

Review

GENETIC RESOURCES OF COCOYAM OF *Xanthosoma Schott* GENUS IN CUBA

Revisión bibliográfica

Recursos genéticos de la malanga del género *Xanthosoma Schott* en Cuba

Marilys D. Milián Jiménez✉

ABSTRACT. Biodiversity conservation is strategic to meet the growing current and future demands of the world's population. The objective of this work is to present the status of the results and advances obtained in the subject of the knowledge of the variability of the species of the genus *Xanthosoma* present in Cuba, the management of their genetic resources, as well as the specific classification of cultivars, to serve as a basis for studies leading to clarify the species taxonomy of the genus *Xanthosoma*. In this review presents the importance of the knowledge of the the genus *Xanthosoma* particularities and of the genetic resources that compose it, as well as its usefulness in elucidating the classification of the cultivated species that make up this important genus of plants.

RESUMEN. La conservación de la biodiversidad es estratégica para satisfacer las demandas crecientes actuales y futuras de la población mundial. El objetivo de este trabajo es dar a conocer el estado de los resultados y avances obtenidos en el tema del conocimiento de la variabilidad de las especies del género *Xanthosoma* presentes en Cuba, del manejo de sus recursos genéticos, así como, de la clasificación específica de los cultivares, para que sirvan como base a los estudios conducentes a esclarecer la taxonomía de las especies del género *Xanthosoma*. En esta reseña se da a conocer la importancia del conocimiento de las particularidades del género *Xanthosoma* y de los recursos genéticos que lo componen, así como, de su utilidad en el esclarecimiento de la clasificación de las especies cultivadas que componen este importante género de plantas.

Key words: diversity, plant genetic resources, taxonomy

Palabras clave: diversidad, recursos fitogenéticos, taxonomía

INTRODUCTION

The conservation of biodiversity is strategic to meet the current and future growing demands of the world population; hence the germplasm banks arise as a response to the need to conserve the plant genetic heritage, which is why they constitute the basis for agriculture dynamic, diversified and sustained. A strategy of this nature has as essential objectives to conserve the variability of each species and

provide the breeders with a set of genotypes for selection programs, establish the representativeness of the collection and identify the duplication of accessions that may exist, among other problems (1,2). The current trends in agriculture are directed towards the search for species that allow a low-cost food supply, protection of natural resources, equity and poverty alleviation. The roots, rhizomes and tubers meet most of these requirements (3) and among them is the taro of the genus *Xanthosoma* (guagüí), whose genetic resources are important for food. This genus is of American

origin, whose distribution extends from Mexico to Brazil and it was cultivated by the aborigines of the Antilles and the rest of the continent before their discovery (4).

At present, its world production is estimated at 4,000,000 tons, concentrated in the central and western zone of Tropical Africa, the Antilles, Venezuela and Oceania.

In Cuba, cultivars of the genus *Xanthosoma* are the most important in the preference of the population and its production has increased in recent years especially because it requires less water in relation to other edible araceae. The nutritional values and

Instituto de Investigaciones de Viandas Tropicales (INIVIT), Apdo. 6, Santo Domingo, Villa Clara, Cuba, CP 53000
✉ geneticamx@inivit.cu

their easy cooking, together with their digestive qualities, make the species of the genus *Xanthosoma*, a product of high demand in the national market, as well as in the diet of hospitals, nursing homes and kindergarten. For this reason, the Ministry of Agriculture, intends to obtain a significant increase in the production of this crop in the coming years, in order to meet the growing demands of the market (5) where a suitable strategy plays an essential role in the genetic improvement of the crop, from a broad source of variability represented by its germplasm (6).

Several attempts have been made worldwide to classify and identify taro germplasm of this genus and one of the first was carried out in Cuba (7), to identify different accessions of the genera *Xanthosoma* and *Colocasia*. Several authors stated that the identification of *Xanthosoma* cultivated species was not clear, although four of them were recognized: *X. atrovirens* Koch & Bouché, *X. caracu* Koch & Bouché, *X. nigrum* (Vell) Manf. (*X. violaceum* Schott) and *X. sagittifolium* (L.) Schott (8-11).

Some cultivars cannot be included in any of these; however, many of these authors agree that for *Xanthosoma*, it is preferable to speak of 'clones of the genus', most grouped in a polymorphic species, *Xanthosoma sagittifolium* (L.) Schott, because of the deficiencies of the existing classification, up to that a modern revision of the genre, clarify the complex taxonomic situation of the same.

Traditionally, the diversity that exists within and between populations has been determined by evaluating its morphoagronomic characteristics (12,13); However, these are not enough to establish

differences between species or between accessions, so more direct genome studies should be used, such as the analysis of the karyotype that allows knowing the number and structure of chromosomes, and the use of biochemical and molecular markers (14-16). These methodologies do not evaluate the effect of the environment on the expression of genes, so they do not substitute, but complement the morphoagronomic characterization and evaluation (17).

In the Research Institute of Tropical Roots and Tuber Crops (INIVIT) a collection of cultivated accessions of taro of the genus *Xanthosoma* has been maintained since 1967, coming from collections, introductions and from the program of genetic improvement of the culture, which constitutes the largest collection of genes of this genus worldwide and one of the highest variability preserved. In this collection, morphoagronomic, cytogenetic, genetic-biochemical and molecular studies have been developed to help elucidate the taxonomic status of the genus, improve the structure of the crop clones, select the best adapted cultivars resistant to the attack of pests and diseases, how to determine the degree of variability in this collection (18).

GENERALITIES OF THE TARO CULTIVATION OF THE GENUS *XANTHOSOMA*

Origin, evolution and geographical dispersion

The taro were the first crops used by man and in Cuba are known by this name the edible species of the family *Araceae* belonging to the genera *Colocasia* Schott and *Xanthosoma* Schott; *Colocasia*, also known as 'islander malanga',

is very old and expanded in the Old World, was introduced into America by European colonizers; *Xanthosoma* ('taro' or 'guagüí') is of American origin, distributed from Mexico to Brazil, and was cultivated by the aborigines of the Antilles and the rest of the continent before their discovery (4).

When the Europeans arrived in America, the taro or guagüí was known from southern Mexico to Bolivia, but probably its cultivation was more intense in the Antilles. The domestication could have occurred in several places, with different materials, and was based on consumption processes when roasting and cooking the rhizomes, which eliminated the irritating substances, calcium oxalate crystals and saponins, which are present in all the parts of the plant (11,19). The taro was domesticated to the extent that its rhizomes enjoyed greater acceptance as food. From America, it took to West Africa, which has been the largest producing region; in it displaces the taro of the genus *Colocasia* for its greater yield, and because it can replace the yams (*Dioscorea* spp.) in the preparation of smash a very popular food in tropical Africa (11).

It is suggested that there are approximately 40 native species of tropical America (10), which are easily distinguished from the clones of the genus *Colocasia* by the peltate leaves of this one. The different species are cultivated for their rhizomes or edible leaves, others for their ornamental foliage that can be even mottled.

In Cuba, the term 'viandas' is used to name, among other vegetables, edible tropical roots, rhizomes and tubers, and within them, plants of the genus *Xanthosoma* and *Colocasia* (20).

There are two species of *Xanthosoma* in Cuba: *X. cubense* (Schott) Schott (endemic) and *X. sagittifolium* (L.) Schott (21); the latter introduced in the country with different common names: yellow taro, white taro and güagüí (22,23). This has traditionally been cultivated by the small Cuban producers, who naturally selected it based on the soil and climatic conditions of the region and extended the clones of the purple group mainly to the mountainous regions of the country, and those of the yellow group to those soils very rich in organic matter (24).

IMPORTANCE AND MAIN USES

The clones of the genus *Xanthosoma* constitute an important crop in several parts of the world like in Africa and South America and in terms of production and need of attention, they gain in popularity because, although the genetic variability at world-wide level is smaller in this genre, they are also, less susceptible to pests and diseases than clones of the genus *Colocasia* (25).

Xanthosoma is the third most important among edible rhizomes, roots and tubers in Central and Eastern Africa and one of the most important as vegetables (26).

In most markets in Latin America, taro of the genus *Xanthosoma* is seen as a superior crop, due to its flavor and texture (11).

With the exception of potatoes (*Solanum tuberosum* L.), sweet potatoes *Ipomoea batatas* (L.) Lam) and cassava (*Manihot esculenta* Crantz), among others, roots, rhizomes and tubers have been relatively little considered from the point of view of food and nutrition. However, accessions of the genus *Xanthosoma* have advantages for their yield and

their contribution of energy as carbohydrates, for their content of carotenes and are only surpassed by cassava in terms of mineral content (3). Its nutritional value is comparable to that of potatoes (*S. tuberosum* L.) and, in addition, flour and starch are obtained from them, which can be used in the textile industries and industrial alcohol. The leaves of some species of malanga or guagüí are important sources of proteins and vitamins (27) and they are used in the preparation of salads. The rhizomes of the malanga or guagüí constitute a valuable food product for the people of many developing countries (in particular of the South-West of Africa); they are consumed cooked or fried and they are very appreciated in the feeding of children and sick people. The cocoyam starch is microgranular, hypoallergenic, high quality and well assimilated. They contain appreciable amounts of vitamin C, which has been estimated between 7 and 9 mg 100 g⁻¹ of fresh material, can also contribute to the requirements of vitamin B, especially as regards thiamine and nicotinic acid (27).

The species *X. nigrum* (Vell) Mansf. (*X. violaceum* Schott) and *X. sagittifolium* (L.) Schott form secondary rhizomes that are used as food. The species *X. jacquini* Schott, known as 'Indian cabbage' in the countries of Central America, has large leaves of which salads are prepared (4). The Cameroonian ethnic groups preparing, processing and consuming *X. sagittifolium* (L.) Schott in many forms: boiled, in smash, among others (28).

The taro market of the genus *Xanthosoma* requires products of high quality and good presentation but, as in the case of other forgotten crops, little effort has been made

to industrialize and diversify it (11). There are many varieties of the genus *Xanthosoma* that differ in adaptation, yield, plant characteristics, rhizome size and flavor. For breeding programs, after creating a germplasm bank, the best cultivars should be selected on the basis of the yield of rhizomes and their starch content. Also the chemical and functional characteristics of the starch must be established to look for specific uses. Equally important is the use of rhizomes in animal production systems (29).

Regarding the average yield, the taro of the genus *Xanthosoma* is not high compared to other roots, rhizomes and tubers. Worldwide, it fluctuates between 5-6 t ha⁻¹ of rhizomes; however, in the countries that employ most modern agrotechnics, the yield can reach between 10 and 15 t ha⁻¹, (30,31).

In Cuba, the white rhizome is eaten by the white cocoyam, while the main rhizome is consumed after subjected to the action of the sun's rays, so that it loses the caustic action. The opposite happens with yellow taro clones in which the main rhizome is preferred and for seeding the secondary rhizomes are used; both are of special and delicate taste (22). The particularity that in the yellow cocoyam, the main rhizome is the edible part, makes it quite similar to the clones of the genus *Colocasia* from the agronomic point of view (18,32).

The agricultural characteristics of the malanga or guagüí have contributed to its increase in Cuba to acquire economic importance. In this sense, the following aspects stand out: a high yield potential (60 t ha⁻¹); resistance to pests and diseases; high conservation power in natural conditions; and the extremely small size of the starch grain (from 1 to 3 µm), which allows

it to be recommended as a food due to its high digestibility (4). The planted area of clones of the genus *Xanthosoma* represents 70 % of the total devoted to the aroids in the country, with an average yield between 10 and 12 t ha⁻¹, (33), and together with those of *Colocasia*, the production in the last period 2006-2007 has reached more than 144 thousand tons. An increase of more than 3 thousand hectares of the area to be planted is foreseen in the next stage (33).

SYSTEMATICS AND DESCRIPTION OF THE GENUS *XANTHOSOMA*

Taxonomic location

Different criteria have been reported for the taxonomic location of the genus *Xanthosoma* (5,8,34,35); however, the most updated seems to be the one published (36). Within the angiosperms or flowering plants, and particularly within the monocotyledonous plants, this author places the cocoyam or guagüí in the following position:

Class: Liliopsida

Order: Alismatales

Family: Araceae

Genus: *Xanthosoma*

Species: *Xanthosoma* spp.

Common name: yautía, malanga (Antilles), macal [Mexico (Yucatán)], quiscamote (Honduras), tiquisque or quequexque (Costa Rica), yautía (Dominican Republic and Guatemala), otóe (Panamá), okumo (Venezuela), uncucho (Peru), gualuza (Bolivia), malangay (Colombia); taioba, mangareto, mangarito, mangarás (Brazil); Chou Caribe (French Antilles); cocoyam, new cocoyam; queiquexque (Mexico), tannia, taniera (Antilles); malanga, guagüí (Cuba) (11,29,37).

In this family very little is known about the botanical delimitation of the genera (8), while the species are not fully identified, especially

for the genus *Xanthosoma*. This author points out that among the edible genera of araceae are: *Alocasia*, *Amorphophallus*, *Aserus*, *Caladium*, *Cyrtosperma*, *Colocasia*, *Monstera* and *Xanthosoma* however, only some clones of the latter, stand out for their higher yield, nutritional value and acceptance.

There is a great diversity of criteria in the specific nomenclature in the genus *Xanthosoma*: *X. sagittifolium* (L.) Schott is the scientific name most used for cocoyam; Engler (19), included *X. caracu* Koch & Bouché and *X. atrovirens* Koch & Bouché in *X. sagittifolium* (L.) Schott.

Engler's descriptions seem to include *X. mafafa* Schott in this complex of species and varieties (19). Some authors recognize six species but for this they mainly use vegetative characteristics (38) *X. atrovirens* Koch & Bouché, *X. belophyllum* Kunth; *X. caracu* Koch & Bouché; *X. jacquini* Schott; *X. maffafa* Schott and *X. sagittifolium* (L.) Schott.

Taking into account the diversity of criteria for the classification and location of clones of the existing genus for these possible species, some authors (39-42) preferred to leave the identification of the accessions at the generic level; however, it was considered appropriate to classify most of the accessions of the Cuban collection of white and yellow mass in *X. sagittifolium* (L.) Schott, (43-45), in correspondence with the appreciable clonal variability within this species found in the Antilles (32), and those of purple, pink and violet mass in *X. nigrum* (Vell) Mansf.

There is also a general consensus that the most cultivated species is *X. sagittifolium* (L.) Schott, of white mass and that there are also other economically important species, *X. atrovirens*

Koch & Bouché of yellow mass, *X. nigrum* (Vell) Mansf of pink or purple mass and *X. caracu* Koch & Bouché of cream white mass (40).

Some authors also differentiate, *X. brasiliense* Engl., as a species that is distinguished by being smaller in height and with upturned leaves, with markedly square basal lobes supported at right angles to the nerve, the secondary rhizomes are very small and are not consumed; they are cultivated only for the consumption of their leaves (10).

In Cuba, studies of the genus *Xanthosoma* indicate that a classification based on one or very few morphological characters does not reflect a solid criterion on the true genetic variability within the genus, considered prominent in the germplasm of the genus *Xanthosoma* in Cuba (18, 22, 45-47).

It is proposed that all species are diploid, with 26 somatic chromosomes (11, 48, 49), although the criteria of most of the authors relate to the great polymorphism that exists between the clones of the genus *Xanthosoma* and although many of them suggest the existence of several species, none had confirmed a definite specific classification. Rigorous studies carried out in the Cuban collection of germplasm of this plant genus for classification have considered cytogenetic, genetic-biochemical and molecular analyzes, which provide new elements for the identification of species (18).

BOTANICAL CHARACTERISTICS OF THE GENUS *XANTHOSOMA*

The cocoyam of the genus *Xanthosoma* has as its main morphological characteristic the sagittal form of its leaves, with spear point and broad basal lobes, separated by a deep slit in the insertion of the petiole with the limb, as well as an accentuated marginal vein (50).

The leaves originate in the bud of the apical end of the main rhizome, with an interval of 15 days on average between the appearance of one leaf and another, are glabrous, simple, heart-shaped or shield.

The petiole is sheathing, with a length between 0.3 and 1.8 m long and with a deep channel (pod) near the base that reaches approximately to the middle of the petiole, is wider at the base than in the part upper, thicker at its center and thin at the edges. It presents an edge whose width can be between 0.3 and 1.0 cm wide.

The rhizome, which is the main stem of the cocoyam or guagüí, is a reserve of nutrients and water. This underground organ can have a cylindrical, spherical, conical or ellipsoid shape; on the outside, it is covered with dark brown cataphylls arranged in a tight form that form a complete ring around the rhizome where the buds are inserted. The mass is usually white, although it may be yellow or purple (4,18). Depending on the cultivar, up to ten or more secondary rhizomes can be produced per main rhizome, which are the most desirable part of the plant and superior from the organoleptic point of view; however, the main rhizome is of equal nutritional value (44).

The cycle of growth of the plant lasts between nine and eleven months; in the first six months the main rhizomes and leaves develop and in the last four months, the foliage remains stable, when it begins to dry, the secondary rhizomes are ready to be harvested (11), usually before the inflorescences appear (49).

The Alismatales order is composed of plants that usually have cyclic flowers homoclamide or naked, trimers or dimers, unisexual or hermaphrodite and

usually actinomorphic, located in inflorescences in spadix, accompanied by a large bract or spathe that usually surrounds them and wrap them to protect them. In the inflorescence the superior flowers are feminine (4). Spadices are rarely fertile and produce few viable seeds (11).

Although there are methodologies for obtaining hybrids, clones that are used as commercial in different regions of the world respond to local ecotypes, and until the early 1980s there were no recommended techniques for hybridization (51). However, it should be noted that the clonal selection made with the use of spontaneous variability, has allowed to have clones with acceptable productive potentials, among them, those with rhizomes of purple mass, in addition to their high yield, have a greater adaptability to diverse edaphoclimatic conditions (52).

PLANT GENETIC RESOURCES: CONSERVATION, CHARACTERIZATION AND EVALUATION

Throughout history, plant genetic resources have contributed to the stability of agroecosystems and have provided the fundamental raw material for the emergence of modern scientific plant breeding; they are the basis of the subsistence of humanity, they supply basic needs and help solve problems such as hunger and poverty. At present, they continue to be the basis of the evolution of crops, as natural resources that have allowed them to adapt to infinity of means and applications that will allow them to respond to new adverse factors that arise in this century (53).

However, they have been lost mainly due to the inadequate use made of them, as well as the destruction of their habitats. Given their vital importance, it is necessary to conserve them for the benefit of present and future generations (17).

Nowadays, the acceptance of the dangers related to the irreversible loss of biodiversity in general and of agrobiodiversity in particular, is growing. It is argued that genetic diversity in farmers' fields is decreasing, partly as a result of the displacement of traditional varieties by modern varieties and introduced crops, in most cases, highly dependent on agricultural inputs and directed to external markets. This situation has led to renewed interest in the collection and conservation of this group of plant genetic resources (54).

It is suggested that diversity be used to indicate the sum of the known and unknown potential genetic information and the variability to indicate a portion of the diversity captured or available. Therefore, the term genetic variability is the one indicated for the study of collections (55).

Plant genetic resources comprise the present genetic variation, and especially useful for the future of humanity. These include traditional varieties and local breeds, commercial cultivars, hybrids and other materials developed through breeding, wild relatives of cultivated species and other materials that could be used in the future for agriculture or for the benefit of the environment (56).

CONSERVATION

The loss of genetic diversity in agriculture reduces the material available for the use of present and future generations. Thus, in this process the path to the possibilities for the development and evolution of different species can be closed. And on the other hand, the increase in uniformity can also cause greater risk and uncertainty (53).

Consequently, plant genetic resources must be conserved, since they constitute the fundamental reason for their possible use as a source of potentially useful genetic variation (56).

Genetic improvement works find limitations in certain areas due to the limited number of existing genotypes; problem that is solved with germplasm banks. Some cultivars are local ecotypes of great value, thanks to their natural rusticity, which represents an advantage in relation to others, typical of different areas (8). The germplasm banks are the best directed effort to collect and maintain the genetic diversity of crops and counteract the constant changes in agriculture, the disturbance of ecosystems and the regression of natural vegetation (57).

To achieve an increase in crop productivity, without degradation of the base of the resources of the agroecosystem, continuous access to the greatest possible genetic variability available for these crops and the wild species related to them is necessary. The threat of genetic erosion led to the first international initiatives for the creation in 1974 of the International Genetic Resources Council (IBPGR), then an independent body that paid tribute to the FAO secretariat, to coordinate an international program on genetic

resources. The practical result of these and other events was an effort to collect and conserve plant genetic resources before they disappeared.

The experts were convinced and had good reasons for this, that they had very little time to collect and safeguard those resources, in order to avoid their disappearance (53).

Field collections play a crucial role in the conservation of materials in natural environments for long periods, in addition to allowing their characterization and evaluation, at least during the first phase, as well as the regular propagation and control of them. The state of these collections varies with the size, reproduction level, origin of the germplasm, its national or institutional character, as well as the objectives of the work (58,59).

Plant species of vegetative propagation, with a long biological cycle and with short-lived seeds (recalcitrant), are usually maintained in field germplasm banks, although it is convenient to use a combination of storage techniques instead of depending on a single. Within this group, the germplasm of roots, rhizomes, tubers, bananas and bananas is conserved *ex situ* using different methods, according to environmental conditions and available means and knowledge. Among the most commonly used techniques are, in addition to the aforementioned gene banks conserved in the field, seed banks, *in vitro* banks and cryopreservation (60).

Although the plants of the field collections are easy to characterize and evaluate, they are also exposed to losses due to the attack of pests and diseases, or to adverse conditions such as drought, floods, fires and wind, among others.

That is why complementary alternative methods such as *in vitro* conservation are perfected and work is being done to improve the appropriate technologies for species with non-orthodox seeds and for vegetative propagation plants. This shows that it is necessary to increase the *ex situ* conservation capacity under profitable conditions (53).

Tissue culture methods offer ways to conserve germplasm of vegetatively propagated species, in a small space, free from attacks by pests and diseases, reduce labor and also facilitate the exchange of germplasm. The tissue used for *in vitro* conservation should allow both its establishment, as a regeneration of plants in a wide range of genotypes and high genetic stability (61). Due to the high risks of loss, a duplicate of each clone should be stored in a regional collection (Latin America, Africa, Asia and the Pacific area) (62). Such purposes begin with the study and preparation of the inventory of existing resources. It is common that most of the samples from germplasm banks have not been properly evaluated, which leads to underutilization of the collections and prevents the full use of their value, resulting in high conservation costs in relation to the benefits obtained (63). It is therefore that it is essential to carry out studies and research that contribute to better understand the true value of the genetic heritage that is conserved. The conservation of germplasm does not only mean storing material for future generations, it implies the handling of information about it, which may be of interest to current and potential users (54).

CHARACTERIZATION AND EVALUATION

The germplasm collections represent a source of useful genes for researchers. However, the management of large collections is a costly and complex activity, particularly to ensure long-term preservation and, on the other hand, the value of germplasm is more noticeable when additional information related to its characterization and evaluation is obtained (16). When a collection of germplasmic material is made, the obvious and necessary step is to make a qualitative and quantitative morphological description for its identification and an adequate evaluation of the genetic material (64).

The measurement of the qualitative and quantitative traits that are transmitted to the offspring of the germplasm in any environment, is known as characterization, allows determining the similarity between the accessions by means of their morphology and studies the variability in the collection. This variability is measured with few or many variables or descriptors, whose data form a scatter of points with a direction or vector, to determine the genetic distances between the accessions, which can be plotted in different ways, but are the dendrograms and the dispersion of points in a Cartesian plane those of easiest interpretation (65).

The characterization of genetic resources is important to identify potentially valuable traits of the samples, as well as to select local varieties that could be used directly by farmers (47).

The characterization of the variability is considered among the strategic lines of research worldwide, because it is a decisive factor in the solution of current and future problems related to

the productivity of cash crops, adaptation to climate change and development of new alternatives in obtaining varieties through the use of traditional and biotechnological methods (66).

THE GERmplasm OF THE GENUS *XANTHOSOMA*

For the genus *Xanthosoma* there is an urgent need to establish living and *in vitro* worldwide for genetic potential to be evaluated in relation to current problems and needs. This means collecting the known cultivars in the New World and in Africa, and exploring northern South America in search of possible wild specimens, primitive cultivars and related species (such as *X. jacquini* Schott) (11).

At the end of the 1990s, 32.5 % of the germplasm conserved in the national cocoyam collection of the *Xanthosoma* genus of Cameroon had been lost due to the attack of pests and diseases and the poor adaptation to edaphoclimatic conditions. In cases like this, if viable alternatives are not developed or applied, the germplasm can be completely lost (46).

The future of cocoyam or guaguí food of exceptional value for its organoleptic characteristics and nutritional properties - is in an expansion of export markets, in the application of technology to diversify its use and in promoting an intensive consumption in the popular diet of the tropical regions (11). This means that the wide genetic diversity present in the genus *Xanthosoma* must be exploited both directly, in the evaluation of cultivars for their resistance to diseases, yield and nutritional value, and in genetic improvement.

All these elements justify the need to direct efforts to deepen the knowledge of such an important plant genus. The experience acquired in Cuba on cultivation, the genetic wealth conserved in the Island and the existence of investigative methods such as molecular ones, constitute important motivations for scientists, breeders and producers of the *Xanthosoma* cocoyam towards the objective of guaranteeing the sustainable management of the existing genetic heritage; which includes a closer approach to the adequate taxonomic location of all the cultivars and to a better knowledge of their characteristics and genetic potential, which allows to achieve their effective use in breeding programs; as well as the improvement of the conservation strategies of the available resources.

USE OF DESCRIPTORS IN THE STUDY OF GENETIC VARIABILITY

In the improvement works it is important the characterization and the stability determination of the characters, that is why a series of descriptors are used such as morphological, genetic-biochemical, cytogenetic and molecular (67,68), each one of them it represents a useful tool, with advantages and disadvantages for the adequate study of plant genetic resources.

When morphoagronomic characterization and assessment data are not sufficient to establish differences between species or between accessions, it is possible to resort to studies close to the genome, such as karyotype analysis, among which determines the number and characteristics of the chromosomes. It is also possible to study the genome directly with the use of biochemical (isozymes) and

molecular markers. However, these methodologies do not evaluate the effect of the environment on gene expression, so they do not substitute-but complement-morphoagronomic characterization and evaluation (17).

MORPHOAGRONOMIC ANALYSIS

One of the essential aspects in the work of characterization of a species, is its description from the point of view of the morphological and agronomic attributes so that the collections of plant genetic resources have practical value (69,70).

The methods for the morphological study are based on the use of descriptors or qualitative characters that can be observed with the naked eye and quantitative that can be measured and expressed in almost all environments. These methods are relatively inexpensive and form the basis of the characterization of the plants in the germplasm banks (63,71-73).

In Cuba, the advantages of morpho-agronomic studies have been demonstrated in the characterization and differentiation of tomato varieties (*Lycopersicon esculentum* Mill.) (74,75), accessions of yam (*Dioscorea* spp.) (76), cassava (*Manihot esculenta* Crantz) (77), sweet potato (*Ipomoea batatas* (L.) Lam. (78), island taro (*Colocasia esculenta* (L.) Schott) (79), and banana and plantain (*Musa* spp.) (80), among other crops.

Although these markers are classical in the evaluations, they can lead to ambiguous considerations and interferences between the marker and the evaluated phenotype and do not represent the true genetic divergence between the genotypes, because the morphological and agronomic characters are highly influenced by the environment (79).

The morphological and agronomic evaluation of varieties, as part of the study of the variability of a crop can be integrated with more direct studies of the genome through cytogenetic analysis, electrophoresis of enzymes, proteins and DNA (81), but can not to be substituted by these methodologies, they do not evaluate the effect of the environment on the expression of the genes (17).

CYTOGENETIC ANALYSIS

Cytogenetic studies have an important role in the knowledge and exploitation of plant genetic diversity, and in particular, in those studies related to the management of germplasm collections.

The correlation between cytology and taxonomy began at the end of the 19th century, when it was found that the species are characterized by a stable number of chromosomes, which can differentiate them from the rest of the species or varieties (79). Some authors have proposed cytogenetic, biochemical and molecular criteria for the detection of genetic changes in the different accessions (80), as a complement to the morphological and agronomic characterization of the collections of plant genetic resources.

In plants, various methodologies are used for the study of chromosomes, ranging from classical methods to the use of molecular techniques. The establishment of ploidy, for example, is usually done by counting chromosomes, in microscopic sections prepared from the apexes of the actively growing roots (81).

Squashing or squash is also widely used to count chromosomes (82).

The chromosomal number is one of the most important descriptors in the characterization works of a germplasm bank of roots, rhizomes and tubers and particularly of the genus *Xanthosoma* where morphological characters such as the color of the mass have been correlated with this descriptor (44,45). A chromosome number of $2n = 26$ is referred to the genus *Xanthosoma* (83); while other authors (32,84) found a similar chromosome number for specimens of *Xanthosoma sagittifolium* (L.) Schott from Tropical America, which was corroborated by others (44,45), who also suggest the existence of $2n = 24$ chromosomes for *X. nigrum* (Vell) Manf (*X. violaceum* Schott), which coincides with that reported in another investigation (85).

Other authors found $2n = 24$ chromosomes in a group of accessions with main rhizomes and secondary rhizomes of pink or purple mass belonging to the Cuban collection of the genus *Xanthosoma* (86,87). Subsequent studies of white mass and yellow mass accessions in this collection showed a chromosome number of $2n = 26$.

The ploidy status of the *Xanthosoma malanga* and its cytogenetic behavior are essential in the improvement programs of this crop, where genetic variability is limited, since male and female sterility prevails, attributed mainly to the different cytogenetic phenomena (88-91). For this reason, the cytological selection of the clones and the identification of the polyploids allow us to plan appropriate breeding systems for this vegetative propagation crop.

This also reveals the causes of sterility and provides an opportunity to evaluate the effect of polyploidy on yield.

ISOENZYMATIC ANALYZES

Having quick and accurate methods to identify varieties that can be combined with the results of morphological analysis, serves to support improvement programs genetic research, determine phylogenetic relationships, assist in the transfer of economic interest traits and, in addition, to avoid the duplication of varieties or clones in germplasm collections (92).

In this sense, isoenzymatic analysis offers an important contribution to research in the field of plants.

The discovery of isoenzymes has since allowed the creation of more efficient biochemical markers than morphological ones, which usually make it possible to distinguish homozygous genotypes from heterozygotes (93-95) and the analysis of their patterns has been for years the most disseminated approach for the study of genetic diversity within and between plant populations, since these have proved to be very useful for the identification and classification of cultivars, evaluation of genetic variability and identification of genotypes, correlation of genotypes with their geographical origin and with important characteristics such as quality, response to attack by pests and diseases, as well as adaptation to extreme environmental conditions (96,97).

The levels at which isoenzymes can be used as a marker system are fundamentally the individual and species levels. This is because the polymorphism of allozymes is frequently found at these levels (98).

The routine use of isoenzyme electrophoresis involves the identification of suitable buffer systems and the staining of enzymes for particular systems and thus determines genetic differences between accessions, valuable information for the analysis of germplasm banks (99). In this way, the genetic distance between individuals and populations or entities can be measured, provided that it is taken into account that they cannot detect all the possible differences at the DNA level, due to the redundancy of the genetic code (100), so that nowadays these studies are complemented with markers at the DNA level (101-103).

Protein markers are also limited by the influence of the environment and the changes that occur at different stages of development, such as mineral nutrition, the incidence of pests and diseases and others that can cause the appearance or disappearance of certain molecular forms (104), so it is evident the need to carry out a rigorous standardization of the technique in order not only to achieve a high electrophoretic resolution, but also to make an adequate interpretation of the results obtained (105). It was possible to detect the presence of possible genetic changes during the tissue culture process of varieties of island cocoyam (*Colocasia esculenta* (L.) Schott) (106). Despite the wide use of this technique in genetic analysis and being considered as a powerful tool in the effective and efficient identification of duplicate material (65), limited information has been found in the literature consulted on the use of the technique of isoenzymes in the genus *Xanthosoma*.

In this sense, it is pointed out that these techniques have been used in Ghana to characterize accessions of *Xanthosoma sagittifolium* (L.) Schott and *Colocasia esculenta* (L.) Schott and at the same time with the use of morphological markers (27,107).

In a quantitative analysis of the isoenzymes was performed the purification and kinetic characterization of the peroxidase and polyphenoloxidases enzymes of *X. sagittifolium* (L.) Schott) and obtained two peroxidase isozymes and three polyphenoloxidases isoenzymes (108). In a work collection with 24 clones of the genus *Xanthosoma*, all with pink or purple coloration in the mass of their main and secondary rhizomes, a marked monomorphism was found in the analysis of the peroxidase isoenzyme pattern, while the esterase pattern was found to be polymorphic (76,77).

MOLECULAR ANALYSIS

It is suggested that the main alternatives for the differentiation of accessions are based fundamentally on the analysis of geographic origin, morphology, karyotype and proteins and isoenzymes (16); however, these criteria, despite their wide use, can be influenced to a greater or lesser extent by environmental factors, by the state of development of the plant and have a limited coverage of the genome, so they reveal only part of the variation genetics.

The above, together with the problem of identifying wild forms, main sources of new genes or identifying commercial materials, leads to the use of molecular descriptors, which constitute a valuable tool for the rapid and accurate identification of these materials for the aforementioned purposes (109-111) and to complement the characterization of cultivated diversity (54).

Molecular marker technology is composed of useful and reliable methods to discern variations within germplasm collections, for studies of evolutionary relationships in populations, as well as, analysis of the origin of the cultivated plants (16, 54, 112).

These techniques have been useful for the estimation of genetic diversity within and between species, as an essential element in the identification and clonal classification, in the construction of genetic maps, ecological studies, monitoring of genetic stability in materials propagated by *in vitro* culture and identification of mutants (113-116).

They have also been used in the identification and selection of genes linked to qualitative and quantitative characteristics of interest for breeding programs through the detection of DNA polymorphism (16,54). In recent years, its use in the conservationist activity of germplasm has increased and it is expected that this trend will continue as new biotechnological products appear and measures are established for their legal production (116).

Among the DNA markers, the RAPD (Random Amplified Polymorphic DNA) technology developed in 1990 (117) from the Polymerase Chain Reaction (PCR) (with non-specific primers) is widely used in genetic research due to its speed, simplicity, low cost and by requiring a small amount of sample for analysis (117-119).

Despite their limitations given their dominant nature, RAPD markers can be used to evaluate the fixation index and genetic parameters of a population when appropriate statistical analysis is used (27).

This advanced technology has been used to eliminate duplicates and estimate diversity among species, as well as to monitor and locate new sources of genetic variation in polymorphism studies and in the characterization of species. It has also been used for the identification of cultivars and for the analysis of the plant genome in population genetics, systematics and phylogeny.

Although the RAPD technique is not always reproducible, it detects a large number of markers and can facilitate gene mapping and identification of hybrids (27).

The diversity and genetic structure were evaluated of 70 accessions of *X. sagittifolium*, (L.) Schott), with the use of RAPD markers in order to obtain information on the genetic diversity of the crop in Ghana, as this is a limitation for the improvement Genetics of the genus *Xanthosoma* in this country (27). The accessions were not grouped in their different geographical regions, which suggest that a germplasm movement may have occurred between these zones. These results showed the usefulness of these analyzes to evaluate the gene flow between species and to study the variability present in the different regions.

GENERAL CONSIDERATIONS

For all of the above, it is necessary to deepen the study of the variability of the genus *Xanthosoma* Schott until achieving a correct specific classification of the cultivars and wild relatives present in the Cuban ecosystems, taking into account that in Cuba, the cocoyam of this genus enjoys the preference of the population and is fundamentally used in the feeding of children, the elderly and the sick with digestive affections.

Research carried out in the Cuban collection of cocoyam germplasm *Xanthosoma* spp., showed the presence in Cuba of five cultivated species of this genus, with an appreciable variability of *X. sagittifolium* L., if it takes into account that its propagation has been through the rhizomes or fractions thereof for a long time, which has caused the loss of their ability to reproduce sexually. The cultivars of this species represent almost the totality of the varietal composition of the different Cuban productive scenarios, which indicates that the improvement has been present, both natural and induced.

Many cultivars rarely or never produce inflorescences. This may be the reason that in the species *X. nigrum* (Vell) Manf. (*X. violaceum* Schott), *X. atrovirens* and *X. brasiliense* the variability present in Cuba is much lower than in *X. sagittifolium*. The malanga (*Xanthosoma* spp.) is an allogamous plant and presents protogyny so it rarely produces seeds. Their inflorescences are formed from the spathe that surrounds the spadix whose structure is distinctive of the araceae. The improvement of cocoyam (*Xanthosoma* spp.) has lagged behind other crops, such as cassava (*Manihot esculenta* Crantz), yam (*Discorea* spp.), potato (*Solanum tuberosum* L.) and sweet potato (*Ipomea batatas* (L.) Lam.).

This is due to the type of inflorescence and the germination problems of the seed. For all the above, it is important to continue providing research, taking into account that the adverse effects of climate change are increasingly notable, therefore the need for potential sources of food that strategically address food shortages will be greater.

CONCLUSIONS

- ◆ The conservation of biodiversity is strategic to meet the demands of the world population, hence the germplasm banks offer a response to the need to conserve the plant genetic heritage.
- ◆ Roots, rhizomes and tubers mostly meet the requirements of species that allow a low-cost food supply, protection of natural resources, equity and poverty alleviation; among them, the cocoyam of the genus *Xanthosoma* (guagüí).
- ◆ In Cuba, cultivars of the genus *Xanthosoma* are the most important in the preference of the population, therefore in the increase of its production an adequate strategy plays an important role in the genetic improvement of the crop from a wide source of variability represented by its germplasm.
- ◆ The taxonomic status of the genus *Xanthosoma* is complex and confusing worldwide despite the various attempts made to classify and identify germplasm.
- ◆ The evaluation of the morphoagronomic characteristics is not enough to establish differences between species or between accessions; It is necessary to resort to more direct studies of the genome, such as the analysis of the karyotype that allows knowing the number and structure of the chromosomes, and the use of biochemical and molecular markers.
- ◆ The studies carried out in the Cuban germplasm collection of the genus *Xanthosoma* Schott represent an international contribution in the subject of the phylogenetic resources of the aforementioned araceae genus de araceae through the integration of morphoagronomic data, chromosomes and isoenzymes.

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