



Short Note

Accessible Morphological and Genetic Markers for Identification of Taioba and Taro, Two Forgotten Human Foods

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Abstract: Some tropical species—such as the domesticated *Xanthosoma sagittifolium* (L.) Schott (Taioba) and *Colocasia esculenta* (L.) Schott (Taro)—have similar phenotypic characteristics, especially in the shape and color of the leaves and petioles which generate uncertainty in their identification for use in human food. This study aimed to analyze the morphological and molecular characteristics of *X. sagittifolium* and *C. esculenta* that may help in the popular and scientific identification of these species. The principal morphological characteristics of *X. sagittifolium* were as follows: leaves with subcoriaceous textures, basal insertion of the petiole, green pseudo-stem in the basal portion with exudate being white and the presence of two collector veins. Distinctive morphological characteristics of *C. esculenta* were as follows: leaves with velvety textures, peltate insertion of the petiole, pink pseudo-stem in the basal portion with pink exudate and presence of one collector vein. The morphological characteristics that can be used to distinguish Taioba from Taro are the basal petiole insertion of the first, against the petiole insertion near the center of the blade of the latter. Molecular analyses using eight Inter-Simple Sequence Repeat (ISSR) molecular markers simultaneously showed distinctive fingerprints for each of the species. These results contribute to the proper identification of the species used as a food source.

Keywords: Araceae; plant genetic resources; tannia; cocoyam; ISSR markers; *Xanthosoma sagittifolium* (L.) Schott; *Colocasia esculenta* (L.) Schott

1. Introduction

The Araceae is a family that consists of 109 genera and approximately 2830 species that are widely distributed, mostly in the tropical, subtropical, and temperate regions of the Northern Hemisphere [1]. The Araceae family is divided into several subfamilies based on the variation of habitats, disposition, and leaf morphology, the structure of the inflorescence and pollen, floral morphology, anatomy, and chromosome number [2]. Economically, the genera *Alocasia*, *Colocasia*, and *Xanthosoma* of this family are important due to their ornamental use or for the starch obtained from their corms [1]. The species *Xanthosoma sagittifolium* (L.) Schott is exclusively of Neotropical origin; however, it has been introduced in many tropical areas and cultivated as a food plant in developing countries, and it

is found naturalized in many places. Approximately 400 million people worldwide now consume its leaves or corms as a staple food [3,4]. On the other hand, *Colocasia esculenta* (L.) Schott is a cultivated species native to tropical Asia and naturalized throughout the tropics, and is valued as a rich source of easily digestible protein [3,5].

In Brazil, the leaves of the species *X. sagittifolium* (taioba) and the rhizomes of *C. esculenta* (taro), both cultivated by family farmers, are used as food in the states of Minas Gerais and Rio de Janeiro [6]. Despite their importance as an energy-rich food and its increased use, there are only a handful of efforts to market their products in the Americas and West and Central Africa, showing the marginalization of these cultivated species and therefore their status as two neglected crops [3,7]. *C. esculenta* has been traditionally grown in South and Southeast Asia and the Pacific region as a valuable staple food, where it is commonly known as taro, dasheen, eddoe [5,7], true cocoyam, old cocoyam [8], and poi [9]. *X. sagittifolium* is referred as yautia and tannia (Caribbean), malanga (Cuba), and cocoyam [9]. In sub-Saharan Africa, both species are known as cocoyam, where their staple root/tuber production ranks third in importance after yam and cassava [7].

There is little information regarding the taxonomy of the above-mentioned cultivated species [3]. Lately, a huge database has been developed aiming at improving the taxonomy of Araceae, including data on chromosome number, morphology, and molecular biology [10]. Molecular studies have been useful in establishing genetic differences between species. One mechanism that has had considerable success in helping to identify different taxa is the use of Inter-Simple Sequence Repeat (ISSR) markers [11].

X. sagittifolium (Taioba) and *C. esculenta* (Taro) are popularly mistaken for one another [5,7] because of their similar phenotypic characteristics, especially in the form and color of the leaves and petioles, which create uncertainty when they are collected for use as food by humans. The research hypothesis is that both species have enough morphological and molecular features that will aid in their characterization. Therefore, this study aimed at the following: (1) to analyze the morphological characteristics of *X. sagittifolium* and *C. esculenta* (Araceae) taxa and highlight those that allow easy differentiation in the field, (2) to evaluate and identify molecular ISSR markers that may help in the identification of these species, and (3) to compare these two species morphologically and molecularly with two additional taxa, *Xanthosoma violaceum* Schott and *Xanthosoma undipes* (K.Koch & C.D.Bouché) K.Koch.

2. Materials and Methods

Samples of *X. sagittifolium* and *C. esculenta* species were obtained from urban (1, 8, 11–14), natural (6, 7), and cultivated (2–5) populations from the states of São Paulo and Minas Gerais (Brazil) (Table 1, Figure 1). Sample data included the sample number, location where they were collected, georeferenced data (GPS), and collection date (Table 1). Additionally, *X. violaceum* and *X. undipes* species were also collected from two urban populations to compare morphology and the genetic profile to the target species (Figure 1). For molecular analysis, 150 mg of leaf tissue was sampled from each of the 14 specimens and stored at -20°C . DNA was extracted following the protocol proposed by Alzate-Marin et al. [12].

Subsequently, plants were described morphologically according to Acevedo-Rodriguez and Strong [13] (data not shown) and prepared for herbarium material. The exsiccates were deposited in the reference collection of the Herbarium SPFR University of São Paulo, Ribeirão Preto, with the following labels: C. Faleiros, P. Sepulveda, and F. Bonifacio (Table 1).

Table 1. Data of specimens studied.

No.	Description	Herbarium Sample	GPS	Local/Brazil	Collection Date
1	<i>X. sagittifolium</i>	C. Faleiros 307	20°33'21.38" S 47°23'56.08" O	Franca/SP	28/05/2013
2	<i>X. sagittifolium</i>	C. Faleiros 308	20°34'13.91" S 47°20'29.29" O	Abaeté-Franca/SP	03/06/2013
3	<i>X. sagittifolium</i>	-	20°34'13.91" S 47°20'29.29" O	Abaeté-Franca/SP	03/06/2013
4	<i>X. sagittifolium</i>	C. Faleiros 309	20°37'29.86" S 47°10'54.44" O	Itirapua/SP	04/06/2013
5	<i>X. sagittifolium</i>	-	20°37'29.86" S 47°10'54.44" O	Itirapua/SP	04/06/2013
6	<i>C. esculenta</i>	-	20°37'16.58" S 47°04'27.15" O	Capetinga/MG	04/06/2013
7	<i>C. esculenta</i>	C. Faleiros 310	20°37'16.58" S 47°04'27.15" O	Capetinga/MG	04/06/2013
8	<i>X. sagittifolium</i>	Sepúlveda P. 752	21°15'03.13" S 47°15'58.28" O	Ribeirão Preto/SP	16/06/2013
9	<i>X. undipes</i>	-	21°09'54.85" S 47°51'40.54" O	USP-Ribeirão Preto/SP	17/06/2013
10	<i>X. violaceum</i>	Sepúlveda P. 749	21°09'54.85" S 47°51'40.54" O	USP-Ribeirão Preto/SP	17/06/2013
11	<i>X. sagittifolium</i>	F. Bonifacio 110	20°53'39.78" S 47°00'41.97" O	São Sebastião do Paraíso/ MG	16/06/2013
12	<i>C. esculenta</i>	-	20°53'42.14" S 47°00'28.10" O	São Sebastião do Paraíso /MG	16/06/2013
13	<i>X. sagittifolium</i>	-	20°53'50.14" S 47°00'26.73" O	São Sebastião do Paraíso/ MG	16/06/2013
14	<i>X. sagittifolium</i>	F. Bonifacio 111	20°53'51.83" S 47°00'16.09" O	São Sebastião do Paraíso/MG	16/06/2013



Figure 1. Images of populations of *X. sagittifolium* (A), *C. esculenta* (B), *X. violaceum* (C), and *X. undipes* (D), which correspond to samples 11, 12, 10 and 9, respectively (Source: Laboratory of Plant Genetics/University of São Paulo, Campus of Ribeirão Preto, Brazil).

For the molecular analyses, 15 ISSR markers were evaluated (UBC 1, 2, 813, 820, 834, 845, 851, 858, 860, 862, 864, 866, 885, 886, 897) according to the official list published by the University of British Columbia in Canada (UCB). The amplification products were separated by electrophoresis on 8% polyacrylamide gel. The results were visualized by staining with silver nitrate according to the protocol of Sanguinetti et al. [14]. Fragments of different mobilities were considered as different alleles. Estimates of the size of the resulting amplification products were performed by comparison with a DNA ladder of 50 base pairs (GE Healthcare, Little Chalfont, UK). The polymorphism obtained by the ISSR technique was tabulated as binary according to the presence (1) or absence (0) of bands. Each polymorphic ISSR band was considered a biallelic locus, with an amplifiable allele and a null allele. GenAlEx 6.5 software [15] was used to generate a matrix of genetic distances according to Nei [16] and Nei's genetic diversity (\bar{H}_e). MEGA 5 software [17] was used to generate a dendrogram of genetic similarity based on the UPGMA algorithm.

3. Results and Discussion

X. sagittifolium exhibited white exudate from the vegetative parts (Figure 2A), green pseudostem (Figure 2B), basal petioles (Figure 2C), leaves with a sagittate form (Figure 2D), two collecting veins at the leaf margin (Figure 2E), and subcoriaceous leaf blades. *C. esculenta* displayed pink or purple exudates from the vegetative parts (Figure 3A), pink pseudostem (Figure 3B), peltate petioles (Figure 3C), velvety leaf blades (Figure 3D), and one collecting vein in the margin of the leaf (Figure 3E).



Figure 2. Images of *X. sagittifolium*. (A) The white color of exudate in stem (arrow); (B) The green color of the pseudostem (arrow); (C) The basal insertion of the petiole in leaf (arrow); (D) The upper leaf and its sagittate form; (E) The two collecting veins at the leaf margin (arrow) (Source: Laboratory of Plant Genetics/University of São Paulo, Campus of Ribeirão Preto, Brazil).



Figure 3. Images of *C. esculenta*. (A) The pink color of exudate in stem (arrow); (B) The pink color of the pseudostem (arrow); (C) The position of the petiole near the center of the leaf blade (arrow); (D) The upper leaf with a velvety texture; (E) the collecting vein (arrow). (Source: Laboratory of Plant Genetics/University of São Paulo, Campus of Ribeirão Preto, Brazil).

Insertion of the petiole and the purple underside of the leaf blade, the leaves with revolute margins, purple petioles, and markedly subtriangular basal lobes were characteristics of *X. violaceum* (Figure 1C). Leaves with involute margins, the apex broadly obtuse to mucronate, and green petioles were features of *X. undipes* (Figure 1D).

For folk taxonomy, the morphological characteristics that can be used to distinguish *Xanthosoma* from *Colocasia* species are from the appreciation of the basal petiole insertion of the first (Figure 2C), against the petiole insertion near the center of the blade of the latter (Figure 3C). The results of the molecular analyses with the 15 ISSR markers show that only one primer did not have any amplification products (UBC 813), while eight primers (UBC 2, 834, 845, 851, 858, 860, 864, 866) produced high-resolution profiles that were selected for the next stage (Figure 4A). From these amplifications, 334 loci were obtained, with an average of 41.75 loci, varying between 22 (UBC 866) and 73 (UBC 2) (Table 2). Of these, 321 (96.11%) exhibited polymorphism and 13 (3.89%) were monomorphic. The results indicated an average of 26.52% genetic diversity among the specimens that were analyzed (Table 2).

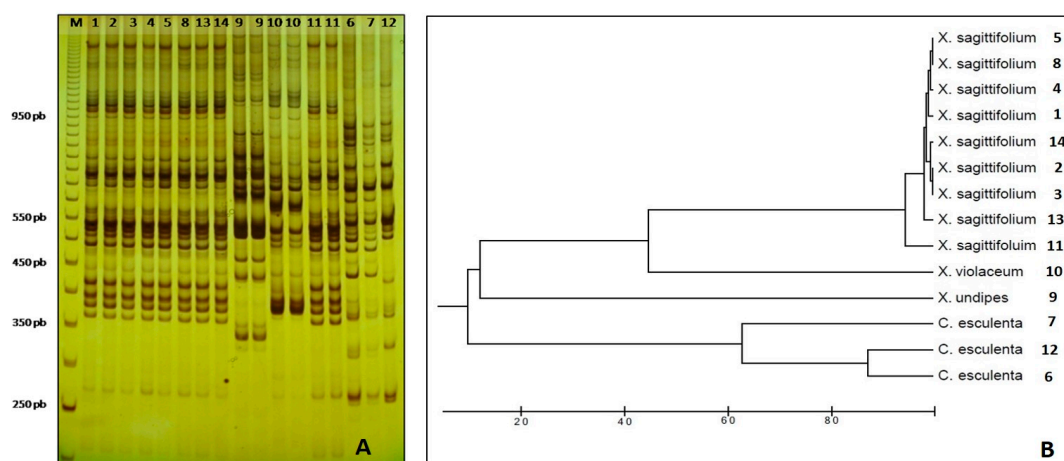


Figure 4. (A) Non-denatured polyacrylamide gel (8%) showing the amplification products related to the ISSR UBC2. The distinctive fingerprint of each species studied is shown. Column M corresponds to a molecular weight marker (GE Healthcare 50 pb). Lanes 1–5, 8–14, and 11 (amplified in duplicate) correspond to the taxon *X. sagittifolium*; channel 9 corresponds to *X. undipes* (amplified in duplicate), channel 10 corresponds to *X. violaceum* (amplified in duplicate). Lanes 6, 7, and 12 correspond to *C. esculenta*. (Source: Laboratory of Plant Genetics/University of São Paulo, Campus of Ribeirão Preto, Brazil); (B) Dendrogram based on the UPGMA algorithm showing the genetic similarity of the specimens of the four analyzed species.

Table 2. Characteristics and sequences of the inter-simple sequence repeat (ISSR) primers used in the genetic analysis of specimens of four species of Araceae. PL = polymorphic loci, ML = monomorphic loci. \hat{H}_e = Nei Genetic Diversity. R = (A,G), Y = (C,T).

Primer	Sequence 5'–3'	N° of Loci	PL	ML	\hat{H}_e
UBC#2	GAGAGAGAGAGAGAT	73	68	5	0.305
UBC#834	AGAAGAAGAGAGAGAGYT	47	47	0	0.270
UBC#845	CTCTCTCTCTCTCTRG	36	36	0	0.237
UBC#851	GTGTGTGTGTGTGTGTYG	37	36	1	0.301
UBC#858	TGTGTGTGTGTGTGTGRT	37	36	1	0.244
UBC#860	TGTGTGTGTGTGTGTGRA	49	45	4	0.240
UBC#864	ATGATGATGATGATGATG	34	33	1	0.266
UBC#866	CTCCTCCTCCTCCTCCTC	22	21	1	0.259
Total		334	321 (96,11%)	13 (3.89%)	-
Average					0.2652

A UPGMA dendrogram was generated based on a genetic similarity matrix (data not shown) which showed groupings of the different species tested (Figure 4B). The analysis by ISSR molecular markers showed low polymorphism among specimens of *X. sagittifolium*, which originated from various collection sites, suggesting that these individuals have a common origin, possibly due to vegetative propagation by producers (Table 3). This result can be illustrated by observing specimens 2–3 and 5–8, which came from different localities, (see Table 1, Figure 4A) but showed identical band profiles for all the primers used. Additionally, three specimens of *C. esculenta* appeared to have higher polymorphism than the nine specimens of species *X. sagittifolium* (Table 3). *X. undipes* exhibited different electrophoretic profiles regarding the other individuals of the same gender. Therefore, it was grouped separately from *X. violaceum* and *X. sagittifolium*.

Table 3. Genetic diversity of specimens of *X. sagittifolium* and *C. esculenta* analyzed using ISSR molecular markers.

Species	N	PL	PPL (%)	Ĥe
<i>X. sagittifolium</i>	9	14	4.19	0.014
<i>C. esculenta</i>	3	86	25.74	0.109

N = number of specimens, PL = number of polymorphic loci, PPL (%) = percent of polymorphic loci, Ĥe = Nei Genetic Diversity.

X. sagittifolium and *C. esculenta* are the most important species of Araceae, with a nutritional value similar to the potato, with exceptional value for food security [3,4,7,18,19]. The usable parts in both species are the subterranean tuberous stems and the young leaves, especially from *X. sagittifolium* [3,4,19]. While there are numerous reports with different molecular markers for *C. esculenta* (e.g., [20–23]), a smaller number were found for *X. sagittifolium* [24–27] and for both species [25,27]. In this work, we characterized for the first time eight ISSR markers that can be used simultaneously in the genetic analysis of *X. sagittifolium* and *C. esculenta* and other related taxa such as *X. violaceum* and *X. undipes*, which can aid in characterization and identification.

C. esculenta has high genetic diversity with diploid and triploid genotypes spread throughout tropical regions [23,28]. As an allogamous and heterozygous species, natural pollination occurs between *C. esculenta* diploid plants; however, its asexual propagation by farmers generates clonal lineages [23]. Similarly, despite being an outcrossing species, *X. sagittifolium* is mostly cultivated by vegetative propagation due to the low viability of seeds produced from natural crosses [3]. Thus, our observations on the diversity of these species are according to their reproductive and cultivation characteristics (Table 3).

The morphologic and genetic characterization of *X. sagittifolium* and *C. esculenta* generated in this study can contribute to their proper identification in field and germplasm banks in Brazil and other places where these species are used as a food source.

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