefore several well-meaning synonyms have recently been created – some of which are philological monsters.

Weeds stick faithfully to us like the plants we have deliberately domesticated. The only difference is that they inadvertently take part in cultivation. The domestication and cultivation activities of humans always influence these voluntary companions of crops. As the actions of humans cause a reaction in nature, they are worthy of study (Hammer and Gladis 1993).

Most of the species regarded as weeds, the total number of which is actually not very high (Sukopp and Scholz 1997), owe their existence and range of distribution to human activity. Some of them are even totally dependent on it. This means that they could not exist – at least not without considerable change – in a world without humans. Many of their highly differentiated ecotypes, which are found nowadays among crops, would disappear (Hammer *et al.* 1982).

Through domestication, i.e. selection by humans, crops differ from original wild populations in morphology and anatomy (Hammer 1984). It is impossible to reconstruct the wild progenitor of barley (*Hordeum vulgare*) from modern breeding varieties of the same species, just as the wild progenitors of poppy cannot develop from the cultivated poppies (*Papaver somniferum*), even if these are found as escaped populations (Hammer and Fritsch 1977). On the other hand, it is, in some cases, possible by investigation to obtain some information on the pathways of evolution from wild species to either weeds or cultivars.

The role of weeds in general

For practical reasons, farmers, foresters, gardeners and different disciplines of science deal with weeds. Plant sociologists, botanists and ecologists have accurately documented the impoverishment of the flora in agricultural ecosystems for several years (Illig and Kläge 1996). These ecosystems will be subject to our further consideration.

Agricultural ecosystems are, of all existing habitats, the ones that have been influenced by humans most intensively. Characteristic changes and adaptations to cultivation are very obvious in these ecosystems. On arable land, evolution has developed considerable diversity in spite of the relative shortness of this period (10 000 years). This acceleration of evolution helped and made it possible for people to create crop plants, but it can be supposed that hardly anybody drew attention to the effects on weeds. Nowadays it is clear that many species have become rare or have even disappeared because of drastic changes in our cultivation of landscapes during recent decades. Traditional plant cultivation was done with a lot of know-how and some tools and farm implements. Today arable areas are usually treated with mineral fertilizers, several agrochemical pesticides, large agricultural machines, short-term crop rotations and crop monocultures. Most of the former diversity of flowering plants has been diminished and today comparatively few species dominate hedges and roadsides. Similar tendencies can be observed with insects and birds.

Such processes of genetic erosion become obvious if a species is totally extinct. But there also has been a large loss of diversity below the taxonomic rank of a species; that is, at the infraspecific level, where several botanical taxa or at least populations can be found. This loss is critical and comparable to the extinction of a species. But hardly anybody is interested in these taxa. Some exceptions exist, of course, where biological scientists are doing research on selected genotypes of weeds (Gladis 1996).

Existing weeds are usually of reduced genetic variability and they are often difficult for farmers to control. Often they possess, or have acquired, resistance to herbicides or they are by other means insensitive to our efforts to eradicate them. But sometimes it is even necessary to cultivate weeds, for example when testing new herbicides, and this is often rather difficult as botanical gardens know by experience.

The evolution of weeds

Weeds and cultivated plants have developed under similar evolutionary processes. Both of them originate from the beginning of agriculture 10 000 years ago when humans started to create environments favourable for useful plants (Hammer 1988). These areas, step by step, developed into agricultural ecosystems. Thus, weeds also found the conditions necessary for their existence (Fig. 1). While the remains of cultivated plants are often found in archaeological excavation sites, remains of weeds are found with less frequency and their existence is often based on indirect evidence (Erskine *et al.* 1994).

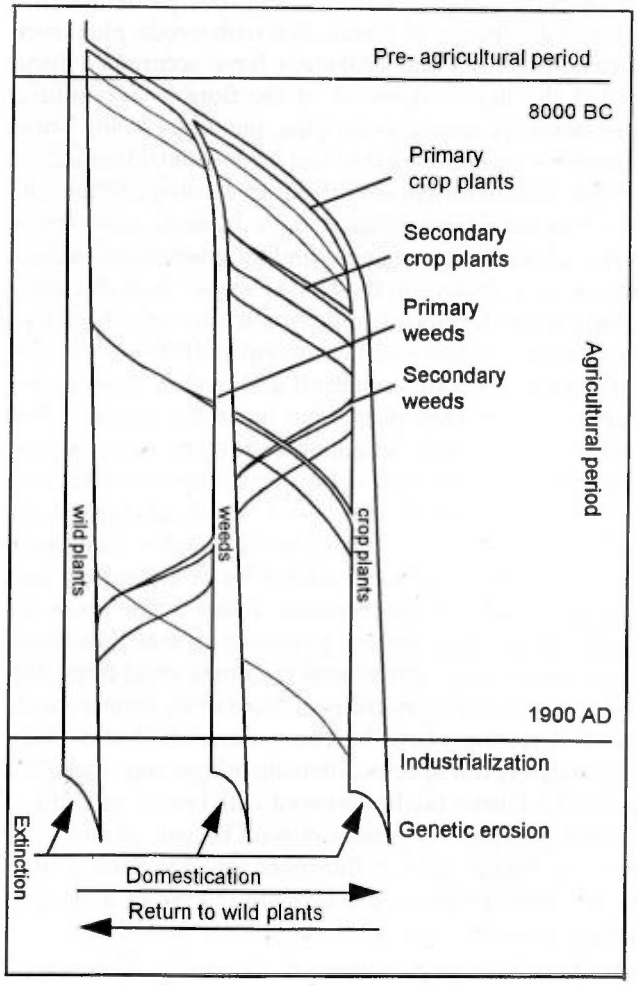

Fig. 1. Plants under human influence and evolution of weeds and crops (after Hammer 1988 and Gladis 1996). Pre-agricultural period = 8000 BC; Industrialization = post-1900.

Primary weeds and cultivated plants both originate from wild species, whose diversification extended after the start of domestication by humans. With selection, cultivated plants developed the characters useful to us. Under this pressure divergent selection of weeds occurred (Kupzow 1980), for example, forming large numbers of small seeds, fast maturation and low growth habit (e.g. shepherd's purse – *Capsella bursa-pastoris* and common chickweed – *Stellaria media*).

After agricultural ecosystems were established, primary weeds also evolved with convergent selection associated with human selection pressure. The result of this process is that weed characteristics have become similar to those of the cultivated plants with which they coexist (Kupzow 1980; Scholz 1996). Typical examples of such characters are the loss of specialized seed-spreading mechanisms, the formation of larger diaspores, the chronological determination of maturity, the loss of mechanical protection and spreading devices, the reduction of toxic or bitter ingredients, and seed and fruit colour different from those of the respective wild progenitor. Thus weeds may develop characters similar to those of cultivated plants. Some examples of weeds which have done so are corncockle (*Agrostemma githago*), rye-like brome (*Bromus secalinus*), flax-ryegrass and bearded ryegrass (*Lolium remotum, Lolium temulentum*) and flax-catchfly (*Silene linicola*) (Rothmaler 1947). Because of similar growth habit and other characters these weeds are extremely well adapted to the cultivated plants with which they grow. Weedy grasses may not have to change too much in order to become similar to cereals, but corncockle and flax-catchfly, both belonging to the campion (Caryophyllaceae) family, have completely changed during evolution in agricultural ecosystems. The convergent adaptation of the reproductive system of both species is obvious by the fact that their diaspores go through harvesting, threshing and cleaning (by winnowing) together with the seeds of the cultivars. The weed diaspores have adapted to the respective crop, and this phenomenon is sometimes called crop plant mimicry (Donald and Hamblin 1983).

As mentioned above, a good example of crop mimicry is corncockle (Hammer *et al.* 1982). Ecotypes adapted to the typical large-seeded cereals of the Mediterranean area also have large seeds (var. *macrospermum*, Fig. 2); the weight of 1000 seeds is nearly as high as that of cultivated cereals. The corncockle populations deriving from emmer fields (*Triticum dicoccon*, a wheat with diaspores consisting of two seeds and their glumes) tend to form seed clusters of mostly two seeds (Fig. 3). Other crops can be found accompanied by specialized types of this species. Some races developed convergently with flax (*Linum usitatissimum* – *A. githago* var. *linicolum*) leading to relatively small and smooth-surfaced seeds, similar to those of flax (Fig. 4). Types specialized for growing in buckwheat (*Fagopyrum esculentum*) have a remarkably short vegetation period like this crop. These examples show that the convergent adaptation of weeds can result in a nearly complete copy of key characters of

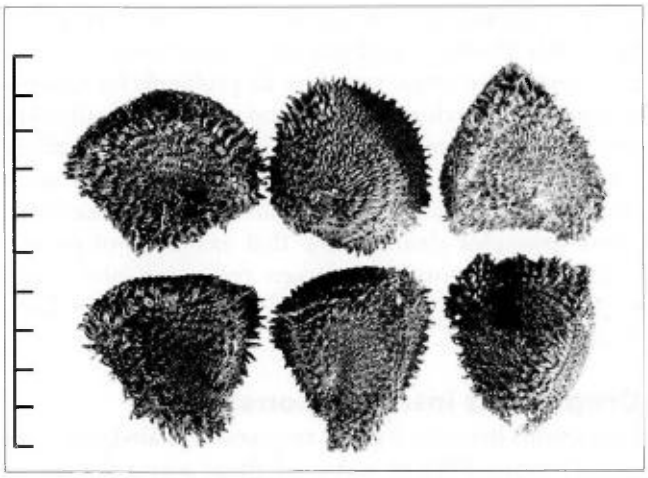

Fig. 2. *Agrostemma githago* var. *macrospermum* (AGRO 51) with large seeds adapted to large-seeded cereals in the Mediterranean. Scale is in millimeters.

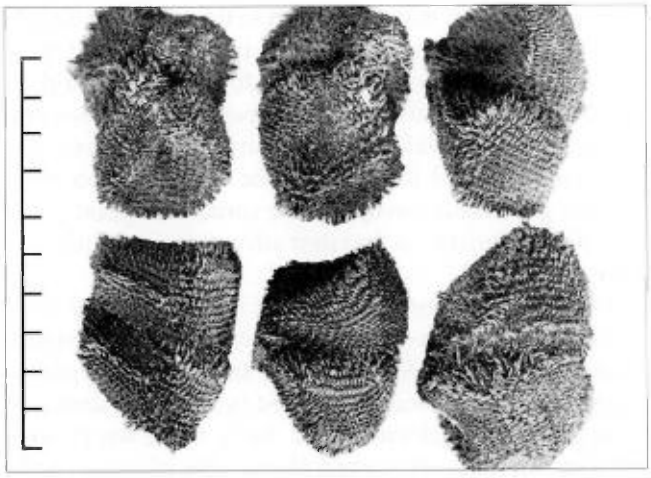

Fig. 3. *Agrostemma githago* (AGRO 51) with seed clusters of two seeds each, similar to the large diaspores of emmer wheat, *Triticum dicoccon*. Scale is in millimeters.

Fig. 4. *Agrostemma githago* var. *linicolum* (AGRO 5) with small smooth seeds (compare Fig. 2) sometimes resembling linseed also in outer appearance (first row, left seed) adapted to *Linum usitatissimum*. Scale is in millimeters.

Fig. 5. Different races of *Agrostemma githago* showing different grades of the domestication syndrome. Highly domesticated races with nearly closed capsules (lower left), races with widely open capsules freely dispersing the seeds (upper left), and races intermediate in capsule opening.

cultivated plants (Fig. 5). The plants show the typical signs of the so-called domestication syndrome (Hammer 1984). Because of their similarity to cultivated plants in many characters, convergent weed types are harvested, stored and sown together with the respective cultivated plant. Their survival even depends on the cultivation of these crops. Thus, corncockle could be used as a crop plant, but the content of saponines in the seeds has, so far, limited this use (Hammer *et al.* 1982). For industrial purposes, on the other hand, the high saponin content causes a special interest in cultivation of corncockle (Doll and Søgaard 1995).

Other weed species have by these means become secondary crops. Gold of pleasure (*Camelina sativa*) passed convergent development in flax fields in South and Central Europe. Rye (*Secale cereale*), one of our most important bread cereals, has been domesticated in barley and wheat fields in Southwest Asia (Hammer 1990).

Unfortunately, the dynamics of these processes are rarely observed. Weeds of the convergent type are in most cases extinct in our agricultural ecosystems. Sophisticated methods of seed separation and herbicides have reduced or eliminated many weed species of the convergent type (Hammer *et al.* 1982). For example, the introduction of grain sorters at the beginning of this century made it possible to eliminate the rough-surfaced seeds of corncockle nearly completely from the harvested crops, even though its 1000-seed weight is similar to that of crop plants (Hammer *et al.* 1982). Special techniques to sort corncockle seeds by treatment with an iron powder that adheres to the rough seed surface, and then removing them with magnetism, has worked well. Flax cultivation nearly disappeared in Western Europe because other fibers were used, and has only recently been reintroduced on a larger scale for oil production. The seeds of corncockle

and other species, which were left in the soil, have died in the meantime, because they do not survive for long (Korsmo 1930). Another strategy for survival could be the change to seed dispersion independent of human cultivation activity. But since the convergent strategy has been so successful for so long, the domestication traits became genetically fixed, and the corncockle plant could not adapt as quickly as the latest advances in agroindustry were introduced.

So it is not surprising that only a few of the many weed species had enough plasticity to adapt to the enormous changes in farming methods. Some small-seeded types escaped from arable land and existed in a manner similar to their progenitors (Hammer *et al.* 1982). Some weeds were even successful as ornamental plants. This, of course, is the exception, but the cultivar 'Milas' of *A. githago* is one example. Ornamental descendants are also found from cowherb (*Vaccaria hispanica*) and thorow-wax (*Bupleurum rotundifolium*).

Thus, the ruderal biotype of corncockle managed to survive, but several others, adapted to the crops emmer, rye, buckwheat, etc. (see Hammer *et al.* 1982), are now seldom found except in areas with traditional agriculture, and especially in those places where the respective cultivated plants are indigenous. Particularly in Central and Northern Europe, many highly specialized weed types have perished together with their respective crops. This genetic erosion is ongoing and several crops are threatened as well as the convergent weeds. Therefore, these weeds have little if any chance to develop further into secondary crops without human intervention, for example, through breeding programmes. In these cases the change from a convergent weed into a secondary crop is often only a small step.

Weeds of the divergent evolutionary type also have sometimes crossed the threshold and became cultivated plants, but compared with the convergent weeds this case is much more seldom. The already mentioned species common chickweed and shepherd's-purse are both cultivated as vegetables. Chickweed is grown in India, and large foliated shepherd's-purse is cultivated in China. Divergent weeds are also strongly influenced by developments in agriculture. Recent populations of common poppy (*Papaver rhoeas*) show much less genetic variability than they did 30-40 years ago in the same fields. This was shown by comparisons of seeds previously collected from fields and stored for a long time (long-term storage without substantical reduction of viability to avoid genetic changes) with recent populations from the same sites (Hammer and Hanelt 1980).

Considering primary and secondary crops we can also define primary and secondary weeds. The latter originate from cultivated plants when characteristics typical of cultivated plants return to characteristics found in the respective wild progenitors. The fragility of the rachis in cereals, causing the spontaneous spread of the seeds, is an example of a fundamental secondary weed trait. Introgressions of the wild characters from closely related wild plants or weeds into crops can have the same result (Hammer 1984). Plants, turned into secondary weeds by these processes, often cause very serious problems for farmers because they are closely related and morphologically very similar to the respective crops. Weedy rice (*Oryza sativa*), for example, with spontaneously shattering seeds, is an enormous problem in East and Southeast Asia (Oka 1988). These examples clearly show that exchange of genetic information is possible between cultivated plants and closely related weeds, and this is sometimes very common (Baker 1991).

Crop/weed interrelationships

Interactions between a given crop and a related weed appear in many different ways. All these aspects belong to the so-called crop/weed complex or crop/weed interrelationships (Pickersgill 1981; Hammer 1991; Harlan 1992). These interactions include:

1. Weeds that are progenitors of secondary crops (e.g. rye, poppy).
2. Secondary weeds that originated from crops (e.g. weedy rice, oats).
3. Weeds and crops that developed more or less simultaneously from one wild species (e.g. millet, tomato). Also, the wild races of barley occurring in open oak forests in the Fertile Crescent have nearly simultaneously developed crop types with a tough rachis and weed types with a fragile rachis. The weed barleys, *Hordeum spontaneum*, are able to intercross spontaneously with cultivated barley, the result of which are secondary weedy barleys (Harlan 1992). It is very difficult and sometimes even impossible to determine the origin of such a plant population (Hammer *et al.* 1985). These weeds must be considered as genetic resources, which are of potential use in breeding programmes in cultivated barley.
4. Some neglected or less important crops can even become feral species (e.g. *Bunium bulbocastanum, Legousia speculum-veneris, Castanea sativa, Malus* spp.). These plants have escaped from arable land and become members of the native flora. The necessary genetic changes can occur supported by the permanent existence of wild ancestors (*Fragaria vesca*) or completely independent (*Coriandrum sativum*).

These four points and other features of the crop/weed complex and its involvement in agricultural ecosystems are often neglected (Hammer 1991). The presence or absence of weed species, their genetic diversity and variability in character expression, allow us to draw conclusions about the state of the crop (local variety, mixture of cultivars, modern cultivar) in which they occur. Thus, they function as indicators of crop types and allow a qualified description of the respective crop (Hammer and Hanelt 1980; Hammer *et al.* 1982). Finally, crop plant research can use weeds for studying the parallel domestication processes in cultivated plants, because both crop and weed have been subjected to the influence of domestication for a

long of time (Harlan 1992). Those weedy plants that have no closely related crop plants, for example the above-mentioned corncockle, are of special interest because they are highly similar to cultivated plants. Genetic introgression of typical crop characters from cultivated plants can be excluded because they do not hybridize with the respective cultivated plants. In those cases the effects of the human selection pressure can be studied (Hammer 1984; Heiser 1988).

The conservation of weeds

Weeds, besides being genetic resources and of evolutionary interest, also are of cultural and historical interest. So there are reasons to preserve weeds, and it seems reasonable that genebanks should do so. Their survival in agriculture is threatened, as is that of the local varieties of crop plants with which they developed (Hammer 1985). Some selected examples of weed accessions in the Gatersleben genebank are given in Tables 1 and 2. Several accessions belonging to the same species indicate a high range of infraspecific variability (Fig. 6). This is obvious from morphological and ecogeographic distances of the single accessions. Molecular studies have just started (Hammer and Lux 1995). The introduction of weed collections into genebanks has been established in Gatersleben, and it is a new activity in crop plant research. Such work has been inspired by the genetic research done by the former director of the Institute for Crop Plant Research, Hans Stubbe (1902-1989) and the botanical systematic works of Rudolf Mansfeld (1901-1960), who, for many years, was head of the taxonomy department and genebank of Gatersleben.

Gatersleben genebank personnel have collected some nearly extinct weed species, which are maintained and multiplied. The final loss of several specialized types could be prevented. Unfortunately, for many species, these activities did not start as early as for corncockle in the 1970s and early 1980s.

A certain increase in accessions can be observed from Table 1 for weeds which are still quite common (e.g. *Avena fatua*) or which have been included in specific studies (*Raphanus raphanistrum*, see Pistrick 1987). Other weeds have become rather rare because of changes in agricultural production, such as weedy types of *Pisum sativum* convar. *speciosum*, which has small seeds, and have been regular components of the weed flora in spring cereals of Poland (Kulpa and Hanelt 1981).

Table 1. Accessions of selected weeds maintained in the Gatersleben genebank. These weeds are closely related to important crops (1984 data cited according to Hammer 1985)

Taxa	Number of accessions 1984	1997
Hordeum subgen. *Hordeum*	215	225
Avena fatua, Avena sterilis	81	151
Pisum sativum convar. *speciosum*	52	53
Vicia angustifolia	142	135
Raphanus raphanistrum	91	167
Sinapis arvensis	10	21
Lycopersicon pimpinellifolium	22	27

Table 2. Accessions of selected weeds maintained in the Gatersleben genebank. These weeds are not closely related to important crops (1984 data cited according to Hammer 1985)

Taxa	Number of accessions 1984	1997
Agrostemma githago	108	210
Papaver rhoeas	35	37
Bromus secalinus	19	26
Lolium temulentum	39	55
Datura spp.	73	186
Hyoscyamus spp.	71	139

A similar picture is shown in Table 2. Besides the increase of material resulting from specific investigations (*Datura* spp., see Hammer *et al.* 1983), a certain decrease in the distribution of a weed can be demonstrated. For *Bromus secalinus* only a few new accessions could be obtained in comparison with the still more common *Lolium temulentum*. For *A. githago* a special programme was initiated, and in spite of the decreasing distribution of this species, additional efforts led to an astonishing increase in the number of accessions of this species. *Agrostemma githago* and other weeds of the convergent type of evolution have to be maintained *ex situ* (Fig. 7) in order to avoid extinction of many forms which are of interest as genetic resources and for crop plant research.

Nevertheless, *ex situ* reproduction and conservation in genebanks, as a matter of fact, often has to be regarded as *ultima ratio*. Complementary *in situ* conservation should be practised, so that the geneflow and evolutionary processes in agricultural ecosystems can continue. In agriculture such on-farm methods depend on the interests and the abilities, including the economical aspects, of cooperating farmers. Farmers, ecologists and genebank personnel should work together and agree on strategies for conservation (Hammer and Knapp 1993; Begemann and Hammer 1994; Hammer and Perrino 1995). This new approach is part of an integrated genebank strategy (Hammer 1996).

Crop/weed complexes have developed in the areas of origin or in the centres of diversity of crop plants (Pickersgill 1981). These places are often located in developing countries and the relationships between them and industrial countries are of importance when discussing problems related to their conservation. Establishing and maintaining crop plant or weed collections in developing countries is often difficult. On the other hand, there can be found a wide range of biodiversity in these countries under sustainable agriculture systems. In industrialized countries this diversity has mostly been lost. Nevertheless, it is sometimes also possible in industrialized countries to

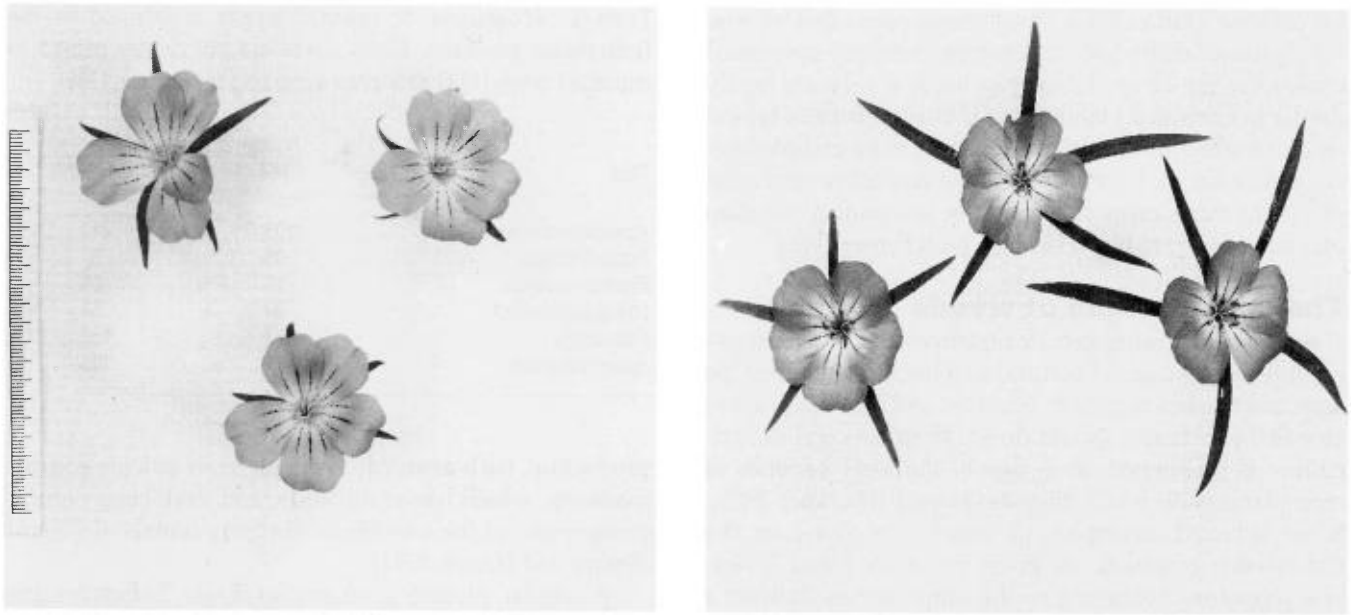

Fig. 6. Variation within the Gatersleben collection of *Agrostemma githago* with respect to flower characters. Flowers with short calyx teeth (left, AGRO 75), flowers with long calyx teeth (right, D 3901).

Fig. 7. Reproduction of *Agrostemma githago* (AGRO 21) in the Gatersleben experimental fields. In its vegetative characters *A. githago* is similar to the neighbouring cereals (crop plant mimicry).

reconstruct how some evolutionary processes developed with the help of secured (e.g. in genebanks) plant material (Hammer 1994). Wild and cultivated oats are another example of a crop/weed complex. They were both still frequently found with high diversity in Germany until the beginning of this century (Kühn *et al.* 1976) and in Slovakia in the 1970s.

Arable land and associated weeds have hardly been typical objects of interest for ecologists up to now. This is changing, and their involvement in this field is increasing (Holzner and Numata 1982). Together with the recent ecological movements in agriculture, they will seek to preserve the traditional cultivated landscapes and the associated agro-ecosystems. The wealth and achievements of our societies are not only defined by the technical innovations but also by our surrounding landscape, which merit careful study.

People have been trying to eliminate weeds for a long time. Therefore, preservation of weeds seems to be a paradox. But several weeds, especially from the crop/weed complexes, have to be considered as important genetic resources and are worth maintaining in *ex situ* (Thompson 1974) and *in situ* programmes. Some of the programmes mentioned for *in situ* conservation caught the public's imagination and the beauty of agricultural ecosystems was also recognized. Some weeds helped to remove political barriers and to open hearts and minds to the necessity of protecting them.[1] In industrialized countries part of the agriculture is now changing towards lower-input systems, as far as fertilizers and pesticides are concerned. This helps to maintain diversity, though it is primarily initiated in order to diminish the overproduction of agricultural goods in our countries. Depending on economic conditions, farming practices may continue to reduce diversity on a large scale. Because of that it is now necessary to complete further research on ideas for conservation and development of biodiversity in agricultural ecosystems, and genebanks could play a role in this process. Weeds have to be considered an important part of biodiversity.

[1] "Our emotions are easily aroused whenever an animal species is threatened with extinction, but who knows, or cares, about the disappearance of the corn cockle, murdered by agrochemical revolution ..." F. Franks (1981).

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Résumé

Les adventices au service de la génétique

Les plantes adventices sont souvent d'importantes ressources génétiques. Elles sont aussi utiles pour les études sur l'évolution en rapport avec la domestication, comme indicateurs des variétés locales traditionnelles et comme matériel de base pour la recherche sur le désherbage. Plusieurs plantes adventices convergentes (mimétisme des plantes cultivées, par exemple *Agrostemma githago*) présentant une grande similitude avec des plantes cultivées sont menacées d'extinction. Ce processus touche en particulier la variation infraspécifique (par exemple, l'adaptation à différentes plantes cultivées). Une approche spéciale a été adoptée à la banque de gènes de Gatersleben pour la conservation *ex situ* des plantes adventices dans le cadre d'une organisation intégrée de la banque de gène. Des exemples de conservation de plantes adventices à la banque de gènes de Gatersleben sont examinés (par exemple, *Agrostemma githago, Papaver rhoeas, Bromus secalinus, Lolium temulentum*). La conservation *in situ* des complexes de plantes cultivées/adventices pose un nouveau défi.

Resumen

Las malas hierbas como recursos genéticos

Frecuentemente, las malas hierbas tienen importancia como recursos genéticos. También son útiles para los estudios evolutivos relativos a la domesticación, como indicadores de variedades locales autóctonas y como material básico para la investigación sobre la lucha contra las malas hierbas. Varias de las malas hierbas convergentes (miméticas de plantas cultivadas, como *Agrostemma githago*), que presentan una gran similitud con las plantas cultivadas, están amenazadas de extinción. Este proceso afecta particularmente a la variación intraespecífica (por ejemplo, adaptaciones a diferentes cultivos). En el banco de genes de Gatersleben se está aplicando un enfoque especial para la conservación *ex situ* de las malas hierbas, dentro del concepto de banco de genes integrado. Se estudian diversos ejemplos de conservación de malas hierbas en el banco de genes de Gatersleben (por ejemplo, *Agrostemma githago, Papaver rhoeas, Bromus secalinus y Lolium temulentum*). La conservación *in situ* de complejos de cultivos/malas hierbas es un nuevo desafío.

Plant Genetic Resources Newsletter, 1997, No. 111: 40-52 ARTICLE

Conservación de germoplasma de moras silvestres (*Rubus* spp.) en la cuenca del río El Palmar, municipio de Ubaque (Cundinamarca, Colombia): I. Distribución y ecología[1]

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Resumen

La transformación de los ecosistemas naturales de los Andes Colombianos significará, a medio término, un riesgo de pérdida de valiosos recursos fitogenéticos de plantas silvestres. Como parte de una estrategia conjunta para su conservación *in situ* y *ex situ*, se realizó a nivel regional de cuenca hidrográfica la exploración botánica y recolección de germoplasma de moras silvestres (*Rubus* spp.). Así mismo, se compiló información sobre el uso popular. Con base en la información cartográfica (1:25.000) y de trabajo de campo, se exploró la cuenca siguiendo un gradiente altitudinal de 1500 hasta 3600 m snm. De cada especie se colectó material vegetativo para el establecimiento, mantenimiento y propagación de una colección de germoplasma *in vitro*, como estrategia de conservación *ex situ*. Se encontraron 10 especies, un conjunto de distribución restringida al piso de Páramo (2800 a 3600 m snm): *Rubus acanthophyllus*, *R. compactus*, *R. floribundus* y *R. macrocarpus*. Un conjunto de amplia distribución en el piso del Bosque Andino y Subandino (1500-3000 m snm): *R. bogotensis*, *R. glaucus*, *R. megalococcus*, *R. urticaefolius*. Se registra una población extremadamente reducida (2700 m snm) con *R. porphyromallus*, y una especie de reciente introducción como *R. idaeus*. Las especies observadas están relacionadas con el hábitat abierto de origen antrópico: bosques y matorrales secundarios o rastrojos, cercas de piedra o linderos, bordes de carretera, taludes y tierras de cultivo abandonadas temporalmente.

Introducción

El diagnóstico más reciente sobre el estado de la biodiversidad en Colombia indica que varios de los ecosistemas de montaña se encuentran seriamente amenazados con alto riesgo de pérdida de los recursos genéticos (Hernández-Camacho 1992; Rangel 1995). A nivel internacional hay un gran interés en la conservación de "los parientes silvestres de las plantas cultivadas" (Hoyt 1992). Las "moras silvestres" (*Rubus* spp.) son los parientes silvestres de la "mora de Castilla" (*Rubus glaucus* Benth.), cultivada comercialmente, según censo de 1983, en un área sembrada de 1170 ha que representan aproximadamente 11 770 t de producción en Colombia (FEDECAFE 1983).

Rubus es el género más grande de la familia Rosaceae con aproximadamente 700 especies a nivel mundial, la mayoría de ellas apomícticas (Romoleroux 1992). Las especies latinoamericanas del género *Rubus* se encuentran agrupadas principalmente en dos subgéneros: *Orobatus* y *Rubus*; en tanto que la especie (*R. glaucus*), considerada un híbrido entre especies de los subgéneros *Idaeobatus* y *Rubus*, pertenece al subgénero *Idaeobatus* (Ballington *et al.* 1993).

Actualmente en los cultivos de "mora de Castilla" (*Rubus glaucus*) se presentan numerosos problemas fitosanitarios causados principalmente por hongos (pudrición del fruto, antracnosis, muerte descendente), que han afectado la economía de los cultivadores de mora en Cundinamarca (FEDECAFE 1983); esta situación revela la importancia de la conservación de especies silvestres como posible fuente de características de resistencia en futuros programas de mejoramiento de las especies cultivadas.

Por otra parte, es muy poco lo que se sabe en Colombia del género *Rubus*, lo cual ya justificaría su revisión completa. Esta problemática llevó a la integración de un proyecto conjunto de investigación entre la Unidad de Ecología y Sistemática y la Unidad de Biología Vegetal de la Universidad Javeriana, para formular un plan de investigación que involucrara estrategias de conservación *in situ* y *ex situ*, para la conservación de recursos fitogenéticos de moras silvestres (*Rubus* spp.). El primer objetivo fue conocer las especies de *Rubus* que se presentaban en la región, y el segundo conocer su distribución geográfica, usos y estado de conservación en el gradiente altitudinal.

Area de estudio

El proyecto se desarrolló en la cuenca del río El Palmar, jurisdicción del municipio de Ubaque, en el departamento de Cundinamarca. La cuenca presenta una extensión aproximada de 11 056,5 ha, se localiza en la vertiente oriental de la Cordillera Oriental, a los 4°24' - 4°37' Lat. N y los 73° 50' - 74° 07' Long. W.

[1] Resultados parciales del proyecto Desarrollo de estrategias *in situ* y *ex situ* para la conservación de la diversidad biológica de moras silvestres (*Rubus* spp.) en la cuenca del río El Palmar, municipio de Ubaque, Cundinamarca, Colombia.

Aspectos geológicos y edáficos

Los suelos se desarrollan sobre diversos materiales geológicos en franjas más o menos paralelas que afloran sucesivamente siguiendo la pendiente principal de la cuenca, de arriba hacia abajo aparecen las formaciones Guadalupe, Chipaque, Une y Fómeque. En la parte alta de la cuenca, con clima de páramo húmedo, aparecen suelos de carácter ándico sobre cenizas volcánicas de origen eólico o entisoles con bajo grado de desarrollo sobre areniscas cuarcíticas. En la parte media de la cuenca con clima frío se presentan suelos de tipo inceptisol y entisoles; en la parte baja de la cuenca con clima templado y contrastes pluviométricos marcados, sobre lutitas o materiales coluviales de textura arcillosa aparecen entisoles, inceptisoles y alfisoles sobre topografías más suaves. El relieve es predominantemente escarpado o quebrado, con pendientes en general mayores del 25% (INGEOMINAS 1990, 1992, 1995).

Aspectos de clima y vegetación

De acuerdo con Hernández-Camacho (1992), los pisos térmicos en el municipio de Ubaque corresponden a los siguientes:

- **Piso térmico templado isomacrotérmico** que corresponde a la cuenca baja. Presenta temperaturas medias anuales entre 18° y 24°C; se presenta desde los 1500 hasta los 1800 a 2000 m snm. Equivale a la Tierra Templada, al piso Premontano (Holdridge 1967).
- **Piso térmico frío o isomesotérmico** que corresponde a la cuenca media. Presenta temperaturas medias anuales entre 12° y 18°C y se extiende entre 1800 a 2000 m snm y llega hasta 3000. Equivale a la Tierra Fría, al piso Montano Bajo (Holdridge 1967).
- **Piso isomicrotérmico** que corresponde a la cuenca alta. Con temperaturas medias anuales entre 6° y 12°C, se distribuye entre 3000 y 3600 m snm en los picos más altos de la divisoria de aguas con el Distrito Capital de Santafé de Bogotá. Equivale a la Tierra Fría (en parte), al Páramo (en parte), y al piso Montano (Holdridge 1967). Según el estudio del DRI (1991), la precipitación promedio anual es de 1056,36 mm. La región presenta un régimen unimodal de lluvias, con una estación lluviosa en los meses de abril a noviembre, y un período seco o de "verano" en los meses de diciembre a marzo.

Aspectos socioeconómicos

Estudios recientes de tipología de la cobertura vegetal y uso de la tierra (Rivera, datos no publicados) en la cuenca del río El Palmar, comprende 2 356,5 ha (21,3%) de vegetación de páramo, 1661,3 ha (15%) de vegetación forestal, 2438 ha (22%) de matorrales y rastrojos bajos, 6705,4 ha convertidos en pastizales y cultivos misceláneos, lo que representa cerca del 60,6% de la superficie transformada en un mosaico de paisaje agrícola y pastoril de los cuales el 15,4% se presenta en el páramo, 70,7% en el piso subandino y 84,6% en el piso subandino.

El municipio de Ubaque está dividido políticamente en 18 veredas, con una población aproximada de 7440 habitantes. El mayor índice de pobreza y a su vez el mayor porcentaje poblacional se encuentra en la parte baja de la zona, debido principalmente al tamaño reducido de las parcelas, a pesar de poseer las mejores condiciones de fertilidad y clima. El 90,4% de los predios no supera las 3 ha y el 72,3% son predios menores de una hectárea, comprendiendo solo el 33,6% de la superficie total del municipio (DRI 1991).

Materiales y métodos

La colección de germoplasma se realizó con la participación de las comunidades campesinas que de alguna manera mantienen estos recursos a través de sus formas tradicionales de uso de la tierra. En este trabajo consideramos que uno de los pasos fundamentales es el contacto directo con las comunidades involucradas, de lo cual en buena medida dependerá el éxito de la conservación de recursos fitogenéticos *in situ*.

Actividades de motivación a la comunidad

El primer paso en el desarrollo de la investigación fue el buscar contacto con la comunidad campesina, con el fin de motivar nuevas actitudes, valores y acciones de la comunidad, con respecto a la conservación de la diversidad biológica de las moras silvestres (*Rubus* spp.). Se realizaron las siguientes acciones:

- Presentación a la comunidad del proyecto a desarrollar en reunión realizada en la alcaldía
- Integración con proyectos locales (DRI-Alcaldía-Comunidad)
- Radiodifusión por la emisora local del Cabildo Verde "La Voz del Bosque"
- Contacto directo y charlas informales (intercambio de saberes) con los propietarios de las fincas y líderes de la comunidad
- Observaciones directas de los vendedores de moras en la plaza de mercado
- Reconocimiento de campo con participación de miembros de la comunidad y estudiantes del colegio y escuelas rurales
- Divulgación a nivel popular mediante jornadas ecológicas y talleres participativos y carteleras sobre biodiversidad y conservación de los parientes silvestres de las plantas cultivadas
- Participación de técnicos de la UMATA (Unidad Municipal de Asistencia Técnica Agropecuaria), en el trabajo de campo y educación ambiental.

Prospección botánica

Para un reconocimiento general de la cuenca, se realizó un muestreo estratificado altitudinalmente, se utilizaron fotografías aéreas (escala 1:25.000) y cartografía disponible del Instituto Geográfico "Agustín Codazzi" (IGAC). Sobre la base cartográfica y el reconocimiento de campo y aspectos logísticos como accesibilidad entre otros, se realizó la colección botánica recorriendo caminos y carreteables de las veredas desde la cuenca baja (1500 m snm) hasta la cuenca

alta (3600 m snm). Con base en la cartografía digitalizada se estableció un retículo de coordenadas UTM de 1 km x 1 km de lado y en el centro de cada sector se indicó con un punto la presencia de la especie observada y/o herborizada, para construir el mapa de distribución (Bolós *et al.* 1994).

Determinación taxonómica

El material botánico se coleccionó según técnicas convencionales de herborización (Forero 1977), con énfasis en las características del hábitat y hábito de la planta, uso actual de la tierra, y abundancia del clon o individuo herborizado (abundante, poco abundante y raro). Las estructuras reproductivas se conservaron en líquido (FAA) para posterior estudio en laboratorio. De cada especie se tomaron muestras de material vegetativo para su conservación *ex situ*. Las descripciones se realizaron en las instalaciones del Herbario de la Pontificia Universidad Javeriana (HPUJ), y posteriormente con estas descripciones en el Herbario Nacional Colombiano (COL) del Instituto de Ciencias Naturales de la Universidad Nacional de Colombia, con base en la colección existente en esa institución. En los dos herbarios mencionados se depositaron duplicados de los ejemplares botánicos.

Resultados y discusion

En la cuenca del río El Palmar se encontraron 10 especies silvestres del género *Rubus* (Tabla 1). Las descripciones de cada una de las especies, se realizaron en base al material coleccionado en la cuenca del río El Palmar. Los caracteres observados fueron de tipo vegetativo y de tipo reproductivo. Pero debido a que no todas las partes de las plantas presentan caracteres constantes, ya que el tipo de hábitat -áreas soleadas y áreas protegidas, la incidencia directa del sol, producen variaciones en el indumento, acúleos, número de folíolos, tamaño, etc. (Monasterio-Huelin 1993), se realizaron descripciones minuciosas con el objeto de mostrar las variaciones de los diferentes ejemplares asignados a cada taxón y facilitar la identificación en el campo.

Tabla 1. Lista preliminar de las especies del género *Rubus* existentes en el Herbario Nacional Colombiano (COL). Se indica con un asterisco (*) las especies registradas en Ubaque en este estudio.

1. *Rubus acanthophyllus* Focke (*)
2. *R. adenotrichos* Schlecht.
3. *R. bogotensis* H.B.K. (*)
4. *R. choachensis* Berger
5. *R. compactus* Benth. (*)
6. *R. coriaceus* Poir.
7. *R. floribundus* H.B.K. (*)
8. *R. gachetensis* Berger
9. *R. glabratus* H.B.K.
10. *R. glaucus* Benth. (*)
11. *R. guianensis* Focke
12. *R. idaeus* L. (*)
13. *R. lechleri* Focke
14. *R. macrocarpus* Benth. (*)
15. *R. megalococcus* Focke (*)
16. *R. porphyromallus* Focke (*)
17. *R. robustus* Presl.
18. *R. rosaefolius* Smith
19. *R. roseus* Poir.
20. *R. trichomallus* Sclecht.
21. *R. urticaefolius* Poir. (*)

Especies registradas en Ubaque

En la distribución y ecología de las especies del género *Rubus*, intervinieron varios factores como son la altitud, clima, sustrato y uso de la tierra. El patrón de uso de la tierra y su tenencia son determinantes en la oferta de hábitats para el establecimiento de las especies de *Rubus*. Desde este punto de vista la cuenca presenta tres grandes ambientes.

Area paramuna: Corresponde principalmente a la transición del bosque altoandino y páramo (*sensu* Cuatrecasas 1958). Comprende 2356,5 ha (21,3%), de los cuales el 15,4% ha sido transformado en pastizales. Las fincas son de gran extensión (mayores de 100 ha) y el sistema de producción predominante es el cultivo de papa con rotación de barbecho para pastoreo (Vargas y Rivera 1991). Se presenta un mosaico de pastizales y fragmentos de bosque altoandino con *Weinmannia tomentosa* y *Drymis granatensis*, el fraylejonal-pajonal paramuno se compone principalmente de *Espeletia grandiflora* y *Calamagrostis effusa*. Estudios detallados sobre este ambiente páramo y vegetación del piso andino se encuentran en Lozano y Schnetter (1976), Schnetter *et al.* (1976), Cleef (1981), Sturm y Rangel (1985), Vargas y Zuluaga (1985), Mora-Osejo y Sturm (1994). Todos los ambientes observados presentan diferentes grados de disturbio por actividades agrícolas, pastoreo, apertura de carreteables, incendios y deforestación. Se observaron las siguientes especies de *Rubus*:

Rubus acantophyllus Focke

Plantas hasta 1 m de largo, postradas, suberectas o péndulas; tallos teretes; ejes vilosos, con pelos glaucos cortos y pelos glandulares rojos largos; con abundantes aguijones anaranjados largos. Hojas simples; estípulas libres, anchamente ovadas, corto-acuminadas, asimétricas, con los nervios teñidos de rosado, con pocos pelos glaucos; pecíolos 1-3 cm largo; láminas ovado-lanceoladas, corto-acuminadas, 4-6,6 cm largo, 2,1-4 cm ancho, en ocasiones con dos lóbulos laterales pequeños, infrecuentemente crenado-serradas, superficie labrada y vilosa, teñida de rosado, por el envés nervios rosados y armados, ocasionalmente con pelos glandulosos rojos. Flores solitarias en los extremos de las ramas, pedúnculos tomentosos, pelos glaucos cortos, pelos glandulosos rojos, largos y escasos, con aguijones; cáliz rojo, tomentoso y con aguijones rojos, lóbulos ovado-triangulares, mucronados, mucros rojos; pétalos rosados; filamentos de los estambres rosados, 5-6 mm largo; pistilos rosados, hasta 5 mm largo, ovarios densamente vilosos; frutos hasta 1,3 cm (Fig. 1a).

a)

b)

c)

d)

e)

f)

Fig. 1. Especies silvestres de *Rubus* recolectadas en la Cuenca del Río El Palmar, Municipio de Ubaque: a) *Rubus acanthophyllus*, b) *R. bogotensis*, c) *R. compactus*, d) *R. floribundus*, e) *R. glaucus*, f) *R. idaeus*.

Distribución y ecología
Planta de hábito herbáceo de tallos radicantes, sin raíces adventicias en el ápice; frecuente hacia los bordes de matorrales de cañadas del subpáramo o en parches de vegetación arbustiva aislados en los potreros. También crece sobre grandes rocas cubiertas de musgos y substrato orgánico, generalmente por encima de los 3000 m snm. Esta especie presenta clones de pequeño tamaño que se extienden por el suelo, casi siempre cubierto de musgos, del cual emergen tallos floríferos que alcanzan 15-50 cm de altura (Fig. 2A).

***Rubus macrocarpus* Benth.**
Arbustos hasta 3 m de alto; tallos teretes o subcuadrados; ejes tomentosos, con pelos largos canescentes; aguijones pequeños anaranjados, pilosos. Hojas compuestas, 3-folioladas; estípulas libres, grandes, anchamente ovadas, corto-acuminadas, vilosas por la haz, tomentosas por el envés; pecíolos 3-6,5 cm largo; folíolos discóloros, ovados, corto-acuminados, 3,8-9 cm largo, 2,0-6,4 cm ancho, vilosos por la haz, tomentosos por el envés. Inflorescencias: pedúnculos tomentosos, brácteas tomentosas, anchamente ovadas, apiculadas, mucro rojo, con pelos glandulares rojos; cáliz tomentoso, lóbulos ovado-triangulares, abruptamente acuminados, ápice subulado; pétalos rosados; filamentos de los estambres rosados, 0,3-0,4 cm largo; pistilos hasta 0,7 cm largo, ovarios densamente tomentosos; frutos hasta 6 cm (Fig. 3c).

Distribución y ecología
Hábito arbustivo, de tallos radicantes y escandentes. Crece preferentemente en ambientes de alta humedad, asociada a matorrales ubicados a lo largo de cañadas o en matorrales que se desarrollan en torno a, o sobre grandes rocas aisladas en el campo, por encima de 3000 m de altitud en la vereda Cruz Verde, o puede presentar plantas de tamaño reducido dentro de bosques secundarios a 2700 m. También se observaron clones creciendo en áreas plenamente abiertas de pastizal, en suelo bien drenado (Fig. 4G).

Area de pastizales para ganadería y cultivos varios del piso andino: Sobre los 2100 m snm hasta aproximadamente 2600-2800 m snm. Comprende 7362 ha (66,6%), predomina la tenencia de la tierra en forma de fincas mayores de 50 ha. Se presentan grandes pastizales de kikuyo (*Pennisetun clandestinum*) que ocupan cerca del 70,7% de la superficie de la cuenca dedicados al cultivo y pastoreo. Se presentan dispersos y reducidos fragmentos de vegetación natural con matorrales altos y fragmentos de bosque andino con *Weinmannia* spp., *Tibouchina lepidota* y *Chusquea* spp., que sirven en parte de refugio a un número mayor de especies de mora, con clones de diverso tamaño, creciendo preferencialmente en los bordes como las siguientes especies:

***Rubus bogotensis* H.B.K.**
Arbustos hasta 4 m de alto; tallos 5-angulosos; ejes finamente tomentosos y débil, densamente cubiertos con pelos glandulares rojos, cortos o largos; aguijones pequeños a grandes. Hojas compuestas, 3-5-folioladas (digitadas); estípulas basipeciolares, estrechamente lanceoladas, vilosas, con pelos glandulares por la haz; pecíolos 2,5-10 cm largo; folíolos subcordados, ovados hasta anchamente ovado-lanceolados, acuminados, 5-15 cm largo, 2-8 cm ancho, vilosos por la haz, tomentosos por el envés, con pocos pelos glandulares rojos por ambas superficies. Inflorescencias grandes: pedúnculos, brácteas y cáliz tomentosos y con pelos glandulosos rojos abundantes; lóbulos del cáliz ovado-lanceolados, abruptamente acuminados, con una arista larga y roja; pétalos blancos hasta blancos y teñidos de rosado; filamentos de los estambres blancos, hasta 3 mm largo; pistilos blancos, hasta 4 mm, ovarios tomentosos; frutos de 2,3 cm (Fig. 1b).

Distribución y ecología
Hábito arbustivo, crece en forma de matorrales aislados haciendo parte de las cercas de alambre en los potreros, en grandes plantas de hasta 50 m de longitud, a lo largo de las cañadas, también en matorrales esparcidos en afloramientos rocosos o en matorrales emergentes dentro de bosques secundarios, desde 2100 hasta 3100 m de altitud (Fig. 2B).

***Rubus compactus* Benth.**
Hierbas hasta 0,40 m de alto; tallos teretes; ejes espaciadamente tomentosos. Hojas compuestas, 3-folioladas; estípulas libres, anchamente ovadas, acuminadas, en ocasiones con la margen superior serrada, glabras por la haz, con pelos espaciados por el envés; pecíolos 2,5-3 cm largo; folíolos discóloros, ovados hasta ovado-lanceolados, corto-acuminados, 3-7,5 cm largo, 2,5-4 cm ancho, haz glabra o con pelos espaciados, envés viloso sobre los nervios. Inflorescencias no observadas (Fig. 1c).

Distribución y ecología
Especie poco abundante, de hábito herbáceo. Crece en pequeños parches de vegetación en el límite de matorrales ubicados a lo largo de cañadas, con suelos muy húmedos, por encima de los 3000 m (Fig. 2C).

***Rubus floribundus* H.B.K.**
Arbustos hasta 4 m de alto; tallos 5-angulosos; ejes vilosos, ocasionalmente con pelos glandulosos rojos y cortos; con aguijones pequeños hasta grandes, anaranjados y vilosos. Hojas compuestas, 3-folioladas; estípulas basipeciolares, largo-lanceoladas, angusti-lanceoladas hasta estrechamente ovado-lanceoladas, vilosas, con pelos glandulares rojos sobre la haz; pecíolos 4-13 cm largo; folíolos ovado-lanceolados, 5-10 cm largo, 2,2-4,6 cm ancho, haz con pocos pelos sobre las nervaduras hasta vilosa, envés viloso hasta tomentoso. Inflorescencias: Pedúnculos vilosos, brácteas anchamente ovadas,acuminadas, glabras; cáliz tomentoso, lóbulos ovados-lanceolados, largos, con una arista negra; pétalos que van del blanco al rosado; filamentos blancos y largos hasta 3 mm ; pistilos blancos hasta 4 mm; ovarios tomentosos; frutos 1,5 cm (Fig. 1d).

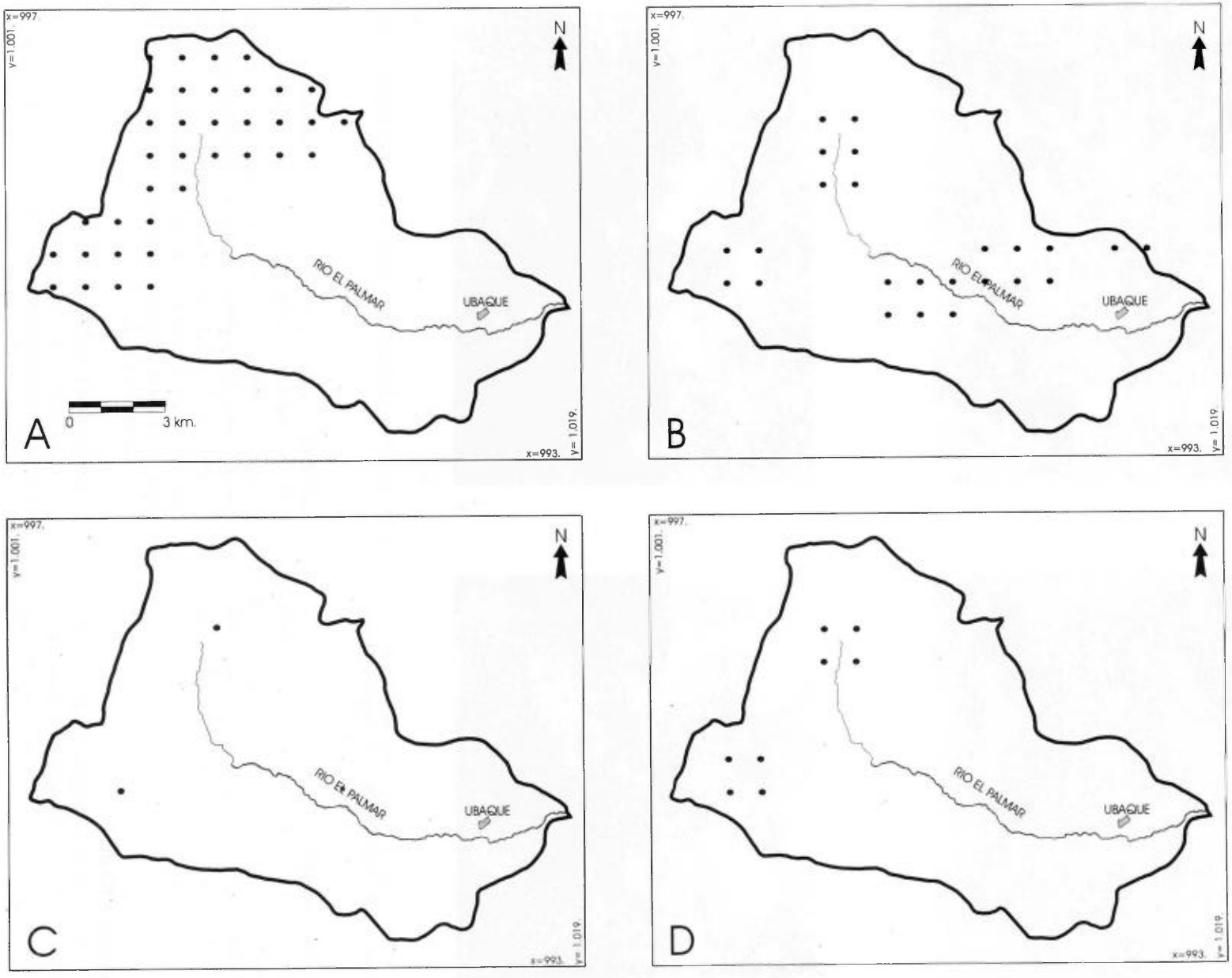

Fig. 2. Mapa de distribución de moras silvestres (*Rubus* spp.) en la Cuenca del Río El Palmar, Municipio de Ubaque: A) *Rubus acanthophyllus*, B) *R. bogotensis*, C) *R. compactus*, D) *R. floribundus*.

a)

b)

c)

d)

Fig. 3. Especies silvestres de *Rubus* recolectadas en la Cuenca del río El Palmar, Municipio de Ubaque: a) *Rubus porphyromallus*, b) *R. urticaefolius*, c) *R. macrocarpus*, d) *R. megalococcus*.

Distribución y ecología

Hábito arbustivo. Crece asociada a grandes matorrales o aislada en potreros cerca del bosque, por encima de los 3000 m de altitud en la vereda Cruz Verde. Su distribución está muy relacionada con la presencia de vegetación secundaria, dentro de la cual llega a formar clones de gran tamaño (Fig. 2D).

Rubus glaucus Benth.

Arbustos hasta 1,5 m de alto; tallos teretes; ejes glaucos y glabros; con aguijones amarillos grandes. Hojas compuestas, 3-folioladas; estípulas basipeciolares, largo-lanceoladas, glabras; pecíolos 3,8-8 cm largo; folíolos ovado-lanceolados, largo-acuminados, 6-9 cm largo, 2,5-3,5 cm ancho, márgenes serruladas, glabras por la haz, blanco-tomentosas por el envés. Inflorescencias terminales, racemoso-paniculadas; pedicelos largos, más o menos aculeados, con pelos glandulosos rojos, espaciados, brácteas ovado-lanceoladas, enteras o lobadas en el ápice, con pelos glandulosos rojos; cáliz densamente tomentoso con pelos blancos, lóbulos ovado-triangulados, abruptamente acuminados, terminados en una súbula, con pelos glandulosos rojos por el envés; pétalos blancos; filamentos blancos, hasta 3 mm largo; pistilos hasta 4 mm largo, blancos, ovarios densamente tomentoso. Frutos hasta 2,6 cm (Fig. 1e).

Distribución y ecología

Hábito arbustivo. Es un cultivar que se constituye en la base de la producción de mora en el país. En la cuenca del río El Palmar presenta algunos clones dispersos originados a partir de antiguos cultivos muy incipientes. Actualmente en la región el cultivo de la mora de Castilla se ha incrementado en 3500 plantas, formando pequeñas parcelas que cubren un rango altitudinal de 1800 a 3100 m snm. Las

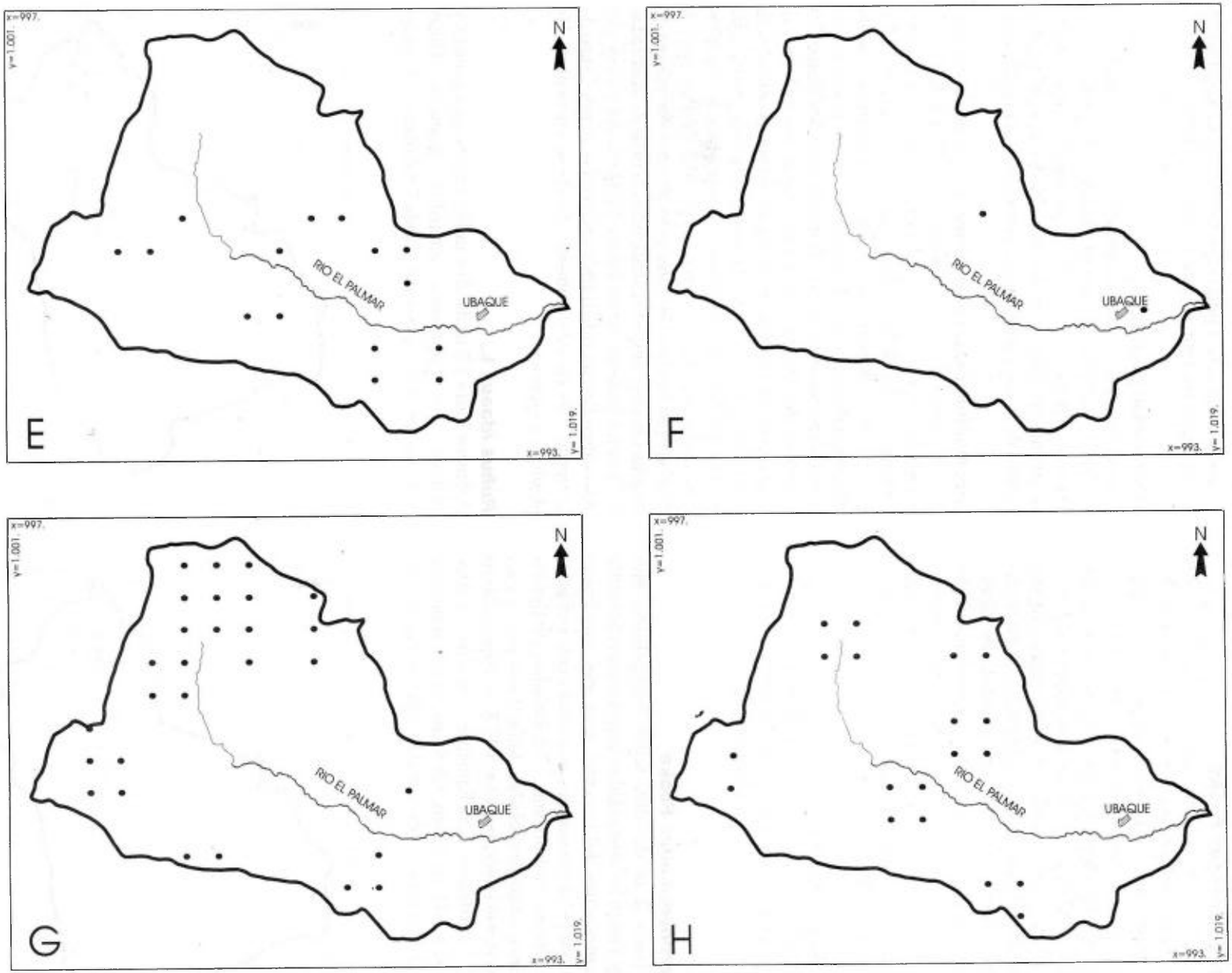

Fig. 4. Mapa de distribución de moras silvestres (*Rubus* spp.) en la Cuenca del Río El Palmar, Municipio de Ubaque: E) *Rubus glaucus*, F) *R. idaeus*, G) *R. macrocarpus*, H) *R. megalococcus*.

semillas diseminadas principalmente por las aves dan origen a plantas que crecen con carácter asilvestrado, aisladas en potreros y bordes de caminos, entre 2000 y 2700 m de altitud (Fig. 5E).

***Rubus megalococcus* Focke**

Arbustos hasta 4 m de alto; tallos 5-angulosos; ejes pubérulos; aguijones grandes. Hojas compuestas, 3-5-folioladas (digitadas); estípulas basipeciolares, largo-lanceoladas, 11 mm largo, 1,1 mm ancho, tomentosas; pecíolos 1,5-9,5 cm largo; folíolos cloróticos por la haz, oblongo-lanceolados, corto a largo-acuminados, 4,2-16 cm largo, 1,6-11 cm ancho, raquis viloso por ambas superficies. Inflorescencias con brácteas estrechamente obovadas, acuminadas, tomentosas, con pelos glandulosos escasos; lóbulos del cáliz tomentosos, ovado-lanceolados, abruptamente apiculados, mucro rojo; pétalos blancos; filamentos de los estambres blancos, 0,5-0,7 cm largo; pistilos hasta 0,3 cm largo, blancos, ovarios pilosos; frutos hasta 1,2 cm (Fig. 3d).

Distribución y ecología

Hábito arbustivo. Se distribuye entre 2500 y 3100 m de altitud en áreas similares a las ocupadas por *R. bogotensis*, aunque el tamaño de sus poblaciones es mucho menor (Fig. 4H).

***Rubus porphyromallus* Focke**

Arbustos hasta 2 m de alto; tallos 5-angulosos; ejes tomentosos, con pelos anaranjados largos esparcidos entre los pelos canescentes del tomento; aguijones anaranjado encendido, con pelos canescentes, ocasionalmente con pelos amarillos. Hojas compuestas, 3-folioladas; estipulas basipeciolares, largo-lanceoladas, tomentosas por la haz, casi glabras por el envés; pecíolos 5-12,5 cm largo; folíolos discóloros, cordados, anchamente ovados, corto-acuminados, 9,5-11 cm largo, 6,8-8 cm ancho, superficie labrada, haz ferrugíneo, envés pardo, vilosos por la haz, densamente tomentosos por el envés, ocasionalmente con pelos amarillos. Inflorescencias: pedúnculos fuertemente armados, densamente tomentosos, brácteas ovado-lanceoladas, densamente tomentosas; cáliz densamente tomentoso, lóbulos ovados, mucronados, pétalos blancos; filamentos blancos, 4 mm largo; pistilos hasta 4 mm largo, ovarios glabros. Frutos hasta 1,3 cm (Fig. 3a).

Distribución y ecología

Hábito arbustivo. Forma matorrales en áreas perturbadas del bosque a 2700 m de altitud en el Alto de Guayacundo. De esta especie, escasa y rara, se registra hasta ahora solamente un clon, y la destrucción del bosque para el cultivo de la papa amenaza su extinción regional (Fig. 5I).

Area minifundista del piso subandino: Ubicada por debajo de 2100 hasta 1500 m snm, comprende 1 338 ha (12,1%). Se caracteriza por el dominio del minifundio con predios menores de 3 ha, de los cuales, según el DRI (1991) el 72,3% son predios menores de 1 ha. Se presentan suelos predominantemente arcillosos, clima con tendencia a seco, vientos fuertes e incendios frecuentes en los pastizales xerófilos de *Melinis minutiflora* sobre las laderas, la vegetación silvestre se presenta en forma de matorrales relegados en las cañadas, barrancos, cercas de piedra y de alambre y sobre afloramientos rocosos (matorrales xerófilos con *Dodonaea viscosa, Clusia* spp., *Myrcianthes* spp. y *Trachypogom ligularis*) se conservan algunos corredores de vegetación muy angostos siguiendo el curso de quebradas y /o entre linderos de las fincas. El 84,6% del paisaje del piso subandino ha sido transformado con un inteso uso de la tierra, y es en este ambiente donde se encuentran las siguientes especies.

***Rubus idaeus* L.**

Arbustos hasta 1,5 m de alto; tallos teretes; ejes glaucos y glabros; con aguijones amarillos grandes. Hojas compuestas, imparipinnadas, hasta 7-folioladas; estípulas

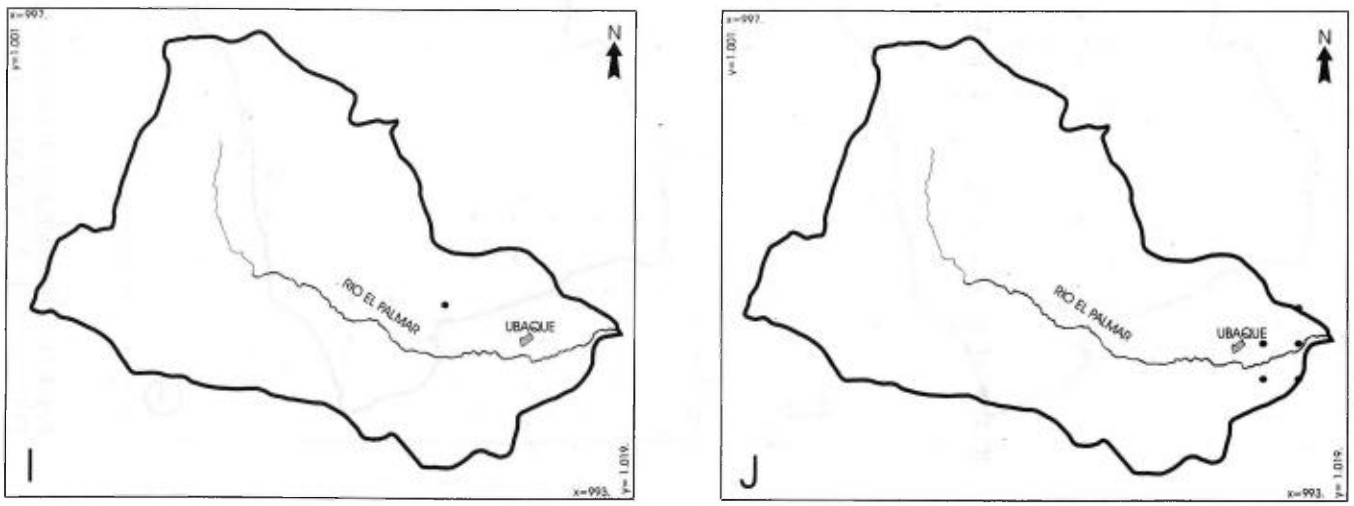

Fig. 5. Mapa de distribución de moras silvestres (*Rubus* spp.) en la Cuenca del Río El Palmar, Municipio de Ubaque: I) *Rubus porphyromallus*, J) *R. urticaefolius*.

basipeciolares, largas, subulado-lanceoladas, tomentosas; pecíolos 2,1-5 cm largo; folíolos discóloros, ovado-lanceolados, corto-acuminados, 2,1-6,2 cm largo, 1,3-5 cm ancho, márgenes serradas, glabros por la haz, densamente blanco-tomentosos por el envés, sin aguijones. Inflorescencias axilares, pequeñas. Pedúnculos tomentosos, armados con aguijones pequeños, brácteas estrechamente ovado-lanceoladas, tomentosas; cáliz tomentoso, lóbulos ovado-triangulares, abruptamente acuminados, mucronados; pétalos rosados; filamentos rosados, 0,3-0,4 cm largo; pistilos hasta 4 mm largo, ovarios densamente tomentosos. Frutos 1,3-1,7 cm (Fig. 1f).

Distribución y ecología
Planta de hábito arbustivo, originaria del este de América del Norte (Whitney 1984), es de introducción y dispersión reciente en la cuenca baja del río El Palmar , donde crece asilvestrada en la orilla de la carretera, a 1500 m de altitud. También se ha encontrado en la vereda Pueblo Nuevo, localizada sobre los 2700 m snm. En este ambiente no presenta buen desarrollo (Fig. 4F).

***Rubus urticaefolius* Poir.**
Arbustos hasta 2 m de alto; tallos 5-7-angulosos; ejes con pelos glandulosos largos rojizos y pelos canescentes cortos o largos abundantes (superficie vilosa); aguijones grandes anaranjados con pelos canescentes, en ocasiones con pelos glandulosos rojos. Hojas compuestas, 3-5-folioladas; estípulas basipeciolares, largo-lanceoladas, 13 mm largo, 1,5 mm ancho, angusti-lanceoladas hasta estrechamente ovado-lanceoladas, discóloras o no, vilosas, con o sin pelos glandulares rojos por el envés; pecíolos 3-12 cm largo; folíolos discóloros, ovados hasta ovado-lanceolados, corto hasta largo-acuminados, 2,3-11,9 cm largo, 2,0-7,2 cm ancho, vilosos por la haz, densamente tomentosos por el envés. Inflorescencias: Pedúnculos tomentosos, con pelos glandulosos rojos, brácteas tomentosas, con pelos canescentes y con pelos glandulosos rojos por el envés; cáliz tomentoso por el envés, con pelos canescentes y con pelos glandulares rojos, lóbulos ovado-lanceolados, abruptamente acuminados, arista roja; pétalos blancos, teñidos de rosado; filamentos de los estambres 0,2-0,3 cm largo, blancos; pistilos 0,2-0,3 cm largo, blancos; frutos hasta 1 cm (Fig. 3b).

Distribución y ecología
Hábito arbustivo. Propia de la cuenca baja - por debajo de 2000 m de altitud, en las veredas Rionegro, San Agustín y Ganco - logra sobrevivir en ambientes que son el producto de la delimitación de los predios: cercas de piedra y de alambre, en donde llega a formar grandes matorrales y en ocasiones crece en potreros o barrancos asociada con otras plantas. Esta especie debido a la severa fragmentación del hábitat encuentra la posibilidad de crecer únicamente en estas áreas marginales, que de ser sustituidas pueden incidir severamente en la distribución y tamaño de sus poblaciones (Fig. 5J).

Uso popular de las especies de moras silvestres

Uso medicinal
En la región las especies silvestres de *Rubus* se conocen localmente con el nombre de "moras", "mora de Castilla" (*R. glaucus*), "zarzamora" (*R. acanthophyllus*, entre otros), "morón" o "mora de oso" (*R. macrocarpus*) y "mora caballuna" o "mora de piedra" (*R. floribundus*).

Algunas especies silvestres (*R. bogotensis*, *R. glaucus*, *R. megalococcus*) son utilizadas ocasionalmente, para elaborar remedios caseros como jarabes de efecto expectorante, mezclando frutos inmaduros y ramas tiernas de mora con otras plantas medicinales como «totes» (*Rhynchospora ciliata*) y «tomillo» (*Thymus vulgaris*), los cuales son sometidos a cocción y posteriormente administrados a los enfermos de «bronquios y pulmones». Según García-Barriga (1974), las especies del género *Rubus* se utilizan en la medicina popular de la siguiente forma: «Las hojas que contienen tanino, los retoños jóvenes, yemas, y botones de estas plantas en decocción para colutorios y gargarismos. Los frutos en jarabe se emplean en la preparación de ciertas fórmulas. Los frutos triturados con panela y carbón vegetal se usan en el "sun" o "sapitos" (*Monilia albicans*) y en aftas. El extracto fluido de los frutos también se preconiza como antiescorbútico, emoliente y antidisentérico.»

Venta de moras silvestres en el mercado local
En el mercado local se vende principalmente *R. glaucus*, cultivo comercial que actualmente se fomenta, y ocasionalmente en épocas de cosecha también se vende la zarzamora silvestre (*Rubus megalococcus*), más preferida que la anterior por su aroma y "goma" que da mayor viscosidad a los refrescos preparados.

Uso alimenticio
En la región un reducido grupo de mujeres se dedica a la recolección de frutos silvestres. En la vereda de Pueblo Viejo (2190 m snm), un clon de gran tamaño (*Rubus bogotensis*) aproximadamente 50 m de longitud y 5 m de diámetro, es aprovechado en época de cosecha de frutos maduros para la venta, alimentación y aplicaciones medicinales. De otra parte, en la vereda de Cruz Verde (3060 m snm) se aprovechan los frutos de *Rubus macrocarpus*, apetecidos por su gran tamaño y sabor dulzón y un poco ácido. Es la especie con los frutos más grandes y de buen aroma (4 cm de largo, 2,5 cm de diámetro aproximadamente). Estas características tan apreciadas en cultivos actuales de la mora de Castilla (*R. glaucus*), han llamado la atención de los horticultores y fitomejoradores desde principios de siglo. Popenoe (1920), en los albores de la agronomía, destacaba el gran potencial de esta especie como recurso fitogenético.

Conclusiones
En la cuenca del río El Palmar del municipio de Ubaque, se encontró una gran riqueza de especies silvestres de *Rubus*,

que representan aproximadamente el 50% de las registradas en el país. En el gradiente altitudinal estudiado se observó una gran heterogeneidad espacial, variación en el tamaño de los clones y su abundancia. De ahí la siguiente clasificación de las especies en dos conjuntos:

1. Conjunto de especies de distribución restringida

	Abundancia
R. acanthophyllus	Abundante
R. compactus	Rara
R. macrocarpus	Poco abundante
R. porphyromallus	Rara
R. idaeus	Rara

Las tres primeras especies son del ambiente páramo (subpáramo), generalmente por encima de los 2900 m snm. Para el caso de *R. porphyromallus* se observó un único clon restringido a una pequeña reserva forestal del Cerro Guayacundo a 2800 m de altura, en el bosque andino. *R. idaeus*, especie exótica, presenta una distribución restringida debido a su reciente introducción en la región.

2. Conjunto de especies de distribución amplia:

	Abundancia
R. bogotensis	Abundante
R. floribundus	Abundante
R. glaucus	Poco abundante
R. megalococcus	Poco abundante
R. urticaefolius	Abundante

Las especies de *Rubus* en algunos sitios sujetos a disturbio o abandono después de ser cultivados, forman grandes masas monoclonales uniespecíficas o pequeños parches dispersos en el paisaje, pero generalmente asociadas a los linderos de fincas, o bosques relictuales. Hacen parte de corredores en una matriz de pastizal o mosaicos de áreas de cultivo. En la vereda Cruz Verde, en la transición del bosque andino y el páramo bajo, se concentra la mayor riqueza de especies. A pocos metros de distancia se presentan clones de aproximadamente 6 especies: *R. acanthophyllus*, *R. bogotensis*, *R. compactus*, *R. floribundus*, *R. macrocarpus*, y dos "nuevas especies" recientemente colectadas (aún sin determinar taxonómicamente), muy afines a *R. acanthophyllus*.

En la cuenca baja el 84,6% del paisaje ha sido totalmente transformado, zona minifundista sometida a intenso uso de la tierra. El hábitat disponible para el establecimiento y desarrollo de las plántulas de *R. urticaefolius*, como cercas de piedra entre las fincas y linderos, zonas de cultivo temporalmente abandonados, son muy escasos y hostiles. Las especies presentan un patrón de distribución muy disperso, formando clones pequeños, que se ven afectados por el desyerbe periódico de las parcelas, lo cual no permite el establecimiento de nuevos "ramets", restringiendo su crecimiento vegetativo. De acuerdo con las observaciones fenológicas (Rivera *et al.*, datos no publicados) algunos clones no llegan a la madurez de los frutos, de esta forma su capacidad de dispersión es limitada.

En cuanto al uso local de las especies, es evidente que representan un recurso ocasional en la alimentación, sin embargo, por su valor nutritivo debido al contenido de calcio, fósforo y vitaminas como se ha indicado en *R. bogotensis* (Góngora y López 1953; Ballington *et al.* 1993) es recomendable en la dieta. Desde el punto de vista medicinal algunas especies son utilizadas para la preparación casera de jarabes expectorantes. Económicamente la recolección y venta en el mercado local de estos recursos silvestres, representa una entrada adicional para la economía familiar. Algunos campesinos consideran las especies de *Rubus* plantas útiles en la recuperación y/o regeneración del hábitat natural y para la conservación de la fauna asociada. El conjunto de especies de este género constituyen un gran potencial genético para el fitomejoramiento, especialmente por las características de los frutos de *R. macrocarpus*, *R. acanthophyllus* y *R. bogotensis*.

Sería necesario coordinar con los recolectores de moras silvestres y la comunidad en general, un plan de manejo de especies silvestres de *Rubus*, para su conservación *in situ* en áreas definidas como reservas genéticas (Lleras 1991); podrían actuar como unidades dinámicas de conservación de la variabilidad genética, donde la mayor preocupación es garantizar que los tamaños y estructura de las poblaciones mantengan la diversidad genética en estado nativo. Por otra parte, consideramos importante realizar estudios farmacoquímicos y bromatológicos para confirmar su utilización medicinal y alimenticia con conocimiento de la composición y propiedades de las especies de *Rubus*.

Las poblaciones de *Rubus* spp. se presentan dispersas en el paisaje agrícola (actualmente ocupa el 60,6% de la superficie de la cuenca), formando parte del borde de matorrales y pequeños fragmentos de bosque andino o pajonales y matorrales de páramo; también en hábitats de origen antrópico, como cercas de piedra, matorrales en los linderos o cercas de alambre que sirven de percha para aves dispersoras y generadoras de clones fundadores en los linderos de pastizales. El flujo de dispersión y su patrón de distribución dependería de la configuración de los fragmentos de paisaje: distancia entre parches de hábitat (Opdam 1988, 1990). Por lo tanto la distribución de *Rubus* spp., está generalmente asociada a zonas de disturbio, sugiere un carácter de especie pionera, facilitadora de la sucesión en sus estadíos tempranos del establecimiento del clon, pero posteriormente parece ser altamente competitiva e inhibidora, convirtiéndose en maleza de difícil erradicación y que compite con pastizales y franjas de cultivo. Las especies de *Rubus* se comportan como especies tolerantes al disturbio, de carácter secundario y heliófilas. Futuros estudios podrían responder si las especies de *Rubus* se ven favorecidas por la fragmentación del hábitat y el disturbio generado por el hombre. Desde la perspectiva de interacciones bióticas, a nivel de comunidad, los clones de *Rubus* representan un nicho-refugio y sustentan comunidades de artrópodos, aves y posiblemente

pequeños roedores. Los trabajos de Whitney (1984) y Stiles (1987), describen la importancia de *Rubus* spp. en la competencia y coexistencia de polinizadores y otros organismos.

La cuenca alta del río El Palmar es la más rica en especies y aún conserva fragmentos representativos de bosque andino y páramo. Sin embargo, la presión de la deforestación (para obtener la leña y expansión de la frontera agrícola en el páramo) se ha incrementado. Por lo tanto se ha indicado a las autoridades municipales y líderes campesinos de gestionar el cambio de esta región como área de reserva municipal y de manejo comunitario para intentar salvar el 40% del hábitat natural en la región y su potencial recurso fitogenético.

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Summary

Botanical survey and germplasm collecting of wild Rubus *species in the Palmar basin, municipality of Ubaque (Cundinamarca, Colombia): I. Distribution and ecology.*

In the medium term the transformation of the natural ecosystems of the Colombian Andes entails a high risk of loss of valuable genetic resources present in wild plants. As part of a strategy which combines *in situ* and *ex situ* conservation, a regional botanical survey was undertaken in the river basin near Ubaque, Cundinamarca, and germplasm of wild blackberries and raspberries (*Rubus* spp.) collected. Information was gathered on local uses of the fruits. As the *ex situ* conservation strategy living material of each species was collected to form part of a germplasm collection for maintenance and propagation *in vitro*. Maps (1:25.000) and previous fieldwork provided sources for a survey of the basin over contours rising from 1500 to 3600 m asl. Ten *Rubus* spp. were recorded. There was a restricted distribution group at the level of the Páramo (2800-3600 m): *R. acanthophyllus, R. compactus, R. floribundus* and *R. macrocarpus*. A broad distribution group was found at the level of Andean and Subandean Woodland (1500-2800 m) including *R. bogotensis, R. glaucus, R. megalococcus* and *R. urticaefolius*, and the recently introduced species *R. idaeus*. The species *R. porphyromallus* had an extremely reduced population (2700 m). The species observed occur in open habitats, with anthropic origin and disturbance: banks, roadsides, stone walls or boundaries, temporarily abandoned farmland and secondary woodland and shrubs or scrub.

Résumé

*Conservation du matériel génétique de la ronce et du framboisier des bois (*Rubus *spp.) dans le bassin du fleuve El Palmar, commune d'Ubaque (Cundinamarca, Colombie) : Répartition et écologie*

La transformation des écosystèmes naturels des Andes colombiennes fait peser à moyen terme un risque de perte de précieuses ressources phytogénétiques issues de plantes sauvages. Dans le cadre d'une stratégie combinant la conservation *in situ* et *ex situ*, l'exploration botanique et la collecte de matériel génétique de ronces et de framboisiers sauvages (Rubus spp.) ont été conduites au niveau régional du bassin hydrographique. Par ailleurs des informations ont été recueillies sur leur utilisation locale. Sur la base d'informations cartographiques (1:25 000) et de travaux menés précédemment sur le terrain, le bassin a été exploré entre 1 500 et 3 600 m d'altitude. La stratégie de conservation ex situ adoptée a consisté à collecter du matériel végétatif de chaque espèce, en vue de créer, de conserver et de multiplier une collection de matériel génétique in vitro. Dix espèces ont été rencontrées : un groupe limité à l'étage de Páramo (2 800 à 3 600 m. d'altitude) : *Rubus acanthophyllus, R. compactus, R floribundus* et *R. macrocarpus* ; et un autre groupe largement réparti à l'étage de la forêt andine et subandine (1 500 à 3 000 m. d'altitude) : *R. bogotensis, R. glaucus, R. megalococcus, R. urticaefolius*. On a observé une population extrêmement réduite (2 700 m. d'altitude) de *R. porphyromallus*, et une espèce récemment introduite, *R. idaeus*. Les espèces observées se rencontrent dans des habitats ouverts d'origine anthropique : forêts et formations broussailleuses secondaires, chaumes, murets en pierre ou autres clôtures, bords de routes, talus et friches temporaires.

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Genetic resources of tree spices and their conservation in India[1]

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Summary
Cinnamon (*Cinnamomum verum* Presl.), nutmeg (*Myristica fragrans* Houtt.), clove (*Syzygium aromaticum* (L.) Merr. & Perry) and allspice (*Pimenta dioica* (L.) Merr.) are the most important tree spices that were introduced into India during the 18th century by the colonial rulers and subsequently became popular there. However, except for cinnamon, no further enrichment of the germplasm of these crops occurred. As a result, the exploitable genetic variability, especially of clove and nutmeg, is rather limited. The Indian Institute of Spices Research (IISR), Calicut, India has started a programme of collecting, conservation, cataloguing and evaluation of these crops. Consequently a sizeable number of germplasm accessions including an endangered nutmeg species, and a dwarf and bushy clove, have been acquired and are being maintained at the National Repository of Tree Spices germplasm in the IISR, Calicut. The variability analysis of the germplasm confirms the lack of adequate genetic variability in nutmeg and clove, which indicates the need for enriching nutmeg and clove germplasm.

Introduction
Spices originating from tree crops are routinely called 'tree spices'. There are 17 tree spices, most of which are grown in India, Sri Lanka, Indonesia, Malaysia, Grenada, China, Myanmar, Vietnam, Tanzania and Jamaica or occur naturally in humid tropical forests of South and South East Asia, Australia, Pacific Islands and tropical America. Among them, clove, nutmeg, cinnamon, cassia and allspice are economically most important. Various plant parts are used as spices: bark and leaves (cinnamon and cassia), flower bud (cloves), aril or mace and seed (nutmeg), leaves and fruits (allspice). The commercial products of these crops are whole spice, ground spice, spice oil, oleoresin, etc.

Cinnamon, clove, nutmeg and allspice, like rubber, were introduced into India by the British during the 18th century. Cinnamon is cultivated in the Indian states of Kerala, Karnataka and Tamil Nadu, and is more prevalent in the hilly regions of the Western Ghats. The oldest cinnamon plantation is the Anjarakandy Estate (250 acres approximately) in the Cannanore district of Kerala, which was planted by the British.

Clove is mainly grown in Kanyakumari and the Nilgiris districts of Tamil Nadu and in a few small areas in Kerala and Karnataka states. Nutmeg cultivation is more prevalent in Ernakulam, Trichur, Kottayam and Trivandrum districts of Kerala and also in a few areas in Tamil Nadu and Karnataka. Allspice is grown in a few gardens of Kanyakumari district. The available information on area and production of the important tree spices grown in India is given in Table 1.

Table 1. Area and production of tree spices in India (1992-93)

Crop	Area (ha)	Production (tonnes)
Clove	1945	1760
Nutmeg and mace	36531	3663
Cinnamon	365	355

Cinnamon
Cinnamon, *Cinnamomum verum* Presl. (Lauraceae), (*Cinnamomum zeylanicum* Blume), commonly known as Sri Lanka cinnamon, is an evergreen tree reaching a height of 6-15 m. It is the oldest known spice and is indigenous to Sri Lanka. Gamble (1925) reported 12 species of *Cinnamomum* from the Western Ghats and adjoining areas, while Kostermans (1983) listed 13 species from different parts of South India.

The species occurring in the Western Ghats and in India, their distribution and description are presented in Table 2. The diversity in these materials is mainly species diversity only. As most of these species occur only in the wild, semi-domesticated genepools of cinnamon do not occur. Owing to severe deforestation, there is every possibility of some species becoming extinct in the near future.

In addition to *C. verum* the other important economic species of this genus are *C. cassia* Presl. Syn. *C. aromaticum* Nees (Chinese cinnamon), *C. burmannii* C.G & Th. Nees (Indonesian cassia), *C. loureirii* Nees (Saigon cassia) and *C. camphora* (camphor).

Nutmeg
Nutmeg, *Myristica fragrans* Houtt. Syn. *M. officinalis* (Myristicaceae), is an evergreen, conical tree reaching a height of about 10 m. Nutmeg is indigenous to the Moluccas islands in Indonesia. Table 3 gives the list of wild and related species of *Myristica* occurring in India. Semi-domesticated genepools of *Myristica* spp. do not exist as most of these species occur in the wild.

[1] Contribution No. 219 of the Indian Institute of Spices Research.

Table 2. The cinnamon (*Cinnamomum*) species occurring in India, their distribution and description

Name	Common name	Place of distrubution	Features
C. filipedicellatum Kostem.	Wild cinnamon	South India, Western Ghats, Anamalais, Nilgiris	Small tree, leaves opposite, with young leaves glabrous or span, tomentous underneath, flowers, glabrous or nearly, pedicels filiform, 5-15 mm long
C. goaense Kostem.	Wild cinnamon	Northwestern Ghats	Leaves opposite with the basal lateral nerves reaching the leaf tip; lower leaf surface glabrous or with microscopical sparse, appressed hairs; fruits ellipsoid with the persistent utmost basal parts of the tepals on the rim of the deep fruit cup
**C. macrocarpum*	Wild cinnamon	South India (Nilgiris, Anamalais)	Tree, bark and leaves giving faint smell of clove and aniseed; breeies large, up to 1" in length, globose oblong, supported by a large thicknened cup and pedicel
**C. malabatrum* (Burm.f.) Bl.	Wild cinnamon	South India, Western Ghats	Leaves with minute hairs mainly on their lower surface, bark pale brown whitish inside; fruit cup shallow, crowned by the complete indurate persistent tepals
**C. nicolsonianum* Manilal & Shylaja	Wild cinnamon	South India	Medium tree, large leaves, very small panicles with very few flowers
**C. perrottetii* Meissn.	Wild cinnamon	South India, Western Ghats	A mountain species with ovate leaves, very densely hairy on both the surfaces, the young emerging ones highly tomentose, more than older ones; bark tasteless
**C. riparium* Gamble	Wild cinnamon	South India, Western Ghats	A graceful little tree, with long lanceolate narrow leaves and bright blue, shining, ovoid berry 0.5" long
**C. keralanese* Kostem.	Wild cinnamon	South India, Western Ghats	Tree with light brown to reddish, odourless and tasteless bark, fruiting panicles pesudoterminal and axillary long, lax, fruit ellipsoid, cupula subobconical, slightly fleshy and not ribbed
**C. tamala* Nees	Indian cassia	North East India	A moderate-sized tree, leaves 3-10", panicles scarcely exceeding leaves, perianth sparingly silky pubescent, lobes deciduous in fruit, fruit small ellipsoid
**C. travancoricum* Gamble	Wild cinnamon	Western Ghats	A small tree, the leaves with basal lateral nerves not reaching the leaf tip; leaves elliptic or subovate elliptic, obscurely acuminate with the undersurface of the young leaves sericeous
Cinnamomum verum Presl. (syn. *C. zeylanicum* Blume)	Cinnamon or Ceylon cinnamon	Sri Lanka	Small tree, bark aromatic, panicles medium-large and many flowers, fruits small, berry ovoid, dark purple
**C. wightii* Meissn	Wild cinnamon	Western Ghats	Bark slimy and without taste and odour; leaves opposite or spirally ar ranged, rigidly coriaceous, broadly elliptic; panicles very short, fruit cup obconical, more or less fleshy, not ribbed
**C. sulphuratum* Nees	Wild cinnamon	South India, Western ghats, Nilgiris	A mountain species with lanceolate elliptic to elliptic leaves, above glossy, beneath initially densely, later sparsely or sublanuginose tomentellous
**C. heynianum* Nees	–	Western Ghats	–
C. gracile Hook. f.	–	Western Ghats	–
C. chemungianum Mohan and	Herry.	Western Ghats	–
C. walaiwarense Kostern.	–	Western Ghats	–

* Endemic species.

Clove

Clove *Syzygium aromaticum* (L.) Merr. & Perry Syn: *Eugenia caryophyllus* (Sprengel) Bullock & Harrison (Myrtaceae) - is an evergreen tree, indigenous to the Moluccas, from where it has spread to many tropical countries. The important species of *Syzygium* occurring in the Western Ghat forests are *S. arnottiamum* (Wight) Walp, *S. cuminii* (L.) Skeels, *S. fruticosum* Roxb.) DC, *S. jambos* (L.) Alston and *S. zeylanicum* (L.) DC.

Allspice

Allspice, *Pimenta dioica* (L.) Merr. Syn. *Myrtus pimenta* L. *(Myrtaceae)* is indigenous to the West Indies and tropical America.

Materials and methods

To collect and conserve the available variability, systematic collecting surveys were made in the forests of Tamil Nadu, Kerala, Karnataka and North East India, for locating the wild races. Cultivated accessions have been collected from growers' fields. Exotic germplasm materials from Zanzibar (clove) and Sri Lanka (cinnamon) also were collected. The collected accessions are maintained at the National Respository of tree spices germplasm of IISR, Calicut and evaluated for yield and quality.

Cinnamon

The characterization of 71 accessions, which include the Sri Lankan introductions and collections from different

Table 3. Wild and related species of nutmeg (*Myristica*) occurring in India

Name	Places of distribution	Brief description
**Myrsitca amygdalina* Wall.	Nagercoil	Glabrous leaves, pale brown on both surface, nerves 8-12 pairs entire, yellow
M. andamanica Hook. f.	Andaman Islands	Glabrous, leaves membranous, nerves 12-15 pairs, bracteole large; apparently a distinct species; but approaches *M. malabarica*
**M. attenuata* Wall	Western Ghats	Branchlets and inflorescences furfuraceously tomentose; leaves pale brown above, nerves 14-20 pairs, fruit beaked, densely fufuraceously rusty tomentose, thin pericarp, aril entire, except towards the lobed apex
M. beddomeii King	Western Ghats	
M. gibbosa Hook. f. & Thoms	Khasia Mountains	Glabrous except the inflorescence and fruit, 15-30 pairs of nerves, aril entire, except at the top
M. glabra Blume	Silhet (Assam), Tinnevelly (Tamil Nadu)	Glabrous, nerves 7-14 pairs, male much branched panicle, fruit 2.5 cm, deeply grooved on one side towards the base, valves thick
M. glaucescens Hook. f. & Thoms	Tinnevelly, Andaman	Branchlets and inflorescence rusty-hairy, nerves 12-20 pairs, very small fruit, aril nearly complete
M. irya gartn. Fruct.	Tinnevelly, South Andaman Islands	Leaves membranous at length coriaceous dark brown above, lighter beneath, nerves 12-20 pairs, male panicles, many flowered, females shorter, perianth larger, fruit 2.5 cm in diameter, glabrous aril thin, entire yellow or reddish
M. kingii Hook. f.	Sikkim Himalaya (3300 m asl)	Branches very stout with rough bark and large scars of fallen leaves, nerves 15-20 pairs spreading panicles sparcely rusty-tomentose, rather stout, thick perianth, staminal column small.
M. longifolia Wall	Sikkim, Himalaya, Assam Silhet and the Khasia mountains	A lofty tree, leaves extremely variable usually linear, nerves 20-40 pairs, male flowers densely tomentose, scarlet within, style long, stigmas small toothed, ovary densely tomentose, fruit tomentose, aril pale, thin, lobed above the middle
**M. magnifica* Beddome.	W. Ghats, parts of Tinnevelly and S. Karnataka	An immense gregarious tree, 30 m high trunks, furnished with numerous aerial roots, starting from the trunk at 3-6m above ground and spread along the ground rising in loops above it; bark purplish black, leaves thick and large with prominent nerves, densely reddish tomentose, smooth above, nerves 20-26 pairs, flowers densely rusty tomentose, fruits densely tomentose, oblong, arillus orange-red and deeply much laciniate
**M. malabarica* Lamk.	W. Ghats (upto 300 m asl)	A tree reaching 15 m height, bark greenish black, leaves smooth, nearly glabrous, nerves about 10 pairs, fruit cylindrical, 5-7.5 cm long, aril yellow, cymes dichasioid up to 20 male flowers and 3 or 4 female flowers; female flowers larger than male

* Endemic species.

places in Kerala and Tamil Nadu (India), was made on the basis of various morphological, yield and quality data. The morphological characters studied included leaf length, leaf width and leaf size index. The yield characters were fresh and dry weight of bark as well as the percentage recovery of bark. Quality traits recorded were bark oleoresin, leaf oil and bark oil. Nine elite lines, identified on the basis of quality aspects, also were studied for yield performance as revealed by their progenies. The colour of young flushes of leaves was correlated to the quality parameters. The chemical nature of pigmentation of young leaf flushes also was studied, and some studies on the floral biology were made.

Nutmeg

Observations were recorded on 28 trees (14 years old) collected from nutmeg-growing belts of Kerala and planted at the Central Plantation Crops Research Institute, Research Centre, Kannara for a period of 3 years (1981-83). Variability and association were determined from the mean data of five characters: fruit number/tree, fruit weight (g), seed plus mace weight (g), seed weight (g) and mace weight (g). Correlation of fruit characters also was calculated. Studies were conducted to determine sex at seedling stage and sex segregation. In a progeny population of 16 elite mother trees from Kallar, Burliar, Balamore (Tamil Nadu), Kozha, Kalady, Moovattupuzha and Kottayam (Kerala), various morphological traits such as plant height, mean girth at 1.4 m; number of main shoots, canopy shape and number of years taken for flowering were recorded. Yield attributes such as mean yield of fruits/tree, fruit weight, seed weight, mace weight, ratio of mace to seed weight, etc. were recorded. Genetic parameters – phenotypic coefficient of variation, genotypic coefficient of variation, heritability (%) and genetic advance – were determined for significant attributes.

Clove

Special efforts were made to identify and collect diverse types from the Ashamboo hills, the southernmost hills of India where clove was first introduced. The distinctly diverse collection of clove includes a dwarf type and a king clove (exceptionally bold and thick buds) whereas a reddish-purple petioled zanzibar type from Zanzibar island and one from Sri Lanka are the only introductions at

present. Elite lines were identified on the basis of yield parameters as per the standard proforma and progeny evaluation undertaken. Observations on seedling characters of the progenies of elite lines also were made.

Results and discussions

The germplasm conservatory at IISR, Calicut consists more than 1000 accessions of tree spices as listed in Table 4.

Table 4. Tree spices accessions maintained at the National Repository of tree spices germplasm, Indian Institute of Spices Research, Calicut

Crop	Exotic	Number of accessions		Total
		Cultivated	Wild and related species	
Cinnamon	14	197	64	275
Nutmeg	0	418	34	452
Clove	2	214	1	217
Allspice	0	137	0	137
Total				1081

Cinnamon

Cinnamon is usually propagated by seeds and is cross-pollinated (Joseph 1981). Ponnuswami *et al.* (1982) reported good variability for growth characters in a 6-year-old seedling population of cinnamon. High coefficient of variation for dry and fresh bark yield per plant, bark oleoresin, leaf oil, bark oil, leaf size index and percentage recovery of bark was observed (Krishamoorthy *et al.* 1992). Association analysis for nine characters in cinnamon revealed significant correlation of fresh weight of bark and leaf oil with dry bark yield. Bark oil was negatively correlated with leaf oil.

Based on quality analysis, nine cinnamon lines have been identified as elite lines and based on comparative field evaluation of clonal progenies of these lines, two lines have been released as national varieties: 'Navashree' and 'Nithyashree'. 'Navashree' has high and stable regeneration capacity (6-7 shoots per year), high bark recovery (40.6%), high yield (average yield 56 kg/ha in the first four harvests) in addition to excellent quality attributes (bark oil 2.7% with a very good cinnamddehyde content – 73%, bark oleoresin 8%, leaf oil 2.8%. 'Nithyashree' also has good and stable regeneration capacity (4-5 shoots per year), high yield (average yield 54kg/ha in first four harvests) coupled with excellent quality attributes (bark oil 2.7%, bark oleroesin 10% and leaf oil 3%, with a very good eugenol content – 78%).

A study on seedling progenies of the above nine elite lines revealed a clear variation in the performance of the progenies (Krishnamoorthy *et al.* 1991).

The colour of young leaf flushes of cinnamon is related to its quality. Krishnamoorthy *et al.* (1988) reported that the ratio of plants having young leaf flushes with purple and purple pigmented ones to green ones was 1:1. Plants with purple leaf flushes recorded about 29% more bark oil, compared with green flushes, showing that flush colour may be taken as a criterion for selection of quality seedlings at the nursery stage. Anthocyanin glycosides present in the cinnamon flushes are cyanidin xyloside and cyanidin galactoside.

Nutmeg

In nutmeg, a tree bearing 1-4 seeds per fruit is one of the valuable accessions besides an endangered species: *M. fatua* var. *magnifica* (Fig. 1). Variability studies in nutmeg are limited (Flach and Cruickshank 1969; Shanmugavelu and Rao 1977; Sriram 1977). A preliminary analysis of genetic variability in 28 trees (14 years old) indicated some variability for fruit number per tree only and correlation analysis revealed a significant negative correlation of fruit number per tree and mace weight; however, seed weight had a positive significant correlation with mace weight.

Fig. 1. Endangered (?) nutmeg species, *Myristica fatua* var. *magnifica*.

In a more systematic study on genetic variability of nutmeg progenies from 16 mother trees of different localities (5 progenies for each mother tree), lack of adequate genetic variability was evident for many of the important attributes. This particular study revealed that even though morphological variation exists for leaf shape, canopy shape, etc. in nutmeg populations, exploitable genetic variation for crop improvement in nutmeg is lacking.

In another study on segregation of sex in 90 progenies, 40 were male, 45 female and 5 were bisexual types.

Nutmeg sex has been a matter of intensive research. Thin layer chromatography of leaf extracts of male and female lines of nutmeg indicated the presence of a steroid degradation compound specific for male lines; b-sitosterol also showed similar degradation. Structural elucidation of this compound was accomplished; it was tentatively identified as 1-dimethyl-2-ethyl-butyl-ketone with a molecular weight of 150 (Zachariah *et al.* 1986).

The main problem facing nutmeg cultivation is the segregation of the seedling progenies into males and females

in the ratio of 1:1. Several authors have attempted to determine the sex of the seedlings based on characters such as leaf form and venation (Prestoe 1984), colour of sprouts, seedling vigour and chromosome morphology (Flach 1966), calorimetric test (Phadnis and Choudhry 1971), shape and size of calcium oxalate crystals of leaves (Nayar *et al.* 1977). The study of seedling characters like sprout colour, days for germination, leaf shape, size, venation, etc. led to the conclusion that none of these characters can be taken as a guide to the sex of the nutmeg seedling (Krishnamoorthy *et al.* 1992). Out of a number of colour tests carried out on the leaf exudates of alcoholic leaf extracts of male and female trees, the ammonium molybdate test alone gave satisfactory differentiation between male and female nutmeg trees.

Clove

Three morphological variants in clove – king clove, dwarf and bushy clove (Fig. 2) and small-leaved cloves – were observed in the Ashamboo hills. A dwarf gene source in clove has important breeding value in developing dwarf genotypes with compact canopy size. Seed and seedling characters of progenies of 14 elite trees revealed no appreciable variation for 100-fruit weight, 100-seed weight, fruit breadth, fruit length, seed breadth, seed length, germination span, germination percentage, root and shoot length and number of leaves of seedlings at 40 days after sowing, 1-year-old and 2-year-old plant height among the progenies of elite trees at the seedling stage (Krishnamoorthy and Rema 1994).

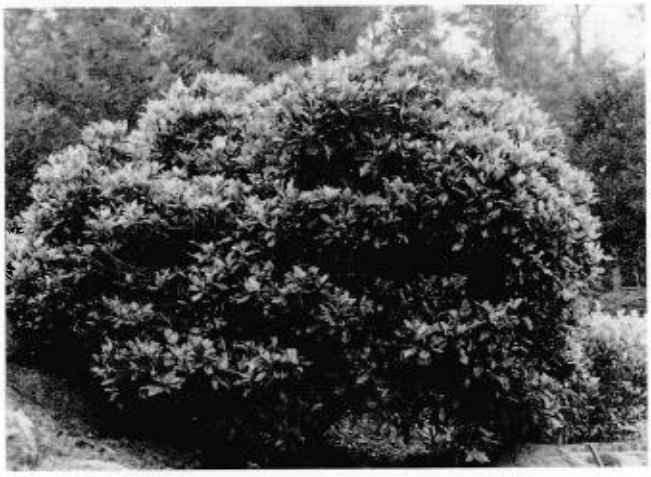

Fig. 2. Dwarf and bushy clove.

Conclusion

The analysis of variability patterns in three of the major tree spices – cinnamon, nutmeg and clove – reveals an interesting pattern. Unlike in cinnamon, the exploitable genetic variability in nutmeg and clove is rather limited. Cinnamon, although indigenous to Sri Lanka, occurs rarely in Western Ghats and this region can be considered as the secondary centre of origin of this crop. Further, the main mode of propagation of the species has been through seeds. The germplasm material of the present study includes collections from the earliest introduction (planted at Anjarakandy Estate Cannanore, Kerala) as well as from subsequent introductions from Sri Lanka done in 1976 and 1979 (105 Sri Lankan progenies of 13 accessions). On the contrary, the nutmeg and clove populations studied have evolved from a narrow gene pool of the original introduction in the 18th century. Thus the entire spectrum of genetic variability in these two crops is centred around these few source trees; there was no further introduction. Furthermore, in perennial tree crops such as clove and nutmeg, there was not much scope for natural selection to act after its introduction to India.

Clove and nutmeg cultivation is spreading to more and more areas in India. Planting materials are generated from the existing population. Even though seed propagation is practised in clove, nutmeg is mainly propagated through vegetative means at present. This will be suicidal for a crop on the threshold of genetic vulnerability.

Future strategy

Upgrading cinnamon quality through interspecific hybridization between *C. verum* and *C. aromaticum* is envisaged at IISR, Calicut. In nutmeg and clove, introduction of exotic germplasm is of utmost importance. At present the crop improvement programme in nutmeg and clove is limited to the selection of mother trees (elite trees), based on high yield and regular bearing habit, by periodical surveys and multiplication of their progenies. In clove, better crop idiotypes having a compact canopy shape which facilitates easy harvesting is one aspect that demands scientific attention. The dwarf gene source located will have a special significance in this context. This important gene source will be utilized in future clove breeding programmes. In allspice, polygamous dioecious is the major problem. The allspice was introduced recently to India from Jamaica probably as a few seedlings and hence the variability in this crop is very less. At present the crop is cultivated in Costa Rica, Gautemala, Mexico and Honduras apart from India. In India, it is cultivated in a few gardens of Kerala, Tamil Nadu and Maharashtra. On plains like Calicut the flowering and fruit set are poor in allspice. Future research efforts in this crop will be oriented towards overcoming this problem.

Note: Detailed tabulated data on the accessions are available from the authors.

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Résumé

Les ressources génétiques des arbres à épices et leur conservation en Inde

Le cannellier (*Cinnamomum verum* Presl.), le muscadier (*Myristica fragrans* Houtt.), le giroflier (*Syzygium aromaticum* (L.) Merr. & Perry) et le poivrier de la Jamaïque (Pimenta dioica (L.) Merr.) sont les arbres à épices les plus importants qui ont été introduits par le colonisateur en Inde au cours du 18ème siècle et s'y sont ensuite répandus. Or, à l'exception du cannelier, aucun enrichissement du matériel génétique de ces plantes cultivées n'a eu lieu. En conséquence, la variabilité génétique exploitable, notamment celle du giroflier et du muscadier, est assez limitée. Le Indian Institute of Spices Research (IISR) de Calicut (Inde), a lancé un programme de collecte, de conservation, de catalogage et d'évaluation de ces plantes. C'est pourquoi un nombre important d'accessions de matériel génétique, dont une espèce menacée de muscadier et une espèce naine et buissonnante de giroflier, ont été acquises et sont entreposées au Conservatoire national du matériel génétique des arbres à épices de l'IISR à Calicut. L'analyse de la variabilité du matériel génétique confirme l'insuffisante variabilité du muscadier et du giroflier, faisant ressortir la nécessité d'enrichir le matériel génétique de ces deux arbres.

Resumen

Recursos genéticos de especias arbóreas y su conservación en la India

La vainilla (*Cinnamomum verum* Presl.), la nuez moscada (*Myristica fragrans* Houtt.), el clavo (*Syzygium aromaticum (L..)* Merr y Perry) y la pimienta de Jamaica (*Pimenta dioica (L.)* Merr.) son las especias arbóreas más importantes que introdujeron en la India los dominadores coloniales en el siglo XVIII y que luego adquirieron gran popularidad en ese país. No obstante, excepto en el caso de la vainilla, no se llevó a cabo posteriormente la mejora del germoplasma de esos cultivos. Debido a ello, la variabilidad genética productiva, especialmente en el caso del clavo y la nuez moscada, es muy limitada. El Indian Institute of Spices Research (IISR) con sede en Calcuta, la India, ha puesto en marcha un programa de recolección, conservación, catalogación y evaluación de esos cultivos. Así, se ha adquirido un número cuantioso de muestras de germoplasma, incluidas una especie de nuez moscada en peligro y un clavo enano y arbustivo, muestras que se mantienen en el depósito nacional de germoplasma de especias arbóreas en el IISR de Calcuta. El análisis de la variabilidad del germoplasma confirma la falta de una variabilidad genética adecuada en la nuez moscada y el clavo, lo que indica que es necesario mejorar el germoplasma de estas dos especies.

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Genetic variation of the acetolactate synthase gene among cultivated *Brassica* species and its use in discriminating accessions of *B. napus* L.

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Summary

The acetolactate synthase (ALS) gene endows herbicide resistance on many agronomically important species. The ALS gene was demonstrated to be a multigene family among *Brassica* species. The objectives of this study were (1) to study variation of the ALS gene among cultivated *B. rapa*, *B. oleracea* and *B. napus*; (2) to determine how the variation is distributed at the species, subspecies and accessions (population) levels, and (3) to evaluate the feasibility of using a genetic marker from the ALS gene family to discriminate accessions *of B. napus*. The polymerase chain reaction (PCR) showed that extensive variation for the ALS gene family exists among *Brassica* subspecies and accessions. The degree of variation differed among species and subspecies. At the subspecies level, variation within *B. oleracea* was lower than that within *B. napus*, and higher than that within *B. rapa*. Significant variation was found among accessions of *B. napus*, indicating that the ALS gene family may be used to discriminate among individual accessions.

Introduction

Acetolactate synthase (EC 4.1.3.18, abbrev. ALS) catalyzes the first enzymatic reaction leading to the biosynthesis of the branched-chain amino acids valine, leucine and isoleucine. ALS genes and proteins were first characterized in *Escherichia coli* and *Salmonella typhimurium* (Lawther *et al.* 1979, 1981; Newman *et al.* 1982; Squires *et al.* 1981, 1983; Wek *et al.* 1985). ALS is a tetramer composed of two large and two small subunits in *E. coli*. The large subunits have a molecular weight of about 60 kd, while the small subunits have a molecular weight of 10 to 20 kd (Grimminger and Umbarger 1979; Eoyang and Silverman 1984; Schloss *et al.* 1985). In the yeast *Saccharomyces cerevisiae*, only one functional ALS gene exists (Falco and Dumas 1985). A small subunit has not yet been identified for ALS in yeast. Biochemical and genetic studies have shown that ALS is the target site of several classes of structurally unrelated herbicides, which include the imidazolinones, sulfonylureas and triazolopyrimidines (Bedbrook *et al.* 1987; Singh *et al.* 1990). ALS genes have been identified in several plants since 1987. Several plant mutants of *Arabidopsis thaliana* (Haughn and Somerville 1986) and *Nicotiana tabacum* (Lee *et al.* 1988) have shown resistance to sulfonylurea and chlorsulfuron. To transfer the resistance to other plants, it is essential to understand not only the ALS gene structure, organization and function but also the variation in agronomically important species.

The genomic clones encoding ALS were first isolated from *A. thaliana* and *N. tabacum* using a yeast ALS gene as a heterogeneous hybridization probe (Mazur *et al.* 1987). Clones were positively identified by the similarity of their deduced amino acid sequences with those of yeast and bacterial ALS isozymes. The tobacco and *A. thaliana* ALS genes have approximately 75% similarity, and encode mature proteins which are approximately 85% homologous. Little similarity is seen between the amino acid sequences of the presumptive N-terminal chloroplast transit peptides. Both genes lack introns. One copy of ALS gene in *A. thaliana* and two copies in *N. tabacum* were found (Mazur *et al.* 1987). The two genes in the tobacco plant are derived separately from its ancestral progenitor species, *N. sylvetris* and *N. tomentosiformis* (Lee *et al.* 1988). Preliminary southern blot analysis of *B. napus* using heterozygous probes derived from *A. thaliana* revealed that more than one gene would exist in *B. napus* (Rutledge and Miki 1988). Wiersma *et al.* (1989) also confirmed that *B. napus* probably contained a more complex genomic complement of four or more genes on the basis of southern blot analysis using an isolated *Brassica* ALS genomic sequences as a probe.

Five *B. napus* ALS genes (ALS1-5) were found through southern blot analysis using controlled hybridization stringences (Rutledge *et al.* 1991). They provided evidence that *Brassica* species contain a ALS multigene family that is more diverse than previously described for *N. tabacum* and *A. thaliana* and established the genomic origins of all five *B. napus* ALS genes. DNA sequence analysis indicates that ALS1 and ALS3 share extensive homology. They probably encode the ALS enzymes essential for plant growth and development. ALS2 has diverged significantly from ALS1 and ALS3 and has unique features in the coding region of the mature polypeptide, transit peptide and upstream noncoding region, which raises the possibility that it has a distinct function. ALS4 and ALS5 have interrupted coding regions and may be defective. Analysis of the presumptive progenitor diploid species *B. rapa* and *B. oleracea* indicated that ALS2, ALS3 and ALS4 originated from the *B. rapa*, whereas ALS1 and ALS5 originated from the *B. oleracea*. Rutledge *et al.* (1991) suggest that the extent of genetic complexity within each ALS family is much greater than anticipated.

The objectives of this study were (1) to study variation of the ALS gene family among cultivated *B. rapa, B. oleracea* and *B. napus;* (2) to determine how variation for ALS gene family is distributed at the species, subspecies and accessions (population) levels, and (3) to evaluate the feasibility of utilizing genetic markers from ALS gene family to discriminate accessions of *B. napus.*

Materials and methods

Plant material

Plant materials investigated in the experiment covered 33 accessions among 21 subspecies from three cultivated *Brassica* species (Table 1). Seeds were sown in sterilized soil in a greenhouse. Leaves were collected at the 4-5 leaf stage, placed immediately into liquid nitrogen and then transferred to –70°C storage before they were lyophilized. No disease symptoms were observed during the growing stage. Insecticide was sprayed on the plants once every week to control pests.

Table 1. Plant materials used in the experiment

Species	Subspecies	Cultivar	Origin
B. napus	*oleifera*	Cascade	USA
		Westar	Canada
		Tobin	USA
		Span	Canada
		Per	Canada
		Bronowski	Poland
		Glacier	Canada
	rapifera	Laurentian	Canada
B. oleracea	*oleracea*	wild cabbage	–
	alboglabra	Bhug-gana	Thailand
	botrytis	All the year round	England
		Cantanese	Italy
	capitata	Wisconsin Golden Acre	USA
		Golden Acre Yellow Re	USA
		Jersey Wakefield	USA
		Gotogon Local	Iran
		Blood Red Early	France
	costata	Couve Nabica	Portugal
	gemmifera	Long Island Improved Catskill	USA
	gongylodes	Early White Vienna	USA
	italica	Texas 107	USA
		Broccolette Neri E Cespuglio	Italy
	medullosa	Giganta	France
	ramosa	New Zealand Thousand Head (seed from 1990)	NZL
		New Zealand Thousand Head (seed from 1992)	NZL
	sabauda	Reed W	USA
	sabellica	Vates	USA
	selenesia	Westland Winter	Netherlands
B. rapa	*chinensis*	Si Yue Man	China
	dichotoma	TL-15	India
	pekinensis	Curled Yellow Leaf	China
	rapifera	Huang Man Jing	China
	trilocularis	T-151	India
	utilis	60 Day	China

Genomic DNA extraction

Leaves were freeze-dried before use. Tissue grinding followed the protocol of Colosi and Schaal (1993). Briefly, for each genomic DNA extract, lyophilized tissue (0.1-0.2 g) was added to a 1.5-ml microfuge tube. Three stainless steel ball bearings (4.0 mm diam.) were placed on top of the lyophilized tissue, the tube closed, and then placed in a liquid N_2 bath for approximately 30 sec. Following removal from the N_2 bath, tissue was ground by vortexing the tube twice (separated by an additional 30 sec N_2 cooling) for approximately 15 sec each. Ball bearings were removed with a large stir bar magnet. Genomic DNA extractions were then performed according to the protocols of Hillis *et al.* (1990).

PCR amplification

The primers used for ALS gene amplification were synthesized according to sequences of ALS genes 1 and 3 (Rutledge *et al.* 1991) by Operon Technologies, Inc., Alameda, CA, USA. The relative positions of primers are indicated in Figure 1. Each primer was 20 nucleotides in length (Table 2).

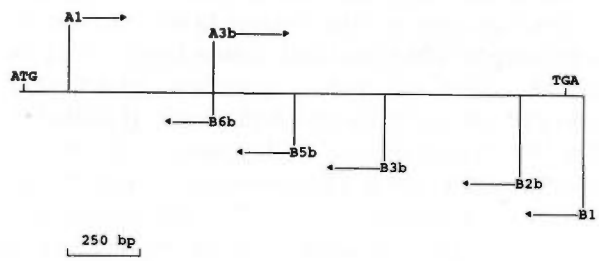

Fig. 1. Relative positions of primers in ALS gene.

Table 2. Primer sequence used in the experiment

Name	Sequence (5'-3')
A1	C C A T C T C C G C C G T T C T C A A C
A3b	G C T T T C T T T C T A G C T A C T T C
A8	C C G A T A C A T C A A A T T C C G C G
B1	A T C A C C A G C A T C A T C T C T C A
B2b	A A C C G A T C T T C C C A T T G C A T
B3b	A A C T G C G C C G C C C A C A T C T G
B5b	A A C T C A T C G T T A C A A G G A T A
B6b	G A A G T A G C T A G A A A G A A A G C

The general PCR protocol (Saiki *et al.* 1988) was followed. Numerous adjustments to standard PCR methods were examined to optimize the assay to achieve necessary reproducibility and reliability. Reaction mixtures (25 µl) contained 10 mM Tris-HCl (pH 8.3); 2.5 mM $MgCl_2$; 0.001% gelatin; 0.2 mM each of dATP, dCTP, dGTP and dTTP (Phamacia LKB Biotechnology, Inc., Piscataway, NJ, USA); 0.4 µM each of 20-mer ALS primer; approximately 25 ng of genomic DNA; and 0.625 units of DNA *Thermus aquaticus*

(*Taq*) polymerase (Perkin Elmer Cetus, Norwalk, CT, USA). Reaction mixtures were incubated in a DNA thermal cycler (Model 9600, Perkin Elmer Cetus, Norwalk, CT, USA) programmed for 45 cycles of 1 min at 94°C, 1 min at 50°C and 2 min at 72°C. Negative controls, the reaction mixtures without genomic DNA, were run with each amplification.

Gel electrophoresis

Amplified products were analyzed by gel electrophoresis in 1.5% agarose in 1xTBE. Molecular size of the products were estimated by utilizing a 1 kb DNA ladder (BRL, Bethesda, MD, USA). The gel was stained with ethidium bromide and photographed with black and white film #667 (Polaroid, Cambridge, MA, USA) under ultraviolet light. Two separate amplification runs were performed on each DNA extraction from each sample.

Data analysis

All the reproducible bands were scored. Similarity between any two samples was calculated according to the equation (Nei and Li 1979):

Index of similarity (I) = $(2*N_{ab}) / (N_a + N_b)$, where

N_{ab} = number of shared fragments between individuals 'a' and 'b',

N_a = number of scored fragments of individuals 'a', and

N_b = number of scored fragments of individuals 'b'.

Genetic distance (D) was calculated employing the equation: $D = -\ln I$.

The UPGMA method (Sneath and Sokal 1973) was used to set up the phenogram based on the calculated genetic distance data.

Table 3. Index of Nei-Li similarity among selected *Brassica* subspecies generated with ALS gene primer pairs (A1xB1, A1xB2b, A1xB3b, A1xB5b, A1xB6b, A3bxB1, A3bxB2b, A8xB6b)

	a†	b	c	d	e	f	g
a	–						
b	0.7021	–					
c	0.6600	0.5854	–				
d	0.3409	0.4286	0.2895	–			
e	0.3409	0.3714	0.3421	0.9062	–		
f	0.4583	0.3077	0.3571	0.5000	0.4722	–	
g	0.3261	0.3514	0.2500	0.5000	0.5000	0.7632	–

† a = *B. oleracea* var. *capitata* 'Golden Acre'; b = *B. oleracea* var. *ramosa* 'New Zealand Thousand Head'; c = *B. oleracea* var. *costata* 'Couve Nabica'; d = *B. rapa* var. *rapifera* 'Huang Man Jing'; e = *B. rapa* var. *chinensis* 'Si Yue Man'; f = *B. napus* var. *oleifera* 'Tanderm'; g = *B. napus* var. *rapifera* 'Laurentian'.

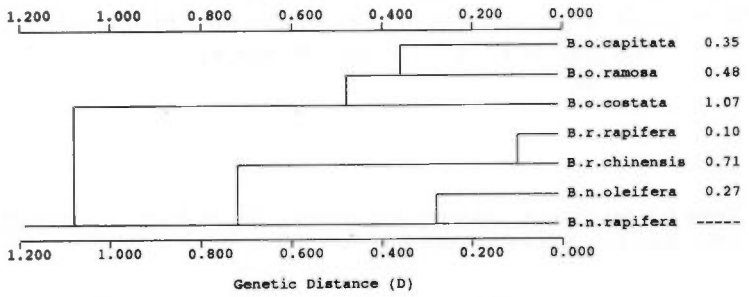

Fig. 2. Relationships between *Brassica* species as determined by UPGMA analysis using Nei-Li similarities computed from PCR amplification data of ALS gene.

Results

Between-species variation of the ALS gene

Three accessions each from different subspecies were used as representatives of *B. oleracea*, two accessions as that of *B. rapa* and another two accessions as that of *B. napus*. Successful and reproducible amplification of ALS gene across the total seven accessions was obtained. Nine primer pairs generated total fragments of 73, 62 and 43 within *B. oleracea*, *B. napus* and *B. rapa*. Of those, 43, 24 and 8 fragments were polymorphic within each species. Nei-Li similarities computed from amplification data were used to determine the relationships between and among *Brassica* species and subspecies by UPGMA analysis. The similarity within species was higher than that among species (Table 3). The subspecies within each of three species clustered together (Fig. 2). *Brassica oleracea* and *B. rapa* had a distant relationship each other, but both were closely related to *B. napus*. This was consistent with the origin of amphidiploid *B. napus* which was assumed to come from the combination between *B. oleracea* and *B. rapa* (U 1935). The phenogram tree indicated that the relationship between *B. rapa* and *B. napus* is closer than that between *B. oleracea* and *B. napus*.

Some banding patterns might indicate the origin of *B. napus*. For example, primer pair A1xB3b produced a 210 bp band in *B. oleracea*, and a 655 bp band in *B. rapa*, while both bands were typically polymorphic among *B. napus* accessions (Fig. 3). Uniform banding patterns were also observed across three *Brassica* species tested. For example, primer pair A3bxB3b generated only two bands with a length of 717 bp and 767 bp in all tested accessions.

Between-subspecies variation of the ALS gene

The genomic DNA of 21 subspecies from *B. oleracea*, *B. rapa* and *B. napus* were amplified with ALS gene primer pairs. The variation level of the ALS gene varied among subspecies of each species. Most primer pairs produce a uniform banding pattern among subspecies of *B. rapa*. The same occurred within *B. oleracea* except A1xB5b which generated polymorphic fragments among both subspecies and accessions of *B. oleracea* var. *capitata*. It was interesting that most primer pairs generated polymorphic banding patterns among the eight *B. napus* accessions investigated (Fig. 3).

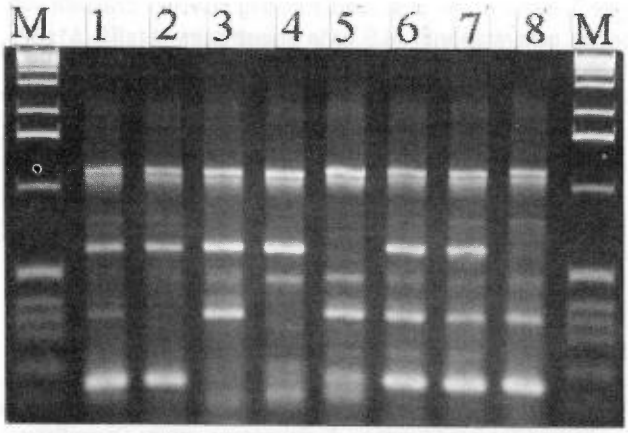

Fig. 3. PCR amplification of *B. napus* accession genomes.

Between-accession variation of the ALS gene

Seven accessions from *B. napus* var. *oleifera* and one from *B. napus* var. *rapifera* were used to investigate the variation of the ALS gene in *B. napus*. In total, 50 fragments were produced using seven primer pairs. Of those, 28 fragments were polymorphic. Nei-Li similarities (Table 4) computed from amplification data were used to determine the relationships between *B. napus* accessions by UPGMA analysis. The phenogram tree clustered seven accessions of *B. napus* var. *oleifera* as one group which joined with var. *rapifera* 'Laurentian' through var. *oleifera* 'Span' (Fig. 4). The results indicated that the ALS gene family may be used to discriminate among individual accessions of *B. napus*.

Conclusion

This investigation demonstrated that (1) extensive variation for the ALS gene family exists among *Brassica* subspecies and accessions; (2) the degree of genetic variation differed among different species and subspecies; (3) at the subspecies level, variation within *B. oleracea* was lower than that within *B. napus* and higher than that within *B. rapa*; (4) significant variation was found among accessions of *B. napus*, and (5) the ALS gene may be used as a genetic marker to discriminate accessions of *B. napus*.

Table 4. Index of Nei-Li similarity among accessions of *B. napus* generated with ALS gene primer pairs (A1 x B1, A1 x B2b, A1 x B3b, A1 xB5b, A1 x B6b, A3b x B1, A3b x B3b)

	Cascade	Westar	Tobin	Span	Per	Bronowski	Glacier	Laurentian
Cascade	–							
Westar	0.8889	–						
Tobin	0.8732	0.8169	–					
Span	0.8333	0.8333	0.8732	–				
Per	0.8571	0.8286	0.8696	0.8286	–			
Bronowski	0.9091	0.8571	0.8158	0.8312	0.8533	–		
Glacier	0.8800	0.8800	0.8108	0.8533	0.8767	0.9250	–	
Laurentian	0.8205	0.8462	0.8052	0.8205	0.7895	0.8193	0.8889	–

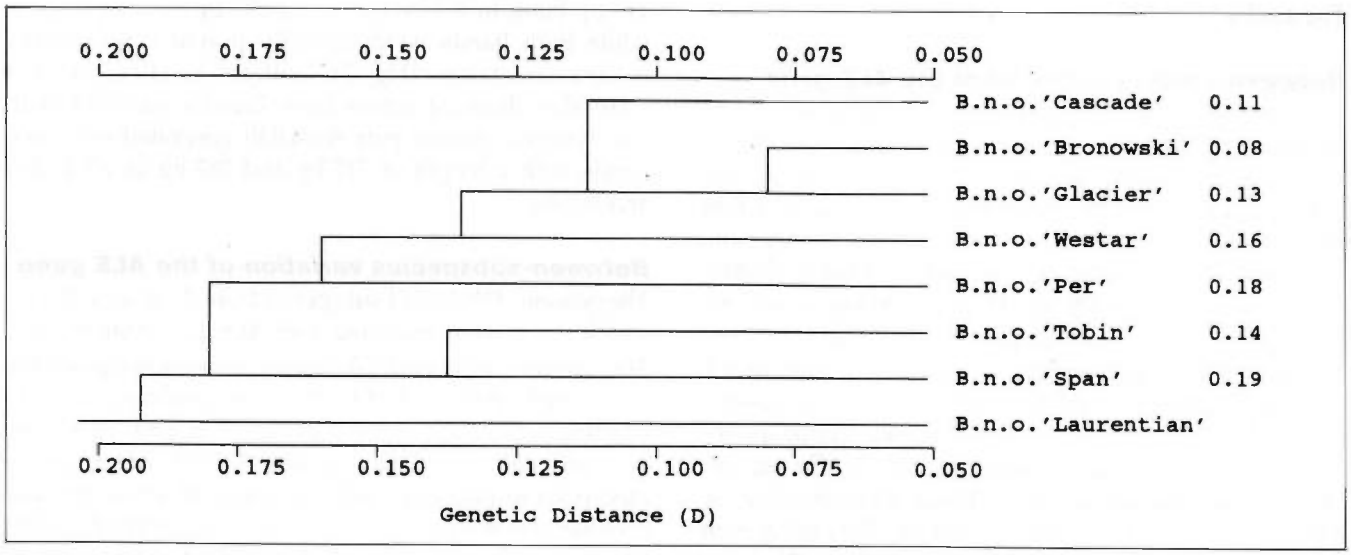

Fig. 4. Relationships between *B. napus* accessions as determined by UPGMA analysis using Nei-Li similarities computed from PCR amplification data of ALS gene.

Acknowledgements

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Résumé

Variation génétique du gène de l'acétolactate synthase parmi les espèces cultivées de brassicas et utilisation pour distinguer les accessions de B. napus L.

Le gène de l'acétolactate synthase (ALS) confère une résistance aux herbicides à de nombreuses espèces d'intérêt agronomique. Le gène ALS s'est révélé être une famille multigènes parmi les espèces de brassicas. L'objectif de la présente étude était 1) d'étudier la variation du gène ALS parmi les espèces cultivées de *B. rapa, B. oleracea* et *B. napus*; 2) de déterminer comment se répartissait la variation aux niveaux des espèces, des sous-espèces et des accessions (population); et 3) d'évaluer la possibilité d'utiliser un marqueur génétique de la famille du gène ALS pour distinguer les accessions de *B. napus*. La méthode PCR a permis de mettre en évidence l'existence d'une variation étendue dans la famille du gène ALS, parmi les sous-espèces et les accessions de brassicas. On a noté une différence dans le degré de variation entre les espèces et les sous-espèces. Au niveau des sous-espèces, la variation était plus faible pour *B. oleracea* que pour *B. napus* et plus forte que pour *B. rapa*. On a constaté une importante variation parmi les accessions de *B. napus*, indiquant que la famille du gène ALS peut être utilisée pour distinguer les différentes accessions.

Resumen

Variación genética del gen de la acetolactato sintetasa entre especies cultivadas de Brassica *y su utilización para distinguir muestras de* B. napus L.

El gen de la acetolactato sintetasa (ALS) confiere resistencia a los herbicidas a muchas especies importantes desde el punto de vista agronómico. Se demostró que el gen ALS era una familia poligénica en las especies de *Brassica*, Los objetivos de este estudio eran 1) estudiar la variación del gen ALS entre las especies *B. rapa, B. oleracea y B. napus* cultivadas; 2) determinar cómo se distribuye la variación a nivel de las especies, subespecies y muestras (población), y 3) evaluar la posibilidad de utilizar un marcador genético de la familia del gen ALS para diferenciar muestras de *B. napus*. La reacción en cadena de la polimerasa puso de manifiesto que entre las subespecies y muestras de *Brassica* existe una amplia variación en la familia del gen ALS. El grado de variación no era el mismo en todas las especies y subespecies. En el nivel de la subespecie, la variación era menor en *B. oleracea* que en *B. napus* y mayor que en *B. rapa*. Se descubrió una importante variación entre las muestras de *B. napus*, lo que indica que la familia del gen ALS puede utilizarse para diferenciar muestras individuales.

Plant Genetic Resources Newsletter, 1997, No. 111: 64-68 SHORT COMMUNICATION

Survey of seed germinability after long-term storage in the Gatersleben genebank

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Introduction

Since the foundation of the Institut für Pflanzengenetik und Kulturpflanzenforschung in 1943, the number of accessions in its genebank has grown continuously up to about 100 000 at present. About 70 000 samples are stored in Gatersleben whereas 30 000 are maintained for reproduction reasons in two outer branches of the genebank.

Because of the increasing size of the collection it was no longer possible to reproduce seed samples frequently. Therefore, cold-storage facilities were erected in 1976. Since that time germination tests have been conducted to control the germinability development of each accession. Viability monitoring, usually assessed by germination tests, should normally be conducted after 10 and 5 years, respectively, for seed samples with high and low initial germination percentages (FAO/IPGRI 1994). At present, more than 14 000 germination tests are conducted per year in the Gatersleben genebank. Because manpower is limited, there is a need to think about an efficient viability control system.

This paper presents an analysis of germination data accumulated in the Gatersleben genebank over 15 years, and stored in the genebank documentation system. On the basis of germination test results, specific intervals for viability monitoring of 12 selected botanical genera or species are suggested. It should be emphasized that this investigation has not been made from a statistical point of view but from a practical one. In this paper practical observations under the set conditions are presented. It is not focused on an individual accession but on a large amount of accessions within one species or genus.

Material and methods

The cold-storage facilities at Gatersleben

It should be pointed out that the Gatersleben genebank as an integrated system (Hammer 1996) includes seed storage and seed regeneration under one roof (Hammer 1993; Specht *et al.* 1995a).

The cold-storage facilities in Gatersleben consist of four chambers. One of them has a storage temperature of –15°C; three are running at 0°C. The present storage temperatures have historical reasons, because in former times better technical equipment was not available owing to economic restrictions. Thus, the design of the chambers allows for the maintenance of the storage temperature for 48 hours (provided that the doors are kept closed) in the case of technical difficulties such as power failure. Since 1976, the technical equipment has been in permanent operation.

The decision where to store seeds of a certain species, either at 0 or –15°C, was made according to the experience in seed longevity, accumulated up to 1976, when all seed samples had to be stored at room temperature. Therefore, seed samples of onion, lettuce, tomato, pepper, legumes, cucurbitaceous and medicinal plants, spices and other minor crops are stored at –15°C. Cereals, grasses and *Phaseolus vulgaris* are kept at 0°C.

All seed drying is done under natural conditions under the roofs of the genebank buildings. This procedure also has historical reasons, because controlled drying facilities were not available. Therefore, all seed samples are brought into the storage vessels during the winter, when the heating conditions provide sufficiently low relative humidity and low seed moisture content. In addition, two silica gel bags (about 100 g) in each storage container keep seed moisture content low. Blue silica gel indicates dry storage conditions and low seed moisture content. If the silica gel turns to pale purple or even white it has to be exchanged and redried, which takes place once or twice in the first year of storage. After that time there is an equilibrium inside the glass jars. The range of the seed moisture content in the glass jars after at least one year of storage in the cold storage chambers of the genebank is given in Table 1. The seed moisture content was determined according to the ISTA rules (Anonymous 1996) in 10 accessions each of the selected genera or species.

Until the end of 1996, there was no dehumidifying equipment in the cold-storage facilities. This situation required hermetically sealed storage containers, which was attained by glass jars, rubber rings and caps fixed with metal clips. The glass jars, which have been proved to be hermetically sealed, were never opened in the cold storage chambers but in a specific room with higher temperature

Table 1. Range of moisture content (MC) in the selected genera or species. In each genus or species the moisture content of 10 accessions was determined, which had been stored for more than 1 year in the cold-storage facilities of the Gatersleben genebank

Species	Seed MC (%) at equilibrium
Aegilops spp.	6 - 9
Allium cepa	7 - 9
Apium spp.	4 - 6
Avena sativa	6 - 9
Brassica napus	3 - 6
Hordeum vulgare	5 - 8
Lycopersicon spp.	3 - 4
Papaver spp.	4 - 5
Phaseolus vulgaris	7 - 8
Pisum sativum	7 - 10
Secale cereale	6 - 9
Triticum aestivum	6 - 10

and less air humidity. Thus, dry conditions could be kept within the glass jars for the duration of storage.

Seed-germination tests

Depending on the size of seed samples, usually 100 seeds are tested according to the ISTA rules (Anonymous 1996) or according to the recommendations given in the Handbook of Seed Technology for Genebanks (Ellis *et al.* 1985). All tests with cereals, peas or beans are made in filter paper rolls on a Jacobsen apparatus. Small-sized seeds are tested in incubators in Petri dishes, usually on top of filter paper.

Germination data

At present germination data of more than 57 500 seed samples are available in the computerized genebank documentation system (Freytag and Knüpffer 1995). Twelve genera or species representing about 30 500 samples have been selected for discussion here. The number of investigated accessions per genus or species and the mean of the initial viability are given in Table 2. For a better understanding of the values, the data for *Hordeum vulgare* are shown in Table 3 as an example.

All 5338 accessions of *H. vulgare* tested so far are divided into six classes according to their time of storage (stored up to x years). All accessions which have been stored for the same period of time are classified into five germination classes according to their germination results (x - y % germinability). The number of accessions per germination class in all figures is expressed as a percentage of the total amount of accessions within that class.

Table 2. Number of investigated accessions and mean of initial germinability before storage of the selected genera or species

Species	No. accessions investigated	Mean initial germinability (%) before storage
Aegilops spp.	1146	60
Allium cepa	331	45-95, depending on the year of harvest
Apium spp.	235	30-55, depending on the year of harvest
Avena sativa	3000	75
Brassica napus	445	99
Hordeum vulgare	5338	94
Lycopersicon spp.	1842	98
Papaver spp.	883	88
Phaseolus vulgaris	3795	98
Pisum sativum	2081	98
Secale cereale	1012	84
Triticum aestivum	10509	93

Results

The first four figures show the germinability of *H. vulgare, Triticum aestivum, Secale cereale* and *Avena sativa* after storage at 0°C. *Hordeum vulgare* (Fig. 1) and *T. aestivum* (Fig. 2) can be stored easily for more than 15 years. Germination of *S. cereale* (Fig. 3) is declining after 12 years of storage. In contrast to these results, many accessions of *A. sativa* (Fig. 4) have low germination results (51-70%) at the beginning of storage and germinability decline is increasing permanently. In *Aegilops* spp. (Fig. 5) stored at 0°C, there is a slight germinability decline. The figures of *Brassica napus* (Fig. 6), *Lycopersicon* spp. (Fig. 7) and *Phaseolus vulgaris* (Fig. 8), all stored at –15°C, and *Pisum sativum* (Fig. 9), stored at 0°C, provide their germinability after long-term storage. In *Papaver* spp. (Fig. 10) stored at –15°C, seed dormancy during the first 2 years after harvest is obvious, and there is a noticeable decline of germination percentages. For *Allium cepa* (Fig. 11) and *Apium* spp. (Fig. 12), both stored at –15°C, a clear statement cannot be given. In the latter genus, germination test results are low, independent from storage duration.

Discussion and conclusions

High germinability is maintained for a longer period in *H. vulgare, T. aestivum, B. napus, Lycopersicon* spp., *P. sativum* and *P. vulgaris* under the Gatersleben storage conditions. Therefore, the need for frequent germination tests is low in these genera. Germinability control can be extended to intervals of 10-15 years. Prolonged test intervals of up to 10 years can also be recommended for *Papaver* spp. and *Aegilops* spp.

Nevertheless, it is necessary to investigate why some accessions of these genera or species possess low germinability after a short duration of storage. One reason might be that they are sensitive with respect to the environment during reproduction as described in the Genebank standards (Anonymous 1994). Another possible explanation is that a general statement for a whole genus is too

Table 3. Data matrix of *Hordeum vulgare*

Germinability (%)	Time of storage (years)											
	≤ 2		3 - 5		6 - 8		9 - 11		12 - 14		≥ 15	
	No.†	%	No.	%	No.	%	No.	%	No.	%	No.	%
0 - 10	2	2	0	0	0	0	4	0	21	2	14	1
11 - 30	0	0	0	0	0	0	4	0	37	3	20	1
31 - 50	0	0	0	0	0	0	12	1	30	3	5	0
51 - 70	3	2	6	1	15	3	25	3	73	6	31	2
71 - 100	129	96	546	99	503	97	1079	96	966	86	1315	96

† No. = number of accessions.

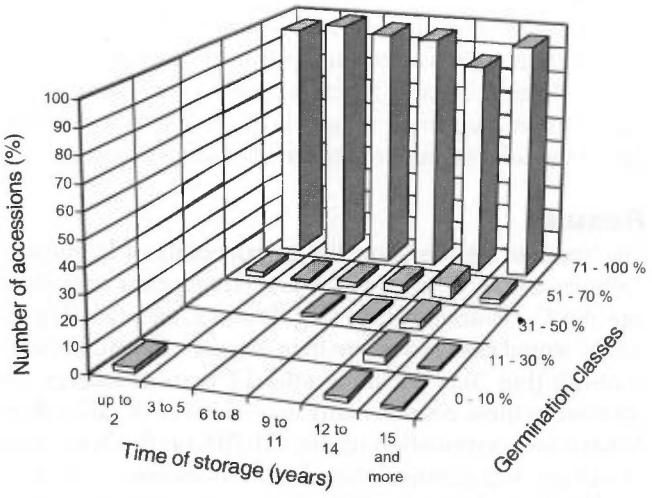

Fig. 1. Germinability of *Hordeum vulgare* after storage at 0°C.

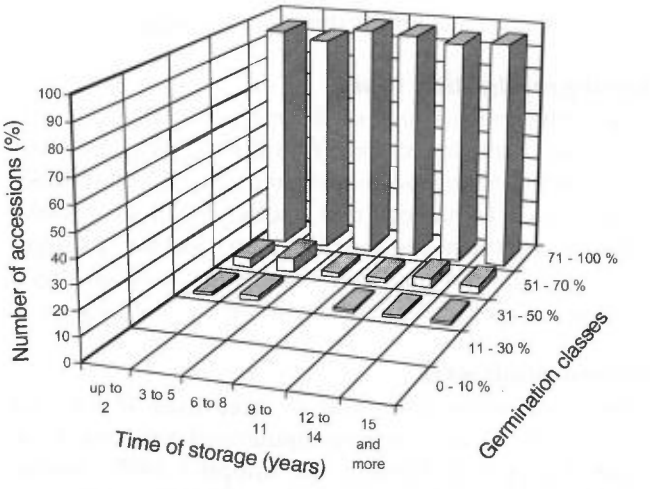

Fig. 2. Germinability of *Triticum aestivum* after storage at 0°C.

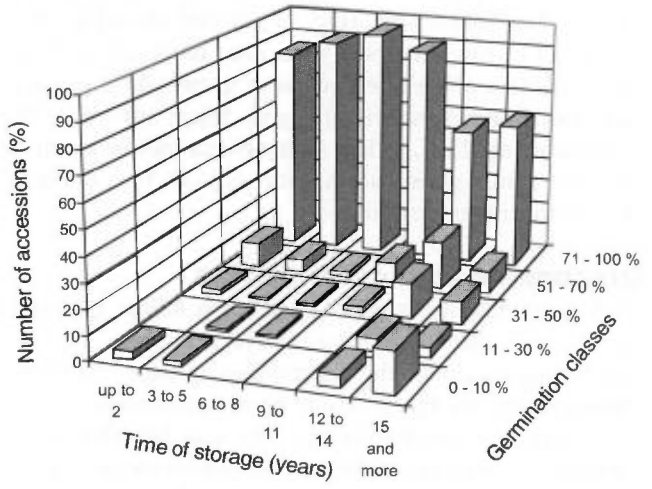

Fig. 3. Germinability of *Secale cereale* after storage at 0°C.

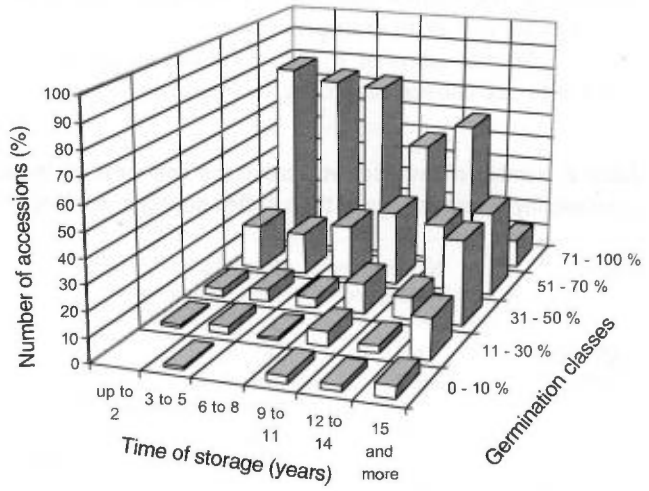

Fig. 4. Germinability of *Avena sativa* after storage at 0°C.

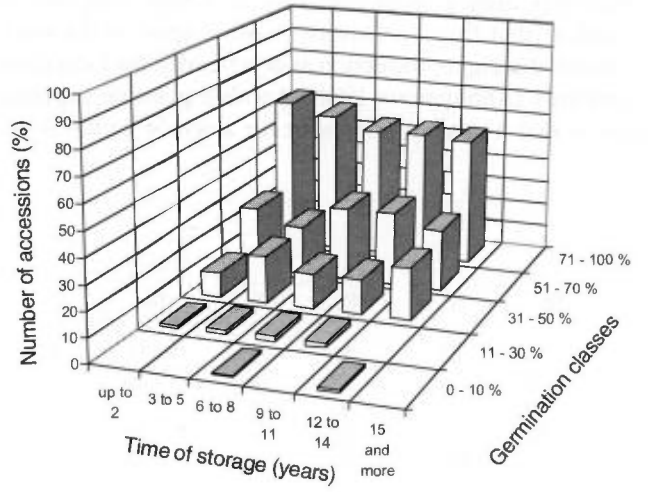

Fig. 5. Germinability of *Aegilops* spp. after storage at 0°C.

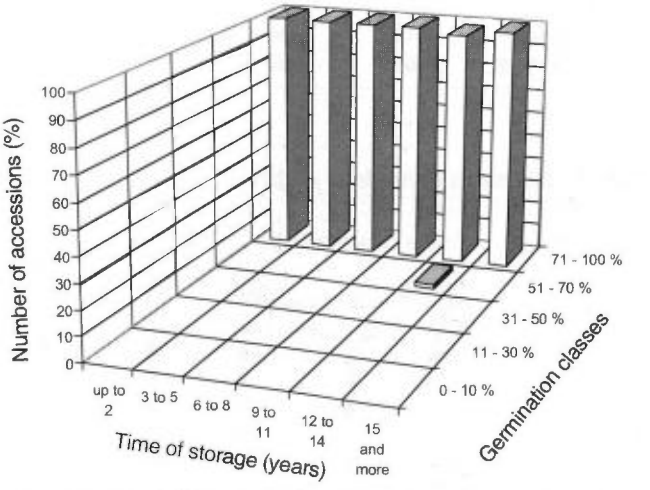

Fig. 6. Germinability of *Brassica napus* after storage at –15°C.

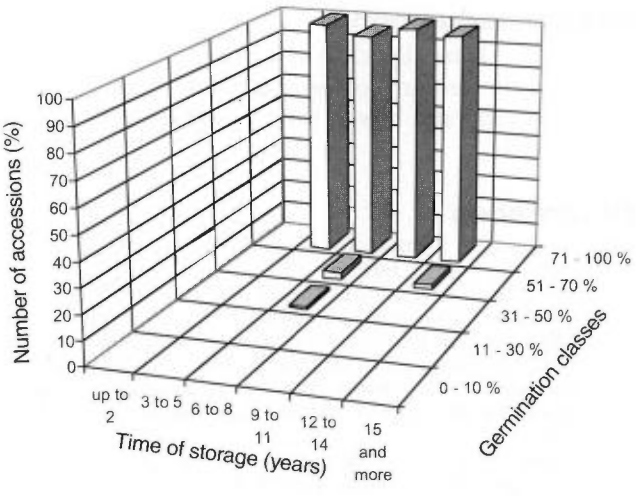

Fig. 7. Germinability of *Lycopersicon* spp. after storage at –15°C.

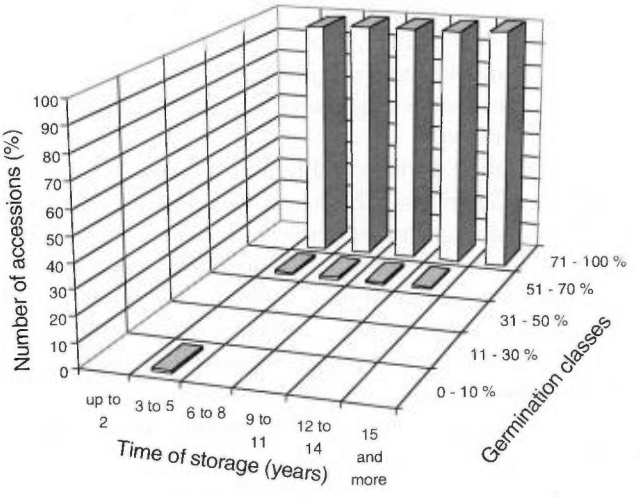

Fig. 8. Germinability of *Phaseolus vulgaris* after storage at –15°C.

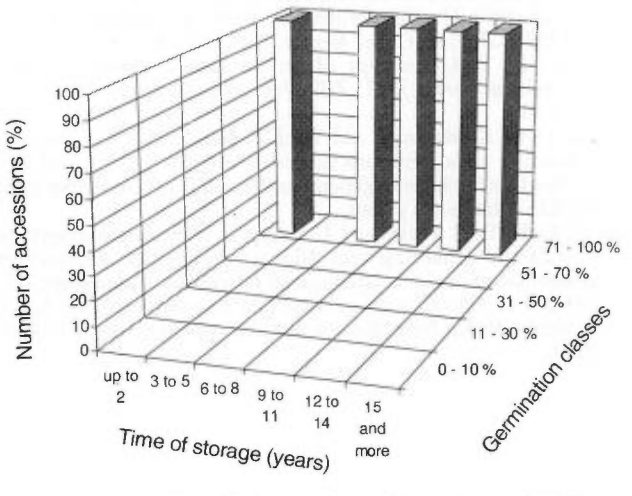

Fig. 9. Germinability of *Pisum sativum* after storage at 0°C.

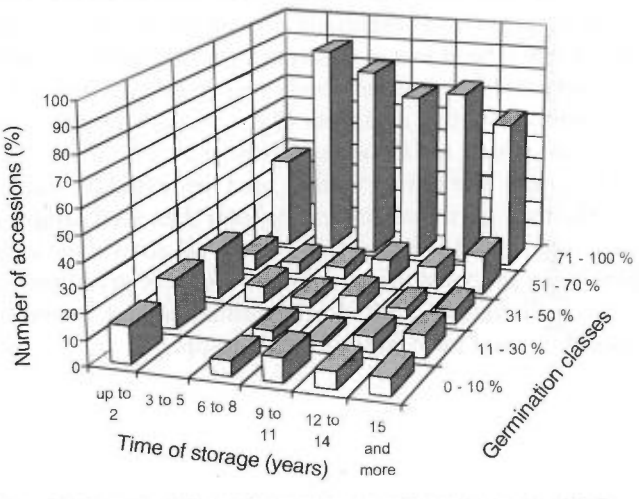

Fig. 10. Germinability of *Papaver* spp. after storage at –15°C.

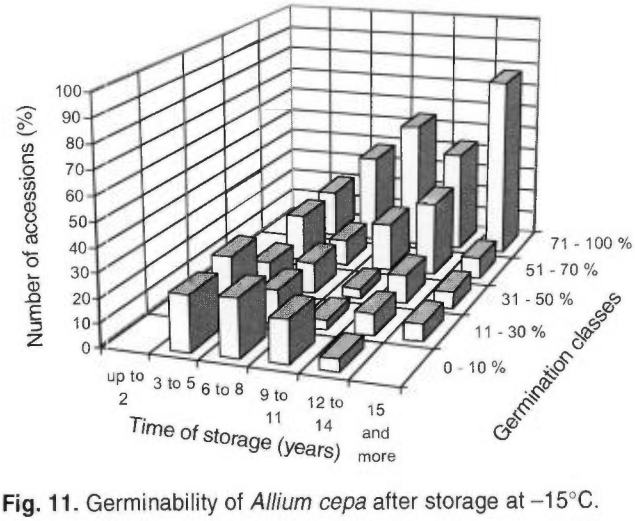

Fig. 11. Germinability of *Allium cepa* after storage at –15°C.

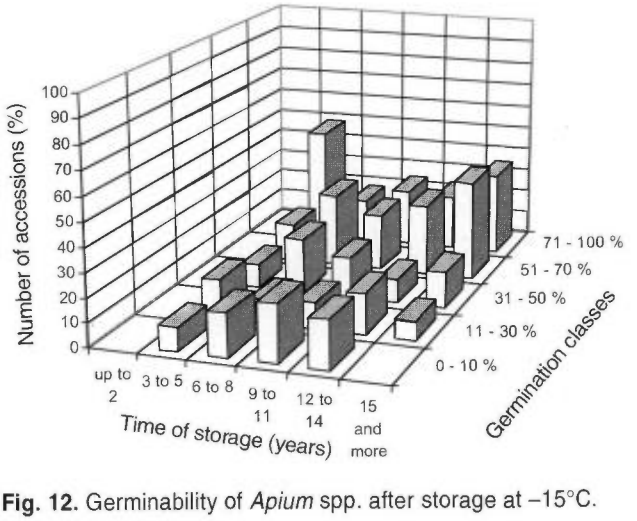

Fig. 12. Germinability of *Apium* spp. after storage at –15°C.

rough and each species has to be considered separately. Particularly in *H. vulgare,* accessions with low germinability were found to have originated from Ethiopia. Therefore, these accessions might need specific handling to prolong their longevity.

Uncontrolled drying conditions are probably a specific problem in Gatersleben. Both lower seed moisture content and lower storage temperature would probably lead to better storability of *S. cereale.* Under the Gatersleben storage conditions, germination test intervals of more than 10 years cannot be recommended for this species.

In the case of *A. sativa,* many accessions have low germination percentages right at the beginning of storage. Their number is increasing permanently. This could be typical for *A. sativa,* but lower seed moisture content and improved storage conditions at –15°C might influence the longevity positively as well and might allow extension of the period between germination tests.

For the two other genera mentioned, a general statement cannot be given. A negative correlation between low initial germinability and cold and wet weather during the growing period was especially proven for *A. cepa* (Specht *et al.* 1995b). Seed lots of this species regenerated in years with dry and hot weather conditions, in comparison with the mean of weather data from 40 years in Gatersleben, can easily be stored for 15 years (Specht *et al.* 1995b). Taking into account that during storage seed lots with low viability lose their germinability much faster than seed samples with high initial viability (Ellis 1982), all accessions with low initial germinability need more frequent germination control. Therefore, individual germination test intervals should be used for *A. cepa* and *Apium* spp.

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Vegetable germplasm collecting in Ghana

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Introduction

Landraces of indigenous vegetables in Ghana are being displaced by introduced improved varieties, a typical example being the tomato. To forestall the loss of the genetic resources of vegetables, a germplasm-collecting expedition was undertaken in the Greater Accra, Volta, Eastern, Ashanti, Brong Ahafo, Northern, Upper East and Upper West Regions of Ghana in October-November 1993.

The collecting expedition

The mission started on 22 October 1993 in the Greater Accra Region and it continued through the Volta, Eastern Ashanti, Brong Ahafo, Northern, Upper East and Upper West regions. A total of 3879 km was covered during the mission (Fig. 1). Collections were made in farmers' fields, gardens, the market and the farm store. Materials collected were landraces and comprised pods, seeds and fruits of the various vegetable species. Collected with each accessions was a set of passport data. These included a collection number starting with the prefix GJ which stands for Ghana-Japan. This is followed by 93, which stands for the year 1993 in which the collection was done, and the actual numbers of the collection which were assigned serially from 001 by the collectors. The scientific as well as the local names of the accessions were recorded. Other passport data collected included the date and the location of the collection and donor of the accession. A total of 324 accessions of vegetable crops was collected (Table 1).

Table 1. List of vegetable germplasm collected from six regions of Ghana

Species	Common name	No. of accessions
Abelnoschus esculentus	Okra	89
Amaranthus sp.		1
Arachis hypogaea	Groundnut	11
Cajanus cajan	Pigeon pea	1
Canavalia gladiata	Sword bean	1
Capsicum annuum	Pepper	28
C. frutescens	Pepper	10
Citrullus lanatus	Neri	3
Hibiscus cannabinus		1
Kerstingiella geocarpa	Kersting's groundnut	7
Lageneria siceraria	Gourd	2
Luffa sp.		1
Lycopersicon esculentum	Tomato	5
Mucuna pruriens var. *utilis*	Velvet bean	5
Phaseolus lunatus	Lima bean	5
Psophocarpus tetragonolobus	Wing bean	1
Sesamum indicum	Sesame	1
Solanum aethiopicum (Gilo group)	Egg plant	61
S. anguivi	Wild eggplant	7
S. erianthum	Wild eggplant	1
S. macrocarpon	Eggplant	4
S. melongena	Eggplant	13
Sphenostylis stenocarpa	African yam bean	4
Urena lobata		1
Vigna subterranea	Bambara groundnut	25
V. unguiculata	Cow pea	36
Total		324

Crop variability

Three genera of the family Solanaceae were collected in this expedition. These are *Solanum, Capsicum* and *Lycopersicon* spp.

A wide range of *Solanum* species was collected. These comprised *S. aethiopicum* Gilo group, *S. erlanthum, S. melongena, S. macrocarpon* and *S. anguivi*. These species exhibited a wide variability in fruit shape, size and colour. Shapes ranged from elliptical to almost globose. Some fruits were smooth, others had longitudinal grooves. The colours of the unripe fruit ranged from cream, green, purple to a combination of these colours. Fruit sizes of the collections varied between 7 mm long and 6 mm wide in *S. anguivi* and 13 cm long and 8 cm wide in *S. melongena*.

The most variable of the edible types of the genus occurred in the Brong Ahafo region. Most of the edible species were found in the markets of the major towns in the region. These species included *S. torvum* which is a wild type but with edible fruits and *S. aethiopicum* Gilo group, *S. melongena* and *S. anguivi* which are cultivated and have edible fruit.

In the Volta region, *S. macrocarpon* is mostly used as a leafy vegetable, whereas in the northern region both the fruit and the leaves of this species are eaten as vegetable.

Solanum erianthum is a wild and non-edible species. A total of 85 accessions of *Solanum* species were collected in the various regions.

Capsicum species

Two species of *Capsicum* were collected and these are *C. annuum* and *C. frutescens*. There was great variability in the fruits of the accessions collected, in length, shape, colour, aroma and taste. The shortest fruit was obtained in the wild bird chili (*C. frutescens*), the longest was the finger-like pepper *C. annuum*. Fruit shape ranged from spindle shape in the bird chilli to round and more or less cylindrical shapes in some *C. frutescens* fruits collected. There was also variability in the colour of ripe fruits collected. Most fruits were red when ripe but some were yellow, cream or purple. The fruits of some accessions had an aromatic smell while others had a pungent smell. All accessions collected had a hot, smarting taste. A total of 39 accessions was collected.

Lycopersicon species

Five accessions of *L. esculentum* were collected. It was the opinion of the collectors that most of the fruits observed in the markets and in the fields and gardens were exotic varieties

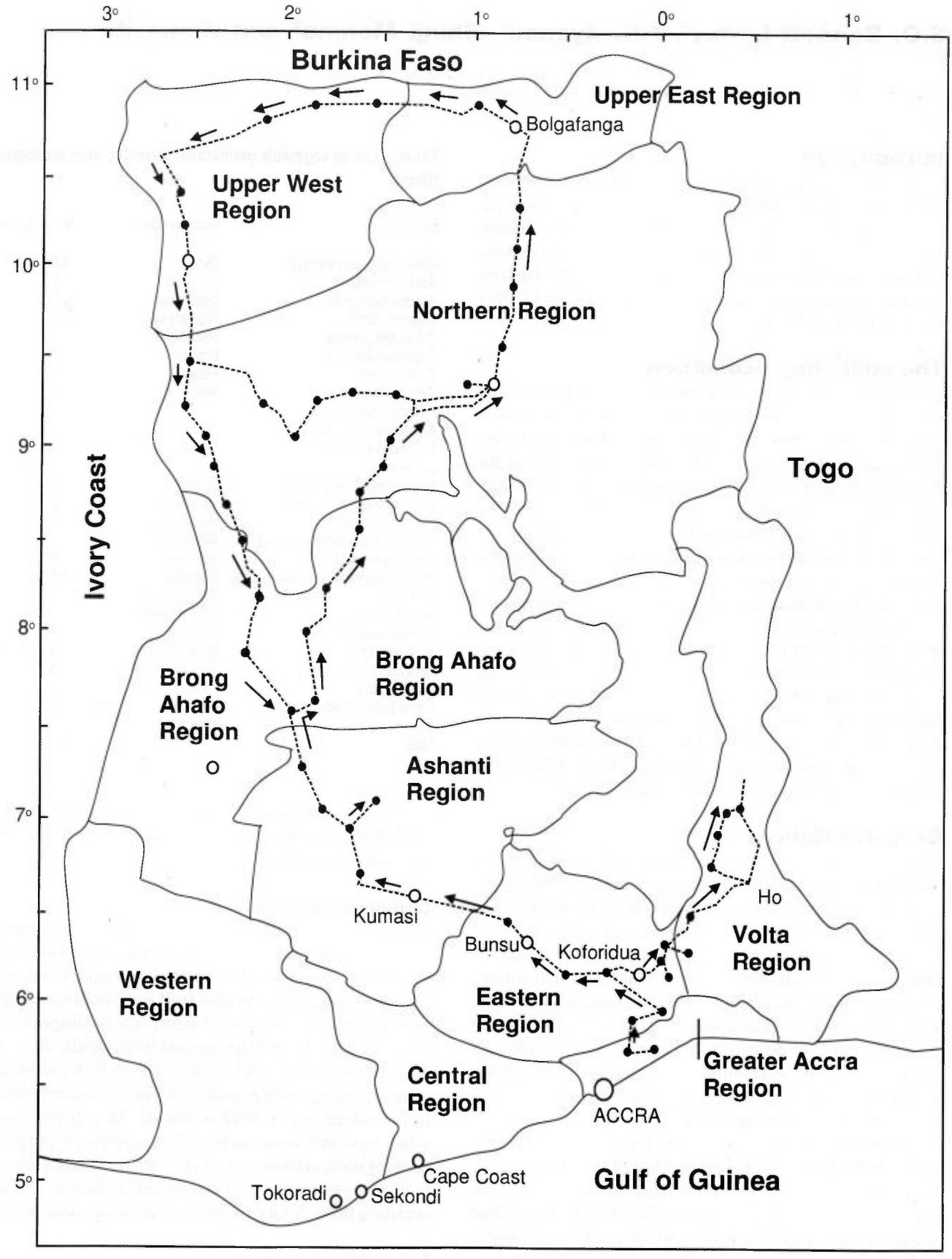

Fig. 1. Vegetable germplasm collecting routes in Ghana, 1993.

introduced from temperate countries for commercial production.

Accessions of the wild species *Lycopersicon pimpinellifolium* were collected. This species had very small but many fruits, which are used for food by several communities.

Malvaceae

The members of this family collected were *Abelmoschus*, *Hibiscus* and *Urena* spp. The most important species of this family collected is *Abelmoschus esculentus*.

Abelmoschus esculentus

Eighty-seven accessions of this species were collected. Throughout the regions visited, this crop was observed to be growing in the home gardens of almost every house. This was moreso in the northernmost regions of the Northern, Upper West and Upper East regions. This is because both the leaves and fruits of okra feature prominently in the diet of the inhabitants of these regions.

The variability observed in this crop is quite enormous, e.g. variability in the period of maturity of this crop. According to the information given by the farmers who donated the specimens, some are early maturing. Their growing cycle is as short as 2 months whereas late-maturing ones take about 6 months. The late-maturing varieties grow very tall, the early ones are relatively short.

Variability was observed in the size of the okra fruit. The length and width of fruits collected ranged between 6 x 5 and 23 x 2.5 cm. Some plants were pigmented (parts of the fruits were red) but most plants were not pigmented (they were all green). Some fruits had short piercing prickles whereas most accessions had none.

Most farmers conserved their okra seeds by hanging the pods above the fireplace where smoke protected them from insect damage. Most samples collected were in this form although a few were harvested from the field or home garden.

Leguminosae

Accessions belonging to this group included cowpea (*Vigna unguiculata*), bambara groundnut (*Vigna subterranea*), groundnut (*Arachis hypogaea*), African yam bean (*Sphenostylis stenocarpa*), lima bean (*Phaseolus lunatus*), winged bean (*Psophocarpus tetragonolobus*), velvet beans (*Mucuna pruriens* var. *utilis*), sword bean (*Canavalia gladiata*), pigeon pea (*Cajanus cajan*) and Kerstling's groundnut (*Kerstingiella geocarpa*).

Cowpea

Thirty-six accessions of this species were collected. Variability was observed in the seed colour of the accessions collected. Seeds ranged from white to grey, brown, red and black. Some were mottled with various colours including brown.

Bambara groundnut

This legume does not seem to be as popular as it used to be some years ago. It could not be obtained in most of the markets in southern Ghana during the period of the expedition. At the Koforidua market only one person had it whereas it was completely absent in the Central Market, Kumasi. One accession was collected in the Juapong market. Most accessions were collected in the northern regions of Northern, Upper West and Upper East regions. A total of 25 accessions were collected. Much variability was observed in seed colour, which ranged from cream, red, purple to black and also mottled with these colours.

Groundnut

Groundnut is second in popularity as a pulse crop in Ghana. Eleven accessions were collected. Most farmers were planting improved landraces of this crop. There was not much variability in the colour and size of the seeds collected.

African yam bean

This is a crop which was collected exclusively in the Volta Regions. This has been consistently so over several germplasm collections. Four accessions were collected, which showed significant variability.

Lima bean

Lima bean is a minor crop in Ghana. Six accessions were collected. A wide range of variability was observed in the seeds of the crop. The colours observed were white, red, mottled and black.

Velvet bean

Velvet bean is a minor crop which is used on the subsistence level. In this expedition four accessions were collected in the Brong Ahafo and Upper West regions. Diversity was observed in the seed colour of this crop, ranging from grey to black.

Kersting's groundnut

This is a minor legume crop which is restricted to the northern regions of the country, that is, northern parts of Brong Ahafo region, the Northern, Upper East and Upper West Regions. The shoot and especially the leaves resemble those of soyabean, whereas the groundnut pods are like those of bambara groundnut and the seeds look like those of cowpea. It is used both for food and medicinal purposes.

This species is facing severe genetic erosion. In this expedition seven accessions were collected. It seems likely that it is being displaced by the more popular legumes like cowpea, groundnut and bambara groundnut.

Storage of germplasm

The germplasm collected is currently in storage at the Plant Genetic Resources Centre, Bunso with duplicate samples sent to the National Research Institute of Vegetables, Ornamental Plants and Tea in Japan. Seeds have been stored in aluminium foil envelopes at –20°C and a moisture content of 6%. The seeds are available to researchers on request.

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Collecting, evaluation and utilization of walnut (*Juglans regia* L.) in Yugoslavia

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Introduction

Walnut growing in Yugoslavia has a long tradition. Because of favourable soil and climate conditions, this species is grown in all fruit-growing regions. There are 1 735 000 fruit-bearing trees in Yugoslavia. The major fruit-growing regions are: Timok, Podrinje, Metohija, Zapadna Morava, Južna Morava, Fruška Gora, etc. The walnut trees are mostly concentrated in the altitudes from 100 to 400 m. In the central part of the country, individual walnut trees occur at 1000 m asl, while in the south the trees can be found even at 1200 m asl. Owing to a continuous generative propagation, the walnut structure is rather heterogeneous.

The importance of walnut in Yugoslavia is best illustrated by 70 settlements whose name is derived from the word 'walnut'. Up to now, walnut trees were propagated generatively, which resulted in a heterogeneity of genotypes representing individual trees. Walnut distribution and structure in Yugoslavia was studied by Jelenkovic (1974) in the region of Zaječar, Rudić (1962) in the Fruška Gora region, Rahović (1959) in Monte Negro. The first extensive studies were initiated in 1973, resulting for the first time in selection of high-quality walnuts. Such selection was repeated in the following 10 years. Additionally, walnut trees were inventoried in different regions with a large number of trees. We inspected over 20 000 trees that included biotypes with characteristics such as nut quality, high yield potential, late flushing, high resistance to diseases, etc. The aim was to select good parent trees for hybridization or introduction of new biotypes.

Material and methods

Biotype collection and evaluation

The first studies were performed in the field. Every biotype with interesting characteristics was observed for several years. If the characteristics were proved during several years, scions were used for grafting and the individuals obtained were planted in collection plantations. The largest collection is in Beška, in the vicinity of Novi Sad. The major criteria for the selection were:

1. Short growing period: biotypes that have a late flushing and early finishing growing period were selected because late spring and early autumn frosts frequently occur in several regions of Yugoslavia.
2. High quality of fruits: the most interesting biotypes are those with 50-58% of kernel, those whose kernels can be easily taken out from the shell and those that have light colour kernels. The flat and light shell has to protect the kernel well.
3. High yield potential: Biotypes were selected that bear fruits on lateral buds, biotypes that bear fruits in clusters and those with high branching coefficient.
4. Low susceptibility to disease agents, particularly to *Gnomonia leptostyla* and *Xantomonas juglandis*: high variability with respect to the characteristics studied was observed.

Results

Beginning of growing period

Well-known varieties and selections were used for the comparison of walnut types from different regions. The most frequently used were the types with early and medium early growing period (approximately 75%) (Table 1). These types are often damaged by late spring frosts. The interval between the beginning of the growing period of the earliest and the latest biotype was 55 days.

Table 1. Relative frequency of walnut biotypes according to the beginning of growing period

No.	Growing period beginning	Characteristic variety	%
1	Very early	Geisenheim 1247	8.1
2	Very early to early	Eszterhazy I	18.5
3	Early	Drjanovski	27.4
4	Early to medium	ejnovo	22.7
5	Medium	Geisenheim 139	12.7
6	Medium to late	Marbot	6.5
7	Late	Franquette	3.6
8	Late to very late	Ronde de Montignac	1.4
9	Very late	Super kasni	0.1

End of growing period

Most of the studied biotypes have a medium-long growing period (approximately 55%), followed by early end of growing period (approximately 28%), while the lowest number of biotypes ends the growing period late (approximately 17%) which reduces the damages caused by autumn and spring frosts (Table 2). A positive correlation exists between the beginning and the end of growing period. The biotypes whose growing period begins late, most frequently end it late also, but there are several exceptions. The Yugoslav variety 'Šampion' has a medium beginning of the growing period and ends it very early. The selection 'Super kasni' has the latest beginning of the growing period (in 1987 it was on 2 July) ending at medium, as do most of the studied walnut biotypes.

Table 2. Relative frequency of walnut populations according to the end of the growing period

No.	End of growing period	Characteristic variety	%
3	Early	Adams	55
5	Medium	Hartley	28
7	Late	Marbot	17

Fruit characteristics

Of the walnut trees studied, the most frequent were the biotypes with small nuts (Table 3). Fruit mass ranged from 3.3 g (biotype V-1) to 29.0 g (biotype 105/76). Type 105/76 belongs to the type 'babac' with the kernel portion only 26.2%.

Table 3. Relative frequency of biotypes according to nut size

Fruit mass (g)	Frequency in population (%)
up to 6	10.1
6-8	18.5
8-10	32.2
10-12	15.2
12-14	12.8
over 14	7.2

Kernel portion

Types with low kernel portion are predominant. Only one-third of the studied walnut biotypes had kernel portion 40% or more (Table 4). The biotype 62/76 had the highest kernel portion (64.3%).

Table 4. Relative frequency of walnut biotypes according to kernel portion

Kernel in fruit (%)	Frequency in population (%)
up to 30	14.7
30 - 40	29.1
35-40	22.0
40 - 45	15.2
45 - 50	9.7
50-55	6.2
over 55	3.1

Distribution of fruit-bearing buds on lateral branches

In most of the studied biotypes, only terminal buds were fruit bearing. Only two types had more nuts on lateral than on terminal buds. Approximately 5% of the biotypes had lateral fruit bearing with fruiting efficiency from 20 to 98%.

Only a few biotypes bore fruits racemosely. We selected nine biotypes that bear fruits in a cluster, four of which are economically significant. Considering racemose types, the variety Tisa has large nuts (even 15.5 g) while the others have rather small ones (under 10 g). The number of nuts in a cluster ranged from 8 (Tisa) to 15 (Medvedja).

Coincidence of male and female flowering

Most of the biotypes (approximately 80%) had protandry flowering, fewer biotypes protogyny (approximately 12%) and a small number homogamy (approximately 8%).

Studying walnut populations, we found some rare forms. The selection 'Bečej' of the laciniate type belongs to such a form.

New varieties

Five selections from indigenous populations were registered as varieties: 'Šampion', 'Srem' (Fig. 1), 'Tisa', 'Bačka' and 'Mire'. Several selections of high quality are in the testing and propagation phases.

These varieties and selection material were used for establishing plantations on over 1000 ha in Yugoslavia. The largest plantation (84 ha) was established in Lipar.

Future work

Priority is given to the propagation of newly selected bio-

Fig. 1. Variety 'Srem' selected from the natural walnut population in Vojvodina.

types and to their planting in a trial nursery for further studies. The study of natural selection should be continued as well as studies on intensification of walnut hybridization. The selections that should be used in hybridization show high frost resistance, high disease resistance, late flushing and normal end of the growing period, high nut quality and high yield potential as a result of fruit-bearing on lateral buds, fruit-bearing in cluster and high branching coefficient.

Conclusion

Our studies, which have been particularly intensive since 1973, proved that the differences between the beginning and the end of growing period of extreme genotypes were

55 and 32 days, respectively. The fruit mass ranged from 3.3 to 29.0 g. Kernel portion ranged from 26.2 to 64.3%. The inflorescence contained maximally 32 female flowers, or 15 nuts per cluster. Approximately 80% of the walnut population has protandry flowering.

It can be concluded that biotypes with poor characteristics dominate but high-quality biotypes can be found as well. Selecting from the existing walnut populations, we have achieved several high-quality selections. Five of them are registered as the first and the only walnut varieties in Yugoslavia: 'Šampion', 'Srem', 'Tisa', 'Bačka' and 'Mire'. These varieties have high yield potential and excellent nut quality and are grown on over 1000 ha.

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Evaluation of local pepper accessions (*Capsicum annuum* L.) in Sadovo, Bulgaria

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Bulgaria is the secondary centre of diversity for *Capsicum* spp. in Europe (Popov 1940). The collection in Sadovo represents the great diversity of *Capsicum annuum* preserved in the country. It includes representatives of eight taxonomy groups, determined according to Popov (1940).

Material and methods

The experimental material was collected during expeditions in different areas and includes 264 local populations. Passport data include data about the area, the village where collecting was done, and the donor's name.

Field testing was done in the experimental field of IIPGR, Sadovo during 1992/94. The evaluation was made according to the international pepper descriptor (COMECON 1986).

The following characters were determined for 20 plants from each accession: (1) biological peculiarities of the plants including the beginning of the different developmental phases, (2) morphological characters - bush habit, colour and shape of the stem, leaves and flowers. Measurements of the fruit were made on 10 mature fruits for each cultivar.

Dry matter content was determined (in %) as an average for 10 fruits. The hygroscopic moisture content was determined by heating the plant material at 102-105°C until constant weight was obtained. The percentage of the total sugar content was determined by the method of Shoorl. The vitamin C content was determined once during every year of the study. The data presented are the average for 3 years. The analysis was made on a sample from each accession at full maturity, using the method of Mury.

The reaction of the local pepper accessions towards *Verticillium dahliae* Kleb. was observed in the field for 3 years. At the end of the growth period (September - October) the percentage of the diseased plants was noted for each population and the index of attack (on a 5-point scale) was determined according to Mc Kinney.

Results

Experience has shown that the best results are obtained in Bulgaria when cultivating local cultivars or when local cultivars (almost 98%) are used as breeding material. This indicates the importance of the local cultivars preserved in the home gardens. The objective of this work was to divide the local forms into taxonomic groups, and evaluate them for a complex of characters so that the best ones could be used as breeding material in the future.

Of the great amount of data obtained, only the results of the biochemical analysis and the field study about the reaction of local peppers to *Verticillium* are reported in this paper.

Table 1. The vitamin C content and chemical composition of local *Capsicum* varieties at full maturity, studied during 1992/94

Variety	Vit. C (mg %)		Dry matter (%)		Sugar content (%)	
	Min	Max	Min	Max	Min	Max
conoides	147.1	279.8	9.2	19.6	3.3	8.1
capia	155.0	238.0	8.9	13.2	4.3	7.1
corniforme	152.7	250.8	13.1	24.7	4.5	7.0
ratundum	130.4	249.7	7.1	15.8	3.8	5.8
dolma	163.7	192.4	8.0	14.4	4.8	7.3
cordatum	165.0	237.8	9.7	11.6	3.9	7.0
shipka	168.1	343.8	11.9	40.1	3.6	8.0
cerasiforme	173.1	227.2	10.9	49.1	3.7	5.8

The results shown in Table 1 reveal that the vitamin C content varies among accessions as well as groups. This suggests that in a larger population this cultivar variation could be far greater. It was interesting to establish whether the morphological characters of pepper correlate with the ascorbic acid content. Thus breeding could be related to the systematic groups and directed towards selection of forms with high ascorbic acid content.

The following conclusions about the different systematic groups can be made from our studies:

1. The small-fruited pepper cultivars - subsp. *microcarpum*, where var. *shipka* and var. *cerasiforme* belong, have the highest ascorbic acid content at full maturity. It ranges from 168.1 to 343.8 mg% in the crude matter. All these cultivars are notable for their small fruits and thin percarp. They have a high dry matter content (40.1%).
2. The big-fruited peppers, belonging to the *dolma*, *ratundum* and *kapia* groups, have a lower ascorbic acid content, ranging from 130.4 to 249.7 mg%. The dry matter content is also considerably lower.
3. The representatives of the group *longum* var. *conoides* have, in spite of their larger fruits, fine meat, a high dry matter content (19.6%) and a high vitamin C content (279.8 mg%). These features are similar to those of the small-fruited peppers, which shows their genetic similarity.

Reaction to Verticillium wilt

Verticillium wilt in Bulgaria appears every year and in some places it affects more than half of the pepper plants (Elenkov and Hristova 1978). There has been no study of the reaction of the local pepper populations to this disease up to now. Our objective was to discover accessions showing resistance in the field.

The tested accessions were divided, according to the results, into five relative groups : immune (index 0%) resis-

tant (0.1-10%); slightly susceptible (10.1-25%); moderately susceptible (25.1-50%) and very susceptible (above 50.1%).

Data from the field observations show that there are no immune forms in the collection in respect to Verticillium wilt. The resistant group includes 24 accessions of var. *ratundum*, 6 of var. *dolma* and 4 of var. *cordatum*. All the rest of the accessions are moderately to strongly susceptible.

Conclusions

The results of the chemical analysis reveal the presence of a positive correlation between the vitamin C and dry matter content and the thickness of the pericarp. There were no immune forms discovered when testing for Verticillium resistance in the field, although accessions from the groups *ratundum* and *dolma* showed higher resistance.

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A proposal for an *Elymus* Core Collection

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Introduction

For a plant breeder or researcher aiming at screening the entire diversity of a species, based on genebank accessions, a trial or study can be problematic with accessions that lack passport data or are incorrectly identified. Also, in some cases, the sheer amount of material precludes its use. This applies to barley (*Hordeum*), for example, where the number of accessions exceeds 400 000 (von Bothmer *et al.* 1992).

As a solution to these problems the concept of a **core collection** has been developed. The original concept was formulated by Frankel and Brown (1984) and Brown (1989a, 1989b).

A core collection consists of a limited set of accessions derived from an existing germplasm collection, chosen to represent the genetic spectrum in the whole collection. The core should include as much as possible of its genetic diversity.

The construction of a core collection has come rather far in some crop species, e.g. sugarbeet (*Beta*), pea (*Pisum*) and barley (van Hintum 1994). The definition of the barley core collection has been made even more precise by van Hintum (1994).

The Barley Core Collection (BCC) is a selected and limited set of accessions, optimally representing the genetic diversity of cultivated barley (*Hordeum vulgare s. lat.*) and wild species of *Hordeum*, and providing well-known genetic standards. The objectives of the BCC are threefold: (1) to increase the efficiency of evaluation and thus of utilization of existing collections; (2) to provide for a manageable and representative selection of the available barley germplasm for use in research and breeding; (3) to provide adequate material for the needs of standardization in scientific work with barley.

In the BCC, accessions have been selected that represent the diversity in barley and these accessions are now being made available. The BCC consists of around 2000 accessions, which is less than 0.5% of the world holding of barley. When the BCC is fully operating it should be easily accessible to all barley germplasm users in the world.

A very important purpose of a core collection is that it provides scientific and agricultural standards for research, evaluation and trials. Furthermore, it provides a well-structured way to obtain access to the relevant parts of the whole collection. This paper is an attempt to enlarge the core collection concept to encompass a genus of wild species, which is not centred around a crop species. It is intended to be a method to help get an overview of the diversity of a large genus, primarily for scientific reasons.

The *Elymus* Core Collection

Elymus is the largest genus, with some 150 species (Lu 1993), in the economically important grass tribe Triticeae, and much of the basic taxonomy and cytogenetics remain to be done before we will have a fair picture of the diversity and relationships in the genus. *Elymus* occurs almost worldwide in extra-tropical areas but its major centre of distribution is in Central Asia. Species of the genus are important as range and forage grasses in North America and in Central Asia. Many species have restricted distribution areas and some are threatened by extinction. The genus consists of alloploids only and shows a large cytogenetic variation. It includes species having two or three of five basic genomes in different combinations (Lu 1993).

Elymus is currently under intense scientific study and one can expect large developments in our knowledge in the coming years. In comparison with the major crop species, *Elymus* is not very well represented in the genebanks. On the contrary, it is often difficult to get access to adequate material. To make the research easier and to enlarge our knowledge of *Elymus*, we suggest that a core collection be set up, representing a majority of the genetic diversity within the genus. The objective of an *Elymus* Core Collection (ECC) is to serve as a readily available standard in research. The expected benefit of this is that most researchers will include species from the ECC in their investigations, which will then provide data over time and locations on a given set of species.

We have envisaged that the ECC should have a hierarchical structure (Fig. 1). The ECC should have one set of

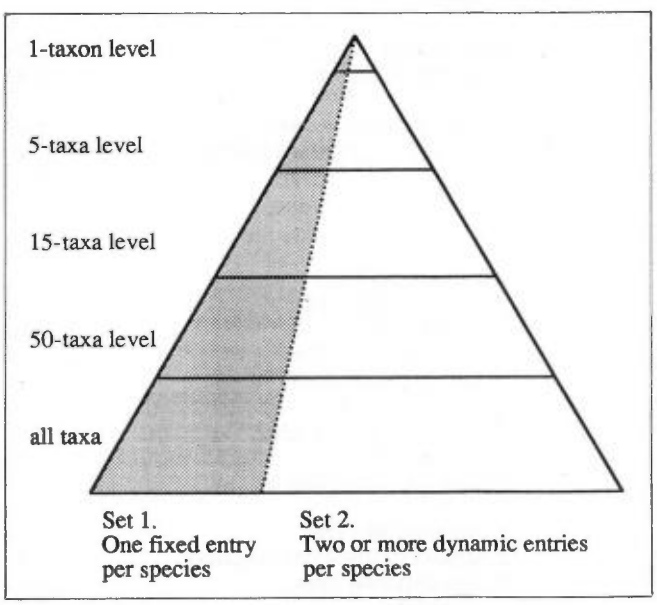

Fig. 1. The hierarchical structure of the ECC combines easy access to adequate material, optimal representation of the genetic diversity and comparability between different studies.

accessions including one accession (entry) per species. The entries in this set will be fixed to ensure comparability. This set should preferably be present in all major genebanks and also be included in all work of individual scientists and agronomists.

A second, larger set will be chosen which includes at least two further entries of each species. Sometimes more than three entries will be included for each species, e.g. in cases where there are several infraspecific units. The additional larger set will be chosen in such a way that the ECC will represent a maximum of genetic variation within the different species. This set should be dynamic with respect to both size and content. This means that when more information about the genetic variation becomes available, a particular entry in the set can be replaced by another in order to maximize diversity and to minimize similarity between entries. Since knowledge of genetic variation in *Elymus* is very scanty the second set has to be chosen mainly on the basis of taxonomic, ecogeographic and cytogenetic data.

At present, it is not realistic or feasible to set up a core collection that includes all the known species in the world. As a start, it is proposed that one-third of the approximately 150 species comprise the major core. Because of the different needs and uses of a core collection in different scientific projects, we find it desirable that there be a hierarchical structure within the ECC. This structure will, only to a limited extent, mirror contemporary taxonomy. The proposed hierarchical levels encompass 1, 5, 15 and 50 taxa, respectively (Table 1, Fig. 1).

Selection of taxa for the ECC

The proposed core collection of *Elymus* is based on the generic delimitation of Dewey (1984) which is a treatment of the genus in a broad sense. Only some minor deviations have been made in light of recent studies (Assadi and Runemark 1995). The most noticeable difference from the treatment of Dewey (1984) is the inclusion of *Elytrigia repens*. Since *E. repens* is the type taxon for *Elytrigia* this generic name is no longer used. We are well aware of the polyphyletic origin of *Elymus*, but the creation of a core collection is an undertaking for practical handling of material and not a taxonomic treatment. The ECC is meant to be a practical tool for studies of the Triticeae in general and *Elymus* in particular. The core collection will, we hope, help clarify the complicated taxonomy and phylogenetic relationships in the current tribe.

As stated above, the species selection is based primarily on three criteria, namely, taxonomic, ecogeographic and cytogenetic data. However, if supporting data are available, they will be included in the decision. The final choice of species will be reached by consensus between all available data. Having levels of the ECC will allow comparison of results between different researchers' studies. For example, in a study of the whole tribe it may, for practical reasons, be necessary to limit the number of investigated *Elymus* species to, for instance, three. If these three species are chosen from the 5-taxa level the comparison with other studies will certainly be easier than if the selected species is a random choice from the whole genus or ECC.

The taxonomic delineation of the genus adopted for the

Table 1. Taxa suggested for inclusion in the *Elymus* Core Collection

Level	Tetraploid, SH genomes[†]	Tetraploid, SY genomes	Hexa/Octoploid
1 taxon	*E. sibiricus* L.		
5 taxa	*E. canadensis* L. *E. glaucescens* Seberg	*E. caucasicus* (Koch) Tzvel. *E. semicostatus* (Nees ex Steud.) Meld.	
15 taxa	*E. enysii* (Kirk) Löve et Connor *E. trachycaulus* (Link) Gould ex Shinn.	*E. antiquus* (Nevski) Tzvel. *E. pendulinus* (Nevski) Tzvel. *E. longearistatus* (Boiss.) Tzvel.	*E. batalinii* (Krasn.) Löve *E. dahuricus* Turcz. ex Griseb. *E. scaber* (R. Br.) Löve *E. repens* (L.) Gould *E. tschimganicus* (Drob.) Tzvel.
50 taxa	*E. agropyroides* Presl. *E. arizonicus* (Cribn. et Smith) Gould *E. caninus* (L.) L. *E. cordilleranus* Davidse et Pohl *E. dentatus* (Hook f.) Tzvel. *E. duthiei* (Stapf.) Löve *E. elymoides* (Rafin.) Sweezey *E. glaucus* Buckl. *E. hispanicus* (Boiss.) Talavera[‡] *E. hystrix* L. *E. mutabilis* (Drob.) Tzvel. *E. nardurioides* (Turca.) Löve et Connor[‡] *E. scribneri* (Vasey) Jones *E. virginicus* L.	*E. abolinii* (Drob.) Tzvel. *E. africanus* Löve[‡] *E. buschianus* (Roshev.) Tzvel.[‡] *E. cacuminus* Lu et Salomon *E. canaliculatus* (Nevski) Tzvel. *E. ciliaris* (Trin.) Tzvel. *E. fedtschenkoi* Tzvel. *E. formosanus* (Honda) Löve[‡] *E. gmelinii* (Ledeb.) Tzvel. *E. grandis* (Keng) Löve *E. panormitanus* (Parl.) Tzvel. *E. serotinus* (Keng) Löve[‡] *E. shandongensis* Salomon *E. yangii* Lu	*E. drobovii* (Nevski) Tzvel. *E. nutans* Griseb. *E. patagonicus* Speg. *E. tenuis* (Buch.) Löve et Connor *E. thoroldianus* (Oliv.) Singh *E. transhyrcanus* (Nevski) Tzvel. *E. tsukushiensis* Honda

[†] The genomically unknown species are placed in suggested genomic group based on morphological features only.
[‡] Currently not available for inclusion in the ECC.

Table 2. Examples of *Elymus* species which have at one time been included in other genera

Segregate genus	Segregate name	Current name
Anthosachne	*A. elymoides*	*E. africanus*
	A. longearistata	*E. longearistatus*
	A. scabra	*E. scaber*
Elytrigia	*Et. arizonica*	*E. arizonicus*
	Et. pubiflora	*E. glaucescens*
	Et. repens	*E. repens*
Goulardia	*G. canina*	*E. caninus*
Hystrix	*H. duthiei*	*E. duthiei*
	H. patula	*E. hystrix*
Kengyilia	*K. gobicola*	*E. batalinii s.l.*
	K. thoroldiana	*E. thoroldianus*
Roegneria	*R. caucasica*	*E. caucasicus*
	R. grandis	*E. grandis*
	R. parvigluma	*E. antiquus*
Semeiostachys	*S. drobovii*	*E. drobovii*
	S. panormitana	*E. panormitanus*
	S. turcazninovii	*E. gmelinii*
Sitanion	*S. elymoides*	*E. elymoides*
Stenostachys	*S. gracilis*	*E. narduriodes*
Terrellia	*T. canadensis*	*E. canadensis*
	T. glauca	*E. glaucus*
	T. virginica	*E. virginicus*

ECC includes a number of species previously referred to in other genera (Table 2). It is desirable for representatives of these taxa to be included in the ECC. Some of the genera included in *Elymus* are *Anthosachne, Goulardia, Hystrix, Kengyilia, Roegneria, Semeiostachys, Sitanion, Stenostachys* and *Terrellia*. No single species can in all aspects be representative for a higher group, except in monotypic genera. To achieve nomenclatural stability, the heart of the core collection should be the taxon that for certain will never be excluded from the genus. Hence, the type taxon of *Elymus* – *E. sibiricus* – is selected for the 1-taxon level. Taxa presently treated in *Elymus*, but which future studies may transfer to other genera, should gradually be added so they are all included at the 50-taxa level.

Species that are taxonomically problematic should normally be avoided in the core collection. An example of such a taxon is *E. trachycaulus* (Link) Gould ex Shinners. This species has a large morphological variation with obscure borders to other species, possibly as a result of frequent hybridization and introgression (Barkworth 1996). The taxonomic status of the different morphological entities is questionable and the species and its allies are in obvious need of a major revision. However, to ignore *E. trachycaulus* would be to ignore a major North American species which is under intense study by both research scientists and plant breeders. Hence, in this particular case it is justified to include it irrespective of the problems.

Some of the names appearing in Table 1 are, for various reasons, illegitimate. This is, however, not the proper forum for nomenclatural changes, and these errors will be corrected elsewhere (Seberg, in preparation; Salomon, in preparation).

The second criterion is the ecogeographic origin of the taxa (Table 3). The vast majority of species, about 90%, are

Table 3. The *Elymus* species grouped according to floristic regions as delineated by Takhtajan (1986)

Kingdom	Subkingdom	Region	Species
Holarctis	Boreal	Circumboreal	*E. sibiricus, E. caninus, E. mutabilis*
		Eastern Asiatic	*E. duthiei, E. formosanus, E. ciliaris,* E. yangii, *E. shandongensis, E. serotinus, E. grandis*
		N. American Atlantic	*E. virginicus, E. hystrix*
		Rocky Mountain	*E. glaucus, E. scribneri*
	Tethyan	Mediterranean	*E. hispanicus, E. panormitanus*
		Irano-Turanian	*E. dentatus, E. caucasicus, E. semicostatus, E. antiquus, E. longearistatus, E. fedtschenkoi, E. abolinii, E. cacuminus, E. canaliculatus, E. buschianus, E. transhyrcanus, E. thoroldianus, E. batalinii, E. drobovii, E. nutans, E. tschimganicus, E. tsukushiensis*
	Madrean	Madrean	*E. arizonicus, E. elymoides*
	Undetermined		*E. canadensis, E. dahuricus, E. pendulinus, E. repens, E. gmelinii, E. africanus, E. trachycaulus*
Australis		Northeast Australian	*E. scaber*
Holantarctis		Chile-Patagonian	*E. agropyroides, E. cordilleranus, E. glaucescens, E. patagonicus*
		Neozeylandic	*E. enysii, E. narduriodes, E. tenuis*

Holarctic. The remaining 10% are essentially confined to two floristic kingdoms, namely Holantarctis and Australis (following the system of Takhtajan 1986). These proportions should be mirrored in the choice of species to be included in the ECC. Likewise, within these areas it is desirable that the species should be selected from as many phytogeographic subkingdoms and regions as possible and in proportion to their numbers. In our case, it means that four South American, three Newzealandic and one Australian species are chosen, which gives a representation of 16%. The South American species are distributed throughout the Chile-Patagonian region but also extend along the Andes and occur as far north as Costa Rica. Material to be included in the ECC will be selected from the extreme south, like *E. glaucescens*, to the Andean *E. cordilleranus*. The Neozeylandic region is a small territory but comparatively rich in *Elymus* species (Connor 1994). *Elymus tenuis* and *E. enysii* were selected from South Island and *E. narduroides* from North Island.

The species from North America constitute about 15-20% of the total number in the genus. In this case, eight taxa (16%) are suggested for inclusion in the ECC. These species are from all four floristic regions of North America, for example, *E. trachycaulus* is found in the Circumboreal region, *E. virginicus* is from the North American Atlantic region, *E. scribneri* is from the Rocky Mountain region, and *E. arizonicus* is endemic to the Madrean region.

The Eurasian part of the Circumboreal region is contributing with three species. These are *E. sibiricus* with an eastern distribution, *E. caninus* with a western distribution, and *E. mutabilis* which is found throughout the area. Apart from *E. caninus* and *E. mutabilis*, only two more species are restricted to Europe. These are *E. hispanicus* and *E. panormitanus*, both of which grow in the Mediterranean region.

The area richest in *Elymus* species is Central Asia, particularly along the rim of the Central Asiatic mountain area (CAMA), see Fig. 2. This area corresponds to the eastern parts of the Irano-Turanian region and adjoining parts of the East Asiatic region. The species that are restricted to the CAMA or that have parts of their distribution in this area constitute half of the total number of species in the genus. Based on the diversity present in this area, it is proposed that seven Eastern Asiatic species and seventeen Irano-Turanian species be included in the ECC. Among these are four species from the western parts of the Irano-Turanian region, namely, *E. caucasicus*, *E. longearistatus*, *E. buschianus* and *E. transhyrcanus*.

Only a few *Elymus* species are indigenous to the tropical areas of the world. These are probably extensions of

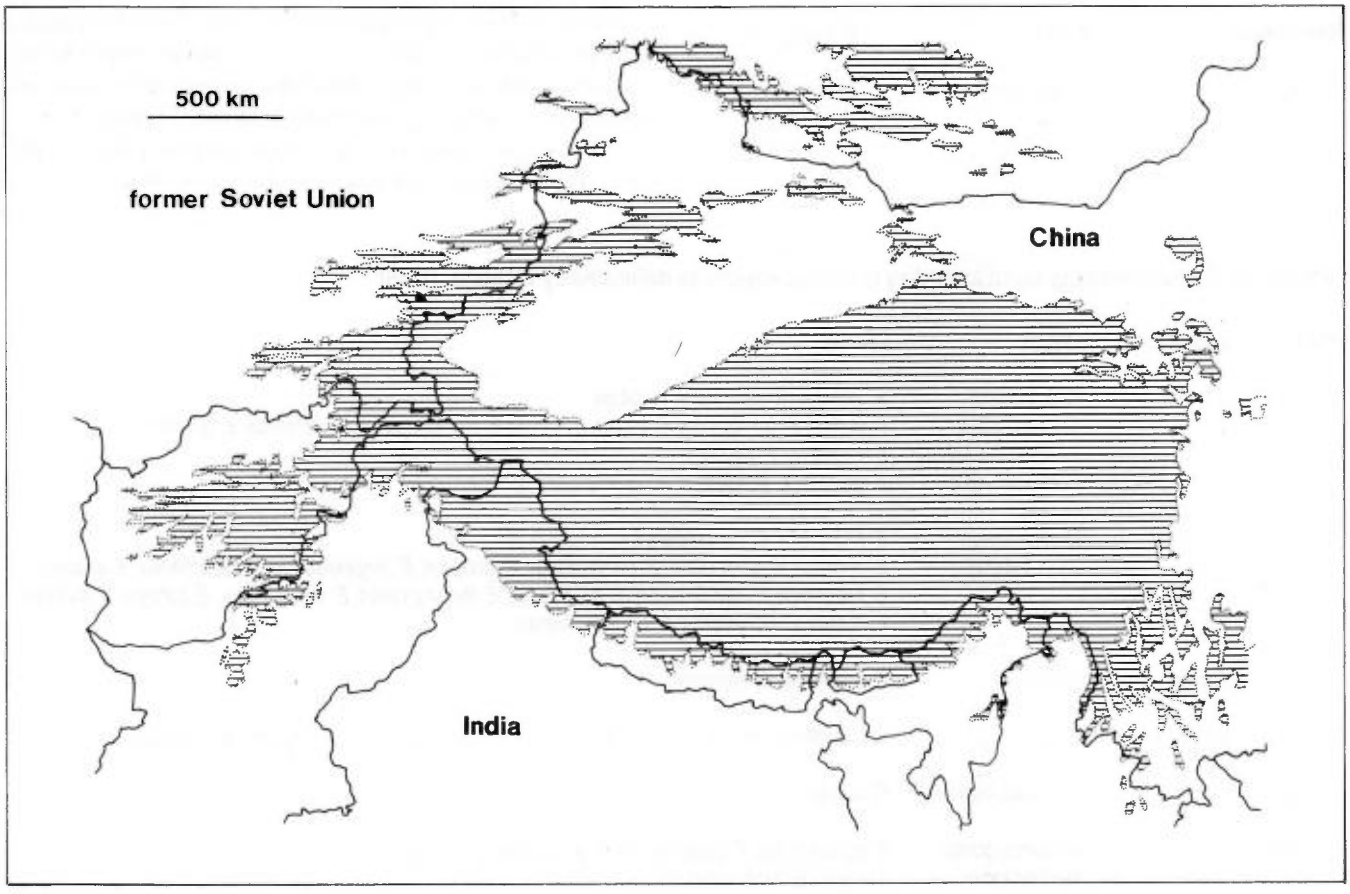

Fig. 2. The Central Asiatic mountain area (CAMA) is the richest for *Elymus* in the world, with approximately 75 species native to this region. The hatched area has an elevation above 2000 m asl.

distributions in nearby non-tropical regions. One exception, however, is *E. africanus* which is endemic to Eritrea in the Sudano-Zambezian region. This taxon is thus isolated from any other *Elymus* species and therefore included in the ECC.

The third criterion used is data obtained from cytogenetic studies, mainly chromosome number and genomic constitution. Tetraploids encompass about 70% of the total number of species in the genus. In the ECC two-thirds of the species included in the 15-taxa level and around two-thirds to three-quarters at the 50-taxa level are tetraploids. The tetraploids have been divided into two cytological groups, namely those containing the SH genomes and those containing the SY genomes (Salomon 1994). About equal numbers of species from the two cytological groups are included in the ECC. Although the latter group encompasses approximately twice as many taxa as the first group, its geographical range is much more restricted. All known genomic combinations among the hexaploid species are included at the 15-taxa level. The hexaploid genomic combinations are SYP in *E. batalinii*, SYH in *E. dahuricus*, SYW in *E. scaber*, SSH in *E. repens* and SSY in *E. tschimganicus* (Lu 1993). At the 50-taxa level, six more hexaploids are added. Furthermore, the rare octoploids are represented by one taxon, *E. tenuis*. The ECC also contains taxa with unknown genomic constitution and in six taxa even the chromosome numbers are unknown. These taxa were selected on the basis of the previous two criteria, i.e. taxonomic and ecogeographic data.

Highest priority will be given to the reliability of the included material, which means that no accession will be accepted as an entry in the ECC unless some basic requirements are fulfilled. These are that the collection must be accompanied by complete passport data and that there is a voucher specimen of the original collection available in a public herbarium. The species presented in Table 1 have been chosen independent of whether they are currently accessible as seed collections or not. Instead, we strongly encourage collection of exactly those species that are still missing. For example, *E. buschianus* is suggested for inclusion in the ECC even though it is currently not available, nor is it likely to be available in near future as it is endemic only to the geologically unstable Caucasus mountains.

How to set up the ECC?

The physical set-up of the ECC is due to a number of practical matters. Though the ECC will only include 50 taxa and around 150 entries, the multiplication of wild species, and *Elymus* in particular, is not a trivial task. The principles for the ECC and the suggested list of species to be included in the ECC were presented and discussed at the 3rd International Triticeae Symposium held at ICARDA, Syria in 1997. At this meeting we will suggest that a working group (or international committee) be set up, the jurisdiction of which will be to achieve a plan for the physical set-up of the ECC. The working group will have to deal with the following issues:

1. Final selection of taxa to be included in the ECC.
2. Screening for which taxa appropriate, well-documented accessions are available to serve as entries.
3. Priorities for collecting complementary taxa have to be decided as well as actual collecting missions carried out.
4. Planning for initial multiplication and characterization and to divide the practical work among research groups and genebanks willing to accept the task.
5. Selection of research institutes and genebanks responsible for distribution, intermediate storage, rejuvenation and further multiplication of the entries.
6. Formulation of procedures for keeping and updating information of the material and for the changes of entries in the ECC in order to maximize the genetic diversity and to minimize similarity between entries.

The easiest way to organize the work would probably be on a regional scale, and we urge anyone interested in participating in this work to notify us.

Acknowledgements

The authors thank Dr Theo J. L. van Hintum, Dr Mary E. Barkworth, and Dr Kevin B. Jensen for their helpful comments which greatly improved the manuscript.

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Plant Genetic Resources Newsletter, 1997, No. 111: 82-83 SHORT COMMUNICATION

Seed quality and seed storage in fruit for short-term conservation of germplasm in Coorg mandarin (*Citrus reticulata* Blanco)

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Introduction

The many species of *Citrus* are distributed predominantly in southeast Asia. In India, diversity is largely found in the northeastern region (Arora 1988) which harbours valuable genes for the genetic improvement of cultivars. Presently, the genetic diversity is being rapidly eroded owing to large-scale destruction of plant habitats for various domestic and commercial reasons.

In fruit crops, fruits generally are collected during plant exploration trips. Later the seeds are extracted and conserved in a genebank. However, if the seed-conservation process in the genebank is delayed, the seeds may die and valuable genetic diversity can be lost. Most citrus seeds exhibit orthodox and a few recalcitrant type of storage behaviour. Kopan (1967) suggested that seeds could be stored in the fruit without loss of viability. Fruit preserves the seeds in natural conditions and prevents their drying out. This paper reports on an attempt to conserve seeds in fruit to maintain seed quality in terms of high viability and vigour until they are separated from the fruit and stored in a genebank.

Material and methods

Fruits of the 'Coorg mandarin' cultivar were collected and stored at 5°C, 15°C and ambient temperatures. No fungicidal treatments were given to fruits during storage. Changes in certain fruit and seed characteristics were recorded at monthly intervals. Seed viability was expressed as percentage of germination. Seeds were extracted from fruits and germinated in rolled towels at alternate temperatures of 20/30°C for 16/8 hours respectively in a seed germinator. One hundred seeds in each replication were germinated in dark conditions. The experiment was a complete randomized design with three replications. Root and shoot lengths were recorded on five randomly selected seedlings. Seedling vigour was expressed in terms of vigour index which was calculated by multiplying percentage of germination by seedling length. The juice content in fruits was measured and pH of juice was recorded. The total soluble solids (TSS) contents in juice were measured with a hand refractometer and expressed in °Brix.

Results and discussion

Mandarin fruits were successfully conserved for 90 days at 5°C and for 120 days at 15°C, compared with 15 days at ambient temperatures. Further, stored fruits showed discolouration, shrivelling and blackening due to heavy infestation of saprophytic fungi. The juice content in fruits decreased, pH and TSS increased with increase in storage period. Fruit drying was relatively faster at 5°C than at 15°C storage as evidenced by a rapid decrease in juice contents in stored fruits at 5°C (Table 1). Subsequently fruits became unsuitable for storage and seeds in stored fruits did not germinate. Likewise seed quality in terms of viability and vigour was tested at monthly intervals in stored fruits at different temperatures. Seeds in stored fruits at low temperatures exhibited higher percentages of germination and vigour. The percentage germination was decreased with an increase in storage period. The loss of viability was greater in seeds stored in fruits at ambient temperature. In total, germination of more than 85% was recorded in seeds of stored fruits at 5°C after 90 days and at 15°C after 120 days compared with about 47% at ambient storage after 30 days of storage (Fig. 1). There was no germination of seeds stored in fruits at 5°C after 120 days of storage. Similarly, high seedling vigour was maintained with low temperature storage (Table 2).

Table 1. Changes in certain fruit characteristics on storage of fruits at different temperatures in Coorg mandarin

Storage temp (°C)	Storage period (d)	Avg. seed wt. (mg)	Juice/fruit (ml)	pH	TSS (°Brix)
Initial	0	110 ± 10	34.6 ± 1.12	3.60 ± 0.06	10.0 ± 0.05
Ambient	30	103 ± 20	8.6 ± 1.08	4.40 ± 0.05	18.3 ± 1.17
5	30	95 ± 15	35.2 ± 0.53	3.77 ± 0.13	12.4 ± 0.00
	60	141 ± 7	7.5 ± 0.35	3.99 ± 0.00	14.3 ± 1.20
	90	118 ± 20	7.0 ± 1.44	4.09 ± 0.06	16.1 ± 1.59
15	30	98 ± 5	34.5 ± 1.77	3.82 ± 0.08	11.3 ± 0.35
	60	150 ± 1	27.5 ± 2.47	3.89 ± 0.16	12.8 ± 0.99
	90	110 ± 7	20.0 ± 4.24	3.90 ± 0.13	12.2 ± 0.14
	120	116 ± 16	7.5 ± 1.77	4.12 ± 0.05	16.3 ± 0.54

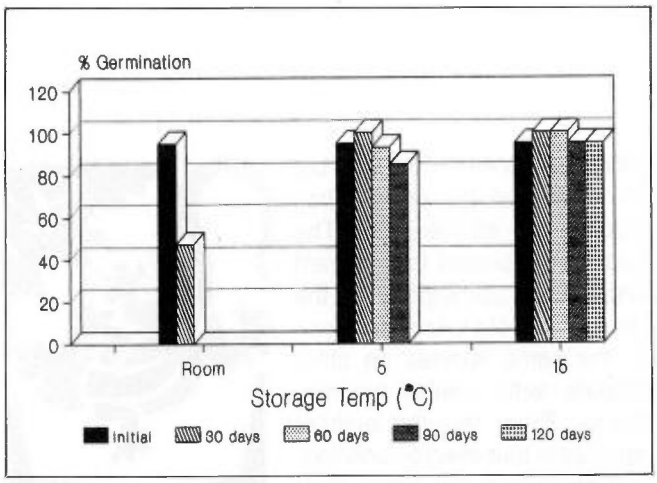

Fig. 1. Seed viability in Coorg mandarin fruits stored at different temperatures.

The fruits showed better fruit quality and high seed quality at 15°C storage and there was 30 days gain in storage life of seeds over 5°C storage. Such a level of temperature is easily available and inexpensive to maintain. It appears that very low temperature was not conducive for fruit storage. The reduction in acidity level might be due to greater accumulation of TSS in fruit. Thus percentage of germination was reduced faster at ambient storage which coincided with a decrease in acidity and an increase in TSS contents, suggesting that low pH and very high TSS might be responsible for decrease in seed viability.

Coorg mandarin fruits were successfully preserved for 120 days at 15°C without any rotting or shrivelling. The seeds in stored fruits showed higher percentage germination and vigour. Subsequently, fruits were unfit for storage owing to chilling injury and desiccation. Kopan (1967) reported that seeds were successfully stored for 30 days without loss of seedling vigour. Similarly in peanut, greater viability was recorded in unshelled seeds than in shelled seeds (Baskin 1979). The fruit provides a congenial atmosphere for short-term preservation of seeds, possibly owing to the prevalence of unfavourable conditions for germination or induction of seed dormancy. In *Brassica cernua*, seeds present in fruits maintained dormancy which disappeared rapidly on separation from the fruit (Tokumasu 1975). Thus storage of seeds in fruits as a low-input storage technique is beneficial for the short-term conservation of germplasm in the form of seeds. It not only preserved the seed viability but also avoided expensive packaging and very low temperatures for maintenance before reaching the genebank.

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News and Notes

Adaptive differentiation

It is a general understanding in genetic resources that populations displaced into a new environment different from the source quickly diverge under the influence of the new set of selection pressures. Despite its fundamental importance, the consequences of colonization for traits of founding populations have mostly been inferred from static distribution patterns, laboratory experiments and accidental introductions. A report by a group of US researchers* describes the results of the first long-term controlled study of colonization. The researchers introduced populations of the lizard *Anolis sagrei* on to small islands from a nearby source and found that they differentiated rapidly, over 10-14 years, in response to different environments. The amount of differentiation corresponded to the degree of difference in the recipient island's vegetation compared with the source. Similar rates of evolution have been detected in other introduced populations, but the nature of the change has rarely been so clear cut. The changes observed in the lizard populations correspond to the morphology of other populations of the same species on other islands with similar environments. From this the authors speculate that macro-evolutionary processes are being illustrated on a microevolutionary scale.

*Losos, J.B. *et al.* 1997. Adaptive differentiation following experimental island colonization in *Anolis* lizards. Nature 387:70-72.

Vavilov-Frankel Fellowships 1998

The International Plant Genetic Resources Institute (IPGRI) has established the Vavilov-Frankel Fellowship Fund to commemorate the unique contributions to plant science by Academician Nikolai Ivanovich Vavilov and Sir Otto Frankel. The Fund aims to encourage the conservation and use of plant genetic resources in developing countries by awarding Fellowships to outstanding young scientists. The Fund will enable fellows to carry out relevant, innovative research outside their own country for a period of between three months and one year. The research should have a clear benefit to the applicant's home country. Awards can be held concurrently with other sources of support. In 1998, a total of US$30 000 will be available for awards, which are intended to cover travel, stipend, bench fees, equipment, conference participation or any other appropriate use. Holders are encouraged to present the results of their research at an international conference. This can take place within one year of termination of the Fellowship.

Applications for 1998 are invited from developing country nationals, aged 35 or under, holding a higher degree in a relevant subject area. Application forms may be obtained from: Vavilov-Frankel Fellowships, IPGRI, Via delle Sette Chiese 142, 00145 Rome, Italy. (Fax 39 6 5750309; Email: e.clancy@cgnet.com). Applications, preferably in English (or accompanied by an English translation), should include a completed application form, brief letter of application, full *curriculum vitae*, research proposal (maximum 1000 words) and letter of acceptance from the proposed host institute. The closing date for receipt of applications is 31 December 1997. The successful applicants will be informed by 31 March 1998 and are required to take up their Fellowships before 31 December 1998.

Earth Summit+5

Five years after UNCED, how far have individual nations and the world community come in implementing Agenda 21? That question was discussed at a high-level Special Session of the United Nations General Assembly from 23 to 27 June 1997. FAO is Task Manager for four of Agenda 21's key chapters - planning and management of land resources, combating deforestation, sustainable mountain development, and sustainable agriculture and rural development. It is actively involved in the implementation of others - such as combating desertification and drought, and conservation of biological diversity - and in broader areas covered by Agenda 21, particularly climate change and energy. FAO prepared for the UN special session a series of Agenda 21 Progress Reports, describing the challenges addressed by Agenda 21, progress since the Earth Summit, key issues to be resolved, future directions, FAO's role and contact points.

Following UNCED, FAO broadened the scope of its intergovernmental Commission on Genetic Resources for Food and Agriculture to cover all plant, farm animal, forest and fisheries biodiversity. The Conference of the Parties to the Convention on Biological Diversity has recognized the Commission's role, given "the special nature of agricultural biodiversity, its distinctive features and problems needing distinctive solutions."

At the recommendation of Agenda 21, the Commission is strengthening FAO's Global System on Plant Genetic Resources. The Commission is the forum for negotiation of revisions, in harmony with the Biodiversity Convention, to the 1983 International Undertaking on Plant Genetic

Resources. Under its guidance, FAO has published the first Report on the State of the World's Plant Genetic Resources and prepared a Global Plan of Action on Plant Genetic Resources that was adopted by 150 governments at an International Technical Conference in Leipzig, Germany in June 1996. It has developed two other key components of the Global System: the World Information and Early Warning System and the International Network of *Ex Situ* Collections under the Auspices of FAO.

FAO is also leading development of the Global Strategy for the Management of Farm Animal Genetic Resources. Work began with a survey in 180 countries that highlighted the gravity of genetic erosion during the second half of the century. The strategy will comprise a country-based structure of focal points, stakeholder networks and FAO's country-secure Domestic Animal Diversity Information System. Also planned are a wide-ranging technical programme, intergovernmental dialogue and policy-making, and expert development of modalities for implementation.

In the fisheries sector, FAO identified in 1992 priorities and general principles for the sustainable use and conservation of the genetic diversity of both natural and farmed aquatic species. Plans of action and guidelines were developed in 1995 through the FAO/Japan Kyoto Conference on the Sustainable Contribution of Fisheries to Food Security, and the FAO Code of Conduct for Responsible Fisheries. With Sweden, FAO also elaborated guidelines for the application of the "precautionary approach" to fisheries management and the use of introduced species.

In the area of forestry genetic resources, FAO co-sponsored in 1995 a series of separate international workshops on boreal zone, North American temperate and European forest genetic resources which could serve as possible "models" for similar discussions in other ecological regions in 1998. Its Committee on Forestry in March 1997 discussed the need to prepare a global plan of action on forest genetic resources, aimed at facilitating a country-driven process to strengthen and streamline future activities in the field. There was no consensus, but some countries considered that FAO should pursue efforts to develop regional plans as a first step to develop a global plan of action.

(This information courtesy FAO at:
http://www.fao.org/WAICENT/FAOINFO/SUSTDEV/EPdirect/EPre0028.htm.)

Reviews

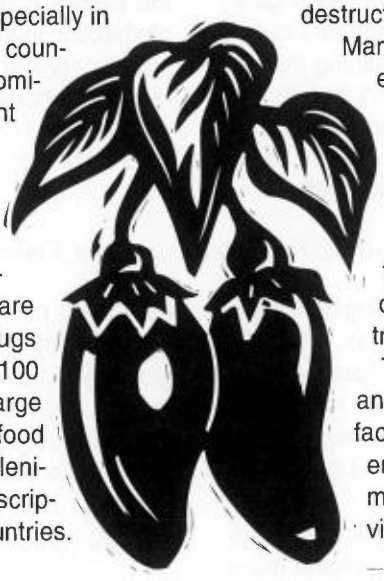
Indian medicinal and aromatic plants facing genetic erosion

Kumar, J. Singh, N.C. Shah and V. Rajan

Hardback. ISBN 81-86943-30-3. Price N/A. 219 pages. Central Institute of Medicinal and Aromatic Plants, Lucknow, India

Plants have been a major source of therapeutic agents for the alleviation or cure of human diseases since time immemorial. The indigenous, traditional or folk medicines have evolved in all the societies in close association with the culture and tradition of human civilization. These traditional systems are playing a major role in the primary medicinal and health care programmes, especially in the developing countries. In the developed countries, where the drugs of synthetic origin dominate the pharmaceutical market, the plant products still represent an important source of prescription drugs.

Plant medicines are proving to be highly effective in treating age-related diseases, including cancer, cardiovascular disorders and dementia. At present, there are about 125 clinically useful prescription drugs worldwide that are derived from around 100 species of higher plants. In addition, a large number of herbal remedies and health food additives involving plant extracts and galenicals are sold as over the counter, non-prescription items, especially in the developed countries.



Bioprospecting of plant species is in progress in many laboratories for the discovery of new pharmaceuticals. Several new plant-based drugs are expected to reach the market in the next few decades. Medicinal plants have been indiscriminately exploited for short-term gains in addition to the loss of biodiversity arising from the extensive destruction of the plant habitats.

Many valuable plants have reached the level of extinction. This book looks at 65 species in India, and presents a systematic account for each, including its botanical name and family, common and trade names, a brief botanical description, flowering and fruiting times, the distribution of the plant in the country on the basis of literature and herbarium collections and the distribution in other countries.

The book is timely as a variety of medicinal and aromatic plants of commercial importance face extinction or severe genetic loss. Too much emphasis has been given to the potential of medicinal and aromatic plant species to provide new drugs and other compounds of com-

mercial interest, yet little or no concerted efforts have been made to conserve most such endangered or threatened species.

Several national and international agencies have taken the lead in and formulating policies and strategies appropriate for conservation of the medicinal and aromatic plants.

The article 7 of the Convention obliges each party to identify components of biological diversity important for their conservation and sustainable use.

The forty-first World Health Assembly called for the establishment of a basis for the conservation of medicinal plants, to ensure that adequate quantities of them are available for future generations. This placed the medicinal plants, their rational and sustainable use and their conservation, firmly in the arena of public health policy and concern. This book is a creditable effort to contribute some fundamental information to the debate.

Amazonia: the fantastic world of the Amazon

By Jorge R. Bodanzky
1996. CD-ROM. UNESCO, Paris.

This CD-ROM (in Spanish, English and Portuguese) sets out to give children an idea of the environment of the Amazon, using photographs, video, sound and words. After a simple 5-minute installation one soon arrives at a colourful view of a jungle scene, with various animals hidden amid the foliage. Clicking on these takes the viewer to a larger photograph and a screen of information. There is a continuous, mysterious soundtrack, sometimes of bird and animal calls, sometimes ethnic rhythms, and sometimes an unidentifiable miasma. Nevertheless, it serves to convey an atmosphere quite effectively. I imagine that any curious, computer-literate child will quickly navigate around the landscape and start exploring the hidden realms of the rain forest. The disc does not actually address 'biodiversity', and only nine entries were listed under 'Flora' in the index, but the paper insert does say that the disc is an introduction to a future 'Encyclopaedia of the Amazon', which, judging from the list of sponsors and contributors, should be very interesting. If you are tired of seeing your children playing violent computer games, then this disc might just be the diversion they need.

(Minimum configuration PC486DX, 50 MHz, 8 MB RAM, 10 MB disk space, Windows 3.1 or above.)

Botanic Gardens in a Changing World

by Christopher Hobson, editor
1996. Electronic. ISBN 0 9520275 3 4. Botanic Garden Conservation International, Richmond, UK

The Third International Botanic Gardens Conservation Congress was held in Rio de Janerio in 1992. The Conference itself yielded much important and interesting information on the role of botanic gardens in global change, genetic resource conservation, horticulture and habitat restoration. However, in an all too common scenario, the BGCI found themselves without funds to publish the proceedings in book form. Undefeated, the group found volunteers to edit and produce the almost 50 papers, and is issuing them in three ways. The first takes the form of two 1.44 MB diskettes with all the papers in Word 6.0 for Windows format, which costs $14 (free to members). Members of BGCI without access to a computer can request paper copies of the material, which will presumably be photocopied. The third form will be via the Internet, on **http:\\www.rbgkew.org.uk/BCGI**. A few copies will be bound and sent to libraries as proof of publication, so that the papers can be cited and mentioned in indexing and abstracting journals. BGCI is to be commended in developing an original dissemination strategy in the face of a resource shortage, to make this valuable material available.

Etymological Dictionary of Grasses

H.T. Clifford
1996. CD-ROM. ISBN 30540-14543-5. $39. UNESCO Publishing, Paris, France

Many scientific terms that are being used are derived from Latin and Greek words. This CD-ROM is the product of 8 years' research into the etymology of all the terms used for naming grasses. The result is an elaborate interactive glossary of more than 13 000 terms and their explanations.

The main part of the work is an interactive dictionary that contains more than 9100 names, thoroughly led back to their roots and explained by a leading authority in the field. An illustrated glossary of basic grass terminology is included. The introduction provides in-depth information on the naming and taxonomy of grasses. A quiz, to test the user's knowledge, adds to the value of the disk for teaching purposes. This CD-ROM is a must for scientists and students and all working in, and concerned with, herbaria.

Information resources on the Internet

The Internet is a vast collection of information, which has been described as a huge library with all the books thrown on the floor. This makes it difficult to obtain information quickly, even with the increasingly sophisticated search engines and web crawlers that are available. Below are listed some interesting addresses related to genetic resources, botany, etc. Please send information on other interesting sites via Email to the Managing Editor of the *Newsletter* (p.stapleton@cgnet.com).

The addresses given here were operating at the time of this issue of the *Newsletter* going to press (September 1997), but as the Internet is a volatile medium, they may not continue to be accurate.

Internet Directory for Botany

An interesting and useful site with mirror sites all over the world. The Internet Directory of Botany is an index to botanical information available on the Internet, compiled by Anthony R. Brach (Harvard University Herbarium, Cambridge/Missouri Botanical Garden, St. Louis, USA), Raino Lampinen (Botanical Museum, Finnish Museum of Natural History, University of Helsinki, Finland), Shunguo Liu (G.F Ledingham Herbarium, University of Regina, Canada) and Keith McCree (Oakridge, Oregon). It consists of two parts, an alphabetical directory and a categorical directory. The alphabetical list was compiled by Anthony R. Brach. It was originally posted on TAXACOM in March 1995. The address is:

http://www.biol.uregina.ca/liu/bio/idb.shtml

Environment News Service

This page provides an international daily wire service of the environment, offering original breaking news plus a comprehensive international events calendar, and a weekly column of opinion. This service provides articles such as: "Genetically altered agricultural products are proven safe, and the United States will continue to press for market access for these foods, U.S. Agriculture Secretary Dan Glickman told the International Grains Council yesterday." at:

http://www.envirolink.org/environews/ens/

Agricultural Research Organizations on the Web

This directory is managed by the International Service for National Agricultural Research (ISNAR). It provides a worldwide directory of organizations and universities working in agricultural research that have a home page on the Web. Currently it holds more than 600 entries in 90 different countries, subdivided by region, at:

http://www.cgiar.org/isnar/arow/arowintr.htm

Global State of the Environment Report 1997

The United Nations Environment Programme recently released this report, the first issue of the biennial Global Environmental Outlook, which is "a snap-shot of an ongoing worldwide environmental assessment process." The executive summary provides a global overview, regional status and trends, regional policy responses, "looking to the future, and the way ahead." The complete report is available online as several HTML files; the addition of a compressed file which could be downloaded would be a nice customer feature. The material is mirrored at different sites around the world. See:

http://grid2.cr.usgs.gov/geo1/

eNetDigest

This site provides a bimonthly electronic guide to environmental, natural resources and agricultural Internet resources from around the world. Sites reviewed include organizations as well as scientific, governmental and political resources. Subscription instructions: to receive the electronic mail version of eNetDigest each week, join the eNetDigest mailing list. We do not distribute your E-mail address to others. Currently the list is maintained manually. For information: send mail to:info@enetdigest.com, and in the subject line, type 'info'. To subscribe: send mail to:subscribe@enetdigest.com, in the subject of the message, type 'subscribe', in the body of the message type: subscribe eNetDigest your E-mail address. Include title and affiliation for the database or complete the form at

http://www.enetdigest.com/index.html

Plant Breeding News

This electronic publication is not meant to be an outlet for research papers. It is for news and discussion of important results, methods, information, inquiries, etc. There are now about 800 subscribers, and the first issue is available by Emailing Plant_Breeding_News@cornell.edu, or PBN-L@MAILSERV.FAO.ORG for extensive discussion and information exchange. For example, one article is entitled: 'Privatization of Crop Seeds: the public aspect', by: Claude André St-Pierre, Andre Comeau and Benoit Gauthier. This is a summary of an article to be published in French and English in Global Biodiversity, Vol. 7(1) in June, 1997.

International Centre for Genetic Engineering and Biotechnology

ICGEB is an international organization established to promote the safe use of biotechnology worldwide and with special regard to the needs of the developing world. ICGEB aims to be a research and training centre of excellence for its member countries, helping developing nations to help themselves and holding out the prospect of advancing knowledge and applying the latest biotechnological techniques in public health, nutrition, agriculture, industrial production of high added-value commodities, environmental protection, remediation and energy-saving. See:
http://www.icgeb.trieste.it/

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. Manuscripts submitted for publication and other communications on editorial matters should be addressed to IPGRI's Editorial and Publications Unit.

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 des Nations Unies pour l'alimentation et 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. Prière d'adresser les manuscrits présentés pour être publiés et d'autres communications sur des questions de rédaction au Bureau de rédaction de l'IPGRI.

Boletín 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, fruto de nuevos trabajos, estudios históricos, revisiones y análisis críticos relacionados con la diversidad genética, investigaciones etnobotánicas y ecogeográficas, estudios de herbarios, actividades 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 volumen anual compuesto por cuatro números, que se publican en marzo, junio, septiembre y diciembre. Se distribuye gratuitamente a las bibliotecas de bancos de germoplasma, facultades universitarias y servicios gubernamentales, centros de investigación, etc. que se interesan en los recursos fitogenéticos. También pueden obtener este noticiario las personas que demuestren necesitar una copia personal.

Tipos de documentos

Artículos: Los artículos divulgarán los resultados de trabajos nuevos y originales que contribuyan de modo importante al conocimiento del tema tratado. Dichos artículos, que deberán tener una longitud razonable, serán examinados por el Comité de Redacción en cuanto a su pertinencia e idoneidad y posteriormente un experto juzgará su contenido y validez científicos.

Comunicaciones breves: Las comunicaciones breves informarán de modo conciso sobre los resultados de trabajos 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 a través del correo electrónico. La versión final debe presentarse como un archivo de correo electrónico o en disquete compatible con el sistema operativo MS-DOS. Los manuscritos para publicar y otras comunicaciones sobre asuntos relativos a la redacción deberán dirigirse a la Oficina de Redacción del IPGRI.

Plant Genetic Resources Newsletter

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