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REVIEW

Germplasm of Cucurbitaceae in Brazil

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ABSTRACT - *The cucurbit species represent an important agribusiness in Brazil. Main species are* Cucurbita moschata, C. maxima, Cucumis melo, C. sativus, Citrullus lanatus, *and* Sechium edule *which were introduced by African slaves and Indians. More recently, European, American and Japanese immigrants introduced some improved genotypes which were spread across the country. There are some public and private breeding programs whose genetic variability is limited. In traditional agriculture however, there is a great genetic variability which gave base to the establishment of four germplasm banks that comprise more than 4700 accessions, involving over fifteen species of which only 25% have been evaluated. Several useful genes were identified. It is necessary to further the evaluation of the rescued genetic variability and to assess the need for new collections in order to have cucurbit germplasm for long-term conservation and for breeding programs in Brazil.*

Key words: genetic resources, traditional agriculture, plant breeding.

INTRODUCTION

The cucurbit varieties on the market in Brazil, although introduced from abroad, play a major role in several production systems in different regions of the country. The annual agribusiness dealing in the species of this family with pumpkins in the first place (*Cucurbita* spp.), melon and cucumber (*Cucumis* spp.), watermelon (*Citrullus lanatus*), and the chayote (*Sechium edule*) is estimated at around one billion Reais (approximately US$ 350 million). In certain specific regions cucurbits are intensely cultivated on minor areas with gherkin (*Cucumis anguria*), which is used as cooked vegetable, smooth loofah (*Luffa cylindrica*) for natural sponge or bottle gourd (*Lagenaria siceraria*) for musical instruments.

The majority of these species were introduced in the country in different periods of history. For example, there are the species of the genus *Cucurbita* originated from America (Purseglove 1974) that were introduced in Brazil by Indians in remote times, mainly *C. moschata* and *C. maxima* (Whitaker and Davis 1962). However, there were introductions of *C. pepo* by European colonizers, probably in the southern region. Similarly, watermelon was first introduced by African slaves three hundred years ago, especially to the Brazilian coastline (Romão 2000). However, there were more recent introductions

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from the USA to the State of São Paulo, to the municipality of Americana in the 50's, and also from Japan through immigrants to the same state. These introductions were dispersed throughout the country (Costa and Pinto 1977).

Melon (*Cucumis melo*) was also introduced by African slaves (Whitaker and Davis 1962) a long time ago and more recently from Spain, in form of the Valenciano melon with fruits of dark green color. A yellow mutant was selected from this variety by the Cooperativa Agricola de Cotia, designated Amarelo CAC tha has been cultivated in several melon-growing areas. It promoted Brazil from an importer to an exporter of this commodity (Costa and Pinto 1977). Cucumber is considered to be originated from India (Lower and Edwards 1986). This is questioned by Whitaker and Bemis (1976) though, since no wild relatives are found in that country. Most likely it was brought to Brazil by African slaves with the other *Cucumis* species, but did not become popular in traditional agriculture. The recent introduction of cucumber probably occurred through European and Japanese immigrants which were origin of the commercial cultivars available.

On the other hand, some species appear to have only been introduced a long time ago. Bottle gourd (*Lagenaria siceraria*) is probably from Africa where it occurs subspontaneously and would have reached the Brazilian coast floating (Purseglove 1974). *Luffa cylindrica* is probably from India but was dispersed across practically all tropical regions of the world (Purseglove 1974). In traditional agriculture bottle gourd is restricted to a few plants around farmers' houses. Gherkin (*Cucumis anguria*) and bitter gourd (*Momordica* spp.), originated from Africa and both of them introduced in Brazil by African slaves, also spread out across the entire northeastern region. However, it is the choyote (*Sechium edule*), a species originated from Mexico and probably introduced by Indians in remote times, used as cooked vegetable (Makishima 1991), which is now cultivated in fourteen States of Brazil with a sizeable fruit production.

Recent introductions of cucurbit cultivars are usually restricted to a few genotypes. They are very uniform and were not developed for the Brazilian farming systems. This creates great demands for an environmental adjustment, especially to minimize biotic stresses in order to attain desirable yields. Besides, the lack of variability in the introduced genotypes reduced the options in terms of different fruit patterns. For instance, the introduced watermelon genotypes were few and only some of them became popular in Brazil, such as the Charleston Gray cultivar, replaced by the Crimson Sweet, both with big fruits, which are still popular on many different markets. However, more recently, there has been a demand for smaller fruits on the external market as well as of some national supermarket chains.

In the case of melon, cultivar Valenciano Amarelo had been predominant in many production areas for years on end, when seed companies recently increased the offer of new hybrid cultivars. Still, the number of genotypes is around a dozen.

For pumpkin only the hybrid cultivar Tetsukabuto is widely used in production areas in the South and Southeast of the country. The hybrid seeds however are imported from Japan (Giordano 1991) until today.

Despite the economic importance of the Cucurbitaceae species in Brazil they were very poorly studied, particularly in terms of knowledge on the genetic resources and plant breeding. More crucial still is the long-term conservation of seeds from accessions, since the Base Collection of Embrapa Recursos Genéticos e Biotecnologia has only one accession of *C. lanatus*, one of *Cucumis anguria*, one of *C. melo* and one of *Cucumis* spp.; three of *Cucumis sativus*; six of *C. pepo* and *Luffa cylindrica*; 22 of *Lagenaria siceraria*; 48 of *C. moschata* and 499 of *C. maxima*, summing up to a total of 558 accessions (Faiad and Bustamante 1999).

In Brazil, many introductions of species of the Cucurbitaceae family were maintained in different production systems and represent an important genetic resource defined here as the biodiversity fraction of current or potential interest (Lévèque 1999). These production systems in many regions of the country represent a valuable germplasm source for many cucurbit species (Queiróz 1993, Barbieri et al. 2004).

GENETIC RESOURCES SPECTRUM OF CUCURBITACEAE IN BRAZIL

Genetic resources are studied in six distinct phases: collection or introduction; multiplication and preliminary evaluation; biochemical, molecular and morphological characterization; in-depth evaluation, short and long-term conservation; and use (Hawkes 1982, Valls 1988). However, as mentioned earlier, cucurbit germplasm is not preserved in Base Collections, which makes cucurbit breeding programs very vulnerable since the genetic variability in different traits (producers' and consumers' demand) is the starting point for any breeding program.

According to Chang et al. (1979), the germplasm spectrum of vegetal species is divided in three different groups: center of diversity; center of cultivation and plant breeding programs (Figure 1).

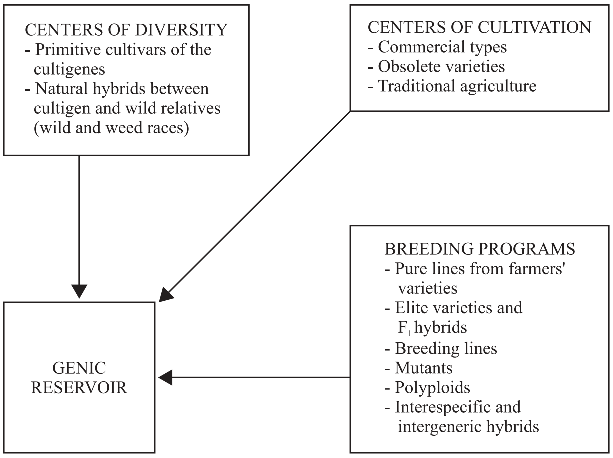

Figure 1. Spectrum of germplasm in a crop species (Adapted from Chang et al. 1979)

In Brazil, where all important species for the cucurbit agribusiness came from abroad, there are no wild relatives of some importance as source of genetic variation.

The breeding programs for cucurbits in the public sector are only too few and do not have an adequate continuity. As reported by Giordano (1991), the first *C. moschata* and *C. maxima* breeding program was realized by the Instituto Agronômico de Campinas (IAC) in the early 40's but unfortunately it was interrupted, although two squash cultivars were released. The Empresa Goiana de Pesquisa Agropecuaria (EMGOPA) also produced a cultivar from a breeding program (Giordano 1991), as well as many braquitic hybrids of *C. moschata* (Peixoto et al. 1992). The Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG) produced the cultivars Lavras 1 and Lavras 2 in partnership with the Universidade Federal de Lavras (UFLA). Embrapa Hortaliças has a cucurbit breeding program, aiming to establish interspecific hybrids of *C. moschata* and *C. maxima*, while a hybrid Jabras was produced with traits of the hybrid Tetsukabuto.

Maluf et al. (1997) have studied sources of resistance to PRSV-w (papaya ring spot virus – watermelon strain), formerly WMV-1 (watermelon mosaic virus 1), the most important potyvirus that attacks several Cucurbitaceae species. They found resistance sources in Brazilian accessions of *Cucurbita moschata* and *C. maxima*, probably from traditional agriculture as well as in the species *C. ecuadorensis* and *C. foetidissima*.

A melon breeding program involving the Embrapa Hortaliças, in partnership with other Embrapa Research Units, has produced a PRSV-w-resistant cultivar some time ago (Pessoa et al. 1988). It was used to develop new hybrid cultivars by the private sector. This program was apparently interrupted, but was resumed with a broader use of germplasm and a greater number of institutions. It has at the moment many lines which are resistant to most melon-attacking potyviruses, as well as experimental hybrids that match the traits of the yellow melon with the cantaloupe melon which are under evaluation (Paiva et al. 2000, 2003). Modolo and Costa (2004) described a gherkin elite line selection from an interspecific cross between *Cucumis anguria* var. *longaculeatus* and *C. anguria* var. *anguria* where mass selection alternating with inbreeding cycles allowed the identification of superior gherkin lines called Paulista Gherkin.

A watermelon breeding program has been developed in the last fifteen years by Embrapa Semi-Árido. It contains lines with different fruit patterns, lines resistant to powdery mildew (*Sphaerotheca fuliginea*) and tetraploid lines (Souza et al. 1999, Queiróz et al. 2001). The development of lines resistant to the main potyviruses of watermelon (PRSV-w, WMV – watermelon mosaic virus and ZYMV – zucchini yellow mosaic virus) is under way (Queiróz et al. 2004). Virus resistance is also being incorporated in the Crimson Sweet cultivar by the Embrapa Hortaliças. Thus, watermelon breeding programs have a reasonable number of pure lines (diploids and tetraploids) and diploid and triploid hybrid combinations.

Despite little information available, it is known that in the private sector some cucumber and melon cultivars were produced (Giordano 1991), mainly. Nevertheless, the fusion in some seed companies brought these programs to a halt. It is therefore probable that some breeding programs of the private sector do not have a collection of superior lines at their disposal. Moreover, the breeding programs of these large companies are, with rare exceptions, based abroad.

Thus, the germplasm in cucurbit breeding programs is to a certain extent limited, be it because of the small number of breeders devoted to the cucurbit species, or because of the interruptions that have hampered private and public breeding programs in the country.

Not with standing, it is in the centers of cultivation, particularly in traditional agricultural areas where the greatest diversity in the species *C. moschata*, *C. maxima*, *Citrullus lanatus* and *Cucumis* (particularly melon and gherkin) is found. Farmers intercrop different food crops with cucurbit according to their intent. There may be areas with sole cucurbit cultivation when farmers are market-oriented and others with few cucurbit plants earmarked for domestic use. This indicates that variability may be found on open markets, booths by the roadside and other selling points in regions where the cucurbit species represent important crops in the traditional agriculture (Queiróz 1993, Barbieri et al. 2004). Subspontaneous plants of *Cucumis* spp. are also found, especially of *Cucumis anguria*, *Momordica* spp. and *Citrullus lanatus* in many parts of northeastern Brazil implying that such plants are submitted to an annual natural selection.

It was therefore the variability in traditional agriculture and of spontaneous cucurbit plants that led to the establishment of the four main cucurbit germplasm banks currently existing in Brazil. The oldest one was initiated in 1960 with first collections at the Universidade Federal de Viçosa-MG, a second one was set up by the Embrapa Hortaliças, Brasília, Distrito Federal, another one by the Embrapa Semi-Árido in Petrolina, state of Pernambuco, and the last one was established by the Embrapa Clima Temperado in Pelotas, state of Rio Grande do Sul (Vieira et al. 1990, Queiróz et al. 1999, Silva et al. 2001, Barbieri et al. 2004). Altogether, over 4700 accessions were collected including several cucurbit species (*C. moschata*, *C. maxima*, *Citrullus lanatus* and *C. melo*). There are also some other species in the germplasm banks such as *Cucurbita ecuadorensis* (source of virus-resistance), *C. pepo*, *C. ficifolia*, *C. lundeliana*, *C. sativus*, *Lagenaria siceraria*, *Luffa cylindrica*, *Momordica* spp., *Citrullus lanatus* var. *citroides* (forage watermelon with virus resistance), and *Citrullus colocynthis* (source of virus-resistance), although some species are underrepresented. In these cases collection or introductions are required.

However, the definition of an accession is different for each germplasm bank and the number of accessions does not give a precise range of the existing variability. According to Vilela-Morales (1988) an accession is the element of the collection comprising different types and genetic combinations and is therefore a sample with more or less genetic variation. The accession should be unique. This would allow no duplication in the collection, which is highly improbable in the case of the cucurbit germplasm banks where seed exchange is a common practice among farmers of traditional agriculture (Romão 2000).

CHARACTERIZATION AND EVALUATION

Some studies on morphological characterization have been conducted with *C. lanatus* (Romão 2000, Silva 2004), *C. moschata* (Ramos et al. 2000), *C. maxima* (Amaral Júnior 1999), and *Cucurbita* spp. (Choer 1999), and an expressive genetic variability was found for many morpho-agronomic traits that are relevant for breeding of the referred species. Assis et al. (2000) have evaluated accessions of *C. lanatus* and *C. lanatus* var. *citroides* using six enzymatic systems. They only found polymorphism when accessions of *C. lanatus* were compared to the accessions of *C. lanatus* var. *citroides*, and did not find differences within the accessions of each species; notwithstanding, the use of RAPD markers revealed great polymorphism among accessions of *C. lanatus* (Silva 2004) and was more discriminative than the morphological characterization. Two hundred and twenty accessions of *C. moschata* were also evaluated through AFLP markers. Polymorphism groups were found, but the variability among accessions of this species proved to be a great deal smaller than shown in previous morphological studies (Ramos 2003).

It has been argued that there is a lack of variability in the Brazilian gherkin (*Cucumis anguria*) and interspecific hybridization with compatible *Cucumis* species has therefore been recommended to increase the genetic variability. However, Queiróz (1993) described three gherkin types found in the traditional agriculture of the Northeast of Brazil: a spineless one, another with scattering thick spines and the last densely covered with thin spines. Some gherkin accessions from the germplasm bank of the Embrapa Semi-Árido were also evaluated. Variability was found for spines, fruit size and seed dormancy. The germplasm bank has over 100 accessions of *C. anguria* though most of them have not been characterized. However, when one considers all rescued genetic variability stored in the germplasm banks, the number of characterized accessions of different cucurbit species is modest, since the total amount of accessions used so far is around 500.

The accessions of *Citrullus* spp were evaluated agronomically and genes were found for resistance to powdery mildew (*Sphaerotheca fuliginea*), to gummy stem blight (*Didymella bryoniae*) (Dias et al. 1999), potyvirus (Oliveira et al. 2002, Queiróz et al. 2004) and foliar diseases (Silveira and Queiroz 2003), besides a great variation in plant and fruit traits (Assis et al. 1994, Ferreira et al. 2000, 2002, Romão 2000, Souza et al. 1999). In fact, as related by Romão (2000) all described genes for watermelon, e.g., Mohr (1986), were found in the traditional agriculture of northeastern Brazil. According to Vieira et al. (1990), the biggest part of the collection of around 300 accessions of *Cucurbita* from the Embrapa Hortaliças germplasm bank has been evaluated for powdery mildew, PRSV-w and *Phytophothora capsici*. In particular for the *Phytophothora capsici*, 150 accessions of *Cucurbita* spp. were evaluated for resistance in the seedling and for fruit flesh and some resistance sources were found (Lima and Henz 1994, Henz et al. 1994).

Moura (2003) evaluated around 100 accessions of *C. moschata* from the Universidade Federal de Viçosa-MG and Embrapa Semi-Árido germplasm banks, for ZYMV virus-resistance. Many of them proved to be resistant and three of them were immune to the inoculated virus. The total carotenoid content was also studied in acession samples from the banks and a range from 26 to 251 $\mu g\ g^{-1}$ was found, even within this small number of evaluated accessions. It is thus probable that an extensive analysis of the *C. moschata* accessions available in the germplasm banks would allow the discovery of accessions with a high total carotenoid content, especially the beta-carotene precursor of vitamin A, which could be important in the recent trend to use functional food.

Some cucurbit species have demonstrated evolutionary dynamics for Brazilian conditions. One species is *C. anguria* that has strong seed dormancy and is found growing spontaneously in the Northeast relying on animals as their spreading agents that eat the fruits and scatter the seeds. *Momordica* spp. can also grow subspontaneously in the Northeast and the fruits open when mature so the seeds are exposed and are easily dispersed by birds and other animals. As a result both species are becoming weed races. This aspect was studied more in-depth for watermelon. Seed dormancy in watermelon accessions was found which made the seeds with this trait stay in the ground despite of regular watering, to sprout only after one to several weeks of delay (Romão 2000). According to Loveless (1983), this can be explained by the existence of germination-inhibiting substances in the seeds that are water-soluble and eventually washed away. This trait has been observed in many watermelon accessions, although its elimination through selection is simple indicating that this trait is controlled by few genes. Another relevant aspect in evolutionary dynamics of the *C. lanatus* in the Northeast of Brazil is the spreading of seeds through a type of maned wolf, a wild canidae that bores holes in the fruits (Figure 2), eats the flesh and scatters the seeds. The spreading of seeds along with the seed dormancy and the seed management by the farmers in the traditional agriculture makes the Northeast of Brazil a center of diversity for *C. lanatus* (Romão 2000).

Figure 2. Watermelon fruits with holes done by a type of maned wolf through which the flesh and seeds are swallowed (Author's photo)

Even then, the total number of evaluated accessions is not more than 25% of those that have already been rescued. Besides, the accessions still need to be evaluated for many other traits of agronomic relevance, e. g., pest-resistance, functional food, among others. Therefore, there is a great need for characterization and evaluation of the rescued genetic variability stored in the cucurbit germplasm banks so far. The necessity of intensifying the cucurbit germplasm collection in some not yet covered selected traditional agriculture areas of importance must also be analyzed to evaluate the risk of genetic erosion in the cucurbit species; this will make cucurbit germplasm available for long-term conservation and for the use in breeding programs which can increase the cucurbit agribusiness in the country.

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Germoplasma de cucurbitáceas no Brasil

RESUMO - *As espécies de cucurbitáceas representam um agronegócio expressivo no Brasil. As principais espécies são* Cucurbita moschata, C. maxima, Cucumis melo, C. sativus, Citrullus lanatus *e* Sechium edule. *Estas foram introduzidas por índios e escravos africanos. Mais recentemente, imigrantes europeus, americanos e japoneses introduziram alguns genótipos melhorados que foram dispersos pelo país. Existem alguns programas de melhoramento públicos e privados, porém, a variabilidade existente nos mesmos é limitada. Na agricultura tradicional, contudo, existe uma expressiva variabilidade genética que foi a base para a formação de quatro bancos de germoplasma com cerca de 4700 acessos, envolvendo mais de quinze espécies, 25% dos quais apenas foram avaliados, tendo-se encontrado vários genes úteis. Torna-se necessário ampliar a avaliação da variabilidade existente e examinar a necessidade de novas coletas para se ter, no Brasil, germoplasma de cucurbitáceas para conservação de longo prazo e para uso em programas de melhoramento.*

Palavras-chave: recursos genéticos, agricultura tradicional, melhoramento vegetal.

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