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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.

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. augulosus 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* (Stevels, 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. ficultures. 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

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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. moscluatus* and *A. manihot* in Thailand (Hamon *et al.* 1987) and in Sri Lanka (Vredebregt, unpublished).

In 1989 the entire collection of more than 2,500 accessions was tranferred 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.

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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. *moschatus* 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 <i>A. esculentus</i> darkish	A. caillei
~	long and curved pedicels:	A. caillei
-	marking of seeds: dense wide	A. esculentus 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. moschatus;
- 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).

<u>A. moschatus</u>

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.

<u>A. manihot</u>

Morpho-phenological characters

The global diversity of this species is more important than any other species. The samples from Sri Lanka (Vredebregt) 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 epycalyx 2 + - 100 anthers per flower

ii) Isoenzymatic characters

The isoenzymatic diversity of this species is, as for *A. moschatus*, 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. esculentus*. 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 (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 Clemsom 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 *Bemissia tabacci*, 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. caillei do manifest symptoms of curling but they have a good tolerance and ensure a reasonable yield. The wild species A. mosculatus 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:

<u>A. esculentus</u>

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.

<u>A. caillei</u>

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. caillei*) 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. moschatus (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. tetraphyllus)

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. moschatus* (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. moschatus* 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. November 1991

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