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INTERNATIONAL CROP NETWORK SERIES. 5

REPORT OF AN **IBPGR** 
INTERNATIONAL
WORKSHOP ON
OKRA
GENETIC
RESOURCES

held at the
National Bureau for
Plant Genetic Resources
New Delhi, India
8-12 October 1990

**INTERNATIONAL
BOARD FOR
PLANT
GENETIC
RESOURCES**

November 1991

INTERNATIONAL BOARD FOR PLANT GENETIC RESOURCES

**REPORT
OF AN INTERNATIONAL WORKSHOP ON OKRA GENETIC RESOURCES**

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**National Bureau for Plant Genetic Resources (NBPGR)
New Delhi, India**

8-12 October 1990

**IBPGR
Rome, 1991**

The International Board for Plant Genetic Resources (IBPGR) is an autonomous international scientific organization under the aegis of the Consultative Group on International Research (CGIAR). The basic function of IBPGR is to foster the collecting, conservation, documentation, evaluation and use of plant germplasm and thereby contribute to raising the standard of living and welfare of people throughout the world. Financial support for the core programme is provided by the Governments of Australia, Austria, Belgium, Canada, China, Denmark, France, Germany, India, Italy, Japan, the Netherlands, Norway, Spain, Sweden, Switzerland, the UK, the USA and the World Bank

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INTRODUCTION

An international workshop on okra genetic resources was convened by IBPGR at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India, from 8 to 12 October 1990, with the objective of encouraging the establishment of an okra genetic resources network. Dr. R.S. Rana, Director of NBPGR, acted as the Chairman. A list of participants is provided in Appendix I.

Dr. R.S. Paroda, Deputy Director-General (Crop Sciences) of the Indian Council for Agricultural Research (ICAR), delivered the inaugural speech and Mr. O.T. Hughes, on behalf of Dr. Axinn, FAO Representative in India and Bhutan, presented the Chairman's remarks.

The Agenda, as approved by the participants, is provided in Appendix II. Participants had the opportunity to visit NBPGR, and subsequently the core collection of okra in the field, on the afternoon of 10 October.

Lead papers were presented which initiated discussions on various aspects of okra genetic resources and these discussions lead to the formation of four Working Groups.

On Friday 12 October the participants considered the recommendations of each Working Group and the final discussions and agreements are reflected in the following report.

Dr. K.L. Chadha, Deputy Director-General (Horticulture) of ICAR, addressed the participants at the Closing Session and gave his concluding remarks.

REPORT

1. Taxonomy

Taking the classification of VAN BORSSUM WAALKES (1966) as the starting point, the Group is of the opinion that available cytogenetical evidence justifies the following amendments:

Classification developed by VAN BORSSUM WAALKÉS (1966)	Classification adopted by International Okra Workshop (1990)
<i>A. moschatus</i> Medikus	<i>A. moschatus</i> Medikus
- subsp. <i>moschatus</i> var. <i>moschatus</i>	- subsp. <i>moschatus</i> var. <i>moschatus</i>
- subsp. <i>moschatus</i> var. <i>betulifolius</i> (Mast.) Hochr.	- subsp. <i>moschatus</i> var. <i>betulifolius</i> (Mast.) Hochr.
- subsp. <i>biakensis</i> (Hochr.) Borss.	- subsp. <i>biakensis</i> (Hochr.) Borss.
- subsp. <i>tuberosus</i> (Span.) Borss.	- subsp. <i>tuberosus</i> (Span.) Borss.
<i>A. manihot</i> (L.) Medikus	<i>A. manihot</i> (L.) Medikus
- subsp. <i>manihot</i>	
- subsp. <i>tetraphyllus</i> (Roxb.ex Hornem.) Borss. var. <i>tetraphyllus</i>	<i>A. tetraphyllus</i> (Roxb.ex Hornem.) R. Graham var. <i>tetraphyllus</i>
- subsp. <i>tetraphyllus</i> var. <i>pungens</i> (Roxb.) Hochr.	- var. <i>pungens</i> (Roxb.) Hochr.
<i>A. esculentus</i> (L.) Moench (including <i>A. tuberculatus</i> Pal & Singh)	<i>A. esculentus</i> (L.) Moench <i>A. tuberculatus</i> Pal & Singh
<i>A. ficulneus</i> (L.) W. & A. ex Wight	<i>A. ficulneus</i> (L.) W. & A. ex Wight
<i>A. crinitus</i> Wall.	<i>A. crinitus</i> Wall.
<i>A. angulosus</i> Wall. ex. W. & A.	<i>A. angulosus</i> Wall. ex. W. & A. <i>A. caillei</i> (A. Chev.) Stevels

It was noted that the previous denominations correspond to the following species:

- * 'Guinean' type of okra = *A. caillei*
- * 'Soudanian' type of okra = *A. esculentus*
- * *A. manihot* var. *caillei* A. Chev. = *A. caillei*
- * *A. caillei* was identified wrongly earlier as *A. manihot* subsp. *manihot* (Flora of Tropical West Africa, Kew Botanic Gardens)

The adoption of this new classification requires the amendment of the determination key of *Abelmoschus* to accommodate the distinction between *A. esculentus* and *A. tuberculatus* as well as the distinction between *A. manihot*/*A. tetraphyllus* and *A. caillei*. To this end the existing botanical descriptors (*A. tuberculatus*, *A. manihot* and *A. tetraphyllus*) need to be compared with the variation in the accessions of the global base collection and other existing collections (and amended whenever possible).

The intraspecific classifications in *A. moschatum* (subsp. *moschatum*, *biakensis* and *tuberosum*), *A. tetraphyllum* (varieties *tetraphyllum* and *pungens*), *A. esculentum* (reported chromosome - race $2n = 62$), *A. angulosum* (purple and yellow flowered types) should receive further attention.

The workshop recommended the preparation and application of a workable determination key for the genus *Abelmoschus* in cooperation with those responsible for national and base collections.

2. Collecting priorities

A. esculentum, *A. tuberculatus*, *A. ficulneus*, *A. tetraphyllum* and *A. caillei* are fairly well represented in existing germplasm collections, however, there is a shortage of *A. manihot*, *A. crinitus*, *A. angulosum* and *A. moschatum*. Therefore, further increase in the representation of species in national and global base collections was recommended with the following priorities:

Species priority	Area priority	
	1	2
1. <i>A. manihot</i> <i>A. moschatum</i> <i>A. angulosum</i> <i>A. crinitus</i>	Indonesia, PNG, Pacific Indonesia, PNG, Pacific India, Sri Lanka, Java India, southern China (Hainan)	Other areas Other areas Rest of Asia Rest of Asia
2. <i>A. ficulneus</i> <i>A. tetraphyllum</i> <i>A. esculentum</i> <i>A. tuberculatus</i> <i>A. caillei</i>	East Africa, Australia Philippines, east Indonesia Northeast Africa --- ---	Asia Rest of Asia Other areas India Central Africa

The workshop recognized that detailed cytogenetical and biochemical analyses are required for all nine species to elucidate further their taxonomic status and species relationships. It recommends basic studies on cytogenetic and biochemical aspects in order to understand the diversity in nine species which is or will be represented in the global base collection. These studies will verify the taxonomy and elucidate species relationships by determining ploidy levels, crossability and genetic diversity, which will contribute to more effective utilization of gene pools.

One Group also felt that genetic diversity studies using morphometric, electrophoretic and possibly molecular techniques in cultivated okra would generate information on the different common okra accessions and on the genetic structure of the populations.

3. Collecting strategies

Participants, while endorsing the general guidelines as given below, emphasized that no recommendation can substitute for the judgement and adaptation of the experienced okra collector to specific field conditions. Some examples of those specific conditions are provided in the notes on collecting, after the main recommendations.

3000 to 5000 seeds from about 100 healthy-looking plants should be collected whenever possible for cultivated types and as many as possible for wild taxa.

Generally, random sampling should be carried out and if necessary, the collector may decide to make a separate collection of plants selected for distinct characters. This sample should be assigned a separate collection number. The number of such distinct samples would depend on the heterogeneity of the material.

As far as possible, okra must be collected during crop-specific expeditions. Choice of sampling sites within a species should be based on the observed heterogeneity of the material. If the population is homogeneous, then the samples should be pooled from different fields and/or backyards at the site of collection. Decisions regarding sampling site and collection must be based on ecological factors, the farming system and on tribal and ethnic factors.

Thin cotton bags should be used for the samples, and as far as possible, fruits should be collected and maintained as such until proper identification. Almost all the passport data should be recorded at the site of collection.

The bulk of the original seed collected should be deposited in long-term storage and used for regeneration only, as long as its viability remains at the appropriate level. If the original sample is small, the first regenerated material with high viability should be deposited in the base collection and a part of this sent to an active collection.

When a national programme does not have a long-term storage facility, arrangements have to be made for depositing the material in the designated base collections. Another set should be sent to the nearest active collection.

4. Storage

The workshop discussed storage problems of okra germplasm seed and participants agreed to the following general guidelines.

All efforts must be made to keep the time lag between collecting and storage of seed as short as possible.

Before seed is prepared for germination tests, all measures needed to break dormancy and/or hard-seededness should be undertaken so as to obtain a truer picture of the viability of the conserved okra seed.

Research should be initiated on the factors contributing to dormancy and hard-seededness in okra seed for long-term storage.

Participants further suggested that long-term conservation should involve both low and ultralow temperature conditions including *in vitro* conservation of vegetatively propagated species, e.g. 'Aibika'.

The Group endorses the existing structure of a base collection in NBPGR, India, and a safety duplication base centre in the USA.

5. Active collections and regeneration

The workshop considered various aspects of active collections and problems of regeneration of rare okra germplasm seed and reached the following conclusions.

The workshop proposed that the following four countries play a leading role as active collections: India, Côte d'Ivoire, the Philippines and Brazil. It was noted that all national programmes holding okra germplasm have the responsibility to regenerate, evaluate and distribute okra germplasm.

The West African collection is one of the most valuable in the system and therefore participants called for international support to regenerate this material. If, for any reason, such international support could not be obtained, the network should identify one or two cooperating centres with the necessary infrastructure (manpower and facilities) for regeneration and storage of this material.

When small samples are collected, the collectors or the sponsoring institute should make arrangements for immediate regeneration of the material within the country of collection or in a country in the region with similar agro-ecological conditions, where facilities to do so exist. Then this material should be deposited at the base collection centre. If any major problems exist in following the above procedures, the network may approach international agencies for help.

Participants called for the active collaboration of all national programmes, including those not present at the workshop, e.g. PNG and USA, with the proposed guideline above.

It was hoped that the active collection with special responsibilities, as mentioned above, or alternatively other active collections, would be able to help with regeneration of material from national programmes which are faced with serious constraints.

The workshop considered the strategies on regeneration as presented in Appendix IV (Regeneration of okra germplasm: V.R. Rao). It recommended that IBPGR finalize, in consultation with the participants of the network, the guidelines for regeneration of okra germplasm seeds for publication and distribution.

6. Descriptors

The Working Group on descriptors and utilization reviewed carefully the IBPGR okra descriptors and, using all the available expertise, proposed several amendments, e.g. reduction of number of descriptor states, or additional parameters for better measurement of quantitative descriptors.

In addition, each descriptor was assigned a priority level (P₁: high priority; P₂: second priority and P₃: third priority) for observation and documentation in an international database.

The workshop recommended that this modified version be circulated by IBPGR for further comment and publication of a revised okra descriptor list. This list will form the basic reference for exchange of data between participants of the network.

7. Utilization

Detailed evaluation of the available germplasm, including wild species, should be carried out by a nodal agency such as NBPGR. Such material may then be distributed at the international level among the participating countries, for multilocational testing and for further crop improvement work.

Feedback from multilocational testing or information on varieties developed in the breeding programmes should be sent regularly to the nodal agency for compilation and distribution to all cooperating/participating institutions.

For effective evaluation by the nodal agency it was suggested that national programmes with large collections of okra germplasm (if they have evaluated their germplasm) should develop a core collection representing the range of variability within their collection. The core collection can then be sent to the nodal agency and to other interested institutions for detailed evaluation and utilization.

For the immediate evaluation of germplasm for economic characters (including biotic and abiotic stresses), the network, with the assistance of IBPGR, should mobilize funding for the nodal agency and 'hotspot' centres.

8. The *modus operandi* of an okra network

Participants emphasized that the costly activities of collecting, documenting, conserving, regenerating and evaluating germplasm can most effectively and economically be performed through collaborative efforts. Countries interested in okra genetic resources were therefore called upon to participate in the activities agreed during the workshop.

Participants recognized that a central database to collate, analyze and disseminate information is an essential requirement for collaborative activities and recommended that an International Data Base for Okra be established in NBPGR with the help, as required, of the IBPGR Regional Office for South and Southeast Asia.

The workshop also agreed that further mechanisms should be established to follow up activities as recommended and to stimulate the building up of a real network.

Two such mechanisms were considered:

- i) the establishment of a coordinating body, the number and status of whose members (e.g. official country representatives, or okra experts acting in an individual capacity) remain to be decided;
- ii) the use of IBPGR Regional Coordinators to follow up workshop recommendations and stimulate liaison of the specialists from their regions with the International Data Base for Okra.

Participants trusted that IBPGR and the International Data Base for Okra (IDBO) would develop the most adequate mechanisms to ensure the future of an okra network.

It was agreed that within the limits of their capabilities and that of their organizations, members should contribute in the following ways:

1. by sharing the data in the IDBO
2. by sharing freely the available okra genetic resources
3. by regenerating, to whatever extent possible, okra germplasm originating in their countries
4. by collaborating in activities of mutual interest which are not within the capabilities of any one national programme alone
5. by the IDBO supplying all available information to all members for further updating

9. Funding

Members are already involved in the expenditure of funds within the framework of their national programmes. The operation of the network should not be regarded necessarily as an additional financial commitment but rather as a means of maximum return on resources which are already committed. However, as the network develops, national programme resources greater than those presently available will become necessary. It is expected that these resources should be sought firstly by the coordinating body from national funding agencies and, if necessary, from regional or international organizations. Although IBPGR will not have the resources to provide significant financial support to the national activities, it will be instrumental in solving bottleneck problems which would impede the activities of the network. IBPGR is eager to provide technical support and encouragement to the okra network and, when unable to provide direct support, it will seek funds from other sources.

SUMMARY OF MAIN RECOMMENDATIONS

Members recommend:

- i) that a workable determination key for the genus *Abelmoschus* be prepared and used for testing;
- ii) that representation of wild species in collections be further enriched according to the agreed priorities;
- iii) that basic studies on all nine species be promoted to deepen knowledge of the genepool and contribute to its more effective utilization;
- iv) deposition of original seeds in long-term storage for later regeneration (or when not possible, seeds of the first regeneration);
- v) that research on factors contributing to dormancy and hard-seededness of okra seeds be undertaken;
- vi) that four national programmes, namely India, Côte d'Ivoire, the Philippines and Brazil, assume a leading role for their regions and provide assistance to other national programmes for regeneration of their okra germplasm when necessary;
- vii) international support for the maintenance of the West African collection which is held in IDESSA, Bouaké, Côte d'Ivoire;
- viii) that IBPGR finalize guidelines for okra regeneration;
- ix) that IBPGR publish a revised list of okra descriptors to be used as a format for international exchange of data;
- x) full evaluation of the available germplasm, selection of useful material for multilocational testing and distribution of information/germplasm to all participating institutions;
- xi) that each active collection having large numbers of okra accessions develop a core collection for further detailed evaluation within the network;
- xii) implementation of an International Data Base for Okra in NBPGR with the help of the IBPGR Regional Office for South and Southeast Asia.
- xiii) that mechanisms be further developed to ensure the development of an okra network.

Additional notes on collecting

India

Okra is cultivated throughout India, but mostly on a small scale in backyards and kitchen gardens. Because of this limitation, it may not be possible to collect the desired sample size from most areas of the country. A few fruits collected from different fields or farmers' stores may, if they are homogeneous, be pooled as a common accession for a site. However, efforts should be made to gather as many fruits as possible from each site.

There are a large number of ecological regions where important variability occurs for both the cultivated and wild types/species. There is an urgent need to collect this germplasm and fruits should be pooled within the species per site. In India, there are regions where different *Abelmoschus* species are concentrated: for example, *A. angulosus* in the southern hills, especially those of Nilgiri and Palni, *A. tuberculatus* in the northern and northwestern plains down to central India, ssp. *tetraphyllus* in the northwest and *A. moschatus* in southern and northeastern regions.

West Africa

Collecting from markets should, if possible, be avoided. In the field, a random sample may be taken (one fruit per plant) from ten plants and/or 3000 seeds per sample if the fruits from different plants can be mixed. Several single-plant samples (five to ten fruits per plant) may also be taken.

In the village farm store, seeds are rarely conserved and in most cases (99.9%) entire fruits are stored by the farmers. Under these conditions, the collector should ask for the vernacular names and a translation of these. The fruits should then be grouped together with the assistance of the farmers, who are usually women. It is a good idea to try to obtain a description of the plant corresponding to a given sample. The reliability of such descriptions should be tested. Different types of *A. esculentus* may then be put together, as may be different types of *A. caillei*. Apparently similar varieties may also be mixed.

If the information is reliable, samples are treated according to the farmers' information and if not, personal judgement should be used to either pool the samples or keep them separate. Samples should be constructed using the farmers' experience and the collector's knowledge.

A. esculentus and *A. caillei* should always be kept distinct and separate.

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Plant Quarantine Division

Sh. Ram Nath
Plant Quarantine Division

Dr. Bhag Singh
Evaluation Division

PROGRAMME

Inaugural Session

Welcome - Dr. R.S. Rana, Director, NBPGR and Dr. J.M.M. Engels, IBPGR Coordinator for S. & S.E. Asia

Introductory remarks - Mr. P.M. Perret, IBPGR Crop Networks Coordinator

Inaugural address - Dr. R.S. Paroda, DDG(CS), ICAR

Chairman's remarks - Mr. G.H. Axinn, FAO Representative to India and Bhutan

Vote of thanks - Dr. R.K. Arora, IBPGR Associate Coordinator for South Asia

Session I - Workshop Agenda and country reports

Chairman: Dr. R.S. Rana
Rapporteur: Dr. V.R. Rao

Proposal and adoption of Agenda

Country reports

Discussion

Session II - Okra gene pools

Chairman: Dr. R.S. Rana
Co-chairman: Dr. J.S. Siemonsma
Rapporteur: Dr. R.K. Arora

Taxonomic review of the genus *Abelmoschus* - Dr. J.S. Siemonsma

Discussion

Knowledge on the distribution of wild species - Ir. J.H.J. Vredebrecht

Discussion

Core collections - Dr. S. Hamon

Discussion

Session III - Evaluation, documentation and utilization of germplasm collections

Chairman: Dr. R.S. Rana
Co-chairman: Dr. V.R. Rao
Rapporteur: Dr. M.N. Koppur

Network of base collections - P.M. Perret

Discussion

Okra descriptors - Dr. S. Hamon

Discussion

Evaluation and cataloguing - Dr. T.A. Thomas

Discussion

Okra database - Dr. J.M.M. Engels

Discussion

Germplasm utilization - Dr. Y.S. Nerkar

Germplasm utilization - Dr. O.P. Dutta

Discussion

Field visit to PEQN (Okra collection) - Dr. T.A. Thomas and Bhag Singh

Session IV - Conservation and regeneration of collections

Medium and long-term storage of okra seeds - Dr. P.P. Khanna

New approaches to conservation techniques - Dr. K.P.S. Chandel

Regeneration problems in okra - Dr. V.R. Rao

Exchange of germplasm plant quarantine - Dr. Ram Nath

Discussion

Working group meetings

NBPGR laboratories and okra field - Dr. T.A. Thomas

Working Group meetings and preparation of the report

Session V - Working Group reports

Chairman: Dr. R.S. Rana

Co-chairman: Mr. P.M. Perret

Rapporteur: Dr. V.K. Mathur

Joint meeting: Presentation of reports by Working Groups

Finalization and recommendations

Session VI - Concluding session

Resume and overview - Dr. R.S. Rana, Chairman, Organizing Committee

Remarks - Dr. J.M.M. Engels

Presentation of the recommendations developed by the Working Groups - Dr. J.S. Siemonsma, Dr. S. Hamon, Dr. V.R. Rao and Mr. P.M. Perret

Concluding address - Dr. K.L. Chada, Deputy Director-General, ICAR

Vote of thanks - Mr. P.M. Perret

COUNTRY REPORTS

Conservation and utilization of okra genetic resources in Sri Lanka

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Okra (*Abelmoschus esculentus* L.) is grown as one of the major vegetable crops in Sri Lanka. Owing to its wide adaptability, it is cultivated in the various regions of the country either as a home garden crop or on a commercial scale. A wide range of varieties, mostly local selections, are cultivated by the farmers. Thus, much diversity exists for this crop in Sri Lanka.

Cultivation of okra

The total land under okra varied from 3296 ha in 1976 to 5230 ha in 1988 showing an increase of about 58.7%. The crop is cultivated during the Maha season (early October to late January) as well as during the Yala season (late March to late July). The extent of land cultivated during the Maha season and Yala season from 1976 to 1988 is shown in Fig. 1. The cultivated area is generally higher during the Maha season than during the Yala season as its cultivation during Maha is scattered throughout the country (Fig. 2). However, during the Yala season its cultivation is restricted mainly to the Wet Zone and certain parts of the Intermediate Zone (Fig. 3).

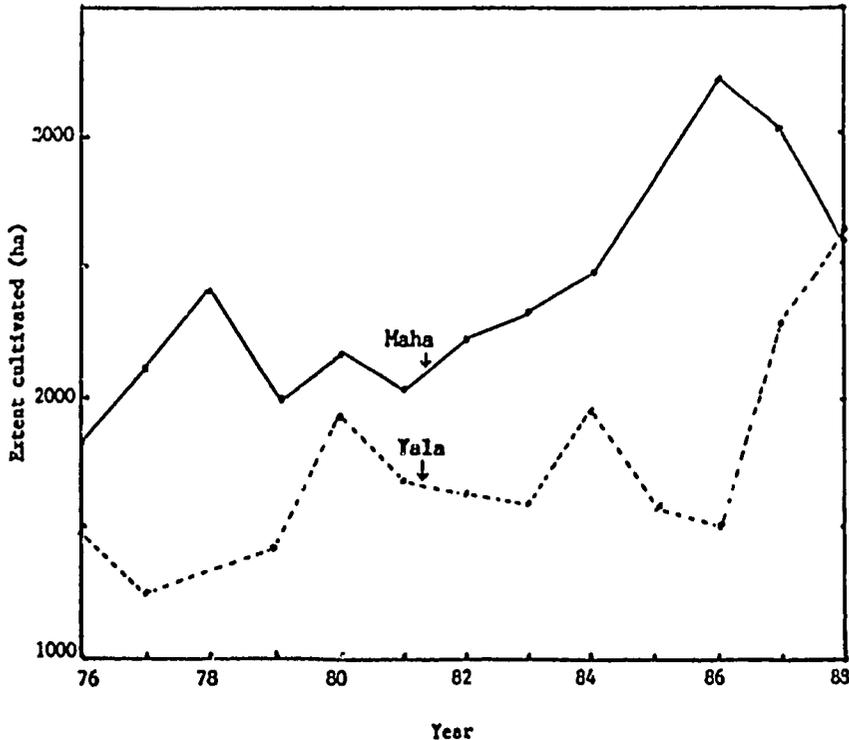


Fig. 1. Amount of land under okra in Sri Lanka, 1976-88

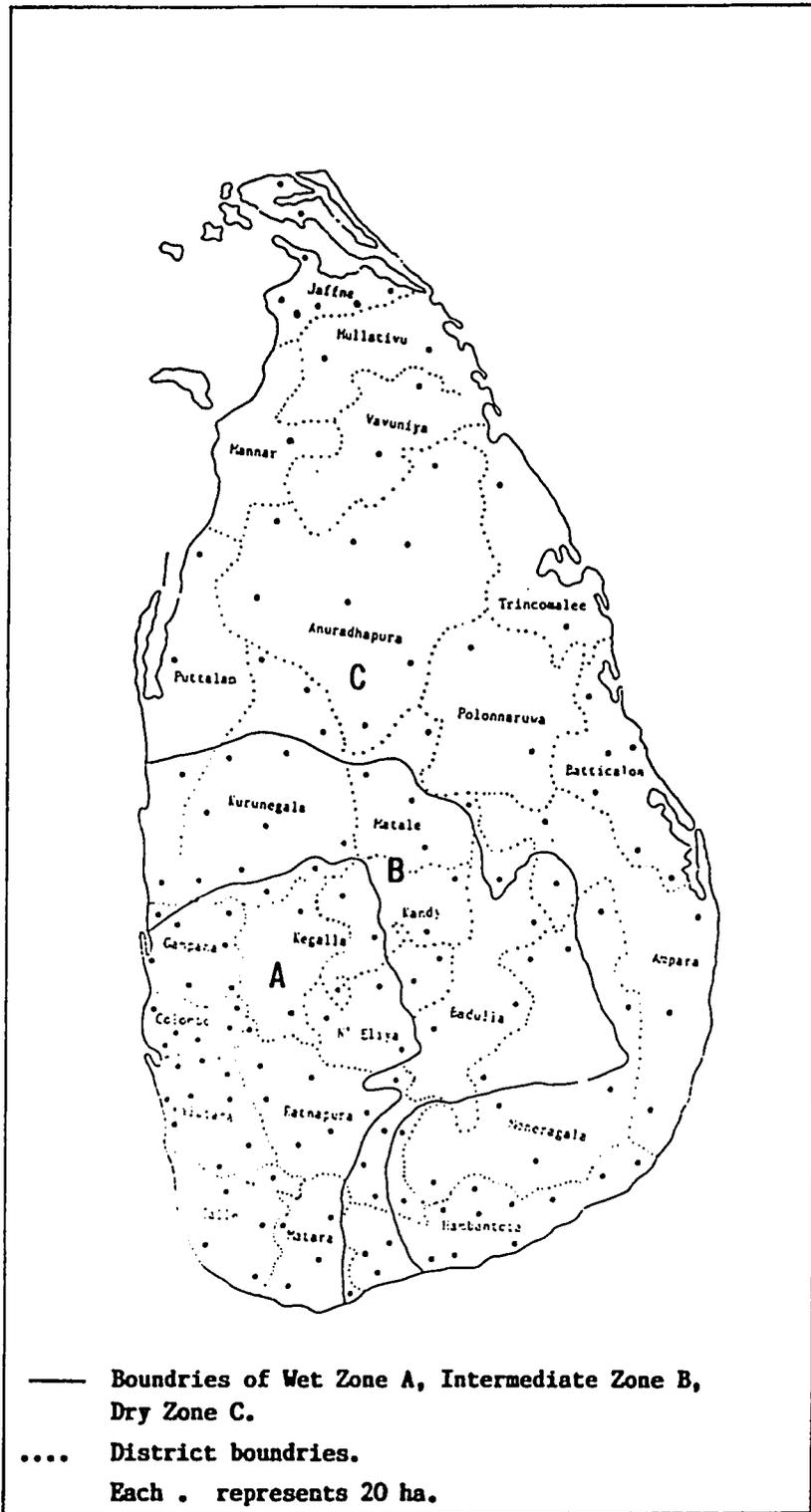


Fig. 2. Extent of okra cultivation in the Maha season (October-January)

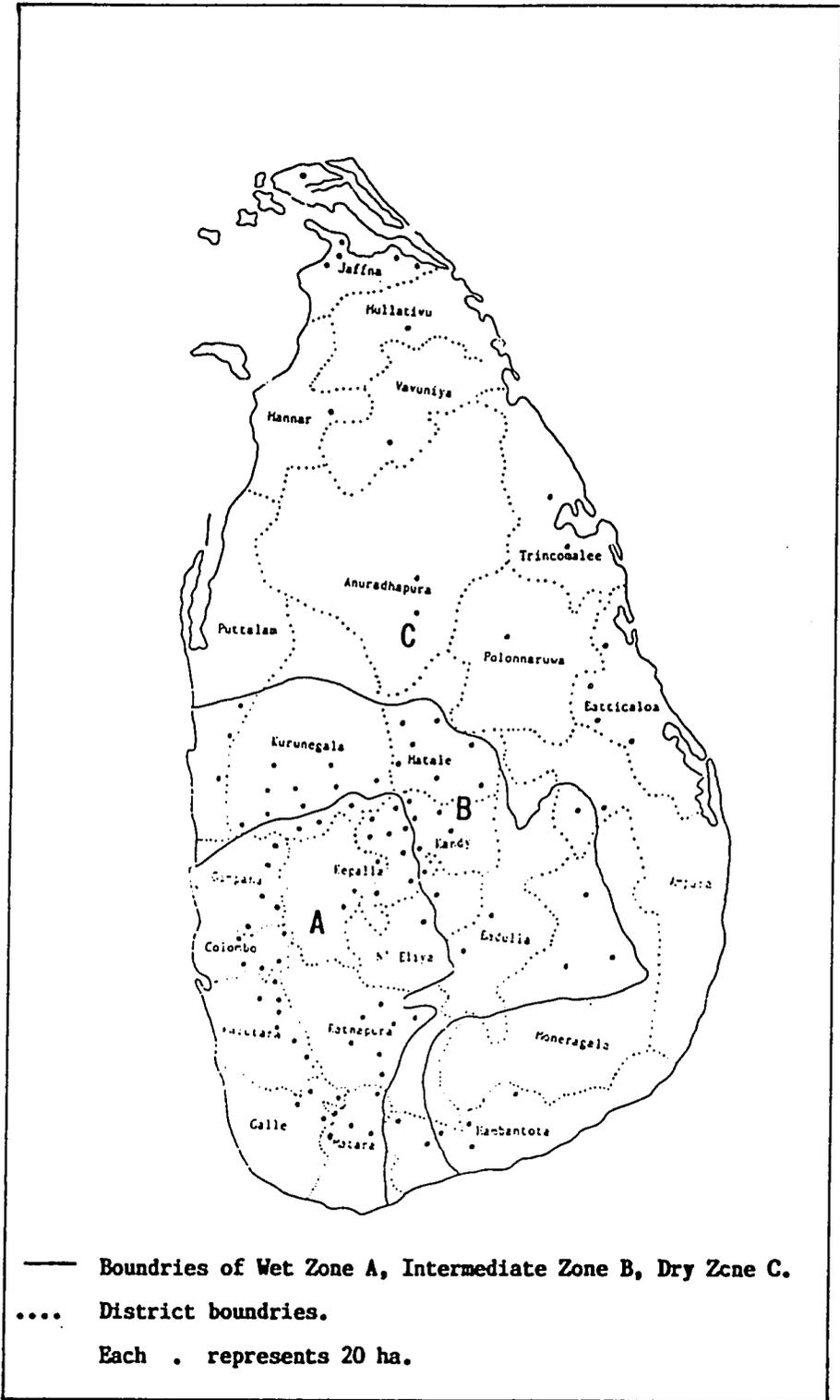


Fig. 3. Extent of okra cultivation in the Yala season (March-July)

In the Dry Zone okra is grown in the highland areas where shifting cultivation is practised. The crop is commonly grown in a mixed cropping pattern between early October to December/January. Most farmers grow the indigenous varieties from the seeds retained from the previous crop and, therefore, much diversity in okra exists in the Dry and the Intermediate Zones. In the Wet Zone the crop is cultivated on a commercial scale during both seasons either in the paddy fields which are left fallow during the Yala season or in the highland areas. However, in certain districts of the Wet and Intermediate Zones, such as Kandy and Matale, okra is mainly grown during the Yala season. In addition to the indigenous varieties, improved varieties are more widely grown in the Wet Zone.

Major problems

Major problems encountered in the production of okra in Sri Lanka are :

- Yellow vein mosaic disease affects the crop in all regions in Sri Lanka. The presently recommended varieties MI 5 and MI 7 had tolerance to the disease at the time of release but this is breaking down now.
- Lack of early maturing varieties (maturing in 75 - 90 days) to fit into the rainfall pattern of the Dry Zone as the crop is grown under rainfed conditions in this region.
- Pod borer (*Heliothis* spp.) damage.
- Susceptibility to powdery mildew (*Erysiphe cichoracearum*).

PGR activities

Germplasm collection

Breeders have collected and used local germplasm of okra in their breeding programmes. However, systematic collecting of indigenous germplasm was started only in 1986. A total of 130 accessions from fourteen districts in the main climatic zones have been collected so far. Fig. 4 shows the locations from where germplasm has been collected. However, much diversity is still left to be collected. The recently established Plant Genetic Resources Centre at Gannoruwa coordinates and organizes all exploration and collecting activities.

Three wild species of *Abelmoschus* have been reported in Sri Lanka. *A. ficulneus* is found chiefly in the drier regions, *A. moschatus* in most areas of the low country and *A. angulosus* in the mid country and lower Montane Zone. Available diversity needs to be tapped for crop improvement needs.

Germplasm multiplication and characterization

The collected germplasm samples are multiplied and characterized. During this activity, seeds are collected from selfed flowers as the crop shows a fairly high degree of outcrossing.

The standard IBPGR okra descriptor list is used in okra characterization; 27 characters are recorded for each accession and so far 47 germplasm accessions have been characterized and the data computerized.

The predominant colour of stem, fruit and leaf was green, but a few accessions were reddish or purple. Most of the accessions showed branching except a few which had a single stem. There were differences in leaf and fruit shape. Most varieties had 5-7 ridges per fruit but in a few varieties the fruit surface was smooth without ridges. The variation observed for some of the agronomic characteristics such as days to flowering, plant height, fruit length, number of seeds per fruit and 1000 seed weight is shown in Table 1.

TABLE 1. Variation for days to flowering, plant height, fruit length, seeds per fruit and 1000 seed weight in 47 indigenous accessions of okra

Character	Range	Mean	C.V. (%)
Days to flowering	45 - 83 days	60 days	13.0
Plant height	72 - 186 cm	107 cm	22.4
Fruit length	19 - 36 cm	23 cm	18.2
Seeds per fruit	50 - 116	77	16.2
1000 seed weight	53 - 76 g	65 g	15.4

Germplasm evaluation

Yellow vein mosaic is a serious disease affecting okra in Sri Lanka. Screening of germplasm collections for this disease is in progress at the Regional Agricultural Research Center in Bombuwela located in the Wet Zone. Out of 30 accessions screened under natural infection conditions in the field, 13 accessions were found to be free of disease symptoms.

Crop improvement/breeding efforts

The Regional Agricultural Research Centre at Maha Illuppallama in the Dry Zone is responsible for Crop Improvement Research on Okra. The breeding programme is aimed at developing varieties with high yield, less pubescence, improved shelf life, less mucilage content and fibre, medium tall height (100-150 cm) and resistance to powdery mildew, yellow vein mosaic virus and pod borer. Several improved varieties such as MI 5, MI 7 and MI 18 have been released by this station. Pusa Sawani, an introduction from India, which is tolerant to yellow mosaic virus, is also being grown and used in the breeding programme. Few selections have been identified having promise of moderate resistance to yellow vein mosaic disease, but these are still in the experimental stage.

Germplasm conservation

All germplasm samples collected are conserved in the Plant Genetic Resources Centre at Gannoruwa. The base collection is presently maintained at a temperature of 1°C, relative humidity 35-40% in vacuum sealed tin cans. The active collection is maintained at 5°C and relative humidity 40% in air evacuated aluminium foils. Short-term storage is maintained at 18°C and 45% relative humidity in aluminium foils.

Studies on okra germplasm in Nigeria

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Introduction

Okra is one of the most important vegetables in Nigeria and is mostly grown as a component in crop mixtures covering around 1.5 million ha of land area annually. Since the early 1970s okra germplasm collection, evaluation and utilization work has been carried out primarily at the Institute of Agricultural Research, Samaru Zaria in Northern Nigeria, the Nigerian Institute of Horticultural Research (NIHORT), Ibadan in Southern Nigeria, and at the University of Ibadan, Ibadan.

The International Institute of Tropical Agriculture (IITA) assisted NIHORT in okra germplasm collection from several West-African countries. Following initial evaluation work, while some 50-60 collections are maintained at these institutes for their breeding and agronomic research, the National Centre for Genetic Resources and Biotechnology (NACGRAB) has some 374 collections under long-term storage.

Some significant findings of the last few years of research work are summarized below.

PGR activities

A germplasm nursery comprising about 300 genotypes from 13 countries and 150 genotypes from parts of West Africa was established at the University of Ibadan. From this nursery, 296 accessions (including 81 Nigerian collections) were studied in detail and 29 cultivars observed. Numerical analysis of the variation patterns apart from revealing significant differences between the exotic and locally collected Nigerian materials, also showed that the range of variability among local accessions was higher and provided further evidence to support the assertion that West Africa is a center of diversity for okra.

Selection of promising types

Three agronomic types (A, B & C) based primarily on flowering habit and plant size were identified. Genotypes belonging to Type A were characterized by early flowering, small stature, few short branches bearing very few extra fruits and long slender fruits with a length to width ratio of 6:1 to 7:1. Genotypes belonging to Type B possessed medium flowering, fairly robust stature, significant number of well developed fruit bearing branches and short fruits with an average length width ratio ranging from 3:1 to 4:1. Type C comprised genotypes which exhibited late flowering, very robust growth habit, large number of well developed branches bearing considerable number of short fruits with length to width ratio rarely exceeding 3:1. All the cultivars with origin other than West Africa belonged to Type A while most of the local materials belonged to Type B or C. Details of characters studied for these three agronomic types are given in Table 1.

Presumably the early flowering day neutral characteristic of the introductions coupled with smaller stature is the end product of both natural and artificial selection pressure on the crop in its evolutionary history after it was domesticated and grown essentially as a sole crop. On the other hand, the medium (facultative photoperiod reaction) to late (short day plants) flowering as well as robust plant type with profuse branching of the local genotypes is a reflection of the primary role of natural selection in okra germplasm in West Africa where the plant is mostly interplanted with other crops and where furthermore for the survival and continuous production of fruits over an extended period, plant height, branching and leaf size become important. While the local genotypes are adapted to mixed cropping situations, they are relatively less productive under sole cropping than the Asiatic genotypes. Strategies for okra genetic improvement utilizing available germplasm have been formulated.

TABLE 1. Characteristics of the three agronomic types of okra

Characters	Type A	Type B	Type C
No. of days to flowering ¹	46 (38 to 52)	74 (52 to 101)	171 (110 to 193)
Flowering period (days)	34 (21 to 41)	35 (25 to 50)	27 (20 to 45) ²
Height at flowering (cm)	40 (26 to 54)	79 (54 to 112)	149 (128 to 166)
Width at flowering (cm)	61 (41 to 77)	84 (50 to 117)	65 (47 to 83)
No. of leaves at flowering	9 (7 to 16)	24 (13 to 45)	70 (41 to 87)
Petiole length of 7th leaf (cm)	21 (16 to 25)	27 (20 to 32)	33 (25 to 40)
No. of branches	5 (0 to 9)	10 (5 to 20)	12 (8 to 17)
Length of branches (cm)	54 (0 to 110)	174 (100 to 300)	180 (140 to 300)
Pedicel length (cm)	3.4 (1 to 5)	2.4 (1 to 3)	6.1 (3 to 8)
Height at maturity (cm)	54 (28 to 75)	100 (58 to 138)	154 (128 to 240)
Fruit length (cm)	13.7 (10 to 18)	9.7 (6 to 14)	8.7 (7 to 10)
Fruit width (cm)	2.2 (2.1 to 2.3)	2.9 (1.5 to 4.0)	3.1 (2 to 4)
Fruit weight (g)	24 (15 to 30)	27 (17 to 35)	22 (17 to 25)
No. of fruits/plant	6.5 (3 to 11)	10.3 (5 to 18)	14.8 (13 to 18)
No. of genotypes in each type	225	58	13

Ranges are presented in parentheses.

¹ When sown during the early cropping season.

² The flowering period for type C would have been longer but for the short period of rainfall during the late cropping season.

Breeding programme

Types B and C of okra correspond to the "Soudanian" and "Guinean" types of okra described earlier in the literature. These have been crossed. In Nigeria Type C (Guinean okra or *A. caillei*) plants are grown on a smaller scale than Type B (Soudanian type or *A. esculentus*). The former generally flowers very late and many are short day plants. When cooked their pods do not produce the thick soup (mucilage) favoured by the consumers. However, the bulk of okra available in the market in the dry season is the Guinean type which tends to live longer and tolerate drought better. Accessions showing resistance/tolerance to yellow mosaic virus have been identified in Guinean types. The hybrids germinate easily and produce vigorous plants. They flower normally but produce less pollen in the anthers. Pollen fertility is less than 40% and seed set is poor (15 to 20 seeds per pod). The viability of seed is also lower and ranges from 25 to 40%. The partial sterility of F1 hybrids coupled with their production of few viable seeds suggests reproductive isolation of these two okra types. Gene transfer however can be effected between them. Through a series of backcrosses it should be possible to transfer some of the characteristics of Type C to the more popularly grown and consumed Type B.

Conservation aspects

The problems of conserving okra germplasm under low-resource conditions at Ibadan have been examined. Trials with seeds stored in cloth bags, desiccators and plastic containers under ambient temperature showed that active collections can be maintained for over 5 years provided seeds entering storage are well dried (10% seed moisture content or less) and seeds undergo minimal absorption of moisture during storage.

Genetic purity of lines put in storage is a major problem. As much as 3% to 27% outcrossing between lines was observed during April (early season) and September (late season) plantings, respectively at Ibadan. Furthermore, in the humid environment of Ibadan seed viability from the crop grown in early season is lower (as low as 38%) while the late season planting results in better seed viability of over 80% in the harvested seed. Seed production in the drier northern Nigeria environment gives a better alternative for germplasm maintenance as well as for commercial seed production.

Other findings

Surveys of viral and fungal diseases as well as insect pests of okra have been conducted and results published in the last few years.

Okra germplasm evaluation and the breeding programme at IPB, Philippines

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Introduction

The Institute of Plant Breeding (IPB) maintains a collection of 703 accessions of okra, the majority of which originate from Brazil, the Philippines and Turkey (Table 1). Part of this collection is used for variety development. Okra in the Philippines is generally cultivated as an annual crop. It flowers within 60 days (earlier during the dry season) from sowing, and harvesting can be done for a period of approximately 60 days before the crop stops producing pods. The number of pods harvested per plant is up to 20, depending on the length of harvesting period. The length of harvesting period is cut short by senescence and stress such as foliage diseases and insect infestation.

Over the years, the okra breeding team at IPB has evaluated germplasm, experimental varieties and hybrids for such special traits as resistance to insects, shade tolerance and suitability as a ratoon crop, in addition to routine evaluation for horticultural and quality traits. These traits are believed to be useful for farming under low input conditions. Some of the findings generated by this long-term, multidisciplinary research work carried out by a team of scientists including entomologists, physiologists and plant pathologists in addition to breeders, are reported here.

Evaluation for insect resistance

The main insect problem in okra production in the Philippines is leafhopper *Amrasca biguttula* (Ishida), which typically causes yellowing of the leaf margins. It is observed to be a major problem throughout the year in many parts of the country, except during wet weather conditions. Serious infestation results in the so called leafhopper burn, which is characterized by cupping of the leaves with burnt margins. The standard commercial variety "Smooth Green" is highly susceptible to this insect.

Evaluation for resistance to leafhopper was done unreplicated in the field using single row plots, 5 meters long. Uniform natural infestation was assured by planting spreader rows of the cultivar "Smooth Green". The field was not sprayed with insecticides and consequently, other insect pests such as aphids (*Aphis gossypii*) and cotton stainer (*Dysdercus* sp.) complicated the observations. At 60 days, leafhopper damage was assessed using a scale from 7 (resistant) to 5 (susceptible). Counts of leafhopper nymphs were taken on three sample leaves per plant and five plants per row to assess the population level of the pest.

So far, 200 accessions have been evaluated over a two year period. Out of this, 5 accessions from the Philippines were rated resistant (Table 2). These accessions showed a low damage rating and low nymph populations (5.8 to 16.0 nymphs per three leaves). The susceptible accession had a high nymph populations (up to 10 times that of the resistant accessions) which is well correlated with the damage rating. Resistance to leafhoppers does not seem to be correlated with aphid resistance.

Evaluation for shade tolerance

The Philippines has more than 3 million hectares of coconut which can potentially be used for intercropping using shade tolerant varieties of food crops, including okra. Thus, IPB's okra evaluation has sought to determine the level of shade tolerance of elite experimental varieties.

TABLE 1. Okra genetic resources held by the National Plant Germplasm Resources Laboratory (NPGRL), Philippines. These include some wild accessions

Source (country/continent)	No. of accessions
Afghanistan	10
Africa	3
Arabia	1
Brazil	330
Bulgaria	1
China	2
Cuba	2
Egypt	6
Ghana	2
Guatemala	2
Honduras	1
India	54
Iran	15
Japan	2
Mexico	3
Nigeria	2
Pakistan	5
Peru	2
Philippines	135
Puerto Rico	1
Syria	1
Sudan	1
Turkey	105
USA	2
USSR	1
Yugoslavia	12
Zaire	2
TOTAL	703

TABLE 2. Field reaction of okra accessions to leafhoppers and aphids (April - June 1988)

Acc. no.	Leafhopper damage rating ¹	No. of nymphs per 3 sample leaves	Aphid population score ²
9	1	5.8	1.8
12	1	7.8	2.2
14	1	4.6	3.0
39	1	7.7	1.0
63	1	16.0	1.8
Mean		8.4	2.0
7	2	33.4	2.0
13	2	16.8	1.8
19	2	11.2	2.8
21	2	15.6	1.4
26	2	22.8	1.0
Mean		20.0	1.8
15	4	40.2	1.4
16	4	16.6	2.6
31	4	42.8	1.6
33	4	27.8	1.0
40	4	31.4	1.6
Mean		31.8	1.6
2	5	67.6	1.0
17	5	35.6	1.4
41	5	61.6	1.0
51	5	43.2	2.0
52	5	70.0	1.0
Mean		49.6	1.3

¹ Rating scale: 1 = resistant, 5 = susceptible

² Score: 1 = low, 3 = intermediate, 5 = high

Advanced experimental lines and hybrids were evaluated for shade tolerance in RCBD yield trials (three replicates) under shaded (42% shade was achieved using black nets) and open conditions. In the dry season 1989 trial involving 18 entries (Table 3), per cent yield reduction due to shade ranged from 31.5 to 71.5%, with a mean yield reduction of 61.0%. Correlation between yield in the open and under shade was significant ($r = 0.56$). These data indicate that the good performing entries in the open were generally the same ones that performed well in the shade. The material tried included 86-46002 and 86-4025 from India (presumably procured through USDA), Hybrid 1, a cross between a Mexican and Indian accessions; 86-4041 from Sudan (route unknown; presumably through USDA); Acc. 19 from the Philippines; and the rest from Brazil, through EMBRAPA.

Evaluation for suitability as a ratoon crop

Ratooning is a practice that can potentially prolong the harvesting period and increase the yield of a given crop while controlling diseases and insects that are associated with maturing plants. However, it is possible that there are genotypic differences in response to ratooning. This possibility was examined in 1989 using 21 experimental varieties (15 hybrids and corresponding 6 parental lines these included Smooth Green, Acc. 14 and Acc. 15 from the Philippines; 86-4028, 86-4029 and 86-4032 from Brazil through EMBRAPA) that were originally planted in April and ratooned in July. The trial was laid out using RCBD with four replicates. The same trial was ratooned at the end of the season and evaluated as a ratoon crop. Spacing was 75 x 30 cm with 2 plants per hill (approximately 89,000 plants per hectare). Ratooning was done by cutting the plants at a height of 30 cm above ground level.

Ratooning caused an average mortality of 21.8% representing plants that failed to produce new branches after ratooning (Table 4). There were significant differences in plant vigor after ratooning and rate of shoot emergence. The ratoon crop flowered at an average of 33.5 days after ratooning in contrast to the first crop which flowered in 57 days after sowing. Harvesting of the ratoon crop was done over a 67 day period from August to November, a longer harvesting period compared to that of the first crop which was done over 42 days. There were significant differences in yield performance among genotypes, some of which exhibited a yield increase and others a decrease over that of the first crop (Table 5). The yield range of the ratoon crop was - 33.6% to 200% of the first crop depending on genotypes. Correlation of yield between the first and ratoon crops was not significant ($r = -0.015$).

These data suggest that the performance of the first crop cannot be used as a measure of the performance of the ratoon crop. Special selection should be done for response to ratooning. Selection criteria that can be used are: survival, new shoot vigor and yield. Previous experiments have shown that yield differences can be detected without harvesting by simply counting the number of pods per plant at maturity.

Other aspects

The passport data for 703 accessions have been computerized using dBase III plus. Characterization for 12 plant characters (plant, stem, leaf and flower) and 9 fruit characters has been completed and is being computerized. The accessions in genebank are stored at -20°C at 6% relative humidity.

TABLE 3. Yield and percentage yield reduction of okra in the open and under partial shade (1989 dry season)

Entry	Yield (t/ha)		% yield reduction
	Shade	Open	
86-46002	6.84 a	14.88 ab	54.0
86-4025	6.68 ab	16.31 a	59.0
86-4034	6.28 ab	12.96 ad	51.5
Acc 19	5.93 ac	8.70 de	31.8
86-45001	5.66 ac	12.00 be	52.8
Hybrid 1	5.39 ad	11.65 bc	53.7
86-45002	4.76 bd	11.14 be	57.3
86-4041	4.19 ce	10.04 ce	58.3
86-45004	4.17 ce	12.26 ae	65.9
Smooth Green (ck)	4.13 ce	13.13 ac	68.5
85-4032	4.10 e	8.13 e	62.2
85-4034	4.09 ce	12.61 ad	67.6
Green Velvet	4.08 ce	10.63 bc	61.6
86-45005	3.96 ce	11.31 be	64.9
86-45006	3.84 ce	11.26 bc	65.9
86-46004	3.93 ce	12.91 ad	69.6
Hybrid 2	3.43	12.04 be	71.5
84-1039-1	2.46 e	8.13 e	69.7
Mean	4.66	11.82	61.0
CV(%)	23.4	18.41	

NOTE: Means followed by the same letter(s) are not significantly different using DMRT.

TABLE 4. Horticultural characters of okra in a yield trial of a ratoon crop (July–November 1989)

Entry	Mortality (%)	Days to flowering	Rating	
			Vigour ¹	Shoot emergence ²
Lines³				
A	21.38	36	2.12 ad	2.50 b
B	40.0	35	2.50 ad	2.62 b
C	22.64	31	3.25 ac	3.75 a
D	19.19	28	3.50 ab	2.88 ab
E	28.72	30	2.25 ad	2.38 b
F	13.39	38	3.12 ac	2.75 ab
Hybrids				
A x B	27.89	38	2.62 ad	2.12 b
A x C	29.01	34	2.25 ad	2.62 b
A x D	18.75	30	3.38 ab	2.50 b
A x E	16.25	29	3.25 ab	2.50 b
A x F	22.83	36	3.00 ac	2.50 b
B x C	18.56	33	3.12 ac	2.75 ab
B x D	33.82	33	2.25 ad	2.39 b
B x E	35.81	32	2.87 ac	2.39 b
B x F	25.31	39	1.75 bd	2.12 b
C x D	20.53	31	3.25 ab	3.12 ac
C x E	15.52	31	3.12 ac	3.12 ab
C x F	10.28	37	3.88 a	3.12 ab
D x E	18.88	33	3.38 ab	2.38 b
D x F	10.22	31	2.88 ac	2.50 b
E x F	8.88	34	3.75 a	2.37 b
Mean	21.80	33.5	2.78	2.56
LSD	-	6.2	0.43	0.25
CV (%)	-	13.0	17.07	10.00

¹Rating: 1 = least vigorous, 5 = most vigorous

²Rating: 1 = slowest emergence, 5 = fastest emergence

³A = Smooth Green, B = Acc 14, C = Acc 15, D = 86-4028, E = 86-4029, F = 86-4032

TABLE 5. Yield of okra when subjected to ratoon treatment (April–November, 1989)

Entry	Yield (t/ha)				% increase/ decrease
	1st crop	Ratoon crop	Total	Difference	
Lines					
A (Check)	9.81 a	6.51	16.32	-3.30	-33.64
B	5.36 de	5.09	10.45	-0.27	-5.04
C	2.88 gh	8.64	11.52	5.76	200.00
D	8.19 ab	6.40	14.59	-1.79	-21.80
E	7.32 bc	6.62	13.94	-0.70	-9.56
F	2.26 h	6.70	8.96	4.44	196.46
Hybrids					
A x B	6.15 cd	4.68	10.83	-1.47	-23.90
A x C	6.20 cd	6.60	12.80	0.40	6.45
A x D	9.34 a	7.48	16.82	-1.86	-19.90
A x E	9.19 a	6.77	15.96	-2.42	-26.30
A x F	5.75 cde	7.82	13.57	2.07	36.00
B x C	4.12 efg	6.31	10.43	2.19	53.16
B x D	6.04 cd	5.68	11.72	-0.36	5.96
B x E	5.39 de	5.91	11.30	0.52	9.64
B x F	2.49 gh	4.85	7.34	2.36	94.78
C x D	5.42 de	7.88	13.30	2.46	45.40
C x E	7.51 bc	8.51	16.02	1.00	13.30
C x F	3.40 fgh	7.34	10.74	3.94	115.88
D x E	6.22 cd	5.95	12.17	-0.27	-4.30
D x F	5.95 cd	7.74	13.69	1.79	30.08
E x F	4.99 def	7.40	12.39	2.41	48.30
Mean	5.90	6.71	12.61	0.81	13.73
LSD	-	2.07	-	-	-
CV	18.37	22.26	-	-	-

NOTE: Means followed by the same letter(s) are not significantly different using DMRT (Duncan's Multiple Range Test). Note that we used DMRT for the first crop and LSD for the ratoon crop. This is because, for this type of experiment, a change in procedure was adopted that took place between the two crops.

Okra germplasm evaluation in Senegal

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Introduction

Okra (*Abelmoschus esculentus* L.) is an important vegetable extensively grown in Senegal mainly during hot and wet season, from April to July under irrigation and from August to November rainfed. The whole production is locally consumed. A wide range of varieties including Clemson Spineless and local cultivars are grown in the Niayes, an area from Dakar to St. Louis, along the Atlantic ocean, with a variable width and a special climate and in other parts of the country. The varieties grown include Puso and Pop 12, selected by ISRA/CDH from 1976 to 1980. Crop improvement studies are under way to evaluate the variability present.

Major constraints

In Senegal, okra faces various constraints, the most important of which are :

- Lack of adaptation to cool weather (19 to 22°C) and short day length, from November to April. Average yields vary from 3-5 t/ha. from November-April, and from 10-25 t/ha between April and November.
- Susceptibility of all cultivated varieties to root nematodes (*Meloidogyne* spp.) and *Fusarium oxysporum* ssp. *vasinfectum*. Other important pests and diseases are easily controlled with chemicals.

Main breeding objectives

The breeding programme aims at finding new material that can be grown throughout the year adapted to cool weather, and types resistant to root nematodes and *Fusarium* wilt. The programme undertaken is as follows:

1987 - 1989: Characterization and evaluation of local and introduced material.

1990 - 1991: Mass selection and crossing attempts between selected genotypes.

1992: Mass and creative selection. For the latter, breeding methods to be used will depend on results expected from evaluation and screening, and the number of genotypes involved.

Germplasm evaluation

From 1987 to 1989, about 400 accessions have been introduced from IIRSDA (ORSTOM, Côte d'Ivoire). In 1987, evaluation essentially consisted of:

- Phenotypic description using IBPGR descriptors of about 200 genotypes including local and new material with Puso and Pop 12 as reference varieties.
- Vegetative growth.
- Flowering and yields compared under hot and cool growing seasons.
- Screening for resistance to *Meloidogyne* spp. using Townsend-Heuberger's method; 91 accessions were inoculated separately with 3 different strains.
- Screening of the same material in Belgium for resistance to *Fusarium* wilt.

TABLE 1. Plant height during hot and cool season

Sowing date	Season	Plant density (pl./ha)	Acreage (m ²)	Month	Plant height (cm)				
					\bar{x}	Min	Max	Puso	Pop 12
07/87	hot	26.667	1560	1st	17.0	5.3	22.5	20.8	18.2
				2nd	54.3	15.7	104.2	60.0	62.0
				3rd	127.8	36.0	211.0	133.0	153.0
12/87	cool	20.000	1560	1st	7.4	3.5	9.6	8.0	6.0
				2nd	21.0	12.0	26.0	21.0	20.3
				3rd	47.4	25.0	89.0	45.0	44.5

In 1988, evaluation mainly consisted of screening for cool weather adaptation of part of the above mentioned material, with Puso and Pop 12 as check varieties. Evaluation in 1989 was aimed at morphological description of new accessions introduced from Côte d'Ivoire.

Among the 91 genotypes tested, eight were resistant to a given *Meloidogyne* species. No accession was resistant to all the 3 strains. The accessions with specific resistance are:

- Accessions 357, 451 and 477 - resistant to *Meloidogyne javanica*.
- Accessions 429 and 479, resistant to *M. incognita*, and
- Accessions 339, 350 and 445, to a new species of *Meloidogyne*.

From 95 accessions screened for *Fusarium* wilt through both natural and artificial infestation only one (acc. 432) was really resistant.

Evaluation studies based on morphological traits of leaf, stem, flower and fruit point out that most of the accessions tested were mainly uniform, though a few exhibited variation in plant height, leaf shape and fruit shape and size. Variability between the accessions occurred.

Of the 172 accessions studied, 54 were *A. esculentus*, 91 *A. caillei*, 7 *A. moschatus* and 14 *A. manihot*. Results are presented in Table 1. The negative effect of cool weather on vegetative growth was evident. Table 2 presents the data on flowering during the two seasons. This pointed out that the number of days to flowering during the hot season is about 42, and anthesis occurs 10 days later. However during the cool season, the time needed from first flowering to anthesis doubles, compared with the hot season (20 days), first flowering and anthesis occurring respectively 5 days earlier and 5 days later.

The average yield of crop grown in the hot season was about 8 t/ha Puso and Pop 12 have respectively yielded 14.7 and 13.6 t/ha. However, in the cool season, only 19 accessions out of 40 produced fruits, with a mean yield value of 1.1 t/ha. It appears that cool weather is not favourable to the okra crop in general as a result of temperature and day length effect on vegetative growth and flowering.

TABLE 2. Days to flowering during hot and cool season

Sowing date	Season	Days to first flowering					Days to anthesis				
		\bar{x}	Min	Max	Puso	Pop 12	\bar{x}	Min	Max	Puso	Pop 12
07/87	hot	42	28	53	30	33	52.8	38	81	42	43
12/87	cool	37	31	40	34	31	57.5	52	63	57	56

Future emphasis

Resistance is already available within the genetic material tested. However, further screening is needed of the newly introduced accessions. It is hoped that horizontal resistance will then be obtained as far as *Meloidogyne* is concerned. Also, the new material will also be tested for adaptation to cool weather and short day length. Further, it is proposed to undertake hybridization work between the accessions selected, including the varieties Puso and Pop 12.

Okra genetic resources in Sudan

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Introduction

Okra is one of the most important traditional vegetables in Sudan. It is used almost in all parts of the country. It is cooked either after being dehydrated or as fresh pods. The dehydrated okra locally called "Waika", is collected from the wild from the rainlands in the central region and from the southern Blue Nile, Kordofan and Darfur.

Okra genetic resources in Sudan are now being eroded due to:

- Expansion of mechanized agriculture in the rainlands of the Central, Eastern and Kordofan regions, to produce sorghum and sesame.
- Cultivation of modern introduced cultivars, and
- Unusual drought conditions.

Collection and conservation of okra germplasm

Efforts to preserve the highly diversified okra germplasm were started after the establishment of the Horticultural Research Section in the Agricultural Research Corporation of Sudan (ARC) in 1965. During the second half of the seventies a programme for collection and evaluation of okra germplasm from the rainlands around Sennar and Central Blue Nile was started at Sennar Research Station in the Central Region. In 1982, an agreement was signed between IBPGR and the ARC/Sudan for the collection and conservation of horticultural crop germplasm including okra. This programme was financed by IBPGR and executed by the research staff of the ARC. Exploration and collection missions were undertaken to parts of Central and Eastern regions in 1982, parts of Kordofan and Darfur regions in the west in 1983 and parts of the Northern region in 1984. In these missions more than 100 accessions of okra including primitive cultivars and wild species were collected, apart from other agri-horticultural germplasm.

132 accessions from okra germplasm are now conserved in the conservation unit, at the Horticultural Research Section at Wad Medani. The equipment for this unit was provided by IBPGR. The material has been stored in sealed laminated aluminium bags in deep freezers at -20°C with seed moisture content equal or less than 7.5%. These included accessions of *Abelmoschus esculentus*, *A. manihot* and *A. ficulneus*.

Multiplication, characterization, evaluation and documentation

In 1986, another agreement between the ARC and IBPGR was signed. The objective was to multiply, characterize and document the horticultural germplasm accessions collected in the previous missions. The programme was started in 1986 and is still going on.

91 okra accessions have been characterized up to now following the IBPGR descriptor list. Data on these accessions are kept in manual records, including collection forms, characterization forms, field books and annual reports.

Diversity observed

A wide range of diversity was observed during collection and characterization between and within the okra accessions collected in Sudan. This variability for different morpho-agronomic characters included observations on:

- Growth habit : erect, medium or procumbent.
- Branching habit: orthotropic, medium or strong.
- Stem pubescence: glabrous, slight or conspicuous.
- Leaf shape: all states according to IBPGR descriptor list.
- Leaf colour: green or green with red veins.
- Red coloration of petal base: inside only or both sides.
- Number of epicalyx segments: 8-10 or more than 10.
- Position of fruit on main stem: erect or horizontal.
- Fruit colour: yellowish green, green, green with red patches or red.
- Fruit length at maturity: less than 7 cm, 8-15 cm or more than 15 cm.
- Fruit shape: all states according to IBPGR descriptor list.
- Number of ridges per fruit 5-7, 8-10 or more than 10.
- Fruit pubescens: downy, slightly or prickly.

Utilization

The okra improvement programme in Sudan has the following objectives:

- Producing cultivars that are cold tolerant for production during winter season (off season).
- Producing cultivars that are resistant to powdery mildew.
- Improving yield and quality for local consumption and export.

In the light of these objectives, 13 lines of okra were collected from the rainlands around Sennar and Central Blue Nile and planted during 1978-79 at Sennar Research Station. These lines were further evaluated in the following years, and three lines from this material were selected for their high yield and superior market quality. Two of them are good for export. These three lines were released by the Variety Release Committee of the ARC in 1987 under the names Rahiba, Higairat and Sennar.

Future studies

Further collections are needed in some of the areas which were explored before because of rich diversity of okra germplasm in these areas, e.g. Blue Nile Province, parts of Kordofan and Darfur regions. Also, more collecting should be done in areas not visited before, e.g. Southern region, the Khartoum area and White Nile Province.

Okra genetic resources in China

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Introduction

Okra is grown as a minor vegetable in China. As summer is a slack season for vegetables, the farmers try to grow okra for market supply. Several wild okra species occur in China but so far these have not been used in breeding programmes.

Distribution

In China the edible okra, *Abelmoschus esculentus*, also called coffee okra, is planted in Hunan, Hubei, Shandong, Zhejiang, Jiangsu, Yunnan and Guangdong provinces and other areas. Its tender fruit is used as a vegetable.

There are several wild species of okra in China. Much variability occurs in *Abelmoschus manihot*, which is considered to be native to the south of China, distributed over Shandong, Henan, Shanxi, Hubei, Hunan, Sichuan, Guizhou, Yunnan, Guangxi, Guangdong and Fujian. A variant of this taxon, covered with long, yellow, sharp hair, occurs in Yunnan, Guizhou, Sichuan, Hubei, Guangdong, Guangxi and Taiwan provinces. *Abelmoschus crinitus* occurs in Guizhou, Yunnan, Guangdong, Hainan island and Guangxi provinces, while *Abelmoschus moschatus* is found in Taiwan, Guangdong, Guangxi, Jiangxi, Hunan and Yunnan provinces.

Among other wild taxa there are two taxonomically different types whose identity needs to be confirmed. These are locally described as *Abelmoschus sagittifolius* which grows in Guangdong, Heinan, Guangxi, Guizhou, Yunnan provinces and *A. muliensis* from Muli country, in the southwestern part of Sichuan in China. Fig. 1 shows the distribution range of cultivated and wild okra in China.

Conservation of okra germplasm

The National Genebank of the Chinese Academy of Agricultural Sciences (CAAS) holds 20 accessions of okra, and 10 more accessions are held in the Genebank of the Beijing Academy of Agricultural Sciences.

Utilization aspect and future emphasis

Okra research work has been just started in China. It is planned to cooperate with other countries and international organizations to promote okra research and production in the country.



Fig. 1. Distribution of cultivated and wild okra spp. in China

Plant genetic resources activities in okra - an Indian perspective

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Introduction

Okra (*Abelmoschus esculentus* (L.) Moench), commonly known as "bhindi", is one of the major vegetable crops of India. It is extensively cultivated during the spring-summer (March-June) and rainy (July-September) seasons for its green tender fruits. Remarkable native diversity in cultivated and wild types occurs in the Indian subcontinent besides the variability observed among introduced cultivars. Improved types cultivated commercially in different areas presently number around 30, of which "Pusa Sawani" is the most popular and widely grown cultivar. The improved types yield about 45-50 quintals per hectare of green fruits, the highest yield reaching nearly 120 q/ha.

The Indian sub-continent is an important centre of diversity for okra. Much effort has been made during the last four decades to enrich its variability which has also been utilized in developing promising cultivars that are adapted to a wide range of agro-climatic conditions and cropping patterns, possess better fruit yield and quality combined with disease/pest resistance. This report highlights the activities carried out in germplasm collection, evaluation, documentation and conservation of okra genetic resources in India. It also over-views species relationships in *Abelmoschus* emerging from Indian studies and presents salient accomplishments of okra improvement *vis-a-vis* effective utilization of germplasm. Being a key research centre where work on okra improvement is being carried out since 1950s, the NBPGR has accepted global responsibility for base collection of okra. The Bureau is a national nodal organization which not only conducts but also monitors all plant genetic resources activities under IBPGR-supported projects on okra.

Distribution of diversity

Eight *Abelmoschus* species occur in India (Table 1). Of these, *A. esculentus* is known only under cultivation. *A. moschatus* occurs wild though it is also cultivated (for its aromatic seeds), while the rest six (refer Table 1) are truly wild taxa. The wild species occupy diverse habitats. Their broad range of distribution in different phytogeographical regions is given in Table 2 based on different floras, floristic accounts, published since the later part of the last century. Main areas of concentration of wild species are as follows:

- Semi-arid tracts of north and northwestern India for *A. ficulneus* and *A. tuberculatus*.
- Tarai range and the foothills of the Himalayas for *A. crinitis*, *A. manihot* and *tetraphyllus* as well as *pungens* types.
- Western and Eastern Ghats and also peninsular tract for *A. manihot* (including *tetraphyllus* type), *A. angulosus* and *A. moschatus*; *A. angulosus* mainly confined to hilly tracts (up to 2000 m) in South India, with only meagre distribution elsewhere.
- Northeastern region for *A. crinitis* and *A. manihot* - mostly *pungens*.

In some of the wild taxa, infraspecific variation exists and has been taxonomically identified as in the *A. manihot* - vars. *tetraphyllus* and *pungens*; in *A. angulosus* - vars. *purpureus* and *grandiflorus*, in *A. moschatus* vars. *biakensis* and *rugosus*, and in *A. tuberculatus* var. *deltoidifolius*.

TABLE 1. Some characteristics of *Abelmoschus* species and their distribution

Abelmoschus species	Somatic chromosome number	Wild (W)/cultivated (C)	Distribution
<i>A. angulosus</i>	56	W	India, other parts of Asia
<i>A. tuberculatus</i>	58	W	India
<i>A. manihot</i>	66	WC	India, other parts of Asia, Africa, Papua New Guinea
<i>A. moschatus</i>	72	WC	India, other parts of Asia, Australia, Africa
<i>A. ficulneus</i>	72	W	India, other parts of Asia, Australia, Africa
<i>A. esculentus</i>	130	C	Worldwide
<i>A. tetraphyllus</i>	138	W	India, other parts of Asia, Papua New Guinea
<i>A. pungens</i>	138	W	India, other parts of Asia
<i>A. crinitus</i>	138	W	India, other parts of Asia
<i>A. caillei</i>	196	WC	India, Africa

Germplasm collection/acquisition

Variability in okra genetic resources, including wild *Abelmoschus* species, has been built up in NBPGR over the last four decades, both by undertaking explorations within the country and through introduction of promising material from abroad. Under an IBPGR-supported project implemented by NBPGR, this activity has been strengthened with emphasis on enrichment of diversity from India and other countries of South Asia. Within India, eight crop-specific explorations have already been organized, exploring and collecting in parts of north, northwestern plains in Punjab, Haryana, Rajasthan, Gujarat and Uttar Pradesh; adjoining foothills in Himachal Pradesh; central and western parts mainly in Madhya Pradesh and Maharashtra; the southern peninsular tract in Karnataka, Tamil Nadu and Kerala, eastwards in Andhra Pradesh and Orissa and the Assam plains and adjoining hilly tracts of the northeastern region. The network of NBPGR Regional Stations in these areas, particularly at Jodhpur, Akola, Trichur, Cuttack and Shillong, actively contributed in this activity planned, conducted and coordinated by the Division of Plant Exploration at NBPGR, New Delhi. The staff working at the Headquarters also participated in this programme.

TABLE 2. Distribution of wild *Abelmoschus* species in different phytogeographical regions of India

Species	Phytogeographical regions ^a							
	Indus Plains 1	Ganges Plains 2	Western Peninsular region 3	Eastern Peninsular region 4	Northwestern Himalayas 5	Eastern Himalayas 6	Northeastern regions 7	Indian Ocean islands 8
<i>A. angulosus</i>	-	-	+	+	-	-	+	-
<i>A. crinitis</i>	-	+	+	+	+	+	+	-
<i>A. ficulneus</i>	+	+	+	+	-	-	+	-
<i>A. manihot</i> ^b	-	+	+	+	+	+	+	+
<i>A. moschatus</i>	+	+	+	+	-	-	+	-
<i>A. tuberculatus</i>	+	+	-	-	-	-	-	-

^a 1. Northwestern semi-arid region; 2. Subhumid northern plains, tarai belt and parts extending to the east; 3. Western ghats and adjoining tract; 4. Eastern ghats and adjoining tracts; 5. Western Himalayas, Himachal-Ladakj; 6. North Bengal, Sikkim and parts of Arunachal Pradesh; 7. Assam and adjoining states; 8. Andaman, Nicobar Islands; Lakshadweep.

^b Includes spp. *tetraphyllus* var. *tetraphyllus* and var. *pungens*.

This collection strategy laid emphasis on the following:

- Areas which still hold landraces diversity in okra so as to fill visible gaps in collections made so far.
- Areas where traditionally grown and locally adapted cultivars are getting replaced because of the spread of high yielding varieties such as "Pusa Sawani".
- Vegetation zones (habitats) where wild species of *Abelmoschus* occur.

At present, the NBPGR holds 1806 accessions of okra genetic resources, represented by eight species, with maximum variability in *A. esculentus*. Other well represented taxa are *A. tuberculatus*, *A. ficulneus* and *A. manihot/tetraphyllus* types. Table 3 lists the current holdings. Exotic collections are mainly from Brazil and a few from Nigeria. Recently, 177 accessions representing the okra "core collection", which was developed in Côte d'Ivoire have been added through the courtesy of IBPGR office, New Delhi and the Seed Handling Unit of IBPGR at Singapore.

Besides NBPGR, some accessions are also held by centres where okra breeding programmes are operating (Fig. 1). Such major centres are: Indian Institute of Horticultural Research (IIHR) Bangalore (125), Punjab Agricultural University (PAU) at Ludhiana (120), Gujarat Agricultural University (GAU) at Junagarh Campus (129), Kerala Agricultural University (KAU) at Trichur (48), Orissa University of Agriculture and Technology at Bhubaneswar (27), Marathwada Agricultural University at Parabhani (60). Small collections are also held at other State Agricultural Universities (SAUs) such as Tamil Nadu Agricultural University, Coimbatore and those at Pantnagar (Uttar Pradesh), Solan (Himachal Pradesh) and Jorhat (Assam). These working/active collections will have material supplied by the NBPGR initially and also the lines generated by the respective centres. Fig. 1 shows the location of NBPGR and its Regional Stations, besides indicating the SAUs and other ICAR institutes actively involved in okra PCR activities.

TABLE 3. Okra germplasm held at NBPGR, India

Species	No. of accessions
<i>A. esculentus</i>	1448
<i>A. tuberculatus</i>	146
<i>A. ficulneus</i>	112
<i>A. tetraphyllus</i>	73
<i>A. manihot</i>	12
<i>A. moschatus</i>	2
<i>A. crinitus</i>	1
Others	12
TOTAL	1806

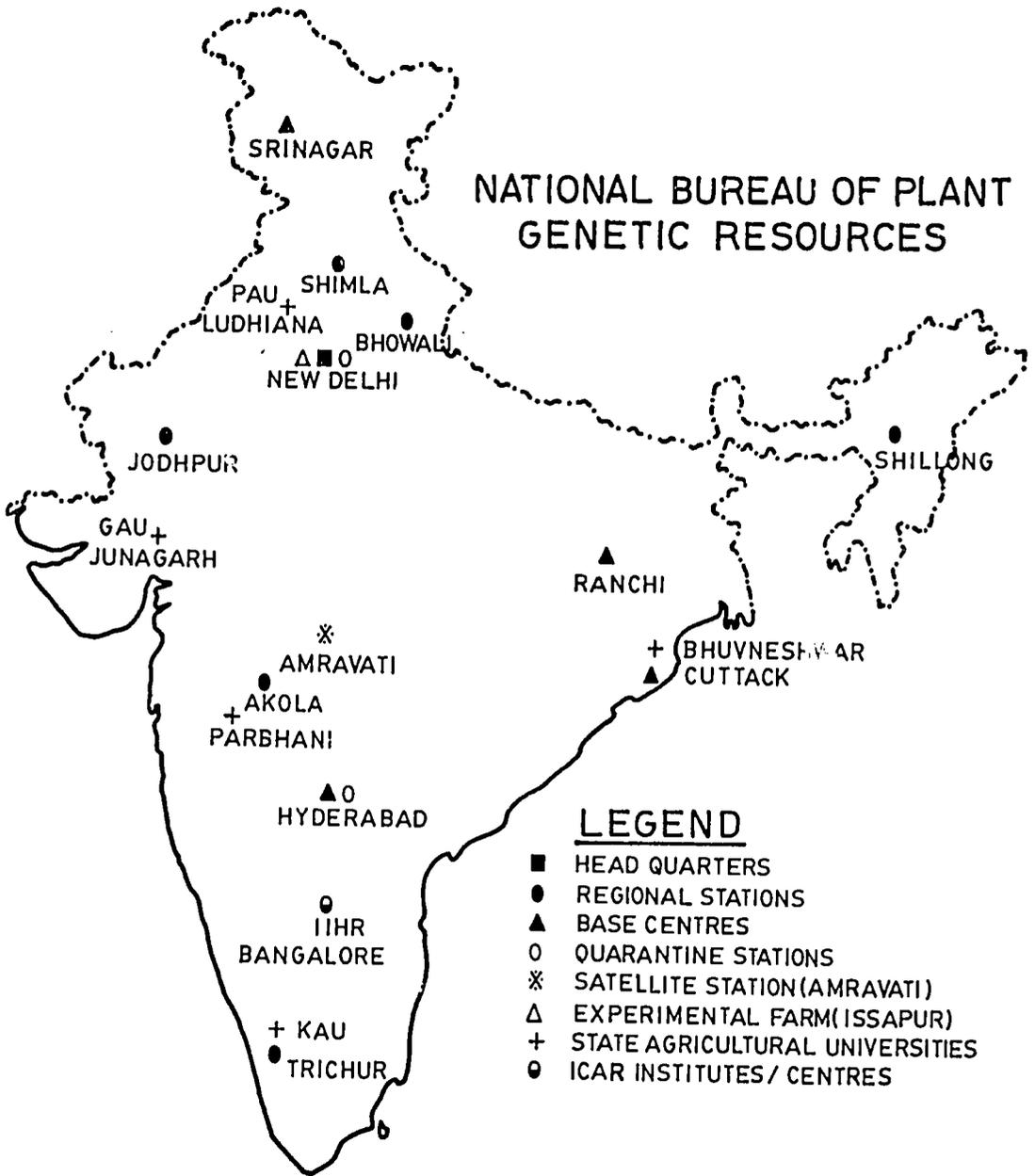


Fig. 1. NBGR, its regional stations and other research institutes/centres engaged in okra improvement work in India

Characterization, evaluation and documentation

The okra germplasm collection with NBPGR is being grown at two locations, namely Issapur Farm, New Delhi and Regional Station, Akola (Maharashtra). The new introductions are first grown in the Post Entry Quarantine Nursery (PEQN) at its Pusa Campus.

The collections are being characterized following IBPGR descriptors. It is intended to evaluate all accessions and document the data for use of okra breeders. Partial success in this effort has already been attained and, based on 2 years' data, 558 accessions have already been evaluated for 45 descriptors. This documented information has resulted in the publication of a "Catalogue on Okra Germplasm" by NBPGR (1990). The evaluated collections include both indigenous and exotic accessions; indigenous variability is represented by 372 accessions from 12 different States of India and exotic variability by 174, of which 160 are from Brazil and 7 from Nigeria. The compiled information points out materials of specific interest to okra breeders. Some noteworthy features are:

- 9 accessions that flowered within 52 days;
- 4 accessions that grow more than 120 cm tall;
- 78 accessions possessing fruit size of more than 20 cm length; IC 3769 had fruits up to 24 cm long;
- 24 accessions having more than 5 primary branches;
- 18 accessions with more than 8 flowering nodes;
- 10 accessions with plant height of more than 120 cm and with more than 15 fruits per plant;
- 20 accessions bearing more than 20 fruits per plant;
- 14 accessions exhibiting maturity within 88 days;
- 9 accessions showing little to moderate susceptibility against YVMV; and
- 8 accessions showing only moderate infestation of fruit and stem borers.

Germplasm utilization

Until 1950, there were no improved varieties in okra. Mostly local cultivars, both 5-edged and multi-edged types, were cultivated. During the 1950s under the leadership of late Dr. H.B. Singh, systematic research work on okra was intensified, not only for building up of germplasm, but also for varietal improvement. As a result, Pusa Makhmali was developed from a collection from West Bengal in 1955 and released for cultivation. In 1960, Pusa Sawani was developed from an intervarietal cross between IC 1542 (symptomless carrier for yellow vein mosaic from West Bengal) and Pusa Makhmali. Pusa Sawani had field resistance to yellow vein mosaic virus and had excellent agronomic characters. After being cultivated for a decade, however, its symptomless carrier reaction to yellow vein mosaic was lost. Hence, a search for resistance was again made by the Plant Introduction Division, IARI (now the NBPGR) and an introduction from Ghana was found to be highly resistant to yellow vein mosaic. It was identified as *A. manihot* ssp. *manihot* and was sent to all major okra research centres for utilization. As a result, many resistant lines were developed during the 1980s, viz. G-2 and G-2-4 (NBPGR), Punjab Padmini (PAU), Parbhani Kranti (Parbhani), and IIHR Sel-4 and Sel-10 (IIHR). Apart from this, NBPGR has also developed Sel-2 from a multiple cross (involving Pusa Sawani x Best-1 x Pusa Sawani x IC 7194) that was released for cultivation in 1985. It is fairly resistant to yellow vein mosaic virus and has long attractive fruits.

TABLE 4. Breeding procedures and varieties developed

Breeding procedure followed	Important variety developed
Pure line selection	Pusa Makhmali (1955)
Intervarietal hybridization	Pusa Sawani (1960) Sel.2 (1985)
Interspecific hybridization	Punjab Padmini (1982) Parbhani Kranti (1985) Sel.4 to 12 (IIHR)
Heterosis	DOH-1, DOH-2, GOH-3, GOH-4 HYB-7, HYB-8, BO-1, BO-2, Vishal, Vijay, Varsha

At present, at the national level, some of the important varieties are Pusa Sawani, Pusa Makhmali, Punjab Padmani, Parbhani Kranti, Co-1 and introductions such as Perkins Long Green, White Velvet and Clemson Spineless. Red Bhindi (Selection AE 106) is a choice variety for kitchen gardens and non-seasonal. Pusa Makhmali and Perkins Long Green are early maturing types. Co-1 is a selection from Red Wonder from Hyderabad. Other varieties include T1, T2, T3 and T4 from Uttar Pradesh; Vaishali Vadhu from Sabour, Bihar; Lam Hybrid from Regional Agricultural Station Lam, Guntum, Andhra Pradesh; Shankerpatti and Sontalotte also from southern India; No.13 from Punjab; besides Satpani, Badanawar, Silari, Patna, Jhabua, Panchdhar, Satshari, Dashdhari, etc. The names are based on place of origin/selection or often on seasonal/agronomic attributes or prominent morphological characters such as number of fruit ridges (when 7, Satdhari; when 10, Dashdhari) and on the names of scientists, e.g. variety Harbhanjan developed by Dr. Y.S. Parmar, University of Horticulture and Forestry, Solan, Himachal Pradesh, mainly for hilly tracts. The overall picture shows that a large amount of available diversity has been utilized effectively including both indigenous and exotic germplasm. The varieties so developed are distinguished by their growth habits as well as variability in colour of stem, shape, surface and length of pod.

The research efforts on okra improvement have proved to be highly rewarding. Different techniques and methods have been employed as is evident from the examples given in Table 4.

Interspecific hybridization has led to large scale variation in okra. Use of related wild species in transferring disease and pest resistance genes to cultivated okra is well illustrated by the work done at NBPGR, IIHR and the Marathwada Agricultural University, Parbhani. Research contributions of Dr. Nerkar (Parbhani) and Dr. Dutta (Bangalore) are presented separately in the proceedings of this workshop. These are significant contributions to okra improvement with emphasis on developing YVMV resistant/pest resistant materials. Work done at the NBPGR, using exotic introductions and selections from indigenous germplasm collections also reflects this emphasis.

Genes for resistance to YVMV and powdery mildew are available in the wild species *A. manihot* and *A. tetraphyllum*; for jassids in *A. moschatum* and for fruit borer in *A. tuberculatum*. Gene(s) for resistance to YVMV of *A. manihot* have been transferred to the widely cultivated variety Pusa Sawani resulting in the development of Parbhani Kranti, resistant to YVMV. The *tetraphyllum* type (*A. manihot* ssp. *tetraphyllum* var. *tetraphyllum*) has been used by IHR. As many as 60 breeding lines and 2 improved okra varieties, Arka Ananika and Arka Abbay, resistant to YVMV have been developed at IHR using interspecific hybridization and back-cross breeding techniques during the last two decades. Also, male sterility has been induced through mutation breeding programmes. Several novel characters leading to enhanced nodal productivity, 'cauliflory', bearing fruits in two flushes, etc. have also been recorded in advanced breeding lines.

Okra manifests hybrid vigour for yield and yield contributing characters. Crosses conducted with some important cultivars revealed that the hybrids AE 9 X H 127 and AE 8 X H 127 manifested maximum hybrid vigour and H 127 proved a good combiner (male parent). Varieties AE 107, Seven Dhari and White Velvet are also good combiners and AE 107, Pusa Sawani, White velvet, Red Wonder and Dwarf Green also appear to be promising parents for heterosis breeding.

Cytogenetical relationships among taxa

On the basis of cytogenetical studies, affinities between cultivated okra and related wild taxa have been worked out. Joshi and Hardas (1950) and Joshi *et al.* (1974) studied meiosis in hybrids obtained by crossing *A. esculentum* (n=65) and *A. tuberculatum* (n=29). They observed that 29 of the 65 chromosomes of *A. esculentum* had complete homology with 29 chromosomes of *A. tuberculatum*. The remaining set of 36 chromosomes (genome Y) of *A. esculentum* showed greater (but still incomplete) homology with 36 chromosomes of *A. ficulneum* as compared to those of *A. moschatum*. It was concluded that one of the parents of *A. esculentum* (n=65) should have been like *A. tuberculatum* (n=29). However, the other genome of 36 chromosomes which possibly was unlike either of the two Indian species, namely, *A. ficulneum* and *A. moschatum*. These studies established that cultivated okra was an amphidiploid (29 T + 36 Y). Another group of polyploid species showing genetic affinity comprises *A. esculentum*, *A. tetraphyllum* and *A. pungens*. The latter two were also found to behave like amphidiploids. Observed chromosomal homologies among different species have been summarized in Fig. 2.

Another species of interest to India is the "Ghana" okra, introduced by the NBPGR. Chromosomal status of this West African "Guinean okra" was studied by Dr. Nerkar and his group working at Parbhani, Agricultural University and crosses were also made between *A. manihot* ssp. *manihot* and *A. tetraphyllum*. Two experimentally synthesized amphidiploids, viz., *A. esculentum* - *manihot* and *A. esculentum* - *tetraphyllum*, were also studied for their meiotic behaviour.

Genetic erosion

Pioneering work done at the NBPGR followed by active breeding programmes undertaken by IHR (Bangalore), MAU (Parbhani), PAU (Ludhiana) and some private seed companies led to development of several improved okra varieties. These varied in maturity from nearly 60 days to around 120 days and were well suited to prevalent agronomic/seasonal patterns. Farmers' seed requirements are generally met by the National Seeds Corporation (NSC), State Seed Corporations and many private leading seed companies. The annual seed production by NSC alone is estimated to be about 7000 tonnes comprising 5000 tonnes of Pusa Sawani, 1500 tonnes of Parbhani Kranti and 500 tonnes of some other varieties. Since 1950s, there has been gradual replacement of okra landraces because of the rapid spread of high yielding and the commercially more acceptable varieties, particularly "Pusa Sawani". "Parbhani Kranti" is now well accepted in Maharashtra and in adjoining areas of Central India. The NBPGR began collecting native variability in okra so as to overcome the growing threat of genetic erosion. IHR is currently supporting collection of okra germplasm in India and neighbouring countries.

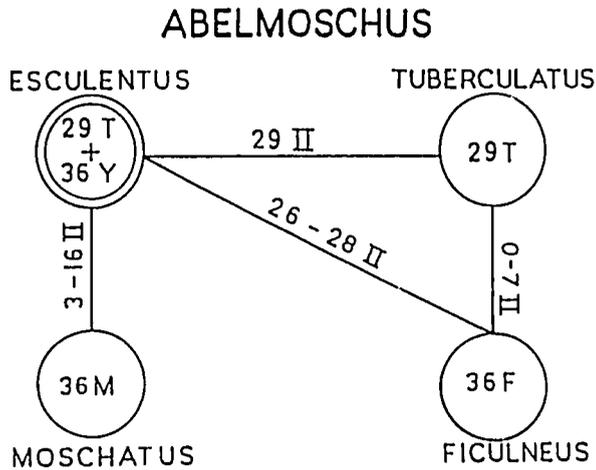
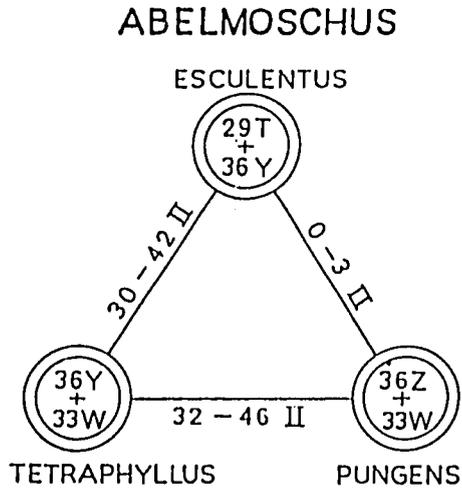


Fig. 2. Bivalent formation indicating chromosomal homologies observed in pollen mother cells of F_1 hybrids involving *A. esculentus* ($2n=130$), *A. tetraphyllus* ($2n=138$), *A. pungens* ($2n=138$), *A. moschatus* ($2n=72$), *A. ficulneus* ($2n=72$) and *A. tuberculatus* ($2n=58$).
Source: Joshi et al., 1974

Conservation of okra germplasm

NBPGR is the nodal organization in India for PGR activities with the national mandate for collecting and conserving seeds of agri-horticultural crops and their wild relatives. It has developed adequate long-term seed storage facilities. Seeds for the okra base collection are first dried to 5% moisture content, heat-sealed in aluminium foils and then kept at -20°C. Seed viability of stored accessions is monitored at regular intervals. Active collections of okra germplasm are maintained at NBPGR and also at several other centres such as those at Parbhani, Ludhiana, Bangalore, Junagarh, Coimbatore, Bhubaneswar, etc. Following an understanding with IBPGR, the NBPGR has accepted responsibility for maintaining a global base collection for okra genetic resources.

Future activities

Many traditionally grown cultivars of okra have already been collected, but efforts to collect more okra germplasm will continue so as to fill gaps identified by the Crop Advisory Committee. Though variability in wild species has been collected, there is an urgent need to collect more diversity in the case of *A. crinitis*, *A. angulosus* and *A. tetraphyllus*, as there is for the *pungens* types. Cytogenetic studies, combined with phenotypic and electrophoretic analysis, will be intensified so as to elucidate evolutionary relationships among *Abelmoschus* species. The NBPGR also proposes to characterize and evaluate all its okra collections during 1991-92 and bring out a catalogue of national holdings numbering over 1800. Efforts are under way to develop a computerized global database on okra genetic resources with the active help of IBPGR. As one of the leading research centres on okra genetic resources and also a key partner to IBPGR's network programmes, the NBPGR envisages developing stronger links with other national programmes with a view to sharing experience, scientific information, techniques and materials. The IBPGR Office for South and Southeast Asia is playing a vital role in stimulating and strengthening these linkages.

Okra in Côte d'Ivoire

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The importance of okra in agriculture

Horticultural crops were traditionally grown by women in limited quantities in the fields of food crops such as rice, yam, and banana. For some years, interest in these horticultural crops has been rising as they have become a safer source of income. They are now frequently grown in pure stands by men. All varieties of this vegetable will often be encountered on the same plot adjacent to another vegetable crop; fields with a mixture of different vegetables are not uncommon.

Okra is cultivated in all climatic zones of Côte d'Ivoire, with more production in the central regions of the country due to dietary preferences. Since it provides a source of income, production is expanding everywhere except where it is inconvenient to crop. Young pods are eaten fresh, dried or in powder for meat or fish sauces. Fruits, which are already somewhat lignified, are cut in slices and consumed in the form of powder after drying. In some regions, fresh leaves are also eaten.

Many varieties can be found in Côte d'Ivoire which differ from each other in earliness, vegetative growth and fruit characters. "Sounde" (very early) and "Gbogboligbo" are the most widespread varieties.

Characterization, evaluation and use

Activities regarding vegetables in the "Institut des Savanes" (IDESSA) were initiated only in 1989 thanks to a project funded by the European Fund for Development. At this early stage, we are focusing on collecting (hot pepper, okra and eggplant) and on the maintenance of the germplasm. No okra breeding programme yet exists.

We hold 226 samples which have been regenerated. During regeneration, the following characters are observed: germination time, date of opening of the first flower, leaf shape and colour, petiole colour, plant habit, stem colour, hairiness, colour of petal base, fruit shape and colour, fruit dehiscence, seed colour and striation. In addition to this collection, IDESSA holds the 2149 okra accessions which were collated by ORSTOM with IBPGR financial support.

Maintenance of the germplasm

These materials, mentioned above, were split in two sets, one stored in a cold room (about 5°C, 60% R.H.) for medium-term conservation, and the other in domestic freezers for long-term storage. For both sets, the seeds were kept in sealed aluminium envelopes.

With frequent electrical power failures, it is necessary to check the seed viability regularly. Unfortunately, the unit lacks basic facilities such as a germinator, petri dishes and absorbant paper. Germination tests have to be carried out in the field, where there is a severe limitation on the number of accessions that can be tested.

General viability of the seeds stored in the cold room

Some germination tests were done in the field, on flower-beds about 1m wide. Whenever possible, 100 seeds were used in each of the 4 replications. Seeds were sown every 4 cm on rows across the bed. Row spacing was about 10 cm. All the flower-beds were watered immediately after sowing and every morning thereafter. Emerged seedlings were counted 7 days and 14 days after planting. The second count was used to determine percentage germination.

TABLE 1. Germination of selected okra accessions from a collection stored in a cold room in Côte d'Ivoire

Accession no.	Germination (%)	Accession no.	Germination (%)
280	42	1987	87
312	85	2134	74
372	4	2137	39
398	75	2142	79
400	32	2146	73
451	69	2148	82
515	0	2150	62
519	52	2151	85
520	45	2163	88
564	72	2165	90
592	59	2169	81
772	67	2171	70
776	75	2173	84
958	43	2178	83
1002	1	2254	76
1004	71	2255	79
1008	11	2270	94
1032	77	2415	52
1070	90	2435	90
1133	83	2649	78
1159	90	2663	78
1175	57	2773	85
1184	37	2808	77
1186	53	3023	81
1228	59	3031	87
1230	82	3104	0
1245	95	3105	0
1247	81	3107	0
1270	75	3108	0
1313	87	3111	88
1317	84	3118	56
1330	91	3124	0
1347	77	3148	5
1354	17	3161	61
1355	89	3165	5
1360	23	3167	84
1372	94	3168	0
1373	70	3243	22
1402	41	3256	2
1404	89	3275	3
1447	8	3291	9
1471	95	3293	3
1472	68	3370	4
1506	61	3380	0
1509	77	3422	12
1521	94	3424	21
1551	75	3425	12
1555	86	3426	11
1577	67	3427	13
1936	89	3428	11

A sample of 100 accessions with sufficient seeds was randomly selected to evaluate the general viability of the collection. There was a wide variation in the germination of the seeds stored in the cold room (Table 1). In the sample tested, 32% of the accessions had 80% germination or more. About 35% of the accessions had between 40% and 79% germination. As much as 25% of the accessions scored between 1% and 39% germination. Finally, 8% of the sample tested did not germinate at all. The seeds may have been still dormant or dead. The sample tested was relatively small. It would be desirable to test the entire collection to justify the need to eventually rejuvenate the seeds. Testing on this scale would be easier not in the field but in a laboratory.

Okra collections in Brazil

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Introduction

Brazilian institutions have gradually, in the past few years, begun to do research on okra germplasm. The germplasm characterization has been done mainly as a support to the breeding programme. It is felt that availability of such data will stimulate further studies, collecting etc. The material was studied mainly at the Itaguaí Experiment Station, PESAGRO-RIO, at Rio de Janeiro State for several years.

Germplasm conservation and holdings

The germplasm collections in Brazil are held by several institutes. Studies have been conducted at Itaguaí Experiment Station from 1983 onwards. The active collection numbers 150 accessions and has been characterized for more than 40 descriptors. The Federal University of Vicosa has the oldest okra collection in the country and its evaluation has been carried out by students, producing graduate theses. Also, Luiz de Queiroz Agricultural School at Piracicaba, São Paulo, holds a small collection and is working on okra breeding. However, for the country, the long-term storage responsibility is with CENARGEN-EMBRAPA, Brasília.

Storage conditions

1. Estação Experimental de Itaguaí (Itaguaí Experiment Station) PESAGRO-RIO: Short-term seed storage, controlled relative humidity. Seeds are kept in wooden and plastic containers. Total 290 accessions.
2. Universidade Federal de Vicosa - UFV: Short-term seed storage at 5°C and controlled relative humidity. Seeds are kept in plastic containers of 4 kg capacity. Total 203 accessions.
3. Escola Superior de Agricultura "Luiz de Queiroz" - ESALQ: Short-term seed storage, controlled relative humidity. Total 170 accessions.
4. CENARGEN/EMBRAPA: Long-term storage at - 25°C.

Germplasm evaluation

At Itaguaí Experiment Station, part of the collection (about 50 accessions) is evaluated once a year. The work is in progress. The material has been characterized for plant, leaf, flower, fruit and seed characters. Data have also been taken on days to emergence/flowering/harvesting; and reaction to diseases and pests. In order to have okra germplasm characterization on large scale it is necessary to involve several institutions like the National Center for Food Technology Research (CTAA) and Itaguaí Experiment Station/PESAGRO-RIO. However meagre financial support limits collaborative studies on the okra germplasm.

A survey done in 1990 showed that okra cultivar Santa Cruz 47 is widely grown in the country. The other cultivars are : Amarelinho, Campinas 1, Campinas 2, Piranema and Chifre de Veado. These cultivars are mainly used as a fresh market vegetable. Recently, work has been done involving food processing technology for canning fruits for export.

DISCUSSION PAPERS

***Abelmoschus*: a taxonomical and cytogenetical overview**

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1. Introduction

This overview aims at summarizing the existing knowledge and various viewpoints concerning the taxonomical status, the phylogeny and species relations within the genus *Abelmoschus*.

The taxonomy of *Abelmoschus* is certainly complex: morphological and ecological observations need to be complemented with cytogenetical information (chromosome numbers, crossing behaviour) for a better understanding of the genetic organization of the genus.

Since 1980, IBPGR has taken the lead in collecting and evaluating germplasm of okra and related species, considerably improving the knowledge on the genus with the ultimate aim to contribute to the improvement of the cultivated species. However, even today, our knowledge is still fragmentary!

2. Bibliographical data

2.1 Taxonomical considerations

2.1.1 History of *Abelmoschus*

The genus *Abelmoschus* may be most easily distinguished from other Malvaceae by the nature of its spathaceous apically 5-toothed calyx that at flowering splits longitudinally along a single suture and that after flowering dehisces circumscissilely, dropping together with the petals and staminal column to which it is adnate basally (BATES, 1968).

The genus *Abelmoschus* was established by MEDIKUS (1787). In his description, he stressed the nature of the dehiscent capsule, but in this respect *Abelmoschus* does not really differ from *Hibiscus*. Therefore, most authors followed DE CANDOLLE (1824) and treated *Abelmoschus* as a section within *Hibiscus*. All *Abelmoschus* species have therefore synonyms in *Hibiscus*.

Abelmoschus was reestablished as a genus by SCHUMANN (1890), based on the caducity of the calyx, but this was generally considered to be a physiological characteristic, not justifying the recognition at the generic level (HOCHREUTNER, 1900).

Later, HOCHREUTNER (1924) discovered the adnation of the calyx to the petals and the staminal column, and his subsequent recognition of the genus *Abelmoschus* has been followed in most recent botanical works.

2.1.2 Number of species

In the few purely taxonomical works on *Abelmoschus*, the number of species varies according to the importance attributed by the authors to certain morphological and ecological characteristics.

HOCHREUTNER (1924), after properly defining the genus, distinguished 14 species and several varieties in *Abelmoschus manihot* and *A. moschatus*.

In his revision of the Malesian Malvaceae, VAN BORSSUM WAALKES (1966) follows a more conservative approach and retains only 6 species with several subspecies and varieties.

The status of the 3 wild species (*Abelmoschus angulosus*, *A. crinitus*, *A. ficulneus*) and the major cultivated species (*Abelmoschus esculentus*) is not contested. Different points of view emerge in the case of the two species comprising both cultivated and wild forms (*Abelmoschus manihot*, *A. moschatus*).

VAN BORSSUM WAALKES (1966) did not make mention of *Abelmoschus manihot* var. *caillei*, described by CHEVALIER (1940) in West Africa as a taxon resembling *Abelmoschus esculentus*, but with large epicalyx segments. It is cultivated in West Africa for its young fruits, in the same way as *Abelmoschus esculentus* (SIEMONSMA, 1982a,b). The variety was recently elevated to a distinct species by STEVELS (1988) as *Abelmoschus caillei* (A. Chev.) Stevels.

Although certain elements of the classification of VAN BORSSUM WAALKES (1966) can be criticized for purely taxonomical reasons, as was done by BATES (1968), I am of the opinion - now that an *Abelmoschus* genepool is being established - that his conservative classification should preferably only be amended on the basis of cytogenetical evidence.

Annex 1 presents the classification of VAN BORSSUM WAALKES (1966) together with a list of synonyms (in *Hibiscus* as well as *Abelmoschus*).

The key to the species, subspecies and varieties is mainly based on:

- * number, dimensions and persistence of the epicalyx segments;
- * form and dimensions of the capsules (including pedicels);
- * characteristics of the indumentum.

Annex 2 summarizes the information presented by VAN BORSSUM WAALKES (1966), amended with data on *Abelmoschus esculentus* and *A. caillei* (*A. manihot* var. *caillei*) from West Africa (SIEMONSMA, 1982b).

2.2 Cytogenetical considerations

2.2.1 Chromosome numbers and ploidy levels

Annex 3 summarizes observations on chromosome numbers in the genus.

The lowest number reported is $2n=56$ for *Abelmoschus angulosus* (FORD, 1938). CHARRIER (1984) and HAMON (1987) reported $2n=38$ as the lowest number, based on observations by SKOVSTED (1935, 1941) for *Hibiscus coccineus* Walter and *Hibiscus grandiflorus* Michx. However, *Hibiscus coccineus*, supposedly a synonym of *A. moschatus* ssp. *tuberosus*, and *Hibiscus grandiflorus*, supposedly a synonym of *Abelmoschus angulosus*, are North American species and do not belong to the genus *Abelmoschus*.

The highest numbers reported are close to 200 for *Abelmoschus manihot* var. *caillei* (SINGH & BHATNAGAR, 1975; SIEMONSMA, 1982a,b).

The numbers reported for *Abelmoschus esculentus* vary greatly. $2n=58$ is reported for a form described by PAL *et al.* (1952) as *Abelmoschus tuberculatus*. VAN BORSSUM WAALKES (1966) includes it in *A. esculentus*, although he notes that it may be one of the ancestors. There are three reports on a chromosome-race with $2n=72$. Most frequently observed, however, is $2n=130$, although DATTA & NAUG (1968) suggest that the numbers $2n=72, 108, 120, 132$, and 144 are an indication of a regular series of polyploids with $x=12$.

The enormous morphological variation in *A. manihot* in a wide sense seems to have a genetic basis, as evidenced by chromosome-counts ($2n=130, 138$) on *A. manihot* ssp. *tetraphyllus*, which are of a higher ploidy level than the more frequently observed $2n=60, 68$.

The references for the other species are less numerous and rather uniform.

TABLE 1. Species and ploidy levels in *Abelmoschus*

Taxa	2n	Ploidy level*
1. <i>A. crinitus</i>	?	1?
2. <i>A. angulosus</i>	56	1
3. <i>A. tuberculatus</i>	58	1
4. <i>A. ficulneus</i>	72	1
5. <i>A. moschatus</i>	72	1
- <i>ssp.moschatus</i> var. <i>moschatus</i>		
- <i>ssp.moschatus</i> var. <i>betulifolius</i>		
- <i>ssp.biakensis</i>		
- <i>ssp.tuberosus</i>		
6. <i>A. manihot</i>	60-68	1
7. <i>A. tetraphyllus</i>	130-138	2
- var. <i>tetraphyllus</i>		
- var. <i>pungens</i>		
8. <i>A. esculentus</i>	(72)-108-144	2
9. <i>A. caillei</i>	185-199	3

* Ploidy level 1: 2n=56-72
Ploidy level 2: 2n=108-144
Ploidy level 3: 2n=185-199

These observations seem to justify the recognition of 3 ploidy levels as suggested by CHARRIER (1984) and some amendments to the classification of VAN BORSSUM WAALKES (1966), which are summarized in Table 1.

2.2.2 Interspecific hybridization

2.2.2.1 Between ploidy level 1 species

Four of the six ploidy level 1 species have been subjected to interspecific crosses, which are summarized in Annex 4.

Viable seed was only obtained in the crosses between *A. manihot* 2n=68 and *A. tuberculatus* 2n=58, and between *A. ficulneus* 2n=72 and *A. tuberculatus* 2n=58. Resulting plants were sterile. Study of the meiosis of the hybrids showed very little affinity between the genomes of the parent species (JOSHI & HARDAS, 1956; JOSHI *et al.*, 1974; KUWADA, 1974).

The artificial amphidiploid 2n=130 between *A. ficulneus* and *A. tuberculatus* (reconstruction of *A. esculentus* 2n=130), realized by JOSHI *et al.* (1974), was genetically unbalanced, being completely sterile.

2.2.2.2 Between ploidy level 1 and ploidy level 2 species

In the search for the parental species of *A. esculentus*, a large number of interspecific crosses have been performed which are summarized in Annex 5.

Study of the meiosis of hybrids between *A. esculentus* $2n=124,130$ and *A. tuberculatus* $2n=58$ revealed the almost perfect pairing of the genome of *A. tuberculatus* with 29 chromosomes of *A. esculentus* $n=62,65$. *A. tuberculatus* is generally accepted as one of the ancestors of *A. esculentus*, apparently an amphidiploid. Concerning the complementary genome, considerable but incomplete pairing was observed with *A. ficulneus*. The possibility of a *A. esculentus* (?) race $2n=72$, as reported by TESHIMA (1933), UGALE *et al.* (1976) and KAMALOVA (1977) should not be completely discarded. It would constitute the most logical source of the missing genome $n=36$.

KUWADA (1957b,1961) obtained a fertile artificial amphidiploid $2n=192$ between *A. esculentus* $2n=124$ and *A. manihot* $2n=68$, called 'Nori-Asa'.

Crosses between ploidy level 1 species and *A. tetraphyllus* are less well documented. PAL *et al.* (1952) obtained sterile hybrids in crosses between different forms of *A. manihot* in a wide sense. No viable hybrid seed was obtained by HAMON & YAPO (1986) in crosses between *A. tetraphyllus* and *A. manihot* s.s. and *A. moschatum*.

UGALE *et al.* (1976) reported on the hybridization of *A. esculentus*(?) $2n=72$ and *A. tetraphyllus* $2n=130$. Almost perfect pairing of the genome of *A. esculentus*(?) with 36 chromosomes of the other species was observed.

2.2.2.3 Between ploidy level 2 species

Viable seed, but sterile hybrids, in the cross *A. esculentus* x *A. tetraphyllus*, were reported by GADWAL (cf. JOSHI & HARDAS, 1976), and HAMON & YAPO (1986). No data on genome affinity are available.

Artificial and spontaneous amphidiploids between these two species have been realized in India in attempts to transfer YVMV resistance to cultivated okra (JAMBHALE & NERKAR, 1981a,b).

2.2.2.4 With the ploidy level 3 species

Hybridization between *A. caillei* and *A. manihot* s.s. (Asian origin) did yield hybrid seed (SIEMONSMA, 1982a,b), although it germinated poorly and showed backward growth (CHARRIER, pers. comm.). Similar results were reported by JAMBHALE & NERKAR (1981a) and HAMON & YAPO (1986).

Crosses between *A. esculentus* and *A. caillei* give viable hybrids, but with strongly reduced fertility (SINGH & BHATNAGAR, 1975; SINGH (In: JOSHI & HARDAS, 1976); SIEMONSMA, 1982a,b; HAMON & YAPO, 1986; HAMON, 1987).

HAMON & YAPO (1986) and HAMON (1987) reported on the cross *A. caillei* x *A. tetraphyllus*. Viable, but sterile hybrids, were obtained. No data on genome affinity are available.

It is interesting to note that the fertile artificial amphidiploid 'Nori-Asa' (between *A. esculentus* $2n=124$ and *A. manihot* $2n=68$) shows morphological characteristics and crossing behaviour rather similar to *A. caillei* (KUWADA, 1957b, 1961; SIEMONSMA, 1982b).

2.2.3 Summary of cytogenetical relations

Cytogenetical relations in the genus *Abelmoschus* are summarized in Fig. 1 (adapted from CHARRIER, 1984).

3. Conclusions and hypotheses

3.1 General

Morphological and cytogenetical evidence support presently the distinction of 9 species in *Abelmoschus*. However, several other hypotheses have been forwarded, which are mentioned and commented upon hereafter.

3.2 *Abelmoschus crinitus* Wall.

Wild species, confined to Asia. Its taxonomical status is not contested. However, no cytogenetical information on its ploidy level or crossing behaviour is available.

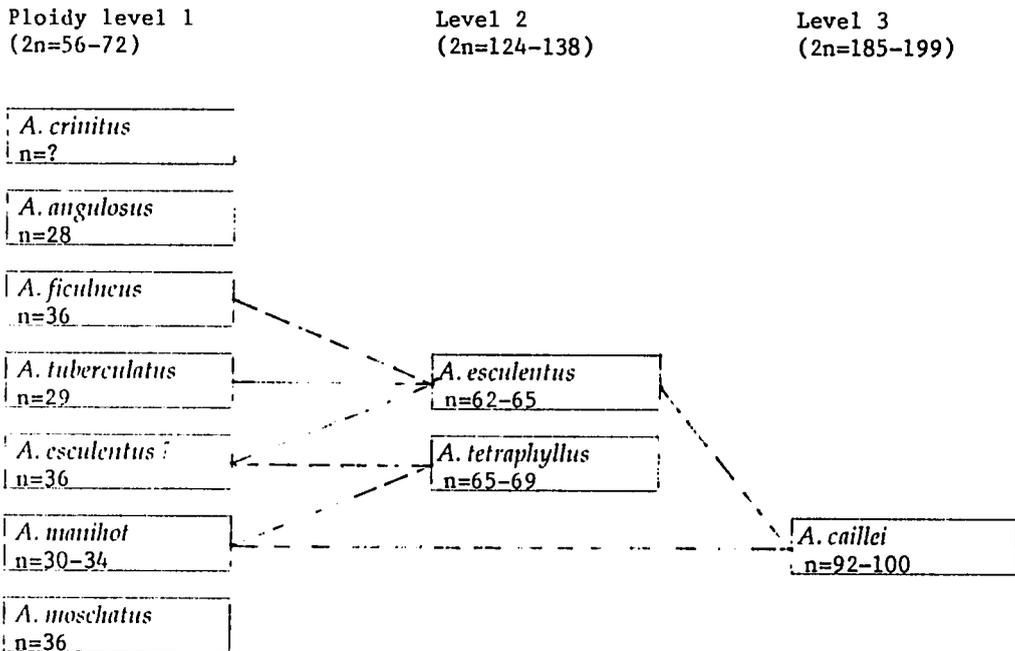


Fig. 1. Cytogenetical relations in *Abelmoschus* (see CHARRIER, 1984).

3.3 *Abelmoschus angulosus* Wall. ex W.& A.

Wild species confined to Asia. One chromosome-count of $2n=56$ (FORD, 1938). *Hibiscus grandiflorus* Michx. ($2n=38$) cannot be maintained as a synonym (CHARRIER, 1984; HAMON, 1987).

3.4 *Abelmoschus tuberculatus* Pal & Singh

This indigenous species from India with $2n=58$ is not available in herbaria, and only few scientists have reported on its performance. The available information suggests it to be one of the parental species of *A. esculentus* $2n=124,130$.

3.5 *Abelmoschus ficulneus* (L.) W.& A. ex Wight

The wild species with the largest distribution, occurring in North Australia, Asia and East Africa. It constitutes the most probable candidate for the other parental species ($2n=72$) of *A. esculentus* ($2n=130$).

3.6 *Abelmoschus moschatus* Medikus

Polymorphic species, cultivated and wild ssp. *moschatus* seems to have pantropical distribution (several South American Flora's mention its occurrence), the other forms are confined to Asia and North Australia.

BATES (1968) proposes to elevate *A. moschatus* ssp. *tuberosus* to specific ranking (correct name: *A. rugosus* Wall. ex W.& A.).

Hibiscus coccineus Walter ($2n=38$) cannot be maintained as a synonym of *A. moschatus* ssp. *tuberosus* (HAMON, 1987), and therefore the hypothesis that *Abelmoschus* ssp. *moschatus* ($2n=72$) might be a polyploid derived from *A. moschatus* ssp. *tuberosus* is highly speculative.

HAMON (1987) proposes to elevate *A. moschatus* ssp. *moschatus* var. *betulifolius* to specific ranking (*A. betulifolius*) on the hypothesis that its morphological characteristics suggest it to be a ploidy level 2 amphidiploid between *A. moschatus* and *A. manihot*.

So far, however, the limited chromosome observations are uniform ($2n=72$). More cytogenetical evidence is needed. Recent collections in Thailand might enable these studies. It is clear that *A. moschatus* still offers much scope for surprises.

3.7 *Abelmoschus manihot* (L.) Medikus

Separated from the wild forms (ssp. *tetraphyllus*), *A. manihot* ($2n=60-68$) appears to be a more uniform entity. A species with uncertain distribution. The Flora of West Africa (HUTCHINSON & DALZIEL, 1958) makes mention of the collection of *A. manihot* ssp. *manihot*, but in spite of many collecting missions, it has not been observed recently.

It remains to be decided where the important leaf-vegetable 'Aibika' (*A. manihot*) from New Guinea belongs. Its appearance is rather different from the typical forms of *A. manihot* in Indonesia (pers. obs.). No chromosome-counts are available to my knowledge.

3.8 *Abelmoschus tetraphyllus* (Roxb. ex Hornem.) R.Graham

Wild ploidy level 2 species ($2n=130,138$) confined to Asia and North Australia. So far, morphologically distinguishable from *Abelmoschus manihot* mainly on the basis of indumentum. More morphological observations are needed.

HAMON (1987) suggests that it might be an autotetraploid originated through chromosome doubling of *A. manihot* ($2n=60-68$). The only reported genome affinity is with *A. esculentus*(?) $2n=72$ (UGALE *et al.*, 1976), which points to an amphidiploid nature.

3.9 *Abelmoschus esculentus* (L.) Moench.

Cultigen ($2n=(72)-108-144$) of uncertain origin, but with pan(sub)tropical distribution. Most probably an amphidiploid of *A. tuberculatus* ($2n=58$) and *A. ficulneus* ($2n=72$). Involvement of a *A. esculentus*(?) $2n=72$ race cannot be completely discarded.

3.10 *Abelmoschus caillei* (A. Chev.) Stevels

A second edible okra species with a distribution limited to West and Central Africa.

Based on chromosome numbers and morphological characteristics, SIEMONSMA (1982a,b) put forward the hypothesis that *A. caillei* might be an amphidiploid of *A. esculentus* ($2n=130$) and *A. manihot* ($2n=60-68$). The fertile amphidiploid 'Nori-Asa' between these two species, realized by KUWADA (1957b, 1961) resembles *A. caillei* in morphological characteristics and crossing behaviour.

The apparent absence of *A. manihot* in West Africa nowadays does not plead in favour of this hypothesis, although *A. caillei* cultivars in Guinea show characteristics (indumentum of seed and fruit) which might derive from *A. manihot* (HAMON, 1987).

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Annex 1. Species classification and synonyms in *Abelmoschus* (VAN BORSSUM WAALKES, 1966)

Species	Synonyms in <i>Hibiscus</i>	Synonyms in <i>Abelmoschus</i>
1. <i>A. moschatus</i> Medikus	<i>H. abelmoschus</i> L.	<i>A. moschatus</i> Medikus
a. ssp. <i>moschatus</i>	<i>H. abelmoschus</i> var. <i>genuinus</i> Hochr.	<i>A. moschatus</i> var. <i>genuinus</i> (Hochr.) Hochr.
var. <i>moschatus</i>	<i>H. pseudo-abelmoschus</i> Blume	<i>A. pseudo-abelmoschus</i> (Blume) Walp.
	<i>H. haenkeanus</i> (Presl) Fern.-Vill.	<i>A. haenkeanus</i> Presl
		<i>A. moschatus</i> var. <i>haenkeanus</i> (Presl) Merr.
d. ssp. <i>moschatus</i>	<i>H. abelmoschus</i> var. <i>betulifolius</i> Mast.	<i>A. moschatus</i> var. <i>longibracteatus</i> Borss.
var. <i>betulifolius</i> (Mast.)	<i>H. longifolius</i> (non Willd.) Miq.	
Hochr.	<i>H. abelmoschus</i> (non L.) Hall. f.	
c. ssp. <i>biakensis</i> (Hochr.) Borss.		<i>A. biakensis</i> Hochr.
d. ssp. <i>tuberosus</i> (Span.) Borss.	<i>H. longifolius</i> var. <i>tuberosus</i> Span.	<i>A. rugosus</i> Wall. ex W. & A.
	<i>H. pseudo-palmatus</i> Span.	<i>A. moschatus</i> var. <i>rugosus</i> (W. & A.) Hochr.
	<i>H. rugosus</i> (Wall. ex W. & A.) Steud.	<i>A. rhodopetalus</i> f. v. M.
	<i>H. abelmoschus</i> var. <i>rugosus</i> (W. & A.) Hochr.	<i>A. sharpei</i> Copel. ex Merr.
	<i>H. rhodopetalus</i> (f. v. M.) f. v. M. ex Benth.	<i>A. todayensis</i> Elmer
	<i>H. sharpei</i> (Merr.) Hochr.	<i>A. brevicapsulatus</i> (Hochr.) Hochr.
	<i>H. subnudus</i> Craib. ex Kerr	<i>A. vanoverberghii</i> Merr.
	<i>H. todayensis</i> (Elmer) Hochr.	<i>A. coccineus</i> Hu
	<i>H. brevicapsulatus</i> Hochr.	<i>A. esquirolii</i> (Léveillé) Hu
	<i>H. esquirolii</i> Léveillé	<i>A. sagittifolius</i> (Kurz) Merr.
	<i>H. sagittifolius</i> var. <i>septentrionalis</i> Gagn.	<i>A. moschatus</i> var. <i>multiformis</i> (non Mast.) Hochr.
	<i>H. sagittifolius</i> (non Kurz) Gagn.	
2. <i>A. manihot</i> (L.) Medikus	<i>H. manihot</i> L.	<i>A. manihot</i> (L.) Medikus
a. ssp. <i>manihot</i>	<i>H. manihot</i> var. <i>genuinus</i> Hochr.	<i>A. manihot</i> var. <i>genuinus</i> (Hochr.) Hochr.
	<i>H. palmatus</i> Cav.	<i>A. manihot</i> var. <i>timorensis</i> (DC.) Hochr.
	<i>H. manihot</i> var. <i>palmatus</i> (Cav.) DC.	
	<i>H. timorensis</i> DC.	
	<i>H. manihot</i> var. <i>timorensis</i> (DC.) Hochr.	
	<i>H. japonicus</i> Miq.	
b. ssp. <i>tetraphyllus</i> (Roxb. ex Hornem.) Borss.	<i>H. tetraphyllus</i> Roxb. ex Hornem.	<i>A. tetraphyllus</i> (Roxb. ex Hornem.) R. Graham
var. <i>tetraphyllus</i>	<i>H. manihot</i> var. <i>tetraphyllus</i> (Roxb. ex Hornem.) Hochr.	<i>A. manihot</i> var. <i>tetraphyllus</i> (Roxb. ex Hornem.) Hochr.
	<i>H. ficuineoides</i> Lindl.	<i>A. ficuineoides</i> (Lindl.) Walp.
	<i>H. notho-manihot</i> f. v. M.	<i>A. luzoniensis</i> Merr.
	<i>H. hostilis</i> Wall. ex Mast.	<i>A. manihot</i> var. <i>luzoniensis</i> (Merr.) Hochr.
		<i>A. mindanaensis</i> Warb. ex Perk.
		<i>A. manihot</i> var. <i>mindanaensis</i> (Warb. ex Perk.) Hochr.
		<i>A. moschatus</i> (non Medikus) Perk.
c. ssp. <i>tetraphyllus</i>	<i>H. pungens</i> Roxb.	<i>A. pungens</i> (Roxb.) Vcigt
var. <i>pungens</i> (Roxb.) Hochr.	<i>H. manihot</i> var. <i>pungens</i> (Roxb.) Hochr.	<i>A. vrieseanus</i> (Hassk.) Hassk.
	<i>H. vrieseanus</i> Hassk.	<i>A. multilobatus</i> Merr.

Species	Synonyms in Hibiscus	Synonyms in Abelmoschus
3. <i>A. esculentus</i> (L.)Moench	<i>H. esculentus</i> L. <i>H. longifolius</i> Willd.	<i>A. tuberculatus</i> Pal & Singh
4. <i>A. ficulneus</i> (L.)W.& A.ex Wight	<i>H. ficulneus</i> L. <i>H. sinuatus</i> Cav.	<i>A. albo-ruber</i> f.v.M.
5. <i>A. crinitus</i> Wall.	<i>H. crinitus</i> (Wall.)G.Don <i>H. cancellatus</i> Roxb.	<i>A. cancellatus</i> (Roxb.)Voigt <i>A. hainanensis</i> Hu
6. <i>A. angulosus</i> Wall.ex W.& A.	<i>H. angulosus</i> (W.& A.)Steud. <i>H. angulosus</i> var. <i>grandiflorus</i> (Thw.)Mast. <i>H. primulinus</i> Alston <i>H. angulosus</i> var. <i>purpureus</i> (Thw.)Mast. <i>H. molochinus</i> Alston <i>H. setinervis</i> Dunn	<i>A. angulosus</i> var. <i>grandiflorus</i> Thw. <i>A. angulosus</i> var. <i>purpureus</i> Thw. <i>A. moschatus</i> (non Medikus)Merr.

Annex 2. Diagnostic characteristics in *Abelmoschus* (VAN BORSSUM WAALKES, 1966; SIEMONSMA, 1982b)

Taxon	Number	Epicalyx segments		Persistence	Shape	Capsule Length(cm)	Pedicel Length(cm)	Indumentum
		Length(mm)	Width(mm)					
1. <i>A. moschatus</i>				through fruit	ovoid-oblong			
a. <i>ssp. moschatus</i> <i>var. moschatus</i>	7-10	8-15	1-2			5-8	3-8	
b. <i>ssp. moschatus</i> <i>var. betulifolius</i>	6-8	17-25	2.5-5			5-8	3-8	
c. <i>ssp. biakensis</i>	8	15-20	3.5-4			5-8	17-19	
d. <i>ssp. tuberosus</i>	9-10	10-25	?			2-5	?	
2. <i>A. manihot</i>	4-8	10-30	5-10	through fruit	ovoid-prismatical	3.5-6	?	
a. <i>ssp. manihot</i>								stems devoid of prickley hairs
b. <i>ssp. tetraphyllus</i> <i>var. tetraphyllus</i>								stems with prickley hairs; epicalyx soft-margined
c. <i>ssp. tetraphyllus</i> <i>var. pungens</i>								stems with prickley hairs; epicalyx hispid-margined
3. <i>A. esculentus</i>	7-15	5-25	0.5-3	through flowering	fusiform-deltoid	5-35	0.5-1.5	
4. <i>A. ficulneus</i>	5-6	4-12	0.5-1.5	through early flower	ovoid	3-3.5	?	
5. <i>A. crinitus</i>	10-16	25-40	0.5-1	through fruit	ovoid-globose	3.5-5	?	
6. <i>A. angulosus</i>	4-5	20-35	10-20	through fruit	ovoid-prismatical	3-5	?	
<i>A. caillei</i>	5-10	10-35	4-13	through early fruit	ovoid	5-25	1-13	

Annex 3. Chromosome numbers (2n) in *Ateleschus*

Species	Chromosome numbers (2n)	Authors	
<i>A. esculentus</i>	‡ 66	FORD, 1938	
	72	TESHIMA, 1933; UGALE et al., 1976; KAMALOVA, 1977	
	108	DAITA & NAUG, 1968	
	118	KRENKE (In: TISCHLER, 1931)	
	120	KRENKE (In: TISCHLER, 1931); PUREWAL & RANDHAWA, 1947; DATTA & NAUG, 1968	
	122	KRENKE (In: TISCHLER, 1931)	
	124	KUWADA, 1957a; 1966	
	126-134	CHIZAKI, 1934	
	130	SKOVSTED, 1935; JOSHI & HARDAS, 1953; GADWAL (In: JOSHI & HARDAS, 1976); GADWAL et al., 1968; JOSHI et al., 1974; SINGH & BHATHNAGAR, 1975	
	131-143	SIEMONSMA, 1982a,b	
	132	MEDWEDEWA, 1936; ROY & JHA, 1958	
‡ 132	BRESLAVETZ et al., 1934; FORD, 1938		
144	DATTA & NAUG, 1968		
<i>A. manihot</i>			
	- ssp. <i>manihot</i>	60	TESHIMA, 1933; CHIZAKI, 1934
		66	SKOVSTED, 1935; KAMALOVA, 1977
	68	KUWADA, 1957a; 1974	
- ssp. <i>tetraphyllus</i>	130	UGALE et al., 1976	
	var. <i>tetraphyllus</i>	138	GADWAL (In: JOSHI & HARDAS, 1976)
- ssp. <i>tetraphyllus</i>	138	GADWAL (In: JOSHI & HARDAS, 1976)	
var. <i>pungens</i>			
<i>A. moschatus</i>	72	SKOVSTED, 1935; GADWAL et al., 1968; JOSHI et al., 1974	
<i>A. ficulneus</i>	72	HARDAS & JOSHI, 1954; GADWAL et al., 1968; JOSHI et al., 1974	
	78	SKOVSTED, 1935	
<i>A. angulosus</i>	56	FORD, 1938	
<i>A. tuberculatus</i>	58	JOSHI & HARDAS, 1953; KUWADA, 1966; 1974; GADWAL et al., 1968; JOSHI et al., 1974	
<i>A. caillei</i>	194	SINGH & BHATHNAGAR, 1975	
(<i>A. manihot</i>	185-199	SIEMONSMA, 1982a,b	
var. <i>caillei</i>)			

Annex 4. Results of crosses between ploidy-level 1 species (positive = viable hybrid seed)

♀ \ ♂	A. manihot	A. moschatus	A. ficulneus	A. tuberculatus
A. manihot		negative (SKOVSTED, 1935; HAMON & YAPO, 1986)	negative (PAL et al., 1952)	positive (PAL et al., 1952) negative (KAWADA, 1974)
A. moschatus	negative (SKOVSTED, 1935; HAMON & YAPO, 1986)		negative (GADWAL et al., 1968)	
A. ficulneus	negative (PAL et al., 1952)	negative (GADWAL et al., 1968)		positive (JOSHI & HARDAS, 1956; JOSHI et al., 1974)
A. tuberculatus	positive (PAL et al., 1952; KAWADA, 1974)	negative (GADWAL et al., 1968)	positive (JOSHI & HARDAS, 1956; JOSHI et al., 1974)	

Annex 5. Results of crosses between *Abelmoschus esculentus* and ploidy-level 1 species (positive-viable seed)

Cross <i>A. esculentus</i> x	Chromosome numbers	Authors	Indicated cross	Reciprocal cross	Bivalents in meiosis
<i>A. tuberculatus</i>	130 x 58	PAL et al., 1952	positive	positive	28.8(2. 29) 27-29
	124 x 58	JOSHI & HARDAS, 1956; JOSHI et al., 1974	positive	positive	
		KUWADA, 1966	positive	positive	
<i>A. manihot</i>	72 x 60	TESHIMA, 1933	positive	negative	0 0-7
	(126-134) x 60	CHIZAKI, 1934	positive	negative	
	130 x 66		SKOVSTED, 1935	positive	positive
			USTINOVA, 1937	positive	negative
			SINGH et al., 1938 ¹	positive	negative
			USTINOVA, 1949	positive	negative
			PAL et al., 1952	positive	positive
124 x 68	KUWADA, 1957a	positive	positive	7	
	HAMON & YAPO, 1966	negative	negative		
<i>A. ficulneus</i>	130 x 72	PAL et al., 1952	negative	negative	27.5 (26-28) ²
		GADWAL et al., 1968; JOSHI et al., 1974	negative	negative	
<i>A. moschatus</i>	130 x 72	SKOVSTED, 1935	positive	negative	8.3 (3-16) ²
	130 x 72	GADWAL et al., 1968; JOSHI et al., 1974	negative	negative	
		HAMON & YAPO, 1966	positive	negative	

¹The article deals with a cross between *A. esculentus* and *A. ficulneus*, but the description of the latter species corresponds to *A. manihot*.

²Hybrids obtained by embryo- and/or ovule-culture.

Taxonomic and ecological observations on species of *Abelmoschus* Medik.

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Background

About 50 species of *Abelmoschus* have been described so far (Charrier, 1984), some of which do not perhaps deserve specific rank (because of synonymy, misidentifications or lowering of rank). In one in-depth study of this Old World genus, van Borssum Waalkes (1966) retained only six species on the basis of epicalyx and capsular morphological characteristics. These are: *A. angulosus* Wall., *A. crinitus* Wall., *A. esculentus* (L.) Moench, *A. ficulneus* L., *A. manihot* (L.) Medik and *A. moschatus* Medik. Out of these, only one species, *A. esculentus*, is fully cultivated, two, *A. manihot* and *A. moschatus*, are semi-cultivated, and the remaining three are truly wild (Hamon and Yapo, 1986). In *A. manihot*, van Borssum Waalkes (1966) identified two subspecies, the subsp. *manihot* (cultivated) and the subsp. *tetraphyllus* (wild). In *A. moschatus*, he recognized three subspecies: subsp. *moschatus*, subsp. *tuberosus* and subsp. *biakensis*. That taxonomic treatment involved perhaps too much "lumping" although it provided an extremely useful clarification. Indeed, some morphological variation as reflected at the population level is too important in certain cases to not be reflected in any taxonomic treatment. Accordingly, Bates (1968) reinstated *A. moschatus* subsp. *tuberosus* as a distinct species: *A. rugosus* Wall. A cultivar group of *A. esculentus* was already mentioned in 1885 by Vilmorin-Audrieux as "a sub-variety in which the seed-vessels are pendent" (p. 357). Later Chevalier (1940) rediscovered this taxon. Siemonsma (1982) started a more elaborate analysis of the situation documented by a nationwide (Côte d'Ivoire) herbarium collection now at Wageningen. This taxon has been recently raised to the rank of distinct species: *A. caillei* (A. Chev.) Stevels (Stevels, 1988).

In order to define the taxonomic relationships between the different species, a survey of 16 herbaria (BM, BR, BSD, BO, CAL, FT, G, K, L, MH, P, PDA, RHT, SING, U, WAG) was carried out. Preliminary results of this survey as well as of field observations made in the areas of distribution of the different *Abelmoschus* species will be reported here.

Results

A. angulosus Wall.

Names: the epithet refers to the angulated epicalyx by which it rather readily can be distinguished from *A. manihot*. No vernacular names have been reported.

Habitat and distribution: it is a montane species living from 750 up to 2,000 masl. It is distributed in the wet and temperate regions, from eastern Kerala (India) down to Bali (Indonesia) through Sri Lanka, northern Burma and Sumatra (see Figs. 1a and 1b). Those territories coincide with the most important tea-growing districts and the areas of rapidly expanding tea production, e.g. in Sri Lanka, tea cultivation is a real threat to this germplasm, particularly to the var. *purpureus* (see below), as it is not very productive.

Intraspecific taxa: two entities are currently recognized at the subspecific rank:

subsp. <i>purpureus</i>	subsp. <i>grandiflorus</i>
a) flower smaller and solitary	in racemes
b) corolla white, later purple	yellow
c) epicalyx smaller	larger
d) more hispid with sharp, bristly hairs	not bristly
e) seed globular	seed reniform
i) up to 2,300 masl	up to 1,300 masl

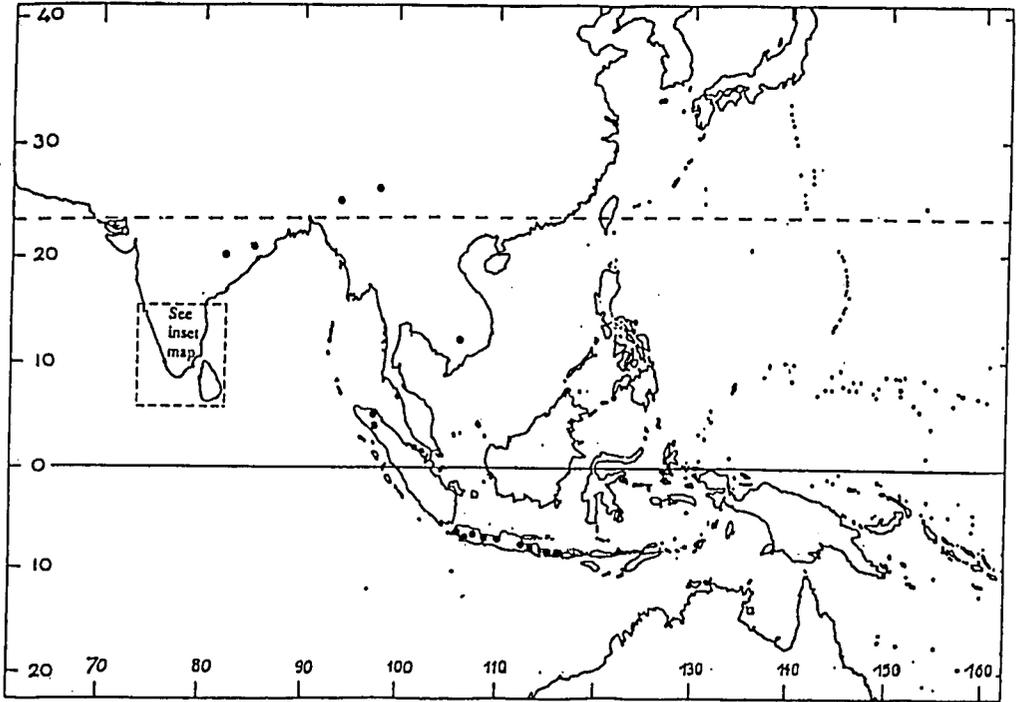


Fig. 1a. Distribution of *Abelmoschus angulosus*

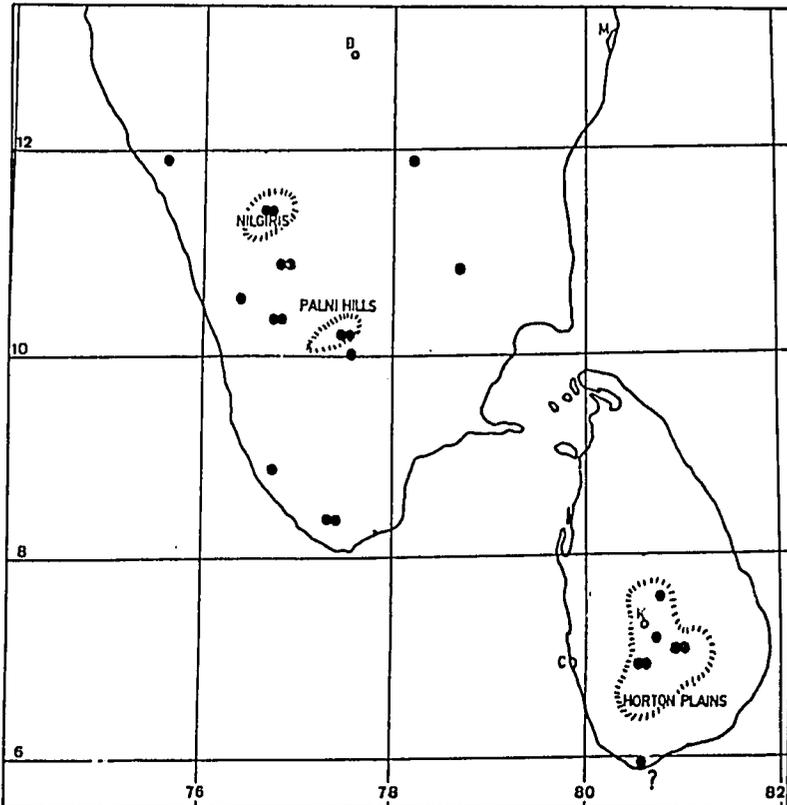


Fig. 1b. Distribution of *Abelmoschus angulosus*

In addition, two varieties are proposed within the subsp. *purpureus*: a var. *purpureus* with solitary flowers (a character not linked to the age of the plants) and a var. *setinervis* (= *Hibiscus setinervis* Dunn.) with flowers in raceme and stiff hairs on the nerves.

Use: it is occasionally used as a hedge plant in Java.

Phenology (it refers here to the period in which flowers can be observed): India (September-February), Java (May-July), Sri Lanka (January-May).

Notes: *A. angulosus* is the only wild *Abelmoschus* species with a pronounced tolerance to low temperatures: the plants withstand light night frosts which occasionally occur above the altitude of 2,000 masl in Sri Lanka.

A. crinitus Wall.

Names: the epithet is derived from the Latin *crinis* meaning long hair, referring to the rather long, linear and narrow segments of the epicalyx. No vernacular names have been reported.

Habitat and distribution: it is distributed from northern Pakistan down to Java (Indonesia) through India, Burma and Thailand, in open forest and vegetation periodically set on fire, in areas subject to a pronounced dry season. Although van Borssum Waalkes (1966) reported it as "a common species in the Indo-Chinese peninsula", the present survey showed that *A. crinitus* is also distributed on the southern slopes of the Himalayas and in western China (Yunnan) (Fig. 2). Its altitude range seems to be from 10 (*van Steenis* 17523, in W. Java) up to 1,400 masl (*C. Baltacharya* 29308, in Uttar Pradesh, India), though van Borssum Waalkes (1966) mentioned it as "lowland" species. Curiously enough, Hamon and co-workers failed to find it in a recent exploration in Thailand (*Hamon et al.* 1987).

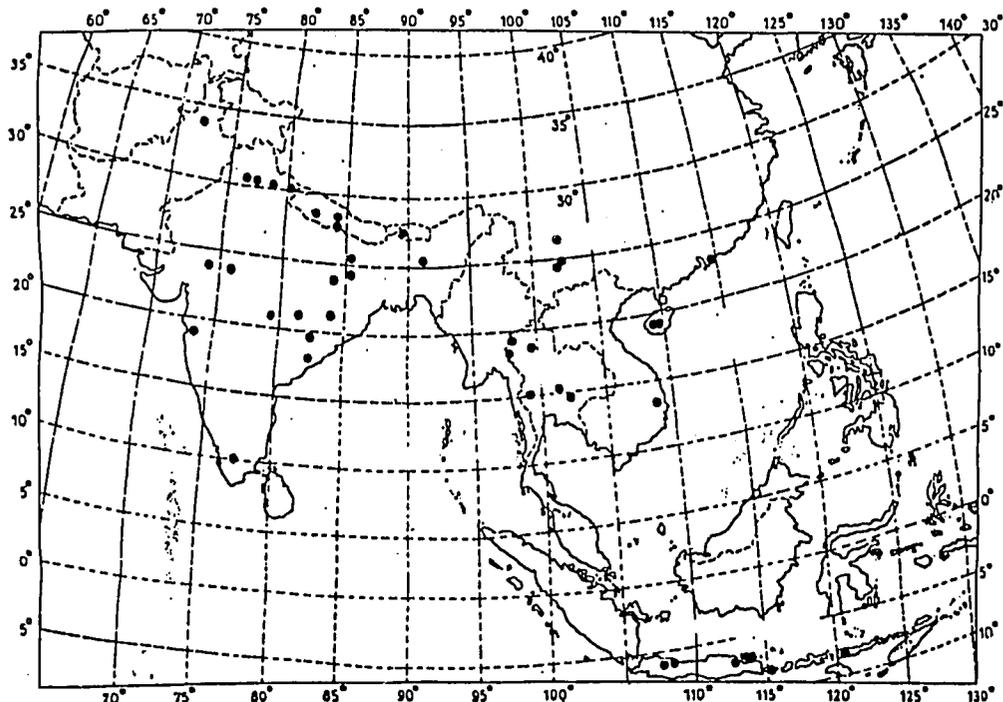


Fig. 2. Distribution of *Abelmoschus crinitus*

Use: root edible; herb used for dysentery.

Phenology: India (July-October), Java (March-April), China (August-September), Thailand (August-November).

Notes: this is a somewhat variable species, particularly in the degree of leaf incision and the density of indumentum. In addition, the length of the epicalyx segments varies: they are mostly longer than the capsule in the types of *Hibiscus cancellatus* and *A. hainanensis*, but may be shorter than the capsule in the type of *A. crinitus*. This hairy *Abelmoschus* species with a tuber-like swollen taproot can survive times of severe drought and even vegetation burnings. The Hainan (China) form of *A. crinitus* has fragrant flowers. It seems that to date only one specimen is present in the national collection of India. Some additional collecting seems thus appropriate in order to know better the agronomical potentialities of this species.

A. ficulneus L.

Names: the epithet has derived from the Latin *ficula*, meaning little fig. It refers to the leaves of this species, which have generally the shape of a reduced leaf of the edible fig (*Ficus carica* L.). Compared with *A. manihot*, *A. esculentus* and *A. caillei*, this most widely distributed wild *Abelmoschus* species is remarkably stable in respect to the leaf shape. Vernacular names are as follows: *samma bende* (Karnataka, India), *daraba bouta* (arab), *rucole dehed* (Somalia), *gurto* (kanuri, Nigeria), *waka afrita* (Sudan) and *nari vendai* (Tamil Nadu, India).

Habitat and distribution: it is the wild *Abelmoschus* species with the widest distribution, from southern Chad (Zolotarevsky c.s. 835) down to central Australia (Maconochie 1275), through Sudan, Ethiopia, Madagascar, India and Timor (Indonesia) (Fig. 3). It is found in lowlands (altitude range: sea level up to 600 m) with pronounced dry season, in open vegetation and on waste land.

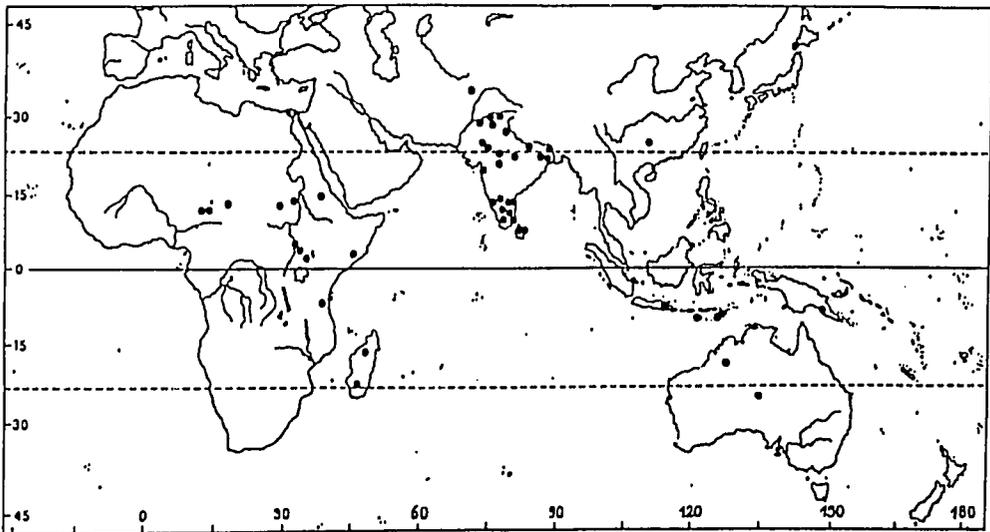


Fig. 3. Distribution of *Abelmoschus ficulneus*

Infraspecific taxa: they will certainly exist, but on the basis of the present observations, no clear-cut definitions can be proposed.

Use: fruits edible; leaves are eaten in times of scarcity as vegetable, and used to clear the sugarcane juice.

Phenology: India (September-January), East Africa (July-November), northeastern Africa (December-May)(fruit), Indonesia (May-July)(fr.), Madagascar (February-May)(fr.), Australia (April-June)(fr.).

Notes: it should receive attention in respect to a collection of wild *Abelmoschus* germplasm for it is possibly an ancestor of *A. esculentus*. Described by van Borssum Waalkes (1966) as an "erect branched underscrub, 0.5-1.5 m" high, I found in Sri Lanka *A. ficulneus* as a purely white flowered creeper hardly 0.5 m high. In India I watched specimens of 1.5 m high, purple flowered. The rich presence of *A. ficulneus* in India combined with the presence of *A. tuberculatus* may be an argument for an Asian origin of *A. esculentus*.

A. manihot (L.) Med. subsp. *tetraphyllus* (Roxb.ex Hornem) Borss. var. *tetraphyllus*

Names: vernacular names were reported as follows: *kembang sampie* (Moluccas, Indonesia), *castuli* (tagalog, Philippines), *ran bhendi* (Thana district, India), *paw fai* (Thailand), *Kastore pukan* (Kangean arch., Indonesia).

Habitat and distribution: this taxon shows a disjunction between its distributional area in South Asia (India, Burma, Thailand) and that in the Southwest Pacific (Philippines, Moluccas, Papua New Guinea) (Fig. 4), probably due to the fact that it needs an annual dry season. It is common in disturbed areas, from sea level up to 400 m.

Use: no particular use has been reported.

Phenology: India (November-April), Indonesia (February-June), Philippines (February-November), Thailand (October), Samoa (August).

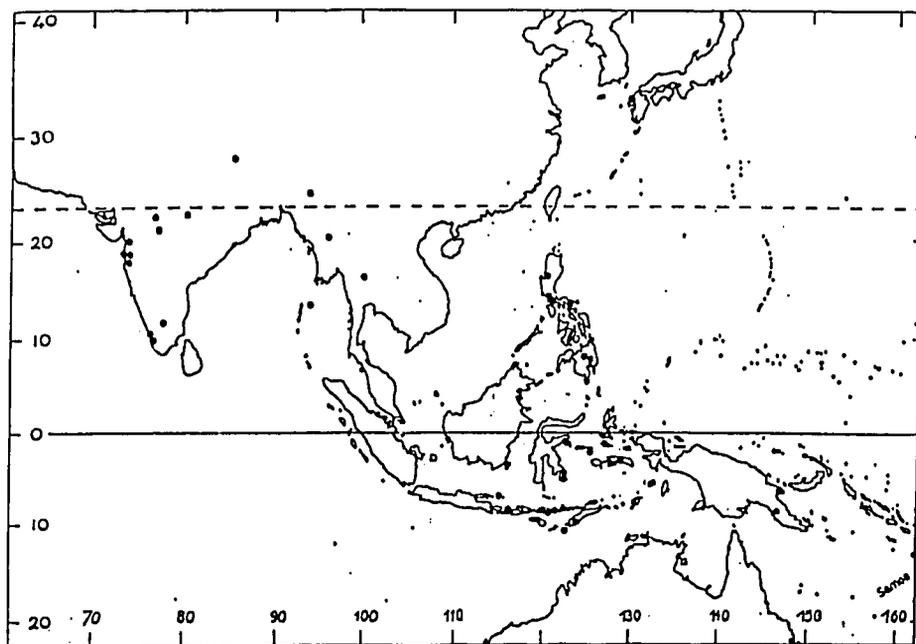


Fig. 4. Distribution of *A. manihot* subsp. *tetraphyllus* var. *tetraphyllus*

Notes: this taxon is important in connection to its counterpart in the classification of cultivated *Abelmoschus*, *A. manihot* subsp. *manihot*. Compared with the variety *pungens* the total number of collections is rather small.

A. manihot subsp. *tetraphyllus* var. *pungens*

Names: the varietal epithet means piercing. Vernacular names are as follows: *si-patjak-rudin* (Sumatra, Indonesia), *pan* (Thailand), *siao-houä-ken* (eastern China).

Habitat and distribution: according to van Borssum Waalkes (1966), it is distributed in Indonesia, the Philippines and Christmas Island. From the present herbarium survey, it appears to be also distributed in Japan, China, India (namely the "hills" in the eastern part), Nepal (in the Himalaya region), the Indo-Chinese peninsula and Burma (Figs. 5a and 5b). It can be found in forest glades, savannahs and abandoned fields, from 400 up to 3,000 masl (Bailey 80, in Nepal).

Use: it is used for rope making; the mucus of the plants is used in preparing paper.

Phenology: India (August-October), Indonesia (March-August), Philippines (August-December).

Notes: this variety, forming shrubs up to 3-4 m high (perennial?), differs not much from var. *tetraphyllus*: it has epicalyx segments with a margin hispid by stiff simple hairs and it is probably somewhat more tolerant to low temperatures. It can therefore maintain itself at higher altitudes, while the var. *tetraphyllus* grows up to 400 masl only.

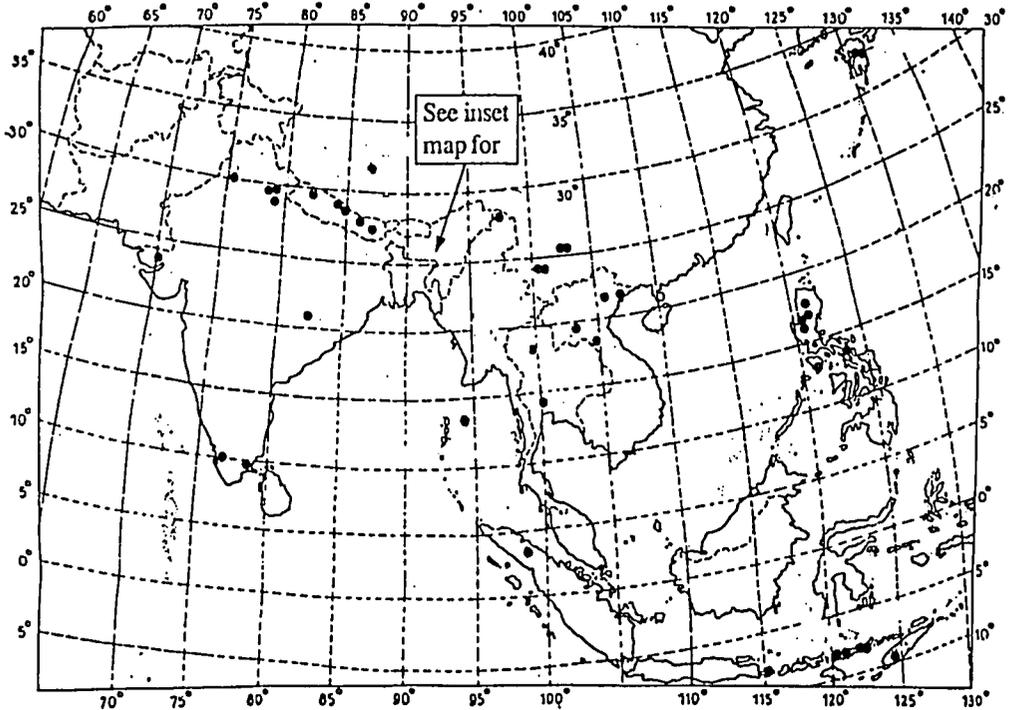


Fig. 5a. Distribution of *A. manihot* subsp. *tetraphyllus* var. *pungens*

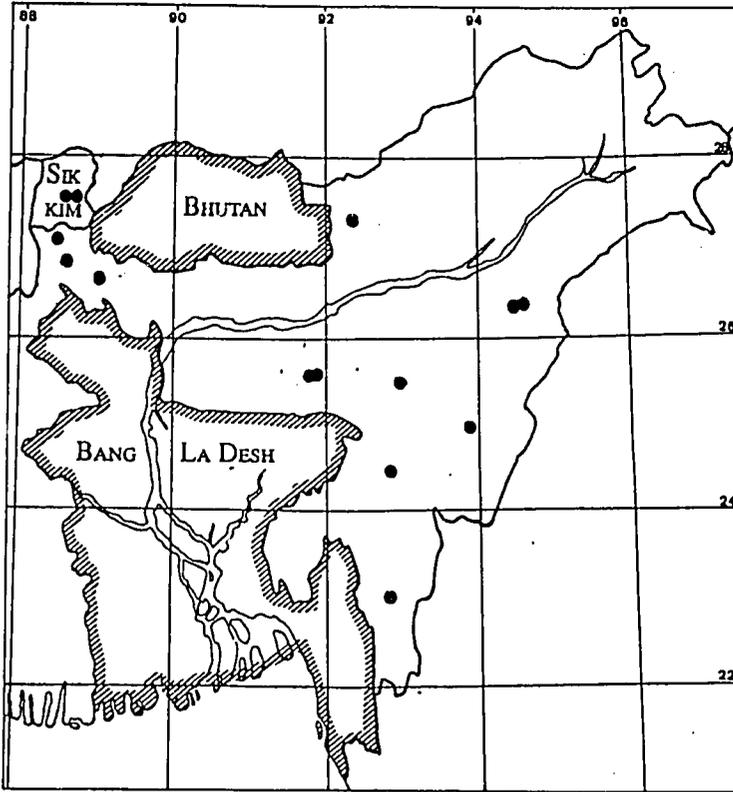


Fig. 5b. Distribution of *A. manihot* subsp. *tetraphyllus* var. *pungens*

A. manihot subsp. *tetraphyllus* var. *megaspermus*

Names: the varietal epithet has been given because of the larger seeds. The vernacular names are: *ran bhendi*, *jangli bhendi*.

Habitat and distribution: it is only reported from the Indian states of Maharashtra, Gujarat and Madhya Pradesh. It can be found on shady hill slopes and along cultivated fields on foot hills.

Use: no particular use has been mentioned.

Notes: it can be distinguished from var. *tetraphyllus* by its smaller ovate-lanceolate distant and caducous epicalyx and its bigger (4-5 mm) and globose seeds. The var. *tetraphyllus* has less epicalyx segments and a persistent epicalyx, while var. *pungens* has reniform seeds with broad sinus and stiff hairs.

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Potential contributions to okra breeding through the study of their genetic resources

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Introduction

Okra is cultivated over the whole intertropical and mediterranean zone for its young fruits which are eaten a few days after flowering. In some regions the leaves are also used for human consumption. This vegetable provides an important input of vitamins and mineral salts, including calcium, which are often lacking in the diet of developing countries.

From 1981 to 1989, IBPGR and ORSTOM contributed to the increase of the genetic resources of this plant held in collections as well as to a better knowledge of the genetic organization within this complex of species. These different activities and achievements will be summarized in this article after a review of the situation existing in the early 1980s. More information can be obtained in more comprehensive articles, e.g. Charrier 1984; Hamon 1988; Hamon and van Sloten 1989.

1. The situation in the early 1980s

1a. The taxonomy

The genus *Abelmoschus*, included in the Malvaceae family by the German botanist Friedrich Medikus at the end of the seventeenth century, had been for a long time classified in the genus *Hibiscus*.

This genus includes, in accordance with the authors, a number of different species. Hochreutiner (1924) mentions 14 species from which two (*A. moschatus* and *A. manihot*) are excessively complex because they are composed of many botanical taxa. Indeed, considering the existing diversity within European herbaria, these two species are the most polymorphic (Hamon and Charrier 1983).

Van Borssum Waalkes (1966) proposed a more restrictive classification including six species divided into two groups:

- the first one includes three species which have cultivated forms (*A. esculentus*, *A. manihot* and *A. moschatus*)
- the second one includes three species, occurring only in wild form (*A. crinitus*, *A. angulosus* and *A. ficulneus*).

Bates (1968) suggested three additional modifications:

- the inclusion of *A. tuberculatus* (which is assumed to be the progenitor of *A. esculentus*) into *A. esculentus*
- the grouping of all subspecies and varieties of *A. manihot*
- the former subspecies *A. moschatus* spp. *tuberosus* to become a new species named *A. rugosus*.

This illustrates the complexity of the genus, which can also be emphasized by the discovery (Chevalier 1940) of an African cultivated species. The latter was rediscovered by Siemonsma (1982 a,b) and described as *A. caillei* (Stevens, 1988).

1b. *Cytogenetic structure of the complex*

The scale of variation in chromosome numbers is important. It goes from $2n = 38$ (*A. tuberculatus*) to $2n = 198$ (*A. caillei*). The species complex can be considered, in accordance with Charrier (1984), as composed of three ploidy levels, but four ploidy levels would also be acceptable (Hamon, 1988). The cultivated species *A. esculentus* would be the result of an amphiploidization between *A. tuberculatus* and *A. ficulneus*. *A. caillei* would result from a hybridization between *A. esculentus* and *A. manihot* (Siemonsma, 1982b).

Differences in chromosome numbers are also reported within the same botanical species. They are of two types:

- the first one can be considered as a wrong estimation in cases of high chromosome numbers (*A. esculentus* with $2n$ from 108 to 144).
- the second type refers to biological differences within species. For *A. manihot* the subspecies *manihot* has a $2n$ number which is between 60-68, whereas the ssp. *tetraphyllus* has a $2n$ number between 130-140. Equally for *A. moschatus*, the $2n$ of spp. *tuberosus* is 39 and the one of spp. *moschatus* is 72.

1c. *Collections and breeding*

At the beginning of the 1980s the most important collection was that of the USDA (Puerto Rico) which consisted mainly of *A. esculentus* accessions from India and the Mediterranean basin; wild forms were scarcely represented (Charrier, 1984).

Breeding activities, at this time, were mainly heritability studies of diverse characters, e.g. yield, height, earliness. However, these studies were conducted with germplasm from limited species, geographic origin and genetic base.

2. **First step: the increase of genetic diversity available in collections**

The IBPGR-ORSTOM okra collection was initially composed of material collected by Siemonsma (350 samples). It reached 600 samples thanks to the duplication of the USDA collection.

A significant step was achieved by a collecting mission in Togo and Benin by Hamon and Charrier (1983) who brought out 700 additional samples. Valuable information on the relative distribution of the two cultivated species, on the diversity of the fruits, the crops cycle, associate crops, etc. was collated at this opportunity. Such information has proved highly relevant for further evaluation.

Multicrop collecting missions organized or supported by IBPGR have completed this collection of cultivated forms, e.g. Sudan (Hassan *et al.* 1983, 1985), Guinea (Hamon *et al.* 1983), Zambia and Zimbabwe (Attere *et al.* 1983).

In 1986 wild forms were not yet represented. This gap was partially covered with a collecting mission for *A. moschatus* and *A. manihot* in Thailand (Hamon *et al.* 1987) and in Sri Lanka (Vredebrecht, unpublished).

In 1989 the entire collection of more than 2,500 accessions was transferred to the "Institut des Savanes de Bouaké" (Côte d'Ivoire). At each multiplication, a duplicate (30 g per accession) was sent to ORSTOM (France) and Fort Collins (USA) (50 to 100 g). In addition, duplicates of the core collection (200 samples representing a maximum of diversity (*vide* core collection concept, Frankel 1983) were distributed on request to various countries.

3. The different phases of characterization of the collection

3a. Choice of a set of descriptors to study diversity

Morpho-phenological descriptors

The selection of a set of descriptors for such material, which was not well studied and for which cultivated varieties are not very polymorphic, created difficulties.

The first set of descriptors was selected from the results of an evaluation for another important Malvaceae: cotton. This set proved very quickly to be inadequate in relation to the morpho-phenological diversity which was observed. A modified set was then proposed by Charrier (1984).

The descriptors were initially proposed for the entire genus. But the morpho-phenological polymorphism of African forms, apart from the co-existence of two cultivated species with very different cycles and structures, revealed very quickly the constraints and limitations of a unique set of descriptors, which was still less valid for wild forms.

Isoenzymatic markers

No publications existed on the use of iso-enzymatic markers for okra. We therefore followed the method recommended by Second and Trouslot (1980) for rice. The major problem was linked with the mucilaginous nature of fresh okra. This was solved by using the embryo, which accounts for almost all of the seed's volume.

Initially, the use of markers was not very satisfactory, which may explain the absence of publications. Indeed the analysis in the first samples of the highly polyploid *A. esculentus* and *A. caillei* showed no diversity. Only the interspecific discrimination between *A. esculentus* and *A. caillei* was possible (Hamon and Yapo, 1985).

Later the introduction of cultivated form from east and southern Africa and from wild forms, as well as the adoption of new techniques, provided more interest for the use of this tool.

3b. The characterization phases

The first phase consisted of the evaluation of the germplasm, which was available in 1982, i.e. the Côte d'Ivoire and the USDA collections.

The second stage focused mainly on the study of the diversity of African forms (west, east and southern). Numerous morpho-phenological characters and iso-enzyme systems were used.

Finally, the third phase consisted of a study of the diversity of wild forms (*A. moschatum* and *A. manihot*) from Thailand and Sri Lanka.

4. Main achievements

4a. Diversity of cultivated forms (*A. esculentus* and *A. caillei*)

Identification and distribution of species

The characterization of all collected samples has shown that the new species *A. caillei* can be considered as endemic to west and central Africa. The identification of one interspecific hybrid from progenies of an accession collected in Sudan does not call this endemism into question.

The sympatry of these two species exists for around three degrees of latitude between the forest and the limits of the Sahelian zone. *A. caillei* disappears completely in the north, at a latitude which fits with the south of Burkina Faso and Niger.

A minimum of experience allows one to distinguish the two cultivated species thanks to their different habits or the observation of the number and width of the segments of the epicalyx. But it is difficult for a non-specialist to recognize the two species only from a mature fruit separated from the mother plant (a situation that always arises when collecting in villages). The observation of a few characters allows, however, a reduction in errors:

- general colouring of dry fruit:
 - pale *A. esculentus*
 - darkish *A. caillei*
- long and curved pedicels: *A. caillei*
- marking of seeds:
 - dense *A. esculentus*
 - wide *A. caillei*
- length of superior fruit more than 20 cm: *A. esculentus*
- width of superior fruit more than 40 cm: *A. esculentus*
- spiny fruit: *A. caillei*

For the use of a classical botanical key, Stevels (1990) should be consulted.

A. esculentus

i) Morpho-phenological diversity

The morpho-phenological diversity of the cultivated species *A. esculentus* is geographically unequally distributed. As observed from the USDA collection, the polymorphism from the Mediterranean basin or from India is low, although some varieties (e.g. Pusa Sawani) are obviously original.

Following our studies, the diversity in west Africa (from Guinea to Benin) is far more important than in any other places. A great number of varietal forms can be found. The polymorphism is expressed through the time length of cycles, the sizes of the plant and the colourings of the organs (especially fruits). Also is to be noted the presence of types selected as associate crops (variety with long stem associated with pearl millet, early variety sown with yams) and the existence of forms adapted to ecological zones located at the limits of the desert (Agadez, Niger) or to very humid zones of tropical forests.

The most important variability, or at least the most visible one, is observed for colouring and sizes of the fruits. Those have a multitude of colours, from white to violet, and can reach sizes unknown in other regions. We can particularly mention two varietal types widespread in Togo and in Benin, i.e. "coue d'Igouti" (up to 6 cm diameter from the green fruit) and "corne d'antilope" (up to 45 cm long at maturity).

The terminology "varietal type" rather than "variety" *sensu lato* is used because the traditional African agriculture has criteria different to European farmers. There are no breeding organizations and seed certification schemes. For a particular region, more or less importance is attributed to okra by the main ethnic tribe, and thus the varietal diversity is proportional (e.g. only two varieties corresponding to one cultivar of *A. esculentus* and one of *A. caillei* for the Ebriés from the south of Côte d'Ivoire and more than 10 for the Baribas of the central region of Benin). The selection is independent in each region (village, tribe) and, if similar schemes are used, this does not imply that samples with two identical names will be similar; at best, it will give some indications e.g. names which indicate that have a length upper to the mean.

ii) Isoenzymatic diversity

The species is characterized by a low level of isoenzymatic polymorphism. The important morphological diversity found in West Africa is not correlated with diversity of isoenzymatic systems. To be noted is more variation (but at a low level) in northeast Africa and southern Africa (Zambia, Zimbabwe) for GOT and SKdH systems. This observation raises a new interest for cultivated forms of this region.

The observed monomorphism is accompanied in some cases with an impressive number of electromorphs (e.g. MdH 26 bands) which outlines, for this complex and duplicated system (Gottlieb, 1982) an additional redundancy linked to polyploidy.

A. caillei

i) Morpho-phenological diversity

This species is later maturing and more photosensitive than *A. esculentus* and thus allows the extension of the production period. They are traditionally denominated dry season okra (which produces in dry seasons to the contrary of the okra of rainy season *A. esculentus*). Thus these two species represent for the farmers two major varieties which are managed independently.

The particular diversity of this species is mainly found in the fruits (shape, appearance, position) and we observed the following:

- a good percentage of varieties with fruits in horizontal position or in falling position in relation to the stem; this is accompanied by a particularly long pedicel, which can be found with the same length only in *A. moschatius*;
- very hairy fruits which can become spiny sometimes accompanied (especially in the central part of Guinea Conakry) with a reddish bloom on seeds. Such a bloom can be found only in a few accessions of *A. manihot*;
- sizes of vegetative organs often bigger than those of *A. esculentus* (height at the end of cycle, diameter of the stem, leaf surface, number of petioles, number of primary fructiferous branches, etc.) which show the high ploidy level of this species.

Up to 15 flowers (thus 15 fruits edible three days later) can be seen on an old plant (which has developed on a fertile soil). In African conditions, it represents the totality of the production of one *A. esculentus* plant, but this has to be counterbalanced by the low potential growing density of *A. caillei* due to the volume of plants and a strong photosensitivity.

Compared with the total existing diversity of cultivated African forms, the specific diversity of *A. caillei* does not appear as extraordinary as judged by Martin (1982) and Siemonsma (1982a).

4b. *The diversity of wild forms (A. moschatus and A. manihot)*

Length of cycle, identification of species and dormancy

These two species have good chances of being perennials and cuttings often have the ability to regrow. This can be attributed to the ecological niche of these plants, which are found along roadsides, in rice fields, in fallow fields and at the edge of the forests. They are therefore subject to periodical destruction of the aerial organs (mowing, burning, etc.).

A. moschatus generally has dense and numerous roots and some accessions have tuberous roots (cf. ssp. *tuberosus*). *A. manihot* sprouts very well and is easily propagated from cuttings.

The two wild species *A. moschatus* and *A. manihot* can be easily distinguished from each other on the basis of the usual botanical criteria, including number and shape of the segments of the epicalyx, shape and aspect of fruit or length of the pedicel. *A. manihot* has also a more important stem diameter, at least in the samples from Thailand and Sri Lanka. Plants are bigger and have more internodes. The leaves have superior sizes. The plant's habit is generally erect, whereas *A. moschatus* has a typically bushy habit. The isozymes (MdH, IdH, PGI) in the seed's embryo allow each species to be identified. The AdH system is useful in discriminating wild from cultivated forms.

The wild traits of the two species are well marked. There is one important dehiscence of fruits at maturity accompanied by a strong seed dormancy. The latter has never been found within cultivated forms. Imbibition of seeds in organic solvents gives good results in breaking this dormancy (Hamon, in preparation).

A. moschatus

i) Morpho-phenological characters

A. moschatus seems, at first glance, to have limited variation, but introductions from Thailand have more diversity than those from the Maldives, which, in our climatic conditions, were late and smaller. The main difference between these two groups consists in the number of seeds per fruit and in the weight of one thousand seeds (Thailand at least 100 seeds per fruit, and 10 g/1000 seeds, Maldives 80 seeds/fruit and 13 g/1000 seeds) and thus is founded on a biological base.

The samples from Thailand can be separated into two maturity groups. The number of seeds per fruit, the width of the fruits and the number of anthers per flower are characters which also separate the early and the late group.

ii) Isoenzymatic diversity

The isoenzymatic diversity of this species is more significant than that of cultivated species. In Thailand, four main groups were distinguished but those could not be linked with the diverse morpho-phenological groupings. The samples from the Maldives, which are more homogeneous from the morphological point of view, are also totally monomorphic except for AdH. They have certainly been introduced, more or less recently, from a narrow genetic base.

A. manihot

i) Morpho-phenological characters

The global diversity of this species is more important than any other species. The samples from Sri Lanka (Vredereg) are very dissimilar to those of Thailand which were also unlike the two previous samples existing in collections (ORS-278 and ORS-592).

A study on morpho-phenological diversity in samples from Thailand (the only ones available in sufficient number) show a diversity which is continuously distributed without well individualized groups. However, two groups can be formed on the basis of the size and number of the segments of the epicalyx.

These segments (on the mean 4 or 5) are longer and covering fruits in most cases, except for a few samples collected in Chang Mai region. The border between the two groups is as follows:

1 +/- 4 cm length for segment of epicalyx
2 +/- 100 anthers per flower

ii) Isoenzymatic characters

The isoenzymatic diversity of this species is, as for *A. moschatum*, more important than that of cultivated forms. Some electromorphs are rare and seem linked with specific origins. Two of the five groups, classified by data analysis, are indeed linked with geographical origin (the samples from Chang Mai in Thailand).

4c. Additional information

The kinetics of selfing

Okra is characterized by an autogamous reproduction, in which allogamy is not excluded (Martin, 1983). Numerous contradictory data are available and we tried to study this topic (two publications are in preparation).

The selfing process was examined on several varieties of *A. esculentum*. We have shown that there are different selfing speeds. However, on the average, an allopollen will be efficient in 70% of cases if it is deposited on the stigmas before 7 a.m., but only has a 0.1% efficiency if deposited at noon. As the okra flowers are very sensitive to the stress induced by emasculation, these results may be advantageously used for hybrid production.

Cruden (1977) suggests that the reproduction system of a species is closely correlated with the parameter $\log(\text{pollen/ovule})$ produced by the flower. Such a criterion has been estimated on a large number of cultivated and wild forms. It can be shown thus that for okra, cultivated species ($\log P/O$ near 200) come into the category of facultative allogamous plants, whereas the wild forms ($\log P/O$ near 220) are closer to the category of facultative autogamous. The observed scale of variation starts from 169 for the variety Clemson Spineless (*A. esculentus*) to 293 for the wild accession ORS-278 (*A. manihot*).

Phytosanitary aspects

The okras, as many cultivated Malvaceae, are very attractive to insects and quite susceptible to fungal and viral diseases.

The use of phytosanitary products allows efficient control of pests and fungal diseases, but there are no chemicals for leaf curl virosis. This disease, which is transmitted by an aleurod *Bemisia tabaci*, follows the same dispersion model for okra as the one described for cassava by Fargette (1986).

The most dangerous phase in West Africa is between February and May. An *A. esculentus* crop sown at this period will, most probably, be completely destroyed. Only one variety can be developed in such conditions (ORS-968) but it is, unfortunately, photosensitive and late-maturing. On the other hand, varieties from *A. cailliei* do manifest symptoms of curling but they have a good tolerance and ensure a reasonable yield. The wild species *A. moschatus* is quite susceptible to this disease, whereas *A. manihot* grows, generally, normally. The latter is therefore a possible source of resistance, especially considering that hybrids from *A. manihot* with cultivated varieties are sometimes possible.

5. Breeding potential

The morpho-phenological diversity of the two cultivated species appears, at least in Africa, as a continuum of complementary forms. This complementarity concerns not only production cycles but also within each cycle a multiplicity of morphotypes.

5a. *Definition and selection of ideotypes*

Our studies show that a cultivar can be defined as the result of a complex function which integrates four main parameters: earliness, duration of phyllocrone (total vegetation time), number of branches and rate of fruiting.

The diversity of eco-edaphic conditions discourages the selection of one variety only, but it favours the selection of a certain number of morphotypes (or ideotypes) well adapted to the agricultural system. Thus we can recommend for West Africa the following:

A. esculentus

An early variety for production at the beginning of the rainy season and in Sahelian zones. It should have a good yield during the first two months and a good tolerance to drought. This ideotype could be selected in research stations, because the very early maturity (30-50 days) does not depend on cultural practices. A simulation of the rainy season can be carried out through more or less frequent irrigations.

A horticultural variety for the urban market. The ideotype is a plant of small size, flowering within 60 days and production will be concentrated in a month with the aim of making maximum use of the sun, minimizing the time spent on harvesting and facilitating marketing. It should give a high response to fertilizers and give a maximum productivity by unit of time/space;

A variety (or more) to be associated with the most important crop of the region (for example sorghum or pearl millet). This ideotype is tall. Its cycle should coincide with the associated crop and it should be adapted to the growing conditions in the region.

A. caillei

A similar scheme could be applied to this species but it would be more difficult to monitor. The monitoring of the cycle of the latest forms (first flowering after 90-100 days) is fragile and may result in the absence of production. However, the early forms which are common near the Guinea-Côte d'Ivoire border (group of varieties type ORS-520 or ORS-2415) can be bred more easily. Their habits are similar to the one of *A. esculentus* and they are not too susceptible to eco-edaphic parameters. They are very productive and provide a good opportunity to increase the production period with only one sowing time.

A foreseeable strategy could consist of the elaboration of a very variable pool, which could ensure under any conditions a good yield from one rainy season to another. This could be a good alternative for this species, which is often grown and consumed by women and used as a supplementary food input. Indeed, these plants are often cultivated near the house or in the garden in association with other crops (tomato, eggplant, peppers, etc.).

5b. *Increasing hybrid production*

Okra has axillary flowers, hermaphrodite and self-compatible. The anthers are dehiscent at the time of anthesis. The reproduction system is however not totally autogamous.

The production of selected hybrids needs a previous emasculation of the flowers. Around one hundred of the etamins located on the staminal sheet around the style have to be eliminated and this is a fastidious and lengthy operation, which implies for many varieties a very strong abscission and the decay of many flowers (up to 90%) before or after pollination.

Hamon and Koechlin (submitted for publication) have analyzed the selfing mechanism, which can be used to improve the hybrid production at the infra and interspecific level. In this case the emasculation is no longer necessary and the hybrid production is markedly improved.

5c. *Potentialities for selection/breeding*

The immature fruits of okra (three-four days after flowering) are what is mainly consumed. Thus in most cases the total production is not dependent on that which has already been realized since the plant does not need to feed the fruit for one month to reach maturity. The latter is therefore not submitted to the negative correlations described by Siemonsma (1982).

The fruit production is a complex function, the two main constraints being the quantity of flowering and the rate of fruiting. The fruiting rate is very dependent on the eco-climatic conditions. The number of flowers which are produced is the parameter which may be most easily monitored.

The production of flowering knots is constant, on the main stem, during the full growth period of the plant. However, it should be noted that the fruit production on lateral branches can often be similar to, and sometimes bigger (e.g. *A. cailliei*) than that on the stem. But it never interferes as a competing factor.

The morphological characters linked with the development (early flowering, number of lateral branches on the stem, diameter of the stem) have a direct bearing on yield. Their combining ability is largely additive and they are therefore good criteria for breeding.

Generally the crosses which express a good hybrid vigour are exceptional especially for the crosses between *A. esculentus*. The differences between the mean of the parents and those of the F1 progenies are below 10%. The characters which were studied, except fruit setting, have a very significant general combining ability. Similarly the specific combining ability is also important. Exceptions can be mentioned for the variables which are linked to yield (number of fruits, number of seeds/fruit and weight of seeds).

5d. *The use of interspecific hybrids*

The hybrid between the two cultivated species

Both cultivated species live in sympatry in a large part of West Africa. Around 1% of fruits harvested by farmers for the next sowing can be identified as natural hybrids between both species.

We can ensure that there is no genetic barrier for F1 production under controlled hybridization. All ovules are fertilized by the pollen of the other species, whatever the directions of the crosses. Seeds germinate and give vigorous and flowering plants, but these are sterile, hence a nearly complete absence of fruits. It was impossible to obtain backcrosses despite the large number of attempts in both directions. It is nevertheless possible to produce F2. This breeding strategy, which is far from easy, deserves to be developed.

Hybrids between cultivated and wild forms

We had a limited pool of wild forms for many years. Only *A. moschatum* (ORS-280 and a few forms from Togo and Benin) and *A. manihot* (ORS-278 and 592) were represented.

Hybrid production systematically failed except with ORS-278. Crosses with this USDA accession were therefore studied.

Hybrids with ORS-278 (*A. manihot* ssp. *tetraplyllus*)

This accession allows the production of interspecific F1, whichever parents are used. All progenies, very vigorous, show without any doubt a hybrid phenotype. They have an exceptional level of fruit production (5 times more than the parents). Fruits are nearly totally deprived of viable seeds. However we managed to produce F2 and F3 due to the large quantity of available F1. There is then an absence of sexual segregation which can be observed (Hamon and Koechlin, in preparation). The backcrosses are also difficult to obtain.

Possibilities provided by the recent introductions

In addition to the poor diversity of wild available forms, hybrid production has for a long time been limited by the susceptibility to emasculation. The implementation of a new technique for hybrid production and the introduction of new accessions (refer to Thailand and Sri Lanka) have extended the possibilities of crosses.

Thus we can find with *A. manihot* some types which behave as ORS-278, others which do not cross at all and also some intermediate forms. For the first time, hybrids with *A. moschatum* (including ORS-3234) can be obtained. They have been produced in limited quantities, but one of the hybrids has the character of hyper-fructification as described earlier with ORS-278. In addition its fruits produce many more seeds.

6. Conclusions and prospects

The availability of *Abelmoschus* genetic diversity has been markedly increased in *ex situ* collections during the last ten years, especially the cultivated forms. The African endemic species is now well represented.

Wild forms are still uncommon, because only the samples from Thailand could be multiplied and evaluated, whereas other species, notably *A. ficulneus*, are still not available. It should be noted in this context that dormancy, which does not exist in cultivated forms, is strong with *A. moschatum* and *A. manihot*. Different methods have been tested for eliminating this dormancy, but there is, at this level, polymorphism. This constraint has to be taken into account at the time of sampling and multiplication.

The evaluation of genetic diversity in cultivated species has shown many important traits:

- the morpho-phenological polymorphism of the main species, *A. esculentus*, is globally rather low, but West Africa provides a great number of varietal forms with differences for earliness, length of cycle, shape and size of fruits, habit, etc. Taking into account the occurrence of *A. caillei*, which is endemic and has specific diversity, West Africa appears as an exceptional and original polymorphic zone;
- the isoenzymatic polymorphism, which is supposed to provide a picture of real genetic diversity, is nearly zero in West Africa and East Africa; the region stretching from Egypt to Zimbabwe has most significant isoenzymatic variability. An in-depth study of this region, notably to the south of Sudan, should be undertaken.

Wild forms, more homogeneous morphologically, include a more important isoenzymatic polymorphism which shows their potential use as a gene reservoir, notably for disease resistance.

All these observations imply a speciation by successive amphyploids within the species complex linked with the reduction of variability.

The breeding of a variety capable of adapting to different zones seems erroneous to us and we would advise instead the selection of ideotypes suited to particular locations and cultural practices. The importance of leaf-curl virus has been demonstrated earlier. This virus can destroy *A. esculentus* if it is sown between February and April.

Generally crosses with high hybrid vigour are rare, but the characters that are linked with yield are good criteria for selection.

The use of wild forms is still problematic. The implementation of a new pollination technique has nevertheless improved the level of interspecific hybrid production. Obtaining later generations offers new avenues for applied research, but the techniques have not yet been well monitored.

Much research needs to be implemented at a fundamental level, to find out more about:

- the genetic organization of the complex. The low availability of wild forms does not yet allow significant progress in this direction;

- the speciation mechanisms: these are specific to the genus *Abelmoschus* and also found in the genus *Hibiscus*. They lead to a considerable increase in chromosome numbers and to a correlative decrease of the genetic variability. This is a very original biological mechanism;
- the genetic origin of *A. caillei*. Its relationship with *A. esculentus* is clear, but the origin of the additional chromosomes (*A. manihot?*) has not yet been identified.

The use of modern techniques, until now hardly used on minor plants, should allow significant progress if the genetic material is available. Molecular markers at the DNA level are already available (De Kochko and Hamon, 1990). The cytofluorimetry of flux, which allows ploidy levels to be studied with great accuracy, is available in a few important research centres.

To conclude, the work undertaken by ORSTOM and IBPGR between 1981 and 1989 has enlarged the genepool available for okra breeding and has deepened our knowledge of the diversity of cultivated forms. The achievements have also raised numerous new questions and it appears that far more research, applied or basic, should be undertaken.

Some proposed procedures for obtaining a core collection using quantitative plant characterization data

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Summary

As well as maintaining a base collection, an optimal plant genetic resources preservation system must take into account the development and use of small collections including a large amount of diversity.

Several procedures have been suggested for obtaining this, ranging from a random sampling of 10% of the samples to sampling based on a good knowledge of population allelic frequencies.

A large part of available data for a crop usually concerns morphological and/or phenological descriptors of a quantitative nature, and in this paper we propose a statistical procedure based on the conversion of the initial variates into new independent ones chosen to represent maximum variability. Then, by a step by step search of the most dissimilar individuals, we are able to choose the number of accessions and/or the percentage of the total diversity to be conserved.

Introduction

The idea of centres of origin and domestication of plants, and the consequences for plant breeding, is mainly associated with the pioneer work of Vavilov (1935), who was also one of the first scientists to collect plant genetic resources on a scientific basis.

Several decades later, prompted by Harlan (1970), Frankel and Bennet (1974) and Frankel (1974), scientists began to study the diversity of natural populations. After early work using morphological characters, isoenzymes were used by ORSTOM to carry out studies on several species, such as *Coffea*, *Oryza*, *Panicum* and *Pennisetum* (Pernes, 1984).

Under the auspices of IBPGR many field collections of cultivated plants of economic importance were carried out. Progressively during the eighties, interest turned to related wild species and to minor crops.

This systematic collection resulted in a number of problems in conservation and management. Curators were faced with massive, sudden and irregular influxes of material, which was kept in cold rooms or in freezers. As underlined by Peeters and Williams (1984), documentation concerning accessions was often lacking. In addition, most collections are now large, difficult to handle and manage, and consequently are underused. Finally, curators are unable to answer specific questions and, as a result, breeders often prefer to use (or build up) a working collection composed of several tens of well-known accessions or varieties.

Frankel and Brown (1984) first highlighted the need for a reference collection containing a good representation of the available diversity, the "core collection". The main objectives are to obtain an accession with a given profile from a reduced set of accessions, or, by default, to guide the breeder within the base collection. These authors stress that the statistical sampling procedures must be correct and ensure representation and preservation of the population genetic structure. It is evident that sampling will lead to a partial loss of diversity, whatever the extent of that loss may be. The questions are what is the real level of the loss and what do we want to retain in the core collection. Brown (1980) related in detail a few examples where allozymic frequencies and the genetic structure of the populations were known. His procedure seemed to be useful because with 10% of the total accessions it was possible to conserve 80% of the allozymic diversity.

The genetic diversity of species is organized on different levels of complexity. When we study one cultivated species using a descriptor list and then consider related wild species, it is frequently the case that their mating systems, ploidy levels, distribution areas and cycle lengths are different (Hamrick *et al.*, 1979). While isozymic markers are often useful, they are not necessarily the best. While in many situations there is a good correlation between allozymic and morphologic polymorphism (Giannisi and Crawford, 1986), this is not always the case (Davis and Gilmartin, 1985). A low level of allozyme variability could be associated with a high level of morphological diversity which is of great interest for the farmer or the plant breeder. In addition, morphological divergence between populations can occur before allozyme divergence. Crawford (1985) gives examples of recent speciations where there is often no or a low level of allozyme difference.

Brown (1989) shows that when the genetic structure of a population is not known, random sampling is better than other sampling strategies and also that if the choice criteria used to select accessions are inadequate, major problems can occur. We suggest here a sampling strategy based on "passport data". The main problem would be that these data are often missing, not homogeneous or valid for all the accessions.

For most tropical crops, detailed data, especially at the biochemical level (isoenzymes, RFLP), are rarely available. Most data are botanical, morphological, agronomical or related to pests and diseases using tools such as the IBPGR descriptor lists.

In most cases, characterization of a collection in developing countries has employed less costly descriptors of this kind, so data refer to quantitative characters such as plant height, flowering period and fruit production and/or qualitative characters such as the shape and colour of different parts of the plant. Quantitative data can, under certain conditions, be analyzed using multivariate analysis such as principal component analysis (Hotelling, 1933). Qualitative data can be analyzed in a similar way by factorial analysis (Benzecri, 1973), where the data are coded by presence or absence. These two procedures are slightly different and, in this paper, we introduce the approach that can be used for quantitative data to show how, by a stepwise analysis which consists of searching for distant individuals, it is possible to assemble a core collection.

Strategy and pathway analysis

Principle

The variability of a population is defined by differences between individuals for one or more characters. To conserve maximum diversity it is necessary to retain the largest differences. We thus first search for the individual which is furthest from the centroid. To build up the working samples, we add those individuals which maximize the inertia of the sample (see below).

Prior data conversion

The initial data set is a table (individuals x variates) where individuals are the accessions and the variates the descriptors. The choice of the metric for distance calculations depends on the nature of the variates. For quantitative data we choose the Euclidian distance weighted by the standard error (to make the relative weight of each variable uniform) or the Mahalanobis distance (1930). For discrete variates, the khi-2 distance or Nei's distance (1972) are most suitable.

The value of a distance is greatly influenced by the number of differences. If these belong to strongly correlated characters (positively or negatively) the effect would be to double the weighting given to a single factor. For this reason the analysis is carried out on standardized principal component scores. This procedure clarifies the structure of the data. New factors, equal in number to the initial number of variables, are chosen in order of decreasing inertia value (% variability accounted for). All are orthogonal to each other. Each individual is then characterized by its factor axis scores. Thus, we have a new data set where variables are independent and where a given individual has a value (positive or negative) relative to the centroid. It may then be possible to calculate distances weighted by the standard deviation of the factor, i.e. the square root of the eigenvalue.

This procedure is very useful for eliminating data redundancy due to the correlation that exists between variates. One remaining drawback is that all factors taken into account are given the same weight. This could lead to bias due to random variation or errors in the initial data set. For this reason we decided to consider only the axes for which the eigenvalue (λ) is greater than 1. Another possibility consists of introducing a dummy variable in the original set and to conserve only the axes for which the eigenvalue is equal to or larger than that for the dummy.

Making up the core collection

For each individual, characterized by a set of axis scores, we calculate the sum (P) of the squares of the standardized coordinates for the k factors selected: $P = \sum x_{ij}^2$. The ratio P/N (where N is the total number of individuals) is the inertia of a given individual. The sum of the partial inertias of the N points is the total inertia (100%). The relative contribution of one individual is the ratio $P/(N \cdot K)$. In the same way, for a subset of individuals, the contribution is equal to $\sum P_i/(N \cdot K)$. This is the selected diversity (SD).

For this selection procedure, we first search for the individual which makes the maximum contribution to total inertia. Then, among the rest, we search for the individual which with the former gives the maximum SD value, and so on. At each step, SD is estimated, so it is possible to stop at any level between a few % and 100% of total inertia. A selection could also be made on a preselected number of individuals.

An example with okra using principal components analysis

1. *Main factorial axis*

The best data set for this purpose is certainly one that contains a maximum number of individuals studied in similar conditions, but for our purpose we have chosen the data for the actual core collection, arbitrarily limited to 152 individuals, and have removed non-quantitative data.

The coded names of the variables are as follows: first flowering day (FFD), plant height (PLHT), number of nodes on the main stem (NNS), stem diameter at the stem base (DIAM), first fruiting node (NIFR), flowering amplitude (FLAM), pod length (LGPD), pod width (WIPD), number of ridges (NBRI), 1000 seed weight (TSW), and seed production (SDPR). The sign (-) just in front of a variable indicates that its contribution to a principal component is opposite to that of the others.

If we retain only axes with a λ value equal to or greater than 1, axes 1 to 4 are valid, if the critical λ value is 0.5, axes 1 to 6 are valid. For axis number 1, major contributions are associated with FFD, NIFR and NNS, followed by DIAM and (-) TSW. For axis 2, major contributions are associated with the following variables: (-) WIPD, LGPD, (-) NBRI; and for axis 3, PLHT, (-) PRDG. Axis 4 is mainly associated with (-) FLAM but also with the remaining part of PLHT and (-) SDPR not accounted for by axis 3.

All 11 original variables are taken into account in the system defined by axes 1 to 4. Several variables are correlated in the same direction for a number of axes. This indicates that some descriptors are less informative than others and that they could be removed. Without going into the details of the analysis, we can see that axis 1 reflects plant precocity, the initial vigour and the ability to produce nodes and light seeds. Axis 2 is mainly associated with pod characteristics, with pod width and ridge number inversely correlated with pod length. On axis 3, plant height and seed production are inversely correlated.

2. *Increase of the selected variability*

At the beginning, the data set constituted 152 individuals. The progression of SD is shown in Fig. 1. Thus we can see that with 15% of the individuals we have selected 30% of the inertia. We reach a level of 50% of total inertia with 30% of the individuals. As a result of the decrease of the slope of the curve, the further addition of new elements becomes progressively less useful.

3. *Position, on the axis, of the selected individuals*

By definition, the axis which corresponds to the higher level of inertia is horizontal and the next vertical. Each such factorial plan is divided in four sectors called, by convention, A (-+), B (++), C (+-) and D (--). Signs in brackets correspond to negative or positive values on the axis, ordered by their decreasing value. For example A (-+) means a negative value for axis 1 and a positive one for axis 2. The distribution of the samples is shown with the total number of individuals by quarter and the relative percentage in the sample.

a) Plan (1 X 2) - A (47, 42.5%); B (31, 41.9%); C (41, 41.4%), D (33, 27.2%).

Selected diversity

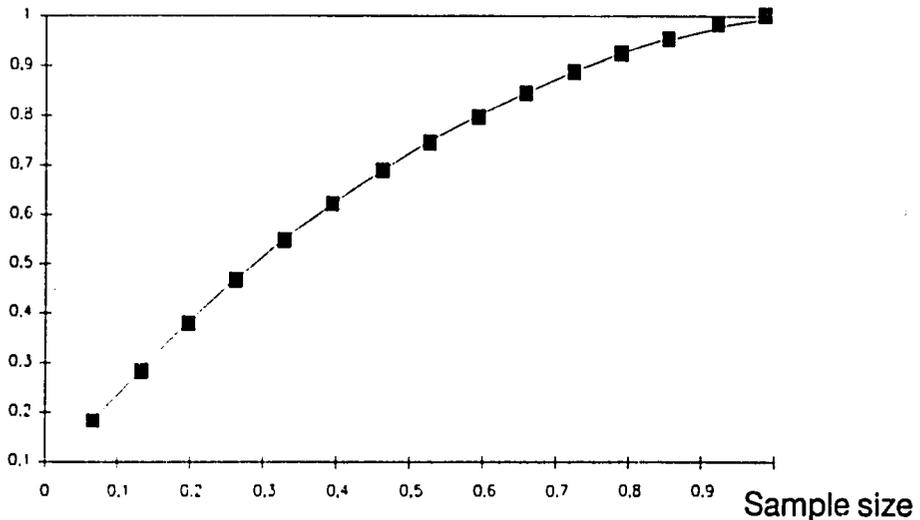


Fig. 1. The relationship between selected diversity and sample size

The sampling result in each quarter, except for D, is well balanced. In Fig. 2, we can see that the sampling is very good in the peripheral area but there is an important zone next to the centre without selected individuals.

b) Plan (2 X 3) - A (45, 33.3%), B (39, 43.5%), C (38, 42.1%), D (30, 36.6%).

Here we also observe a low density of selected individuals near the centre and a small disequilibrium in the quarters, where the limits are 33% and 43%.

4. *The picture given by clustering analysis*

The step by step analysis of each factorial plan gives an interesting but restricted view. A more general appreciation is obtained by a clustering analysis made on the first 4 factorial axis. The clustering procedure is the variance criteria on weighted Euclidian distances.

The dendrogram is characterized by a division into 3 main clusters. The following symbols are used: C = cluster number, S = number of sub-cluster in a given cluster, I = number of individual in a cluster and P = the sampling percentage in a cluster. The dendrogram, when the total number of selected individuals is limited to 60, can be summarized as follows: (C1-S7-136, P 30.5%); (C2-S20-144, P 52.3%); (C3-S14-172, P 36.1%). Thus, we observe that when a cluster becomes complex ($S_7 < S_{14} < S_{20}$) the sampling percentage increases (31, 36, 52). If the total number of individuals is limited to 30, then the breakdown is C1 (20%), C2 (56%), C3 (24%) and again the choice is made according to the internal structure of the cluster.

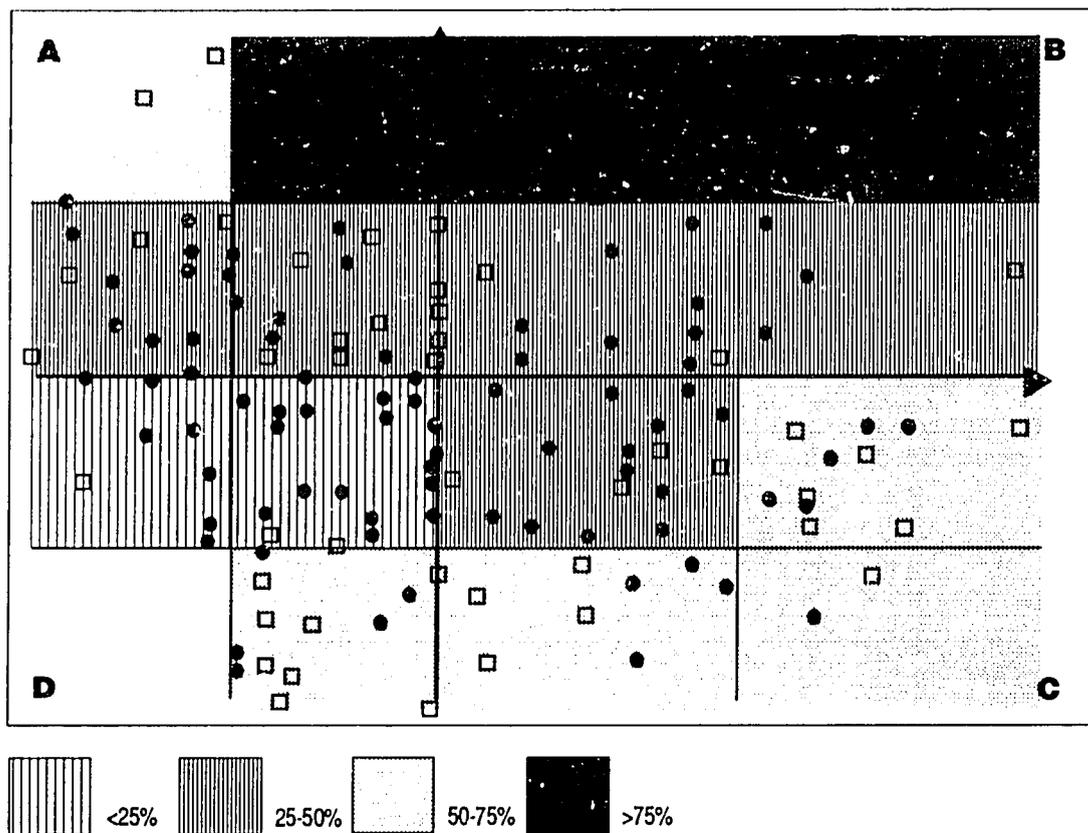


Fig. 2. PCA scatter plot showing position of individuals with reference to principal components I and II. Squares represent individuals selected by the inertia procedure

Discussion

A method has been described which permits statistical selection of a sample of a given set of individuals, based on selecting maximum variability using quantitative data.

First, the initial data set (accessions x descriptors) containing more or less correlated variables is transformed to an independent axis system where individuals are characterized by their scores defined on each axis. We then identify a sample which contains the maximum existing variability by a step by step aggregative procedure. Choice criteria allow us to fix the number of individuals or the percentage of the total variability retained in the sample.

In the example studied, this method gives a sample which is equally distributed in the sectors defined by the factorial plans. However, the percentage of selection is greater in areas further from the factorial axes. The clustering analysis, which gives a more synthetic view, shows that more individuals are selected from the more complex clusters.

This procedure can be criticized on the following points:

- contrary to random sampling, it does not take into account the central area of diversity. The clustering analysis gives a first partial answer. However, it seems likely that it will be easier to regenerate the diversity of the central part using peripheral individuals than the converse;
- it is also possible to argue that the heritability of the variables used is not well-known. Such criticism is open to experimentation but does not affect the selection procedure *per se*. However, when the level of heritability of characters used is known, it is better to use those with high heritability.

The method described provides a possible way of improving the way the choice of a core collection is made. To obtain a really efficient method qualitative data should also be included in the analysis.

**Potential and limitations of standardized descriptors
for the genus *Abelmoschus* (okra)**

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Summary

The limitations of a set of standardized descriptors are becoming apparent during trials for the following reasons:

- there is monomorphism within a specific species
- there are descriptors which are not planned but interesting
- descriptor states are lacking
- the absence of guidelines for selecting a subsample of descriptors

In this paper we make comments and proposals (on the basis of the existing set of descriptors) that should allow a set of descriptors to be elaborated in the future that is less constraining and better suited to the purpose of describing existing diversity and its organization.

Introduction

The management of crop genetic resources is not limited to the storage of seeds only. In such cases, collections become quickly unusable, thus it is essential to describe, as best as possible, samples to implement a reliable information basis for their effective use. We will try to issue comments on a general level, but many remarks will of course be linked with observations from West Africa.

Okra is an annual, herbaceous plant. The monoic flowers are self-compatible. The rate of allogamy differs according to varieties and ecological conditions (Martin, 1983; Hamon et Koehlin, submitted a,b).

Seeds are kept in fruits for cultivated forms with the exception of those from very wet, forest zones. These fruits, when collected, are sorted individually into groups of similar fruits or put together in a basket (Hamon and Charrier, 1983). There is no information available on the parents, or even on the mother plant. In principle, one sampling per fruit should be done. The starting set would then include between 50 and 150 half-sister seeds.

Often many fruits are put together in order to reduce the number of samples and to increase the number of seeds. This practice induces irreversible mistakes such as the mixture of species or varieties.

In this paper we will, on the basis of our experience (Hamon, 1988; Hamon *et al*, in press) analyze critically the list of descriptors which was proposed by Charrier (1984) and used as a reference by IBPGR. We will underline the reliability of some of those descriptors, the limits of others and the unusefulness of some of them in specific conditions.

Comments and proposals (descriptors)

1. *The key steps of characterization*

The characterization of samples or the evaluation of their diversity includes many key steps; these are mainly:

1.1 "Passport data". Data linked to the origin of the germplasm

In the past these data were often missing (Peeters and Williams, 1984). Data about the country of origin, collector's number, geographical coordinates and sometimes ecological data of the collecting sites are now available for recently collected accessions, but information on their role in local agricultural practices is often lacking. But excess of information at different levels (store, backyard primitive cultivar, advanced cultivar soils type) is often difficult to interpret.

1.2 The description of the sample at the collecting stage

This is the most important step. Inaccurate or incomplete information will have irreversible consequences.

The description of an okra specialist may be quite different from that of a generalist who does not know the diversity of the genus. At a simple level, confusing *A. esculentus* with *A. caillei* is common for at least three reasons: 1) *A. caillei* is not yet well known; 2) African farmers consider *A. caillei* as a late type of *A. esculentus*; 3) there are no traits of classical botany which can be applied to the pods (Stevens, 1988).

2. Identification of the accession

For easier reading, we have not respected the order of descriptors in Charrier (1984); however, we will systematically refer to its numbering.

2.1 (D 1.1.1) Accession number

The accession number is unique and should also be employed during evaluation. Other passport data do not need to be referred to, but the corresponding file will be consulted, if necessary. However, the geographical coordinates should be included, as they will serve as a variable for analysis.

2.2 Coordinates of collection sites

- (D 2.2.4.) Country of origin

The international code proposed by IBPGR (1976) is used

- Geographical coordinates of the collecting site

(D 2.2.7.) Latitude, (D 2.2.8.) Longitude

2.3 (D 1.5) Scientific name

The name of the species as provided by the collector and included in the passport data should be distinguished from the identification of the evaluator. Experience shows that the co-existence of two cultivated species in Africa is an important source of mistakes; similarly, the taxonomy within *A. moschatum* and *A. manihot* is not yet very clear.

Proposal

1) *A. esculentus*, 2) *A. caillei*, 3) Natural hybrid between *A. esculentus* and *A. caillei*, 4) Mixture of *A. esculentus* and *A. caillei* in the same sample, 5) *H. sabdariffa* (often included as samples), 6) *H. cannabinus* (often included as samples), 7) *A. moschatum*, 8) *A. manihot*, 9) Unknown, 10) *A. ficulneus*, 11) and 12) and following numbers: not yet designated.

N.B. Fruit shape, seed striation and zymograms are very reliable identifiers.

2.4. The homogeneity of the accession

The homogeneity of the sample is commonly accepted as a basic starting point. This is often not the case, notably in Africa, and this should be noted as soon as the evaluation allows. The level of heterogeneity can be linked with the status of the sample, but it is quite often the case that many characters segregate. It is up to the evaluator to fix limits in a global manner (see below) or for some characters (refer further for each character).

1) Yes (variety of fixed type), 2) No (heterogeneity but same species), 3) No (heterogeneity with two different species), 4) No (two different species plus hybrids).

3. Quantitative characters linked with growth

3.1 General remarks

A certain number of rules should be respected for the characterization to be useful. These are:

- describe accurately the site, including data on sowing period and pluviometry;
- use well-known standards, at least two (or three) for each species;
- carry out evaluation separately for each species but include in each evaluation the standards from other species in order to create a reference basis;
- indicate on how many plants the observations were made.

Around 30 plants should be enough, in the first evaluation phase, to provide a good picture. If the sample is obviously not a fixed form or an interfecondation, there are no real solutions except to create subsamples and purify them later on.

3.2 Variables for vigour and growth

Three variables are retained. These are:

- D 6.1.2. Maximum plant height (cm)
- D 6.1.9. Maximum numbers of internodes
- D 6.1.3. Stem diameter at the base (mm)

These three descriptors will cause problems if the conditions of observation are not precisely defined. Indeed:

- 1) the diversity of growth cycles within the same species is important. Okra growth is continuous until death. Thus, maximal value should be estimated on dead plants. This implies numerous visits and risks of errors;
- 2) it is more difficult to compare *A. esculentus* with *A. caillei* in West Africa because the former ends its cycle when the latter starts to flower; wild species have cycles of undetermined duration and are more or less perennials.

In conclusion, there is therefore a need to identify the species of the sample quickly and to proceed accordingly with separate trials.

The best time to observe *A. esculentus* is around 80 to 90 days after sowing. At this time, most *A. caillei* plants will still be growing. The optimal time for this species, which has slower growth, is around 100-110 days. Finally, the speed of growth of wild species is much slower. There is therefore a need to be extremely careful when making these observations.

3.3 Variables for habit and plant structure

Plant structure and habit are characters which are taking different modalities. With *A. esculentus* there is no real problem, because there are only a few branches, which can be clearly seen at the base. They are easy to count and their length can be measured. There are more difficulties with *A. caillei* whose structure is much more branched with eventually secondary branches. The same occurs with most of *A. manihot*; it becomes nearly impossible to observe number and length of branches of *A. moschatatus*, which is bushy.

These characters are nevertheless interesting because, beyond a simple description, they serve as reference parameters for the yield. Indeed, the fruit yield on the stem is little influenced by the production on branches, which for *A. caillei* and wild species is often of greater importance.

Proposals

- Plant habit

1) Unique orthotrop axis, 2) Dense branching at the base followed by an orthotrop axis without branches, 3) Base without branching but densely branched apex, 4) Densely branched all over the plant

- Length of the branches

The number of branches can be counted but the length should be estimated, because of the time taken for measurement, in the case of many accessions

0) No branch, 1) Rarely more than 10 cm, 2) Frequently more than 10 cm.

4. *Inflorescence and fruit*

4.1 General remarks

The flowering of okra starts with the emergence of a first flower which may be followed, depending on the species, by a new flower every day, or a minimum of one new flower per week, continuing until the end of the cycle.

A flower opens during the morning, closes in the afternoon and the corolla will fall the following morning. The young fruit will grow very quickly and can reach, for *A. esculentus*, several centimeters in a few days.

The observation of flowering characters requires a lot of work if results are to be precise (in terms of days). Observations of terms of weeks after flowering will reduce the workload.

The observation of pods, which, unless exceptions, are homogeneous, is important. Observations should be done on fruits which are representative and not on the ones which are obviously misshaped.

The actual codification is too restrictive. The diversity observed in Africa shows that more importance should be given to traits which are used in breeding or at least for variety identification. Measurements should be done on completely mature and dried fruits.

4.2 Flowering and fruit earliness

Four descriptors are retained to assess flowering and fruit earliness; these are:

- D 6.2.1. First flowering
- D 6.2.2. First flowering node
- D 6.2.3. First fruit-producing node
- D 6.2.X. Flowering span

N.B. 999 is noted if no flowering occurs

The flowering span is a new descriptor which could be interesting in that it provides an index of grouped flowering. This can be difficult to assess, because it requires daily visits, but its observation can be simplified by a weekly registration of the production of young fruits. The observation of the first 10-15 plants per accession is possible.

4.3 Fruit shape

Five descriptors are selected as follows:

- D 4.2.8. Fruit length at maturity
- D 4.2.X. Fruit width
- D 4.2.11 Number of ridges per fruit
- D 4.2.6. Position of fruits on the main stem
- D 6.3.1. Weight of 1000 seeds

N.B. The number of ridges per fruit and their position on the main stem are useful only for cultivated forms and in this case require the selection of descriptor states. The number of ridges is always five for wild forms. This descriptor is an exception to the quantitative continuity, as the number of ridges is either zero if these are not well marked, or it varies between 5 and 12. There are nearly always variations on the same plant, thus assessment is difficult and the formation of classes should be preferred.

Proposed descriptors

D 4.2.11. Number of ridges per fruit (simple genetic character with intermediate heterozygotes)

1) Smooth fruits or ridges unmarked until the base, 2) 5 ridges, 3) Frequently more than 5 but less than 9, 4) Frequently more than 9

D 4.2.6. Position of fruit on main stem

Homogeneity: 1) Erect, 2) Intermediate, 3) Horizontal, 4) Slightly falling, 5) Totally falling. *Heterogeneity:* 6) Presence of 1 and 2, 7) Presence of 2 and 3, 8) Presence of 3 and 4, 9) Presence of 4 and 5

5. Colouring of diverse plant organs

5.1 General remarks

Colour traits are not easy to assess due to uncertainties with colour variations, even in a homogeneous accession. This applies not only to green and red variations, but also to all other colour characters, except for the internal flower spot. The superimposition of one colour upon another is common with okra, the green or red background being influenced by other colours. The ideal would be to print a scale of colour references on paper in order to avoid observations being influenced by the previous accessions. There is also a need for staff with a very reliable sense of colour and its gradations. Thus, except for clear cases, there is a need to be prudent and to do two evaluations. It should not be forgotten that, due to the complexity of the genetic base of the pigmentation (Mehetre *et al.*, 1980), it is rare to find a totally homogeneous accession. Accessions should be noted as heterogeneous when differences are marked beyond the level of gradations.

5.2 Colouring of vegetative organs

4 characters are selected. These are:

D 4.1.4.	Stem colour
D 4.1.6.	Lamina colour
D 4.1.X	Leafrib colour
D 4.1.Y	Petiole colour

- D 4.1.4. Stem colour

Homogeneity: 1) Green (unless special cases, gradations will not be noted), 2) Green with clearly noticeable red spurs, 3) Anthocyanic (red or purple) even if there are some green spurs. *Heterogeneity:* 4) Rather red, 5) Rather green, 6) Complete segregation

- Leaf colour

Leaf colour is assessed from that of lamina and ribs. Lamina is of green colour, but eventually, in some cases (plants with slightly anthocyanic stems) red spots may appear on aged leaves. It is better not to note this, as the stage of appearance of this phenomenon is difficult to assess. Only important red spots, which are rare, should be noted.

For the ribs we can distinguish the following sections:

- the point of convergence of the ribs (foliar spots); the basal half of the ribs; the distal half of the ribs

Quantification is very difficult. It is preferable to limit ourselves to the presence of anthocyanic pigmentation on basal and/or the distal half.

- D 4.1.6. Lamina colour

1) Green, 2) Green with some red veins, 3) Green with important red spots

- D 4.1.X. Rib colour

Homogeneity: 1) Totally green, 2) Red at the ribs' joining point, thereafter totally green, 3) Green with some red spots widespread on the lower half of the ribs, 4) Green but with plenty of red spots all along the rib, 5) Ribs nearly totally red. *Heterogeneity:* 6) Heterogeneity (1+2), 7) Heterogeneity (2+3), 8) Heterogeneity (2+4 or 5), 9) Complete segregation

- D 4.1.Y. Petiole colour

1) Green, 2) Red above but green below, 3) Red on both sides

5.3 Flower and pod colour

Three descriptors are selected. These are:

D 4.2.5. Petal blotch

D 4.2.7. Fruit colour

D 4.2.X. Colour of the darkest ridges

- D 4.2.5. Petal blotch

Okra flowers all have a red petal blotch in the centre of the flower. Nevertheless, the blotch on the external face may be absent. This character is simple monogenic; we will therefore note the three following cases:

Homogeneity: 1) Internal, 2) External. *Heterogeneity*: 3) Segregating

- D 4.2.7. Fruit colour

A large scale of colours and gradations is involved in addition to the usual difficulties associated with the observation of colour markers. This is particularly true when red and green are together or when diverse nuances of green must be described. The diversity of fruit colour reaches its maximum in West Africa.

It is important, before starting the notations, to become acquainted with the descriptor states. It would certainly be most useful to build a file of colour references.

N.B. The numeration follows its gradual implementation, hence its apparent disorder. Reordering of the descriptor states could be useful.

1) Whitish green to white, 2) Common green, 3) Green background plus more or less red spots, 4) White background plus more or less red spots, 5) Red, 6) Green towards black, 7) Light green but not white (1+2), 8) Mixture of (2+3), 9) Violet to purple, 10) Mixture of (2+6), 11) Dark green but not black, 12) Mixture of (3+5), 13) Mixture of (6+5), 14) Water green (characteristic of Sudan), 15) Pink, 16) Mixture of (3+6)

- D 4.2.X. Darker colour of fruit ridges and spines on fruits and seed hairiness

1) No hairs, 2) Slightly hairy, 3) Very hairy, 4) Slightly spiny, 5) Numerous spines, 6) Spines on fruits and seed hairiness, 7) Seed hairiness but no spiny fruits

6. *Electrophoretic markers*

The use of enzymatic diversity for varietal identification has been used for many crops. With regard to okra, the low level of polymorphism (Hamon, 1988) shows that this technique, using classical systems, only allows (with a few exceptions) the two cultivated species *A. esculentus* and *A. caillei* to be distinguished.

For wild species we only have data for *A. moschatum* and *A. manihot* (Hamon, 1989). These results show, however, that the level of diversity for these species is clearly superior and that these markers could be useful.

7. *The descriptors at a species level*

We have not examined, in this paper, descriptors that are used at the species level, i.e. those which do not vary within a given species.

The example of zymograms, as outlined above, is a particular case. These observations will be made at the stage of species identification (refer to para 2.3) and could eventually be included within passport data.

8. *Non-independent descriptors*

We cannot develop the problems linked with non-independent descriptors, some aspects of which have been treated in other papers (Hamon, 1988); nevertheless, knowledge of these may allow substantial time-savings when making observations.

Descriptors are often presented as autonomous elements and this is inaccurate. Indeed, numerous quantitative descriptors are correlated with each other because their purpose is to:

- assess, in a different way, the same biological phenomenon;
- assess different aspects of the same organ.

Similarly for the qualitative variables, information on one may allow, with good probability, the state of another descriptor to be guessed at; this applies for many colour descriptors.

Discussion

We have presented in this paper the potential and limits of a set of standardized descriptors for the genus *Abelmoschus*. Our work is based on the initial list proposed by Charrier (1934), which was not considered exhaustively, but the most used and most easily assessable descriptors in that list were picked out.

We have proceeded by regrouping descriptors which can be measured or observed simultaneously. Their number is reduced and in some cases this number could be further reduced when some quantitative descriptors are correlated or when qualitative ones are not independent.

The most important recommendations we would like to make are the following:

- make sure species are correctly identified before being characterized (this can be done at the time of collecting or performed on the seeds with the help of zymograms);
- compare only what can be compared, i.e. distinguish in time and in the field the characterization of each species. Be careful not to plan so many observations that they become unmanageable;
- select in respect of the evaluation site some standards (two very different per species) which will be systematically included in all trials. This will allow more reliable comparisons;
- check the genetic homogeneity of the sample: autogamy and varietal fixation are far from being the rule. About ten artificial selfings should be performed in each sample: this means about 1000 seeds.

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Okra germplasm build-up and evaluation

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Okra is a popular vegetable grown throughout the country, but researchers have only turned their attention to germplasm since the middle of the twentieth century and before 1946 there were hardly any germplasm collections in India. Local types and a few exotics were grown, which included White Velvet, Clemson Spineless, Perkins long green, Best I, Best II, Redwonder, Barielly Wonder, Indian long green, Panchdhar, Satdhari, Dashdhari, Jabura, Silari, Satpani, Badnawar, etc.

Work on okra germplasm was initiated at the Indian Agricultural Research Institute, New Delhi, during 1946 under an ICAR project which assembled some popularly grown cultivars and wild relatives from different regions. These were evaluated for useful traits, particularly economic traits, as a result of which Pusa Makhmali was identified in 1950 as the first improved variety in India, developed under the leadership of Dr. H.B. Singh, from an accession from Sabour in Bihar, which was released for large scale cultivation in 1955. Although it was a very good variety suited to both seasons, it was susceptible to yellow vein mosaic virus, which was a limiting factor in okra cultivation. The existing germplasm was thoroughly screened and one accession, I.C. 1542 from W. Bengal was identified as field resistant. It was crossed with Pusa Makhmali in 1952 and 4 promising selections were made in 1955 which were further evaluated. In sel-7, which was most promising, further selections were made and tested and 7-11 with attractive fruits were identified in 1959 as Pusa Sawani, which was released for large scale cultivation in 1960. This was the first okra variety developed with resistance to YVMV (symptomless carrier from IC. 1542) and it spread like wild fire throughout the country, causing great gene erosion of the valuable landraces from major okra growing regions.

During 1961 to 1974, okra germplasm was built up by the erstwhile Plant Introduction Division (the present NBPGR) during multicrop explorations and also specific crop explorations for guar and legumes, and it assembled germplasms, particularly from the north and northwest regions. By the end of the 1960s, the resistance of Pusa Sawani was broken down, and efforts were made to look for new sources of resistance in cultivated okra as well as related species. Introductions from Ghana (*A. manihot* ssp. *manihot*) and Japan were found to be resistant, as was *A. tetraphyllus*. These were sent to the main centres working on okra improvement in the country, from which a number of new, resistant, promising varieties were developed, viz. Punjab Padmini and P-7 from PAU, Ludhiana, Parbhani Kranti from Parbhani; Sel-4 and 10 from IIFR, Bangalore, G-2 and G.2-4 from NBPGR.

Jassids also had become a major problem and some multiple crosses had been made to improve Pusa Sawani in the early 1970s (Pusa Sawani x Best-I) x (Pusa Sawani x I.C.7194 - an accession from Punjab highly resistant to jassids) and 6 selections were made by Dr. Singh and sent for testing to all centres from which Sel-2, Sel 2-2, Sel 6-1 (released as Harbhajan in Himachal Pradesh for low hills) and Sel 1-1 (released as Gujarat Bhinda-1 in Gujarat) were identified for large scale cultivation. Other improved varieties developed include Sel-13 in Punjab (1966), T-1 & T-2 in Uttar Pradesh, Shankarpalli, Somalkotta and CO-1 in Tamil Nadu, developed from germplasm.

From 1976-1988 NBPCR strengthened the okra germplasm during multicrop explorations and the All India ginger survey and second finegrid survey for guar (both undertaken by the author). About 1000 accessions, which were initially evaluated for a few important agronomic traits, were assembled. As per 1980 the All India Vegetable Crops Improvement Project demanded a descriptor list and NBPCR developed a descriptor list for 10 *klurif* vegetable crops (Thomas, 1981), one of which is okra. IBPGR published a descriptor list for okra in 1984. NBPCR has evaluated the germplasm using NBPCR descriptors and a catalogue on 558 accessions (based on two years' data) was published in 1990 for 45 descriptors. This is the first okra catalogue published, as far as I am aware. Germplasm collections are also being built up at IHR, Bangalore (125), PAU, Ludhiana (120), GAU, Junagadh (129), OAUT, Bhubaneswar (27) and KAU, Vellanikkara (48).

During 1989 further efforts were made to assemble the okra variability from the region by crop specific collection with IBPGR support and 473 cultivated and 296 wild species accessions were collected (mainly belonging to *A. tuberculatus*, *A. ficulneus*, *A. manihot* var. *tetraphyllus* and *A. moschatus*) by 8 explorations in different places and 45 in Bangladesh during 1990. These along with 177 IBPGR core collection accessions from Côte d'Ivoire are being evaluated for 47 descriptors. 1989 collections include 41 from North Kerala, East and South Tamil Nadu and Andhra Pradesh; 53 from Kerala, S. Karnataka and South Tamil Nadu; 61 from Meghalaya and Assam; 129 okra, 114 *A. tuberculatus*; 84 *A. ficulneus*; 32 *A. manihot* var. *tetraphyllus* from Gujarat and Rajasthan; 19 from Orissa, 71 okra, 16 *A. ficulneus* and 5 *A. tuberculatus* from Western Ghats; 82 okra, 20 *A. moschatus* and 3 *A. manihot* var. *tetraphyllus* from Uttar Pradesh.

Evaluation

Germplasm stored is of no use unless it is properly evaluated. Hence, proper descriptors and descriptor states need to be used for characterization. Important descriptors and descriptor states followed are given below:

1. Plant characters: habit, height, branching, pigmentation, hairiness, node number, thickness of stem and fruiting node.
2. Leaf: size, shape, lobing, margin, tip, veins, petiole.
3. Flower: type, colour, hairiness, type of calyx and epicalyx, stigma type and shape.
4. Fruit: size, colour, shape, position, hairiness, pedicel size, fruit weight.
5. Seeds: shape, colour, hairiness, size seeds/pod, dormancy.
6. Maturity: days to flowering and maturity.
7. Tolerance to stresses: salinity, wetness, shade, drought (1-9 scale).
8. Resistance to pests and diseases: all major ones (1-9 scale).
9. Chemical evaluation: oil content, protein %, gossipol %.
10. Biotechnological evaluation: isozyme evaluation.

The NBPCR is evaluating germplasm at its station at Trichur and Akola (320 for 33 descriptors), particularly the Nigerian and Brazilian materials). 27 were also evaluated at Bhubaneswar for 10 descriptors and 48 at Vellanikkara for 15 descriptors.

A number of promising accessions were identified for various traits, particularly for yield contributing characters. One accession from Nigeria, 4 from Brazil and over 2 dozen promising indigenous ones were identified. Also some germplasm suited to different regions was identified from multilocation evaluation. A good range of variability is observed with regard to plant height, branching, number of nodes, position of first fruiting node, leaf size, pigmentation, internodal length, stem thickness, number of ridges of fruits, fruit colour, days to flowering and maturing, tolerance to stresses and pests and diseases. Chemical evaluation needs to be done, and this was planned for 1990. Collecting from remaining areas in India and also from Sri Lanka, Nepal and Burma is planned for 1990-91.

Evaluation in other countries

The USA has 1688 accessions at Griffin, but evaluated only 224 for 11 descriptors in 1965 and 285 for 29 descriptors in 1981; Japan has evaluated 29 accessions for 12 descriptors (1964); Côte d'Ivoire, 314 accessions for 7 descriptors (1982); in Nigeria all germplasm has been evaluated for 15 descriptors (1982); and in Papua New Guinea all germplasm has been evaluated for 18 (mainly leafy) descriptors. Now most of the centres are following the IBPGR descriptor list for germplasm evaluation. This aspect needs more attention, if the assembled germplasm is to be properly utilized.

Future thrust

1. Evaluation of all germplasm for all traits to be done at the national centre for germplasm.
2. A core collection to be identified for each national collection based on passport and characterization data, which should be further evaluated in multilocation tests to identify useful germplasm for utilization.
3. Germplasm from core collections to be increased for exchange.
4. There is a need to assemble all wild okra germplasm variability in each country and evaluate it properly.
5. Studies for better maintenance and regeneration procedures need to be developed.
6. Chemical evaluation and evaluation for stresses to be given more attention.
7. All existing collections to be increased and conserved in the base collection along with passport and characterization data.
8. Generated information on germplasm to be processed and catalogued and also supplied to national database for documentation and utilization.

Data management within the okra genetic resources network

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Introduction

If the okra germplasm is to be made available to participating countries, it is a prerequisite that each network participant has ready access to information on all accessions conserved by the network. Each participant should be able to retrieve information on the location, origin, agronomy, taxonomy and other relevant characteristics of the accessions. To enable such a system to function, one institution within the network must assume a coordinating role. This institution will coordinate information compilation, maintenance and dissemination of the requested information to other participants.

This paper describes some of the aspects regarding data management within a potential okra germplasm network.

Okra genetic resources data

IBPGR descriptor lists are widely used to describe germplasm and to facilitate the effective management of germplasm information. The following classification of descriptors in these lists is now in use:

1. Passport

These include accession identifiers, collecting and other data regarding the 'history' of the accession. The collector of an accession is responsible for recording most of this data, whereas the curator assigns the accession number and maintains other names or numbers given to the accession by the donor, if the accession was acquired from sources other than an original field collection.

2. Characterization

These are descriptors for characters that are highly heritable, can easily be seen by the eye and are equally expressed in all environments. This makes the characterization descriptors very useful for quick identification of accessions.

3. Evaluation

These include traits usually influenced by environmental factors.

4. Management

These are descriptors which are used by the curator to assist in the management of germplasm collections. They consist of information which is indispensable for the management of accessions in medium- and long-term storage, for the multiplication and regeneration of material, and for the exchange of accessions.

Workshop participants should agree on descriptors that are vital for database management in a network, i.e. passport data, characterization descriptors - a minimum set? - and, if deemed necessary, some evaluation descriptors. Management descriptors are mainly of local importance and only commonly shared descriptors need to be standardized.

Details of the aforementioned descriptors have been published by Charrier (1984) and IBPGR (1989).

Identification of accessions

To avoid potential confusion regarding the identification of material maintained by participating institutions, it is necessary to provide some basic information about each accession maintained in the database. The greater the detail about the material, the more likely it will be that confusion during communication about individual accessions will be avoided. The most reliable characters to use for this are characterization. However, using only these will not always provide the ability to discriminate between accessions that closely resemble one another.

Another possibility could be the registration and maintenance of all the accessions existing in all the member countries of the network at a central place (i.e. base collection) along with a comprehensive description made at (a) standard location(s). This would provide standardized information and ensure a high degree of data reliability.

An information network for okra germplasm

The institution that coordinates information on all the accessions known within the network will be expected to develop and maintain a central database. The participating national institutes' databases will form the building blocks. This database should contain the following information for each accession maintained in the network:

- passport data
- characterization data
- evaluation data

All known okra germplasm programmes are encouraged to participate in the information network. It will be necessary first to document the passport information of their respective national collections and subsequently to send these to the coordinating institute or central database (CDB). Respective data files are compiled into several levels of verification and formatting of the data is needed before redistribution to the participants. This database will be periodically updated through the participants.

For the central crop database to function as the principal information source of the network, the commitment of all participants to give high priority to the documentation of their collections and to share information with the coordinating institute is needed. This commitment must include the regular updating of the data files and redistribution of the information by the coordinating institute. Subsequently, data on characterization and evaluation can eventually be added to the central database which will improve its usefulness with regard to duplicate identification establishing collecting priorities, coordinated characterization/evaluation trials and maintenance of collections.

In order to enable the above to function properly it is required that:

- the format for the exchange of data be defined;
- a set of standard passport descriptors be agreed upon by all participating members and the agreed rules strictly followed;
- further descriptors for use in the central database be standardized. Also exact and accurate descriptor definitions must be provided. This includes where, how and on which parts of the plant the descriptors have been collected;
- all the participating national databases be fully committed to updating and submitting their data sets in timely fashion to the CDB;
- an *ad hoc* committee or a standing committee function as a scientific body to give leadership to the network.

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The use of related species in transferring disease and pest resistance genes to okra

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Okra (*Abelmoschus esculentus* (L.)) is an important vegetable crop in the tropical and subtropical parts of the world. It is normally grown in the monsoon and summer seasons in India, although it can be grown all the year round in the eastern and southern regions of the country.

Yellow Vein Mosaic Disease

Okra incurs severe losses in India due to the yellow vein mosaic (YVM) disease transmitted by white fly (*Besimia tabaci* Gen.). Pusa Sawani, the tolerant cultivar, was developed using a resistant gene from the strain I.C.1542 (Joshi *et al.*, 1960). Pusa Sawani had stabilized okra cultivation in the 1960s and early 1970s. However, later Pusa Sawani has been severely affected by YVM. There is no source of resistance to YVM in the cultivated okra; some related species are resistant to the disease (Sandhu *et al.*, 1974; Arumugam *et al.*, 1975; Thakur, 1976; Arumugam and Muthukrishnan, 1978).

Three YVM resistant taxa, viz. *Abelmoschus tetraphyllus* Wall. (2n=138), *A. manihot* (L.) Medik. ssp. *manihot* from Ghana (2n=194) were crossed to *A. esculentus* (L.) cv. Pusa Sawani (2n=130) reciprocally during the monsoon season of 1976. The F₁'s raised in summer 1977 were partially fertile or completely sterile. The partially fertile hybrids were advanced to subsequent generations directly or after one or three backcrosses to Pusa Sawani. Amphidiploidy was induced in the three hybrids by colchicine treatment of seeds or vegetative buds. Amphidiploids were also crossed to Pusa Sawani. Every generation was grown under epiphytotic condition of the disease and only resistant plants were backcrossed to Pusa Sawani and/or selfed. The artificial epiphytotics were created by growing infector rows of Pusa Makhmali, a variety highly susceptible to YVM, around the plot. The material free from disease symptoms under field conditions was further screened artificially in the glasshouse employing the bud graft technique (Noordam, 1973). In all the generations selections were made on the basis of resistance to YVM, seed fertility and tenderness of fruit.

The hybrid *A. esculentus* × *A. manihot* was partially fertile (5.90% seed fertility). Resistant segregants from the F₃ generation could not be carried further due to complete seed sterility and failure to obtain backcrosses. However the backcross of F₁ hybrid to Pusa Sawani was successful. Improvement in fertility and restoration of the desirable traits of Pusa Sawani were sought through 1 to 3 backcrosses followed by selection. Some of the segregants in the BC₁ F₄, BC₂ F₄ and BC₃ F₂ generations were resistant to YVM, and had about 58 to 88% seed fertility and desirable fruit characters of Pusa Sawani. An induced amphidiploid of this cross was backcrossed to Pusa Sawani, but the BC₁ plants were sterile and could not be further backcrossed to Pusa Sawani.

The hybrid *A. esculentus* × *A. manihot* ssp. *manihot* had 7.07% seed fertility. Backcross of F₁ hybrid to Pusa Sawani was successful but did not show promise in BC₁ F₂ and BC₂ generations. An attempt to backcross the induced amphidiploid was not successful. A resistant segregant from F₂ generation had improved seed fertility (71.67%) and desirable traits. The F₆ lines derived from this plant had fixation of morphological traits of Pusa Sawani and resistance to YVM.

The hybrid *A. esculentus* × *A. tetraphyllus* was completely seed sterile and attempts to backcross the F₁ to Pusa Sawani met with failure. Backcrossing the induced amphidiploid to Pusa Sawani could not be carried beyond BC₂ due to sterility.

Yellow vein mosaic resistant and fixed lines, five derived from the cross *A. esculentus* cv. Pusa Sawani x *A. manihot* and one line from the cross *A. esculentus* x *A. manihot* ssp. *manihot*, were evaluated in yield trials along with two check varieties (Pusa Sawani and Sel. 2-2). The trials were conducted at three locations over a period of four years (1982-85) during the monsoon and summer seasons. One promising line was further tested in mini-kit trials on farmers' fields and officially released for commercial cultivation in the country by the name Parbhani Kranti. Fruits of Parbhani Kranti are dark green, smooth, tender and slender. On average, Parbhani Kranti, Pusa Sawani and Sel.2-2 yielded 115, 107 and 116 q/ha green fruits respectively in the monsoon season, and 83,76 and 78q/ha respectively in the summer season. However, 10-19% fruits of Pusa Sawani and 19-22% of Sel.2-2 turned yellow due to YVM infection in the monsoon season and thus were of poor marketable quality. In the summer season, 40-43% fruits of Pusa Sawani and 60-64% of Sel.2-2 were infected with YVM. Plants and fruits of Parbhani Kranti were completely free from YVM.

The segregation pattern for reaction to YVM indicated that disease resistance in *A. manihot* and *A. manihot* ssp. *manihot* was controlled by a single dominant gene in each taxon (Jambhale and Nerkar, 1981). The resistance was of the symptomless carrier type. The line derived from the cross of Pusa Sawani to *A. manihot* ssp. *manihot*, however, has been showing mild YVM symptoms in the last few seasons. Singh and Thakur (1979) have also reported on the nature of resistance from these species.

The amphidiploid of the cross *A. esculentus* x *A. tetraphyllus* is being used by Dutta (referred by Charrier, 1983) for transferring YVM resistance to cultivated okra.

Powdery mildew

Powdery mildew caused by *Erysiphe cichoracearum* DC. is another disease of okra which sometimes results into substantial losses. No source of complete resistance to powdery mildew seems to be available in the cultivated okra (Prabhu *et al.*, 1971; Jhooty *et al.*, 1977; Joi and Shende, 1979). Prabhu *et al.* (1971) observed resistance to powdery mildew in the wild species *A. moschatus*, *A. manihot* and *A. pungens*. Joi and Shende (1979) reported *A. manihot* and *A. tetraphyllus* to be immune to this disease.

We screened some cultivars, wild species, interspecific hybrids and derivatives using the infector row technique in the monsoon season of 1981. The material was sown in the first week of July. Infector rows of Pishor local, a broad leaved, highly susceptible variety, were planted after every 10 rows and around the plots. Powdery mildew outbreak occurred in the last week of August. The infector rows completely succumbed to the disease. The species *A. tetraphyllus*, *A. manihot*, *A. manihot* ssp. *manihot* and *A. tetraphyllus* were immune, highly resistant and moderately resistant respectively. Similar was the reaction of the amphidiploids of these crosses. One yellow vein mosaic resistant line (no. 155) from the interspecific cross was found to be highly resistant.

The line no. 155 was further crossed to the cultivars Pusa Sawani and Pishor local to study the nature of inheritance of resistance to powdery mildew. Studies made in the F₂ and backcross generations of these crosses revealed that powdery mildew resistance is controlled by a single incompletely dominant gene. This gene is being transferred to Parbhani Kranti, the YVM resistant variety. Work has also been initiated to transfer the gene(s) for resistance from the immune species to Parbhani Kranti.

Vein enation

A. manihot ssp. *manihot* is highly susceptible to vein enation and leaf puckering caused by a virus. Though Indian varieties are tolerant to this virus, the lines derived from interspecific crosses involving *A. manihot* ssp. *manihot* may be susceptible to this virus.

Parawilt

A new wilt disease is being observed in okra in recent years. The symptoms of this disease are: dropping of leaves, epinasty and wilting from top. The causal organism is not known. However, it is suspected that a flagellate protozoa may be involved. Pusa Sawani and Parbhani Kranti are susceptible to this disease. Similar type of parawilt has been reported in cotton. Sources of resistance to parawilt of okra may be present in the wild species.

Sucking pests

Sucking pests like jassids (*Empoasca* sp.) are a menace to the okra crop, especially in the early growth period. Screening of the germplasm has revealed that the degree of tolerance of jassids varies among the different cultivars (Sandhu *et al.*, 1974). The species *A. moschatum* due to its hairiness, was found to be highly resistant to jassids. The cross between *A. esculentum* and *A. moschatum* was obtained only through ovule and embryo culture (Gadwal *et al.*, 1968). The hybrid turned out to be totally seed sterile. However, attempts need to be made to overcome the sterility barrier.

The present cultivars are susceptible to aphids (*Aphis gossypii*). *A. manihoti* is resistant to aphids.

Fruit borers

Spotted boll worm (*Earias fabia*) and pink boll worm (*Platyedra gossypiella*) cause considerable damage to the fruits. Chemical control measures of the pests are risky due to the residues on the fruits. Fruits of *A. tuberculatus*, Pal *et al.*, (1952) have tubercles on the surface and the fruit wall is tough. This renders the fruit resistant to borer infestation. *A. tuberculatus* has been identified as one of the two genome donors of *A. esculentum* (Pal *et al.*, 1952). Hybrids between the two species, though readily obtained, are sterile. Kuwada (1961) obtained an amphidiploid of this cross with partially restored fertility. Thus there is scope to overcome the sterility barrier.

White fly

White fly (*Bemisia tabaci* Gen) is a vector of okra viruses. However, it can become a pest as has been experienced in cotton. The wild species should be screened for resistance to white fly.

Red mites

The cultivated varieties are highly susceptible to small red mites which cause substantial yield loss in certain seasons. The wild species can be donors for resistance to the red mites.

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Okra germplasm utilization at IIHR, Bangalore

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Introduction

Okra, or lady's finger (*Abelmoschus esculentus*) belonging to the family Malvaceae is an important vegetable crop valued very highly for its mature, tender and green fruits in India. The fruits are eaten mainly boiled in culinary preparations as sliced and fried pieces. It is also sliced and dried in summer for year round consumption. Okra fruits are rich in calcium (90 mg/100 g fresh weight) and provide a valuable supplementary item in the tropical diet which is basically starchy in nature and lacking in calcium and iron. Fresh okra fruit has good export potential. Of the fresh vegetables exported, mainly to the Middle East and Gulf countries, okra accounts for approximately 60 per cent. The crop is cultivated throughout India mainly during spring-summer and the rainy season. It is susceptible to drought and low temperature and prefers hot tropical weather for normal growth.

During the recent past, okra cultivation in India has received a great set back due to the heavy incidence of Yellow Vein Mosaic Virus disease, spread by an insect vector - white fly (*Bemisia tabaci*). The loss in marketable yield has been estimated at 50-94% depending upon the stage of crop growth at which the infection occurs (Sastri and Singh, 1974). In the Indian subcontinent, the virus is now widespread in the subtropical region during the rainy season (June-September) and the tropical region during spring and summer (February-June).

Breeding okra for high yield, quality and resistance to Yellow Vein Mosaic Virus (YVMV)

The popular okra variety Pusa Sawani, developed earlier as tolerant to YVMV, lost its tolerance during late sixties. Since there was no other cultivar tolerant to YVMV to replace this variety, a systematic breeding programme was initiated at IIHR, Bangalore, during 1969, to develop okra varieties having high yield, good quality and resistance to YVMV. The source of resistance to YVMV could not be located in the cultivated species *Abelmoschus esculentus*. However, in the wild species *Abelmoschus manihot* ssp. *tetraphyllus* var. *tetraphyllus*, a true source of resistance was isolated during the early seventies at IIHR, Bangalore.

Gene introgression: through interspecific hybridization of *A. esculentus* x *A. manihot* ssp. *tetraphyllus* var. *tetraphyllus* unilaterally, followed by the synthesis of induced amphidiploids using colchicine and backcrossing with the cultivated parent, it was possible to isolate, in the advanced progenies of Amphidiploid BC₃ F₁₀, recombinants highly resistant to YVMV combined with high yield and good horticultural qualities (Fig. 1). As many as 60 advanced breeding lines and 2 improved varieties (Arka Anarika and Arka Abhay) resistant to YVMV have been developed at IIHR, Bangalore (Dutta, 1984).

Cytogenetical studies in okra

The chromosome numbers of *A. esculentus* and *A. manihot* ssp. *tetraphyllus* var. *tetraphyllus* were confirmed as n=65 and n=69 respectively. Meiotic studies in the interspecific hybrids revealed the chromosome number 2n=134 forming 36IIs + 62Is. Studies on the microsporogenesis of the interspecific hybrids revealed the occurrence of First Division Restitution (FDR) in PMCS during anaphase-I resulting in the formation of dyads and 2n gametes. Studies of megasporogenesis revealed the degeneration of megaspores of the linear tetrad and formation of empty ovules. The sterility observed in the interspecific hybrid was thus solely due to the failure of the development of female gametes. The induced amphidiploids showed regular meiosis forming 134IIs and were fertile. Meiotic studies of amphidiploid backcross generations showed the formation of triads, pentads and polyads which apparently contributed to almost complete sterility. Meiosis in 10 advanced generation breeding lines was normal. The chromosome number varied from n=60 to n=72 (Suresh Babu, 1987).

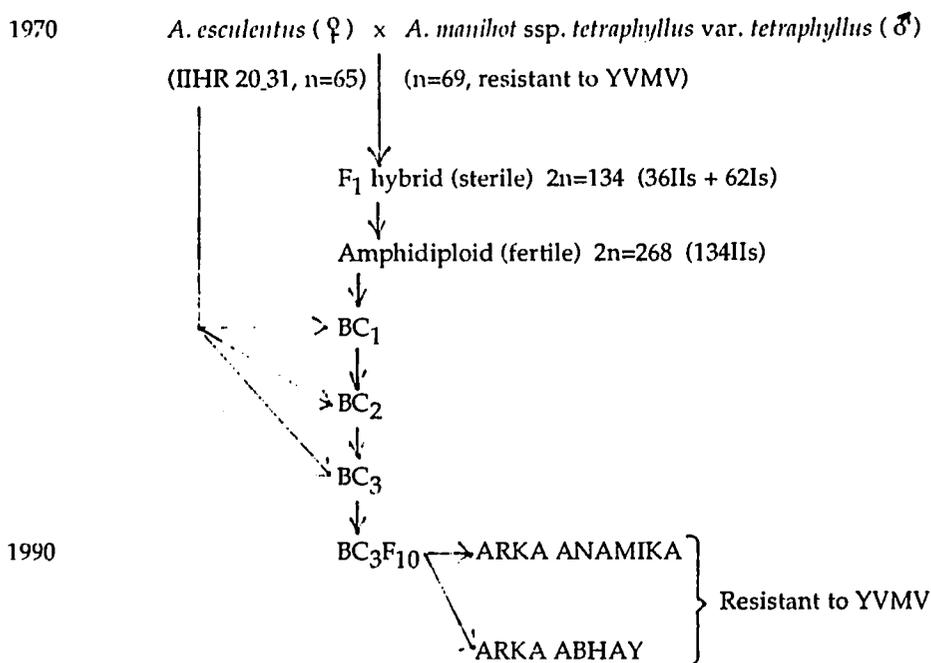


Fig. 1. How two YVMV varieties were bred at IIHR

Genetics of resistance to YVMV in okra

Resistance to YVMV in advanced generation lines of okra was controlled by two pairs of genes. Resistance is imported only when at least one pair of genes is in homozygous dominant condition. Intermediate expression is seen when both genes are in a heterozygous condition (Sadashiv, 1988).

Induced male sterility in okra

Genetic male sterility in okra has been induced through mutation breeding using gamma-rays. The character is governed by a single recessive gene when present in a homozygous condition. The male sterility character can be exploited for hybrid seed production by hand pollination. In comparison with fertile lines, approximately 70% saving in time and manual labour can be achieved in the production of hybrid seed using male sterile lines of okra (Dutta, 1971).

Novel character expression in okra

As a result of transgressive segregations in the amphidiploid BC₃ F₁-F₈ generations, various novel characters such as enhanced nodal productivity bearing multi fruits at each node, cauliflory, modified branching pattern, bearing fruit in two flushes instead of a normal single flush, have been recorded in okra.

Future thrust

Efforts are being made to breed okra varieties with multiple resistance to YVMV, Enation Leaf Curl Virus, nematodes and *Fusarium* wilt, both for the market and the processing industry.

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Germination tests for medium- and long-term storage of okra seeds

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The maintenance of high viability and germination potential of germplasm in long-term storage is one of the basic requirements of conservation and a major genebank activity. The supply of germplasm with high genetic integrity to the crop scientist user is essential. Maintaining such high viability requires careful and planned operations to be carried out in the genebank. Although apparently simple, these activities are complicated and require perfect planning, systematization and the unstinting service of the genebank managers.

Abelmoschus esculentus (L.) Moench belongs to the family Malvaceae. The fruit is the edible part, which is a ridged capsule. The seeds are round and exhibit orthodox/desiccant tolerant storage characteristics.

Prescription for germination test

The prescribed germination test conditions for *Abelmoschus* are as follows:

	ISTA	ADSA
Substratum	BP: TP, S	BP
Temperature	20/30°C (16h/8h)	20/30°C (16h/8h)
Duration of test	21 days	14 days

Hardseededness and dormancy

Apparently most annual cultivated *Abelmoschus* produces few hard seeds. However hardseededness can be a problem for seeds of *A. esculentus*, causing slow and erratic germination. Seeds at 13% moisture content tend to show little or no hardseededness; but once the seeds have been dried to 4-6% moisture content, hardseededness becomes prevalent. This suggests that hardseededness is a potential problem in seeds dried to low moisture content for long-term storage (Ellis *et al.*). In addition to hardseededness, seed dormancy is an added problem in germination tests of *A. esculentus*.

Some successful dormancy breaking treatments

Ellis *et al.*, in their Handbook of Seed Technology for Genebanks, have suggested some successful dormancy breaking methods for *A. esculentus* seeds. These are as follows:

Constant temperature: 15^o-25^oC, 25^oC, dark.

Alternating temperature: 15.5^o/29^oC, 15.5^o/32^oC (night/day) pre-soak; 24h.

Removal of seed covering structures: part of the testa

Scarification: concentrated sulphuric acid; 30 min.-3h.

Sulphuric acid 50%, 1h;

Concentrated HCl, 0.5, 1h

50% 30 min.

Concentrated nitric acid 30 min.

50% nitric acid 1h.

Acetone 95% 5 min.

Acetone 30 min.

Alcohol 95% 5 min.

Alcohol 0.5, 1h.

While monitoring the accessions of the released varieties of okra at NBPGR Genebank, it has been observed that accessions of okra which had registered an initial viability of above 85% had become hardseeded to the extent of 78% on storage after reducing the moisture content. Hot water treatment at 80°C for 4-5 min. could reduce hardseededness considerably and this could be followed by complete removal of hardseededness by mechanical scarification (rubbing against sand paper). Scarification with concentrated sulphuric acid for 15 min. was found to be successful to the same extent in restoring germination but seedling growth was restricted.

Distribution of hard seeds for genebanks

In commercial seed testing it is often the case that treatments to remove hardseededness are not applied. Instead, at the end of the germination test, the (irreversibly) hard seeds are identified and their proportion reported. It is often assumed that the hard seeds are viable. Consequently the proportion of viable seeds may be reported as the sum of the proportions of germinated seeds and hard seeds.

There are thus three alternative ways of dealing with the problem of hardseededness:

- treat all seeds prior to the germination test;
- treat only those seeds that remain hard after some time in the germination test has elapsed;
- do not to treat the hard seeds at all but only report their proportion.

The last option can be discounted for the purposes of genebank management. It is essential that all seeds stored in genebanks are able to germinate.

Where samples of seeds from accessions containing hard seeds are distributed, it is important that the customer is made aware of the problem of hardseededness and provided with details of the treatments required both to remove hardseededness and to humidify the seeds. Otherwise the customer/user scientist may not be able to produce seedlings from the distributed seeds.

In general it is not suggested that the seeds should be treated for hardseededness and humidified before distribution. This is because the humidified seeds will age much more rapidly than dry seeds and hence there may be some loss of viability if sowing is delayed, particularly where ambient temperatures are high. On the other hand, when it is known that no delays are likely to occur and that the seeds are to be sown out immediately upon arrival, then prior treatment to remove hardseededness and humidification could be allowed. An effective link between the customer and the distributor is therefore essential.

**Conservation strategies: a holistic approach
with particular reference to the *Abelmoschus* genepool**

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Introduction

Significant progress in the conservation of plant genetic resources has been made in the last two decades. The number of fully operational genebanks has grown exponentially and now numbers well over 100. During the same period, research has been initiated to solve the conservation of species that do not fall into the category of 'orthodox' seed-producers, i.e. vegetatively propagated crops and species that produce so-called 'recalcitrant' seeds. Protocols have been developed for the maintenance of tissue cultures in *in vitro* active genebanks and, more recently, considerable progress has been made in cryopreservation where liquid nitrogen is used as a storage medium.

These *ex situ* approaches to conservation clearly meet certain needs but a conservation strategy should be based on the genepool, not on technology that may or may not be the most appropriate for the case in question. As will be shown in this paper, the needs of a genepool are very unlikely to be met by a single approach to conservation. It is more likely that a balanced application of technologies will be needed including both *in situ* and *ex situ*. Within the latter category, a further balance needs to be made between seed, field genebank, *in vitro*, pollen and, perhaps in the future, DNA and gene storage. The different approaches complement each other, balancing their respective disadvantages.

Conservation methods

Depending on biological factors, on the available technical support and infrastructure, on the number of accessions at hand and the function of the genebank, one will arrive at a combination of conservation methods for a given genepool. The methods listed in Table 1 are not all suitable for long-term conservation. The majority are, in fact, only suitable for short- or medium-term conservation and some others, such as cryopreservation and DNA storage, need further research to be safely utilized for long-term conservation. At present, only seed storage and, although hardly used so far, pollen storage are safe and suitable methods for adequate long-term conservation of genetic diversity. In the case of *in situ* conservation, the naturally occurring species are allowed to evolve in their 'ecosystem'. If proper monitoring is available, this will be an adequate form of dynamic conservation. (Further definitions of conservation methods and other terms are given in Annex I).

A conservation strategy for *Abelmoschus* spp.^{1/}

The starting point for devising a conservation strategy for any crop is knowledge of its genepool. The various technologies should then be evaluated for their suitability to conserve parts of the genepool, bearing in mind the factors relating to the taxonomy and reproductive biology of the crop. Moreover, the conservation strategy chosen should be durable but flexible, responding to changing needs and technological developments. It should be economically and biologically sustainable and capable of supporting crop improvement efforts from the present into the future.

^{1/} The National Plant Germplasm System (USA) and the National Bureau of Plant Genetic Resources (India) have both accepted global responsibility for the conservation of okra germplasm within the existing global network of base collections (IBPGR, 1991). No regional base collections have been officially 'designated'.

Table 1. Methods used for germplasm conservation and the corresponding categories of plant genetic resource (PGR)

Method	Predominantly conserved PGR categories
Biosphere reserve	Ecosystem/biodiversity
Nature reserve	Specific habitat/wild and/or weedy species genepool
Gene sanctuary	Ecosystem (specific)/wild species genepool
On farm conservation (mass reservoirs, bulk hybrid populations)	Agro-ecosystem/landraces
Botanical garden/herbal garden/arboretum	Wild species, obsolete cultivars, tree crop germplasm
Field genebank	Wild species, vegetatively propagated crops, tree crop germplasm
Plant organ storage	Vegetatively propagated crops (mainly in the form of roots, tubers, suckers and bulbs)
Seed storage	All plant species which produce fertile and orthodox seeds
Pollen storage	In principle all species which produce long living pollen (mainly binucleate)
<i>In vitro</i> storage	Wild and cultivated species which produce recalcitrant or no seeds, vegetatively propagated crops, disease-free germplasm
Cryopreservation	Germplasm mentioned under points 8, 9 and 10 which tolerate cryopreservation
DNA and gene libraries	All germplasm

Taxonomy and ecology of the genus

The genus *Abelmoschus* has been taxonomically divided into varying numbers of species (Charrier, 1984). Four species -- *A. esculentus* and *A. caillei* (okra), *A. manihot* (aibika) and *A. moschatus* (musk mallow) -- are cultivated or have cultivated forms with centres of diversity in Africa and Asia. At least four wild species (*A. ficulneus*, *A. crinitus*, *A. angulosus* and *A. tuberculatus*) are recognized, of which *A. ficulneus* and *A. tuberculatus* are related to *A. esculentus* (Charrier, 1984). This fairly diverse genepool is confined to the tropical and subtropical belts and includes the wild species, weedy forms and the four cultivated species. It occupies habitats and grows in agronomical conditions varying from subtropical desert to seaside conditions, and from forest regions to hilly areas from 750-2000 m altitude.

Reproductive biology

Abelmoschus spp. are predominantly annual. Owing to their floral morphology and the absence of a self incompatibility system, they are generally regenerated through selfing. However, depending on the species or variety, season and location, varying degree of outcrossing (up to 60%) occurs in okra. Bees (*Apis mellifera* and *A. cerana*) appear to be the main vectors of pollen. Such a level of outcrossing will maintain a considerable amount of heterozygosity and heterogeneity, eventually resulting in off-type segregants during repeated multiplication cycles. It is generally argued that extreme care is necessary in regenerating okra, minimizing the number of field rejuvenation cycles to avoid further deterioration of the original population, and genetic drift over space and time.

Although *Abelmoschus* spp. produce viable orthodox seeds, some species are predominantly vegetatively propagated. This fact, plus the problems in regeneration described above, highlights the importance of exploring additional *ex situ* methods to complement seed storage for genetic conservation of the okra genepool. The pollen of *Abelmoschus* spp. is generally short-lived. *In vitro* culture, however, appears to be relatively unproblematic. The different conservation methods are examined further below.

Seed storage

Physiologically well developed and healthy seeds of cultivated and wild *Abelmoschus* species should be deposited in base collections, adequately duplicated for safety reasons under favourable long-term storage conditions (see Annex I). At the National Bureau of Plant Genetic Resources (NBPGR), okra seeds are preserved in sealed, laminated aluminium foils and kept in cold storage (in the genebank) at -20°C with a seed moisture content of 7.5%. Seeds of *A. esculentus*, *A. manihot* and *A. ficulneus* appear quite amenable to this procedure.

In establishing an okra seed collection, factors related to dormancy and hard-seededness must be determined to allow long-term storage (see Khanna, 1991). Also, other alternative strategies for seed conservation such as cryopreservation may be explored simultaneously. Preservation of seed can be carried out at ultra-low temperatures in deep freezers (-80°C) and cryogenic containers cooled by liquid nitrogen (-196°C). It is necessary to take into account interactions between seed size, dormancy and hard-seededness that may affect viability. Available data indicate that average seed longevity is directly proportional to seed size. Also it has been shown that large- and black-seeded genotypes have better storage potential than small-seeded types under standard base storage conditions, while the reverse is the case in cryopreservation. Seeds of okra cultivars have been successfully cryopreserved at NBPGR-NPPTCR. In the case of wild species, where availability of seeds may be a limiting factor, the possibility of *in vitro* conservation techniques needs to be considered selectively on a case-by-case basis.

In vitro conservation

The logical methods for *in vitro* active conservation and *in vitro* base conservation are slow growth storage and cryopreservation respectively. Maintenance of *Musa*, sweet potato, ginger, yams and *Allium*, *Colocasia*, *Xanthosoma* and other crops under slow growth using minimal media and low temperature incubation has shown a considerable amount of progress (Withers, 1987; 1991). Attempts to cryopreserve these cultures for long-term conservation have met with varying degrees of success (Chandel and Pandey, 1991), indicating that, for the moment, only *in vitro* active conservation is readily available for use in genebanks. A model *in vitro* active genebank for cassava is already being operated jointly by CIAT (Cali, Colombia) and IBPGR (Chavez *et al.*, 1987).

More research is needed to establish reproducible cryopreservation methods for *in vitro* base conservation. Cryopreservation of plant cell suspension cultures and production of somatic embryos from freeze preserved cells were first reported by Quatrano (1968) and Nag and Street (1973), respectively. Since then extensive studies have been conducted on the cryopreservation of plant systems (Kantha, 1985; Withers, 1980; 1987; 1991). This research has shown that cryogenic storage in liquid nitrogen (-196°C) provides ideal conditions as the plant material is held in a state of suspended animation. The technology is applicable to seeds, pollen, embryos and embryonic axes, as well as cells, meristems and shoot-tip cultures. Although an emerging technology, cryogenic storage has made impressive progress so far as can be judged from the numerous reviews that have appeared in recent years (Bajaj, 1976; Finkle *et al.*, 1985; Kantha, 1985; Steponkus, 1985; Withers, 1980; 1987; 1991).

Protocols for the cryopreservation of *in vitro* cultures of several crops (cassava, potato, strawberry etc.) have been explored. Results have been most satisfactory for cell suspension cultures but all culture systems have survived cryopreservation. Survival levels of up to 95% have been reported. Duration of storage is not an issue. The main scientific challenges relate to the satisfactory cryopreservation of organized cultures. Embryos may be more amenable to cryopreservation than shoot-tips.

Successful examples of somatic and zygotic embryo cryopreservation also exist in the literature. For example, somatic embryos of carrot gave 80% survival, with proembryos undergoing normal embryogenesis, callusing and formation of normal plants. In *Citrus* species, cryopreserved nucellar embryos proliferated to form pseudo-bulbils and shoots. The cultures were frozen both rapidly and slowly in liquid nitrogen with cryoprotection using DMSO and sucrose. Recently, cryopreservation of tea embryonic axes has been demonstrated to be feasible (Chaudhury *et al.*, 1991).

Successful desiccation and freeze preservation of embryonic axes of trifoliolate orange (*Poncirus trifoliata*) has also been achieved and, after one year's storage in liquid nitrogen, the material was retrieved and plants were established using appropriate *in vitro* protocols (Radhamani and Chandel, submitted).

In the case of okra, preservation of immature excised embryos and ovules could be of great practical significance particularly in interspecific hybridization. This can help in establishing F₁ hybrids and overcoming problems of sterility and post fertilization barriers. For example, hybridization of *A. caillei* and *A. esculentus* can be easily achieved, but the hybrids are partially sterile.

Pollen preservation

Abelmoschus pollen is generally short-lived. Pollen and anther preservation can aid crop improvement strategies both through enabling crossing to be carried out with pollen collected in different locations/times and coupled with *in vitro* culture technology for the production of homozygous diploids.

Pollen preservation could be particularly useful in the case of wild species of okra. Successful examples of pollen preservation exist in temperate fruits (plum, peach and apricot) and among tropical fruits (*Citrus*, papaya and grape). In *Citrus*, pollen was reported to be kept viable for four years, while in papaya and grape, pollen remained viable for almost five years. Among vegetable crops, tomato, eggplant and onion pollen were also cryopreserved for well over four years (Chandel and Pandey, 1991). *Capsicum* pollen was cryopreserved for two years. These examples clearly suggest the practical utility of pollen storage, especially in liquid nitrogen.

Conclusions

Seed storage will continue to be the predominant conservation method for the okra gene pool. However, applications can be identified for pollen preservation, and *in vitro* conservation involving both embryos and vegetative tissues. The possibility of and extent of *in situ* conservation of the wild species need further study. More research is needed to realize the potential of these alternative approaches and design a conservation strategy that uses them in an integrated way.

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Definitions

Some of the major terms used in discussions of conservation methods and strategies are defined as follows:

Active collection

A collection of seed accessions for medium-term storage (temperature regime between 0 and 10°C and frequently under controlled relative humidity of ca. 35% in "open" containers). The genebank holding an active collection is assumed to take responsibility for regeneration, multiplication, distribution and related quarantine issues, evaluation and documentation. This category was previously termed 'working collection'.

Base collection

A collection of seed accessions for long-term conservation (temperature regime below 0°C and preferably -18°C or less); sufficient and properly dried seeds (3-6% moisture content) are stored in hermetically sealed containers. The centre holding such a collection is assumed to conduct viability tests, to document the relevant information and to administer other matters, in particular to link with active collections for regeneration and evaluation.

Duplicate collection

Such a collection is a duplicate set of accessions maintained as a base collection elsewhere and is kept under similar storage conditions. The only purpose is to have an insurance against accidental loss of material from the base collection.

'Designated' base collection

A genebank which has accepted responsibility for the conservation of a given crop on a global or regional level.

Field genebank

A collection of vegetatively propagated accessions which are maintained under actual field conditions and is, from a conservation point of view, comparable to an active collection.

***In vitro* genebank**

This is the 'counterpart' of an active seed collection where accessions are maintained as cultures under laboratory conditions. The distinction is being made between an *in vitro* active (slow growth) and *in vitro* base (cryopreservation) genebank.

***In situ* conservation**

The activity of conserving plant genetic resources 'on site', where they naturally occur.

***Ex situ* conservation**

The activity of conserving plant genetic resources 'off site', not in the original or natural environment (for instance as seeds, pollen or *in vitro* tissues in a genebank).

Biosphere reserve

An area of terrestrial or coastal environment which is representative of one of the world's natural or biogeographical regions. It is conceived as an open system of conservation in each area of undisturbed natural ecosystems and surrounded by areas of sympathetic and compatible land use. They thus constitute important reservoirs of genetic material for long-term conservation.

National park

The long-term conservation efforts to protect plant genetic resources diversity is only one of the major objectives. Frequently, the protection of specific animal species in their natural environment is a more prominent goal, allowing a varying degree of use by local inhabitants.

Gene sanctuary

An area for the conservation of particular species or groups of species because they are particularly important, or because they are rare and not adequately represented in existing national parks or nature reserves.

Regeneration of okra germplasm

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Introduction

In most cases the initial sample received by any genebank consists of a limited quantity/number of seeds, sometimes a single fruit of okra. So, for the purposes of storage and distribution for utilization, it is necessary to multiply/regenerate the seed samples. Further regenerations will be required due to loss in viability of conserved seed or reduced seed stocks in the genebank. It is generally agreed that for most species an accession has to be regenerated when the viability falls below 85%. For species like okra, which appears to have a problem of poor germination (Hamon and van Sloten, 1989), regeneration may have to be carried out much more frequently. Regeneration is also required when the stock for an accession falls below the quantity required for at least 3 regenerations.

The genetic integrity of the accession and the variation within the accession must be preserved through various regeneration cycles. In this type of static conservation in seed genebanks, some changes in gene and genotype frequencies are inevitable, but the resultant disturbance of genetic structure and loss of alleles have to be minimized by adopting correct regeneration procedures. These include (a) avoiding contamination through pollen, seed and mutation and (b) avoiding genetic deterioration and loss of alleles due to genetic drift and shift. These should be main objectives of any good regeneration strategy (Breese, 1989).

There is a need to distinguish between regeneration (rejuvenation) and multiplication since this would help to reduce the frequency of regeneration (Breese, 1989). Regeneration, with all the stringent measures to maintain genetic integrity of accessions and to conserve the diversity within an accession, should be carried out only when the viability has been reduced to a particular germination standard observed in a genebank and/or when seed stocks are depleted to a critical level. For regenerating under these conditions seed from the base collection (if possible original seed) must be used for planting. In contrast, multiplication is mainly for seed increase for distribution for evaluation/characterization and/or utilization and is not for long term conservation. For this purpose stringent regeneration measures may not be necessary and seed for planting may be drawn from active collections.

Breeding systems and genetic structure

Okra, owing to its floral morphology and absence of self-incompatibility, produces much of its seed through selfing (Hamon and van Sloten, 1989). However varying degrees of outcrossing occur in okra, depending on variety, season and location - ranging from 0 to 60% (Chandra and Bhatnagar, 1975; Martin, 1983) and insects (bees - *Apis mellifera* and *A. cerana*) appear to be the main vectors of pollen (Tanda, 1985; Akorda, 1986). This level of outcrossing can result in maintaining some amount of heterozygosity and heterogeneity and also may be the reason for occurrence of off-type plants during multiplication. The heterogeneity may be interspecific, intraspecific or partially intraspecific referring to only a few traits (Hamon and van Sloten, 1989). Under these conditions an okra accession which is homogeneous is rare and extreme care will be necessary when regenerating okra accessions.

Very little information on okra genetic structure exists. A major study by Hamon and van Sloten (1989) on the genetic diversity in the West African okra collection basically describes the interspecific variability and also stresses the confusion that exists in correctly identifying the taxonomic groups in okra. The allopolyploidy automatically introduces a complication in regeneration practices by increasing the number of plants that have to be grown to adequately maintain phenotypic correspondence over generations (Breese, 1989). Electrophoretic work done so far indicates that there is hardly any variability within the cultivated species and this does not correspond with the phenotypic diversity observed for the species. Much more work will be necessary to understand the genetic structure of okra and in the mean time, necessary improvements to regeneration practices have to be undertaken.

In farmers' fields, as against the situation in a research station, insect vectors of pollen for okra might actually be promoting cross pollination to a greater extent than usually expected for okra. This hypothesis is corroborated by a fairly high amount of hybrid vigour found in certain crosses (Charrier, 1984) though expression of heterosis is very rare. Such high levels of outcrossing lead to a further complication in regeneration procedures required for maintenance of diversity in okra. Would the usual practice of selfing be enough to maintain okra germplasm or would it lead to inbreeding depression and subsequent loss of genes/alleles in later generations? At the moment there does not appear to be enough data to decide these questions. In the meantime it may be essential to follow some sort of controlled pollinations of plants within an accession by growing in isolation and using insects. However, the resources needed for such modifications in regeneration procedures may be prohibitive. In addition, continuous flowering may make bagging for selfing difficult.

Number of regenerations

Generally, how quickly the material is used and viability of stored seed determine the frequency of regeneration. Base collections, which generally receive material from other centres, do not need frequent regeneration if the original seed sample is large. On the other hand, the active centres, which generally interact with the other crop improvement disciplines, use the material much faster and the frequency of regeneration will be high. The risks due to genetic drift, shift and contamination could be minimized by restricting the frequency of regeneration. This is possible by producing large quantities of high quality seed from the initial multiplication/regeneration and conserving it in long-term storage.

Monitoring of viability of the seed in storage, an essential activity of any genebank, helps to reduce the frequency of regeneration by identifying accessions that have a lower germination standard. Generally okra accessions exhibit varying levels of initial germination (Hamon and van Sloten, 1989) and setting a germination standard appears to be difficult. Under these conditions the regeneration frequency is expected to be high.

Regeneration strategy

Isolation requirements

As cross pollination of up to 60% has been reported in okra and it appears to be dependent on variety, season (Aken'Ova and Fatokun, 1984) and location, the outcrossing rate for a given regeneration site, as well as the appropriate season (when the outcrossing rate is lowest) has to be determined before extensive regeneration is undertaken. Insects appear to be the main vectors of pollen in okra so intensive pesticide application may result in reduction of cross pollination. However, in the farmers' fields, where most okra has been collected, one can expect a fairly high amount of cross pollination due to less frequent use of pesticide. Is there also a need to consider growing okra accessions in isolation and to promote random mating if the outcrossing rate exceeds 50%? These are some of the questions that need to be answered in order to develop correct strategies.

Seed handling techniques

Most of the following considerations are general and apply to other crop species also. Seed packets for individual accessions have to be prepared. It is always better if the field layout and planting plans are prepared ahead and only plot numbers are marked on the packets. Ideally, it is recommended that the individual plot (accession) is labelled in the field, at least by plot number. This would greatly help in avoiding 'label mutations' and would also help at the time of harvest.

The quantity of seed required for storage determines the type of harvesting. If all the seed that is produced is needed, all the fruits are harvested from each plot (accession), bulked (if the bulk method is followed, see Methods of Regeneration), threshed and cleaned for storage. If only part of the produce is required, it is necessary to collect at least a few seeds (or a few fruits to thresh later) from each plant in the plot. This will be a good representative sample of the plot.

From a seed health point of view, it is always better to rogue out infected plants and collect seeds from healthy plants. However, there is a danger of losing some diversity if such roguing is carried out. So prophylactic measures to check infection by disease-causing organisms will greatly reduce the risk of reducing the population size and counter the selection pressure exerted by pests and diseases. Plant protection practices will also help in producing healthy and clean seed as required for conservation.

Methods of regeneration

For a mostly inbred species like okra, two methods can be used, the pedigree method and the bulk method (Breese, 1989). The pedigree method involves selfing by bagging of individual plants within an accession and maintaining these selfed subsets as component lines. Here the objective is to conserve within-population diversity and this is suitable for highly polymorphic okra accessions. However, more information is needed on the effects of bagging on seed production in okra. Also the feasibility of bagging has to be examined. But in general the bulk method, which aims to conserve between-population variability, may be followed since the effect of drift will be less. The pedigree method is labour intensive and would require maintenance of subset lines separately. If the outcrossing rate is negligible, even the bulk method would amount to selfing of individual flowers. Obviously the exact method will depend on the information available on the genetic structure of the accessions being regenerated and on the rate of outcrossing for okra at the site of regeneration. If the outcrossing rates are as high as 20% or more then there is need to take a completely different approach. The accessions will have to be isolated (by distance, barrier crops or in a glasshouse or screen house) and outcrossing may have to be promoted using honeybees to allow for random mating. Again the question here is how important is it to maintain the okra germplasm in this manner?

Population size

The size of population at the time of collection of the accession has a bearing on deciding the population size for regeneration. For some okra accessions which may be from a single fruit that was originally collected, growing a few plants will be sufficient, and this will mainly depend on the quantity of seed required. However, the population size at the time of collection would generally be larger than a single plant, which would contribute to the heterogeneity of the accession, and would require growing a greater number of plants to regenerate the seed.

Basically, the seed that is taken out for planting to rejuvenate an accession is a sample of a larger quantity of seed in store. This can lead to sampling error which results in random changes in gene frequencies and loss of some alleles (genetic drift). In the context of genetic resources conservation there is no way that this effect can be avoided. However, it can be minimized by making the sample size as large as possible, thereby increasing the effective population size.

As already indicated, though okra is a predominantly self pollinating species, it can retain a high amount of variability (heterogeneity). In heterogeneous populations, in order to preserve genotypes or genes at a particular frequency, the amount of seed required for regeneration is dictated by the original frequency. For an inbred species like okra, the number of plants required would be double that of a complete outbreeder to maintain alleles at any given frequency. If we assume the original population has genes of frequency ≥ 0.05 then it would take 60-75 plants to retain this frequency with a high degree of confidence in the next generation (Breese, 1989). So, it is recommended to raise a plot of about 50-75 plants per accession, depending on the resources of the genebank. This number is based on the assumption that there will be no loss of individuals contributing to reproduction due to pests, diseases, competition and other factors.

The number of seeds needed for planting also depends on the viability of conserved seed. Care should be taken that the loss in viability will not result in poor and patchy stands in the regeneration field. Compensation for loss in germination percentage has to be made so as to maintain 50-75 plants for harvesting.

Selection

It is best to regenerate an accession in the area of its original habitat to reduce the effects of natural selection. In most cases, however, this may not be possible for practical reasons and will be very expensive. So the best solution is to reduce the effect of natural selection, if any, by increasing the survival so that all or most of the plants in an accession reproduce and contribute to seed production. Survival can be enhanced by providing optimum growing conditions, such as optimum temperature, photoperiod, protection against biotic and abiotic stresses, breaking dormancy, etc.

High plant density may significantly affect survival of plants through competition resulting in seedling mortality (differential survival) and in reducing plant size, thus impairing reproductive efficiency of less competitive plants. This is probably an important consideration in the regeneration of accessions of *A. caillei*, due to its large plant size. It is essential to maintain optimum inter- and intra-row spacing so that all the plants survive and contribute to seed production.

The available information on differences between the yields of individual okra plants is limited. If differences in seed production are significant then it will be essential to avoid individual plants contributing to the harvested sample in proportion to their seed yield as this would result in a genetic shift. The equal contribution of each individual within the sample will ensure the conservation of allelic frequencies as in the earlier or original sample; this is particularly necessary if the accession is heterogeneous to the extent that within the accession variability is highly significant.

Optimal cultural conditions are essential to provide high quality and healthy seed, a prerequisite for any type of storage as well as for distribution. It is desirable to group the accessions according to maturity growth habit or other characters to facilitate cultural operations and harvesting of material at the most appropriate time. Plant protection is essential to avoid incidence of pests so that no selection is exerted and population size is maintained, but roguing and collecting seed only from healthy plants may be necessary.

It is more difficult to counter the effects of genetic shift (due to natural selection) than genetic drift. However, natural selection acts over time, and a number of regenerations may be required before its effects can be felt. So, if the frequency of regeneration is kept to a minimum, the effects due to shift could be minimized.

Concluding remarks

The suggestions made above are based on the assumption that most of the regeneration will be carried out under field conditions. However, if regeneration is carried out in containment, suitable alterations have to be made, especially for those that relate to field plot maintenance. The major objective of this paper is to raise a number of questions - original sampling, outcrossing, polyploidy, inter-accession variability, etc. - in relation to regeneration of okra germplasm. The little information available on all these aspects indicates a need for changing regeneration practices, at least for the heterogeneous okra accessions and in locations where outcrossing rates are very high.

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Germplasm exchange and plant quarantine

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Availability of genetic diversity is basic to any crop improvement programme as it provides the desired genes to the breeder to develop crop varieties with high yield potential and with tolerance or resistance to various biotic/abiotic stress conditions. International cooperation has been very helpful in this respect and a wide variety of genetic materials are being transported from one country to another. The germplasm exchange activity has received a tremendous boost through the untiring efforts of the International Board for Plant Genetic Resources, established in 1974. However, unrestricted and unregulated plant introduction activity may result in inadvertent introduction of serious pests to a country leading to severe crop losses. Because of many unpleasant experiences in the past, almost every country involved in this activity has formulated its own quarantine regulations to regulate the inflow of plant material with a view to preventing the introduction of exotic pests. Depending on the level of pest introduction risk and the crop spectrum within their countries, governments either prohibit or permit the introduction of plant material with or without certain specific conditions.

Germplasm exchange and quarantine in India

In India, plant quarantine regulations are framed and enforced under the provisions of the Destructive Insects and Pests Act (DIP Act) of 1914. Under the latest "Plants, Fruits and Seeds (Regulation of Import into India) Order", 1989, conditions have been laid down for import of exotic plant materials. The main features of this Order are:

- all consignments entering the country must be accompanied by an import permit to be issued by a competent authority in India;
- no consignment shall be permitted into India unless accompanied by a phytosanitary certificate issued by the official plant quarantine service of the exporting country;
- seeds, plants, planting materials for propagation/planting purposes shall be imported only through the ports of Amritsar, Bombay, Calcutta, Delhi and Madras;
- soil, plant debris accompanying seed/planting materials shall not be permitted;
- seed/planting materials that need to be grown in post-entry isolation shall be grown in post-entry isolation facilities approved by the competent authority.

Responsibility for enforcing these regulations rests with the Plant Protection Adviser to the Government of India. However, as NBPGR has been designated as the nodal institute in the country for exchange of plant materials for research use, the Director, NBPGR has also been empowered to enforce plant quarantine regulations in respect of germplasm materials for research purposes. The procedure for import of seed/planting material for research purposes has been published in the June 1990 issue of the NBPGR Newsletter. As per the new procedure, all applications for import of seed/planting material for research should be addressed to the Director, NBPGR, in prescribed proforma for the issue of an import permit. The import permit so issued should be forwarded to the exporting agency and the import permit and the phytosanitary certificate must accompany the consignment for clearance by the customs department. All consignments must be addressed to the Director, NBPGR, for quarantine clearance.

At NBPGR, the Division of Germplasm Exchange is responsible for the exchange of plant materials, the Division of Plant Quarantine handles quarantine clearance of the materials under exchange. This Division has trained scientific and technical staff in the disciplines of entomology, nematology and plant pathology, and has well equipped laboratories and isolation growing facilities to meet quarantine requirements.

Quarantine considerations for okra germplasm

Out of a large number of insect pests recorded on okra, only about half a dozen feed on flowers and capsules and only *Ephesttia cautella* and *Spermophagus albosparus*, which attack seed, are of quarantine importance. In the case of plant parasitic nematodes, species of *Meloidogyne*, *Pratylenchus*, *Rotylenchulus*, *Radopholus*, *Tylenchus*, *Scutellonema*, *Hoplolaimus*, *Tylenchorhynchus*, *Rotylenchoides*, *Aphelenchus*, *Basiria*, *Helicotylenchus*, *Xiphinama*, *Hemicriconemoides* and *Macroposthonia* have been reported on okra. While none of them is reported to be transmitted through seed, soil clods mixed with seeds can easily introduce these nematodes. While a number of viruses have been shown to infect okra under experimental conditions, only yellow mosaic, yellow vein mosaic and leaf curl viruses infect the crop under natural conditions and are serious. Fortunately, none is seed transmitted. Out of a large number of fungal pathogens, the following are reported to be seed transmitted:

- pod spot, blight	<i>Ascochyta abelmoschi</i>
- fruit rot	<i>Choanephora cucurbitarum</i>
- wilt	<i>Fusarium oxysporum</i> f.sp. <i>vasinfectum</i>
- root, collar rot	<i>Fusarium solani</i> f.sp. <i>hibisci</i>
- die-back, top blight	<i>Colletotrichum gloeosporioides</i> <i>C. dematium</i>
- charcoal rot	<i>Macrophomina phaseolina</i>
- grey mould	<i>Botrytis cinerea</i>
- leaf spot, blight	<i>Bogryodiplodia theobromae</i>
- blight (bacterial)	<i>Pseudomonas syringae</i> pv. <i>syringae</i>

While the majority of these pathogens are widely distributed, one must keep in mind that new and more virulent forms could be as important as new pathogens. Soil clods mixed with seeds can bring in pathogens not reported to be seed-borne. From the quarantine angle, most of these pathogens could be easily detected by using one of the other seed health testing methods. Not much information is available on pests of wild species of okra and therefore all okra germplasm introductions must be subjected to detailed quarantine checks using seed health testing methods before release from quarantine for further exploitation. The seed should not be given any pre-export pesticide seed dressing as this interferes with the detection of seed-borne pests. Seed treatment without knowledge of the health status is undesirable from a quarantine point of view.