

# Angolan vegetable crops have unique genotypes of potential value for future breeding programmes

## AUTHORS:

José P. Domingos<sup>1</sup>

Ana M. Fita<sup>2</sup>

Maria B. Picó<sup>2</sup>

Alicia Sifres<sup>2</sup>

Isabel H. Daniel<sup>3</sup>

Joana Salvador<sup>3</sup>

Jose Pedro<sup>3</sup>

Florentino Sapalo<sup>3</sup>

Pedro Mozambique<sup>3</sup>

María J. Díez<sup>2</sup>

## AFFILIATIONS:

<sup>1</sup>Department of Biology, Science Faculty, University Agostinho Neto, Luanda, Angola

<sup>2</sup>Institute for the Conservation and Improvement of the Valencian Agrodiversity, Polytechnic University of Valencia, Valencia, Spain

<sup>3</sup>Center for Genetic Resources, University Agostinho Neto, Luanda, Angola

## CORRESPONDENCE TO:

María José Díez

## EMAIL:

mdiezni@btc.upv.es

## POSTAL ADDRESS:

Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Edificio 8E, acceso J, 3er piso Camino de Vera s/n Valencia, Valencia 46022, Spain

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A survey was carried out in Angola with the aim of collecting vegetable crops. Collecting expeditions were conducted in Kwanza-Sul, Benguela, Huíla and Namibe Provinces and a total of 80 accessions belonging to 22 species was collected from farmers and local markets. Species belonging to the Solanaceae (37 accessions) and Cucurbitaceae (36 accessions) families were the most frequently found with pepper and eggplant being the predominant solanaceous crops collected. Peppers were sold in local markets as a mixture of different types, even different species: *Capsicum chinense*, *C. baccatum*, *C. frutescens* and *C. pubescens*. Most of the eggplant accessions collected belonged to *Solanum aethiopicum* L. Gilo Group, the so-called 'scarlet eggplant'. Cucurbita genus was better represented than the other cucurbit crops. A high morphological variation was present in the *Cucurbita maxima* and *C. moschata* accessions. A set of 22 *Cucurbita* accessions from Angola, along with 32 *Cucurbita* controls from a wide range of origins, was cultivated in Valencia, Spain and characterised based on morphology and molecularly using a set of 15 microsatellite markers. A strong dependence on latitude was found in most of the accessions and as a result, many accessions did not set fruit. The molecular analysis showed high molecular variability and uniqueness in the collected accessions, as shown by their segregation from the set of global controls. In summary, the material collected is quite valuable because of its uniqueness and the potential of the breeding characteristics it possesses.

## Introduction

Angola is a country with enormous agricultural potential, with vast cultivable and underutilised areas, excellent water resources and a large variety of climates and ecosystems. However, huge challenges still exist with regard to food security and the high levels of poverty among the rural population. Approximately half the Angolan population live in rural areas, and more than 85% of growers are small farmers that cultivate crops for self-consumption. Landraces adapted to their specific agro-climatic conditions and requiring low inputs are maintained by these growers. These varieties are quite valuable as they present unique adaptations to specific cultivation systems and soil types.

The typical Angolan diet is based on the consumption of various legumes, cereals and roots, such as cassava, millet, sorghum, beans, sweet potato, peanut, rice, wheat and potatoes. The cultivation of vegetable crops is scarce, which contributes to a low intake of vitamins and minerals among the population. Some initiatives are currently being developed to increase the cultivation of vegetables. One example is the urban and peri-urban agriculture implemented in the neighbourhood of Luanda. This is playing a crucial role in ensuring the population's access to high-quality fresh food. However, in order to increase the benefits of this and other initiatives, and facilitate a more market-oriented production system, it is crucial that they employ more suitable varieties that will maximise yields and adapt to specific conditions. At present, Angola has alternative varieties of vegetable crops such as pumpkin, eggplant, pepper and watermelon. However, little information is available regarding the genetic resources of these crops or of the adaptability of breeding cultivars to suit specific Angolan growing conditions.

In 1991, The Center for Plant Genetic Resources of Agostinho Neto University (CRF-UAN) in Luanda started a programme with the purpose of collecting and conserving the genetic resources of food-plant landrace species. The CRF-UAN currently holds a collection of 4100 accessions, of which only 8.8% are horticultural vegetable crops. All of the accessions are preserved under long-term conditions with the aim of characterising them and incorporating them into breeding programmes. Owing to economic restrictions and a lack of technicians, only 7% of all the accessions have been characterised further, and only 4% have been assessed for genetic diversity using molecular markers. Of these characterised accessions, none have been horticultural crops.

The CRF-UAN is aware of the importance of conducting detailed studies of the collection, as their utilisation in plant breeding requires a profound knowledge of their morphological, agronomical and genetic characteristics. A joint project between the Instituto de Conservación y Mejora de la Agrodiversidad Valenciana of the Universitat Politècnica de València (COMAV-UPV) and Agostinho Neto University in Luanda was initiated in 2012. The project was funded by the Agencia Española de Cooperación Internacional. The global aim of this project was to strengthen the use of Angolan vegetable genetic resources, with the understanding that in order to do so, it would be necessary to further characterise these resources. A second objective was to provide a fully equipped molecular laboratory to the Centre for Genetic Resources of Luanda and to train its staff in collecting, morphological characterisation and molecular techniques, as well as in the use of vegetable genetic resources in breeding programmes.

We describe a collecting expedition conducted in 2012 in four Angolan provinces with the aim of enriching the vegetable collection of the CRF-UAN of Luanda. Additionally, we conducted a molecular study of the collected *Cucurbita* material, using microsatellite markers, to obtain information about the genetic variability of the Angolan *Cucurbita* accessions and their relatedness to a set of reference accessions from all over the world.

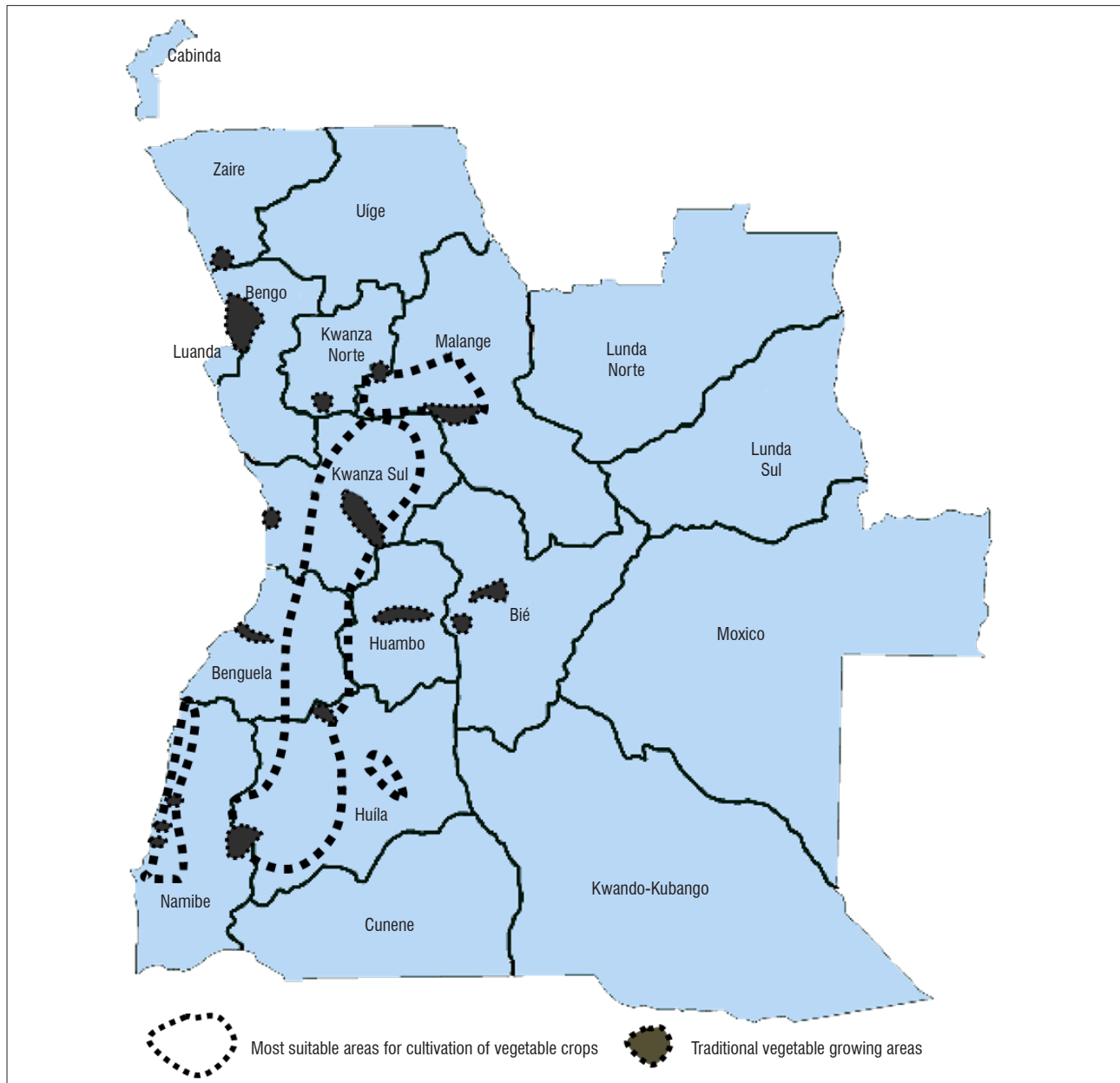


Figure 1: Areas visited during the collecting expedition.

## Material and methods

### Areas surveyed

The provinces of Kwanza-Sul, Benguela, Huíla and Namibe were all visited during the collecting expedition. These areas are part of the seven traditional areas of vegetable production, and are among the 5 most suitable areas for the cultivation of these types of crops in Angola. All 4 provinces are situated along the western strip at altitudes between 1000 m and 1200 m, and the period of exploitation of crops lasts about 6 months, from May to October<sup>1</sup> (Figure 1). According to Diniz<sup>1</sup>, Lombe, in Malanje Province, and Cela, in Kwanza-Sul Province, both at an altitude of 1200 m, supply vegetable products during the dry seasons and even for part of the rainy season. Other growing areas are distributed throughout the centre and south, especially in the areas connected by the Benguela railway (Catabola, Chinguar, Bela Vista and Vila Verde) and in the southern regions of Lubango-Humpata-Chibia and Matala-Capelongo.

Kwanza-Sul encompasses the vegetable-producing areas of the south shore of the Kwanza river (Libolo-Amboim) and the transitional central-

northern region that comprises a large part of the strip that has a tropical, hot and semi-arid climate, with a rainy season of about seven months (October-April). The provinces of Benguela, Huíla and Namibe belong to four agricultural areas: the south shore, the transitional central-western region, the transitional central-southern region and Gambos, where the climate is similar to the humid heat of the interior, semi-arid high plateau, according to Thornthwaite's classification.<sup>2</sup>

The collecting expedition was conducted jointly by Angolan and Spanish researchers. In all provinces, previous contacts established with the local agricultural agencies facilitated direct contact with the farmers and the collection of accessions on their own farms. Other accessions were collected in local markets. In all cases, passport data included in the Multicrop Passport Descriptor List<sup>3</sup> were recorded for each collected accession. The Centre for Plant Genetic Resources of Luanda holds seed samples of all the collected accessions. Some were sent to the Genebank of the COMAV-UPV for further characterisation and molecular analysis in collaboration with the Angolan researchers.

**Table 1:** *Cucurbita* spp. accessions collected in Angola and used in the morphological and molecular diversity assay

Species	Accession (code used in Figures 3–6)	Collection site	Latitude	Longitude	Altitude (m)	Collection source
<i>C. maxima</i>	ANG01 (ma01)	Porto Amboin, Kwanza-Sul	101135S	0133117E	34	Local market
<i>C. maxima</i>	ANG08 (ma08)	Ebo, Kwanza-Sul	105123S	0143847E	1192	Local market
<i>C. maxima</i>	ANG11 (ma11)	Kibala, Kwanza-Sul	103842S	0145747E	1387	Local market
<i>C. maxima</i>	ANG12 (ma12)	Kibala, Kwanza-Sul	103842S	0145747E	1387	Local market
<i>C. maxima</i>	ANG13 (ma13)	Kibala, Kwanza-Sul	103842S	0145747E	1387	Local market
<i>C. maxima</i>	ANG14 (ma14)	Kibala, Kwanza-Sul	103842S	0145747E	1387	Local market
<i>C. maxima</i>	ANG17 (ma15)	Kibala, Kwanza-Sul	103842S	0145747E	1387	Local market
<i>C. maxima</i>	ANG18 (ma18)	Kibala, Kwanza-Sul	103842S	0145747E	1387	Local market
<i>C. maxima</i>	ANG38 <sup>a</sup> (ma38)	Baia Farta, Benguela	125651S	0130534E	7	Seeds from farmer
<i>C. maxima</i>	ANG44 (ma44)	Baia Farta, Benguela	123832S	0131638E	7	Local market
<i>C. maxima</i>	ANG50 (ma50)	Cacula, Huila	141507S	0140223E	1577	Orchard
<i>C. maxima</i>	ANG52 (ma52)	Cacula, Huila	143258S	0140439E	1577	Bought from road vendor
<i>C. maxima</i>	ANG53 (ma53)	Cacula, Huila	143258S	0140439E	1577	Bought from road vendor
<i>C. maxima</i>	ANG54 (ma54)	Cacula, Huila	143258S	0140439E	1577	Bought from road vendor
<i>C. maxima</i>	ANG73 (ma73)	Chibia, Huila	151242S	0134423E	1527	Local market
<i>C. maxima</i>	35040 (ma35040)	Kwanza-Sul	–	–	–	Local market
<i>C. moschata</i>	ANG03 (mo03)	Gabela, Kwanza-Sul	105211S	0142538E	1077	Small village
<i>C. moschata</i>	ANG04 (mo04)	Ambuíla, Kwanza-Sul	105211S	0142538E	1077	Small village
<i>C. moschata</i>	ANG15 <sup>a</sup> (mo15)	Kibala, Kwanza-Sul	103842S	0145747E	1387	Local market
<i>C. moschata</i>	ANG16 (mo16)	Kibala, Kwanza-Sul	103842S	0145747E	1387	Local market
<i>C. moschata</i>	35039 (mo35039)	Kwanza-Sul	–	–	–	Local market
<i>Cucurbita pepo</i>	ANG58 <sup>a</sup> (pe58)	Humpata, Huila	150237S	0133025E	1872	Small village

<sup>a</sup> Accessions tested with the complete set of microsatellites

### Plant material

Accessions of 22 different species were collected. Those belonging to the genus *Cucurbita* were used for the genetic diversity analysis. In total, 22 *Cucurbita* accessions from Angola were assayed, along with 32 *Cucurbita* spp controls from a wide range of origins, representing most of the cultivated and wild species of the genus.<sup>4</sup> Seeds of the control accessions were supplied by the United States Department of Agriculture (USDA), the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), the COMAV and the Vavilov Institute of Plant Industry (VIR) genebanks (Tables 1 and 2).

### Morphological characterisation

A total of 22 accessions from Angola (three plants per accession) were cultivated in greenhouses at the UPV. According to a first tentative classification based on seed morphology and fruit pictures taken during the collection, all of these accessions except one belonged to the species *Cucurbita maxima* and *C. moschata* (Table 1). The following plant and flower descriptors were recorded: days to male/female flowering (measured as the number of days from sowing to the opening of the first male/female flowers), node in which the first male/female flower appeared, days to fruit setting (number of days from sowing to the first fruit set) and presence of leaf spots (0 absence, 1 presence).

### Isolation of DNA and Simple Sequence Repeat analysis

DNA was extracted from the young leaves of 3 plants per accession using the Cetyl Trimethyl Ammonium Bromide method with minor modifications (Table 3).<sup>5</sup> DNA concentrations were measured with a NanoDrop 1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA) and adjusted to 50 ng/μl. Twenty-six microsatellite *loci* were selected from those previously identified in an *in silico* screen of an Expressed Sequence Tag (EST) collection of *C. pepo*.<sup>6</sup> These Simple Sequence Repeats (SSRs) were previously validated in a *C. pepo* collection,<sup>7</sup> and in the present study were further validated in a wide range of *Cucurbita* species. First, the amplification of the whole set of SSRs was checked in a subset of 5 accessions belonging to various accessions of the main cultivated species (*C. pepo*, *C. moschata* and *C. maxima*), including the parentals of the *C. pepo* genetic map from which these SSRs were derived.<sup>5</sup> All the SSRs that amplified in the three species and which indicated variability were analysed in the full collection. Amplifications were carried out in 15 μl reactions containing 25 ng template DNA, polymerase buffer, 2 mM MgCl<sub>2</sub>, 200 mM dNTPs, 160 nM labelled forward primer, 200 nM unlabelled reverse primer and 0.5 U Taq polymerase. Polymerase chain reaction (PCR) was performed using a cycling profile of one cycle at 94 °C for 3 min, one cycle at 94 °C for 30 s, 65 °C for 30 s and 72 °C for 30 s, plus 9 cycles of amplification with a descending annealing temperature of 1 °C per cycle, followed by 20 cycles at 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s with a final extension at 72 °C for 10 min. The forward primers

were 5' labelled with FAM, PET, HEX and NED to allow for amplicon detection after separation on an ABI 3100 capillary DNA sequencer (Applied Biosystem Inc, Foster City, CA, USA).

#### Data analysis

A Factorial Correspondence Analysis (FCA) was conducted with the SSR data using the Genetix 4.05.2 programme.

## Results

### Accessions collected in the visited areas

A total of 80 accessions were collected: 13 in Benguela, 30 in Huíla, 32 in Kwanza-Sul, and 3 in Namibe (Table 4). It was not possible to explore the Bibala or Camacuio municipalities, the most important agricultural areas of the Namibe province. Even though no collecting activity was planned in Luanda, 2 accessions were collected in this province.

**Table 2:** *Cucurbita* spp accessions from different origins included as controls in the molecular analysis

Species	Accession (code used in Figures 3–6)	Country of origin	Source
<i>C. argyrosperma</i>	PI 438547 (argy47)	Belize	ARS-GRIN
<i>C. argyrosperma</i>	PI 451712 (argy12)	USA	ARS-GRIN
<i>C. cordata</i>	PI 653839 (cord)	Mexico	ARS-GRIN
<i>C. ecuadorensis</i>	PI 432443 (ecua)	Ecuador	ARS-GRIN
<i>C. foetidissima</i>	PI 442191 (foet)	Mexico	ARS-GRIN
<i>C. lundelliana</i>	PI 540898 (lund)	Honduras	ARS-GRIN
<i>C. maxima</i>	AN-CU-59 (maSP)	Spain	COMAV-UPV
<i>C. maxima</i>	CATIE-9824 (maCO)	Colombia	CATIE
<i>C. maxima</i>	SUD-CU-6 (maAR)	Argentina	COMAV-UPV
<i>C. maxima</i>	VAV-1860 (maAU)	Australia	VIR
<i>C. moschata</i>	AFR-CU-1 (moMO)	Morocco	COMAV-UPV
<i>C. moschata</i>	AN-CU-45 (moSP)	Spain	COMAV-UPV
<i>C. moschata</i>	CATIE-7223 (moPA)	Panama	CATIE
<i>C. moschata</i>	KUROKAWA (moJA)	Japan	COMAV-UPV
<i>C. moschata</i>	NIGERIAN LOCAL (moNI)	Nigeria	COMAV-UPV
<i>C. moschata</i>	PI 264551 (moGU)	Guatemala	ARS-GRIN
<i>C. moschata</i>	PI 369346 (moCR)	Costa Rica	ARS-GRIN
<i>C. moschata</i>	PI 381814 (moIN)	India	ARS-GRIN
<i>C. moschata</i>	PI 419083 (moCH)	China	ARS-GRIN
<i>C. moschata</i>	PI 458746 (moGU)	Guatemala	ARS-GRIN
<i>C. moschata</i>	PI 482527 (moZB)	Zimbabwe	ARS-GRIN
<i>C. moschata</i>	PI 498429 (moCO)	Colombia	ARS-GRIN
<i>C. moschata</i>	PI 512150 (moMX)	Mexico	ARS-GRIN
<i>C. moschata</i>	PI 543218 (moBO)	Bolivia	ARS-GRIN
<i>C. okeechobensis ssp. martinezii</i>	PI 512105 (okee)	Mexico	ARS-GRIN
<i>C. pedatifolia</i>	PI 442290 (peda)	Mexico	ARS-GRIN
<i>C. pepo</i>	CATIE-11869 (peGU)	Guatemala	CATIE
<i>C. pepo</i>	UPV-196 <sup>a</sup> (peSPV)	Valencia, Spain	COMAV-UPV
<i>C. pepo</i>	MU-CU-16 <sup>a</sup> (peSPM)	Murcia, Spain	COMAV-UPV
<i>C. pepo</i> var <i>fraterna</i>	PI 532354 (peMX)	Mexico	ARS-GRIN
<i>C. pepo</i> var <i>ozarkana</i>	PI 614701 (peUS)	USA	ARS-GRIN
<i>C. scabridifolia</i>	PI 532391 (scab)	Mexico	ARS-GRIN

<sup>a</sup> Accessions tested with the complete set of microsatellites

**Table 3:** Microsatellites tested in five accessions of *C.pepo*, *C.maxima* and *C.moschata* (marked with the letter 'a' in Table 1 and Table 2).

Unigene in transcriptome version 1 <sup>a</sup>	Unigene/SSR number in transcriptome version 3 <sup>b</sup>	Expected size (bp)	Observed size (bp)	Amplification	Polymorphic
CUTC004307	CUUC107629/ SSR2993	100	120-145	yes	Yes
CUTC001906	CUUC87397/ SSR 1124	198	200-255	only <i>C. pepo</i>	only <i>C. pepo</i>
<sup>†</sup> CUTC004158	CUUC96784/ SSR 1755	188	200-204	yes	yes
CUTC005739	CUUC85492/ SSR 1020	194	200-204	only <i>C. pepo</i>	only <i>C. pepo</i>
CUTC004399	CUUC62112/ SSR 147	165	175-200	yes	no
CUTC004782	–	164	175-200	yes	no
CUTC005800	CUUC64302/ SSR 397	189	200-240	no	unclear
<sup>†</sup> CUTC006703	CUUC61907/ SSR 122	123	145	yes	yes
<sup>†</sup> CUTC008357	CUUC109093/ SSR 3236	136	156	yes	yes
<sup>†</sup> CUTC006209	CUUC119994/ SSR 5054	200	200-300	yes	yes
CUTC018879	CUUC98150/ SSR 1863	164	175-200	yes	no
CUTC012342	CUUC97483/ SSR 1806	182	200-204	yes	?
<sup>†</sup> CUTC009316	CUUC104381/ SSR 2559	300	320	yes	yes
<sup>†</sup> CUTC009760	CUUC62185/ SSR 2870	159	179	yes	yes
CUTC022867	–	140	145-175	yes	unclear
CUTC011336	–	149	145-175	no	no
<sup>†</sup> CUTC017708	–	223	250-300	yes	yes
<sup>†</sup> CUTC002749	CUUC65488/ SSR 603	183	175-200	yes	Yes
<sup>†</sup> CUTC020992	CUUC96401/ SSR 1725	156	175	yes	yes
<sup>†</sup> CUTC023363	–	244	264	yes	yes
<sup>†</sup> CUTC008722	–			yes	yes
<sup>†</sup> CUTC007942	CUUC118813/ SSR 4820	152	175-200	yes	yes
<sup>†</sup> CUTC006891	CUUC96843/ SSR 1759	154	145-175	yes	yes
<sup>†</sup> CUTC004991	CUUC114609/ SSR 4102	173	175-200	yes	yes
CUTC009607	CUUC108020/ SSR 3041	146	145-200	yes	?
<sup>†</sup> CUTC008409	–	107	120-145	yes	yes

<sup>a</sup> These SSR loci were previously validated in a *C.pepo* collection in Formisano et al.<sup>7</sup> This column shows the name of each SSR according to this publication (based on a v1 assembly of the *C.pepo* transcriptome available in the cucurbigene.net database).

<sup>b</sup> This column indicates the unigene name in which each SSR marker is located and the SSR number according to a new assembly of the *C.pepo* transcriptome (v3 available at cucurbigene.net). Details about the gene annotation, repeated motif, etc. are available in the cucurbigene database.

<sup>†</sup> SSR selected for genotyping the whole collection

Some of the accessions were later subdivided as they were composed of a seed mixture of different types, primarily of those belonging to the *Capsicum* genus. Most of the accessions belonged to the Solanaceae (37 accessions) and Cucurbitaceae (36 accessions) families, as they were the focus of the project. The same solanaceous and cucurbit types were found repeatedly in different local markets and in farmers' fields. To avoid too much redundancy, we collected only representative accessions of each type.

During the trip, we gained a good idea of the current status of horticulture in these provinces. After many years of continuous conflict, many of the traditional varieties that were the focus of this project, mainly solanaceous and cucurbitaceous crops, had become extinct. This was especially evident in tomato, melon, watermelon and cucumber

crops. Only landraces of *Cucurbita* species and some types of African eggplants continued to be cultivated extensively. *Cucurbita maxima* and *C. moschata* were the most abundant species, although we cannot disregard the cultivation to a higher extent of certain species of *Lagenaria* genus in other areas that we were unable to visit.

The most predominant types of pepper, locally called 'guindungo',<sup>8</sup> were the small-sized ones, some of which were only 1 cm long. They are sold in local markets as a mixture of different types, including various species, mainly *Capsicum chinense*, *C. baccatum*, *C. frutescens* and *C. pubescens* (Figure 2). This is why almost half of the pepper accessions were subdivided into different types. Most of the 12 eggplant accessions collected belonged to the *Solanum aethiopicum* L. Gilo Group, the so-called 'scarlet eggplant' and 2 accessions belonged to the wild species *S. lichtensteinii*.

**Table 4:** Areas surveyed and number of accessions collected

Province (total number of accessions collected)	Localities	Species (Number of accessions)
Luanda (2)	Luanda	<i>Citrullus lanatus</i> var <i>citroides</i> (2)
Kwanza-Sul (32)	Honga, Zambia, Kondé, Demba, Kibala, Kizenza, Gabela	<i>Arachis hipogea</i> (2), <i>Capsicum</i> spp. (8), <i>Cucurbita</i> spp. (15), <i>Lagenaria siceraria</i> (1), <i>S. aethiopicum</i> (4), <i>S. melongena</i> (1), <i>S. lycopersicum</i> (1)
Benguela (13)	Catumbela, Dombe Grande, Bahía Azul, Bahía Farta, Chongoroi	<i>Capsicum</i> spp. (5), <i>Cucumis sativus</i> (1), <i>Cucurbita</i> spp. (3), <i>Solanum melongena</i> , <i>S. aethiopicum</i> and spp. (3), <i>Solanum lycopersicum</i> var. <i>cerasiforme</i> (1)
Huíla (30)	Sonso, Cacula, Matala, Lubango, Huíla-Humpata, Mulenga, Ontimpe, Chibia, Strada de Leba, Sierra de Leba	<i>Allium cepa</i> (1), <i>Capsicum</i> spp. (5), <i>Citrullus</i> var <i>citroides</i> (3), <i>Cucumis anguria</i> (1), <i>Cucurbita</i> spp. (7), <i>Daucus carota</i> (1), <i>Lactuca sativa</i> (1), <i>Lagenaria siceraria</i> (1), <i>S. aethiopicum</i> (2), <i>S. lycopersicum</i> (1), <i>S. l.</i> var. <i>cerasiforme</i> (3), <i>Solanum lichtensteinii</i> (2), <i>Vigna unguiculata</i> (2)
Namibe (3)	Guirau de Lima, Kanbongue	<i>Capsicum</i> spp. (1), <i>Citrullus lanatus</i> var <i>lanatus</i> (1), <i>Cucumis melo</i> (1)



**Figure 2:** Accessions collected in Angola. From left to right and from to top to bottom: pepper: ANG74, ANG22, ANG33, ANG34, ANG45, ANG75; eggplant: ANG24, ANG31, ANG42; *C. moschata*: ANG04, ANG09, ANG15; *C. maxima*: ANG12, ANG17, ANG73, ANG14, ANG50; *C. pepo*: ANG58.

A few accessions of *S. lycopersicum* var *cerasiforme* were also collected. Some of these, which were round or plum-shaped, were bought in small local markets; others were found growing as weeds close to commercial tomato fields. Five accessions of *Citrullus lanatus* var *citroides* were also collected in Luanda and Huíla Provinces.

### Morphological description of the *Cucurbita* spp. accessions collected

The collected accessions of *Cucurbita* showed high variability in the morphological characteristics of their fruits, such as fruit size, shape (flattened, round, oblong or elongated), rind colour (white, cream, green or orange) and presence/absence of ribs, stripes and spots (Figure 2). Flesh colour was also variable (yellow or from light- to dark-orange).

The cultivation of the plants at the UPV permitted the taxonomic classification of the accessions. Depending on the seed, leaf and flower morphologies, the presence/absence of spots on the leaves and the plant growth habit, it was possible to classify the accessions as *C. maxima*, *C. moschata* or *C. pepo*. The peduncle morphology was also studied in the originally collected fruit to confirm the specific classification of the *Cucurbita* accessions. Accession ANG37 was composed of a mixture of *C. moschata* and *Lagenaria siceraria* seeds and was separated into two accessions. Many of the accessions cultivated in the greenhouses of the UPV did not set fruits, as a result of a very delayed flowering, probably because of not being adapted to Spain's latitude (39°28'11"N). This

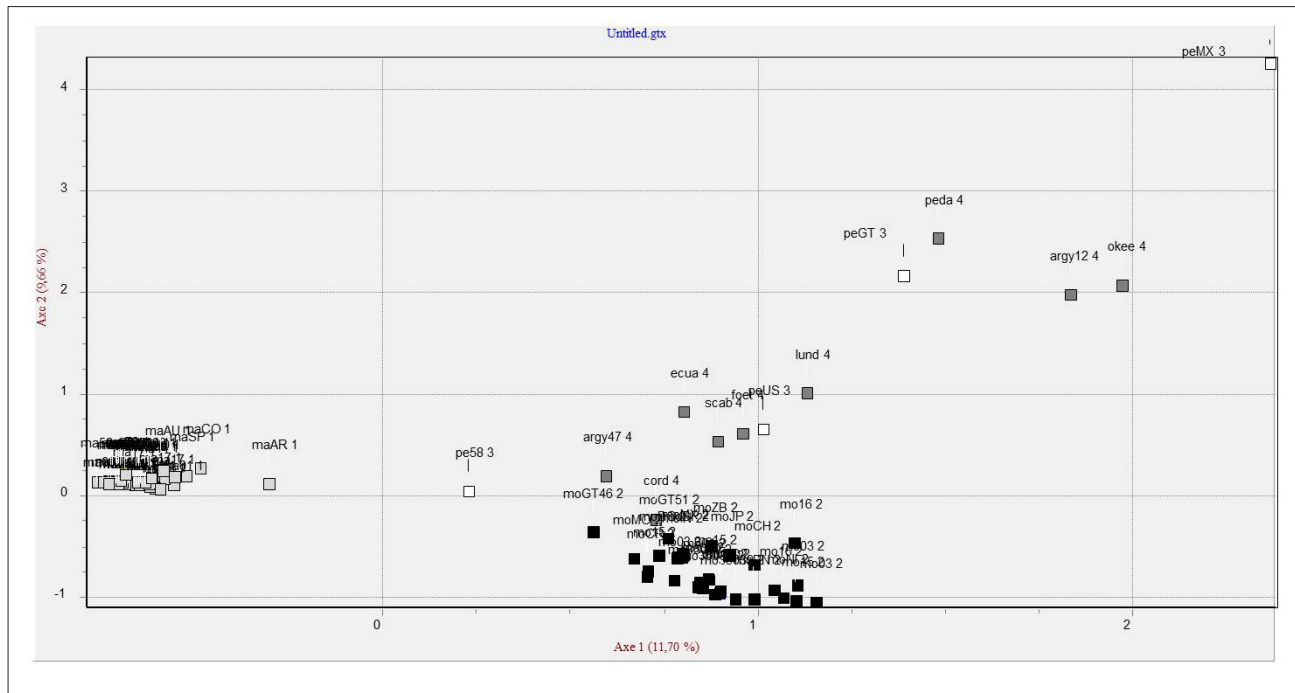
was more noticeable in the *C. moschata* species, in which 35% of the plants did not flower, compared to 0,05% in the case of *C. maxima* (Table 5). A total of 4 accessions of *C. maxima* developed female flowers, which allowed the regeneration of three accessions, whereas only one accession of *C. moschata* developed female flowers. Most of the other accessions of the COMAV, USDA, CATIE and VAVILOV collections, which came from different origins and belonged to these and other species (*C. pepo*, *C. maxima* and *C. moschata*), produced both male and female flowers under the same conditions, showing their complete adaptation after several years of cultivation in Valencia. In general, the fruits obtained from the Angolan accessions did not look like the original fruits collected. Also, the different plants of the same accession that set fruit revealed the existence of some segregation for morphological traits of the fruits. This means that the collected accessions are heterogeneous and they belong to highly heterozygous populations, probably due to cross-pollination between plants cultivated by different growers.

### Genetic diversity of *Cucurbita* species

In the FCA performed with the SSR results, the *Cucurbita* accessions from Angola were mostly clustered into two groups with the reference accessions of *C. maxima* and *C. moschata*, thus confirming the morphological results that assigned most of the accessions to these two species (Figure 3). The accession ANG58, belonging to the species *C. pepo*, is located between the *maxima* and *moschata* groups. The rest of the accessions appeared disperse and more distant.

**Table 5:** Number of days from transplant to male and female flowering in Angolan accessions (mean of three plants)

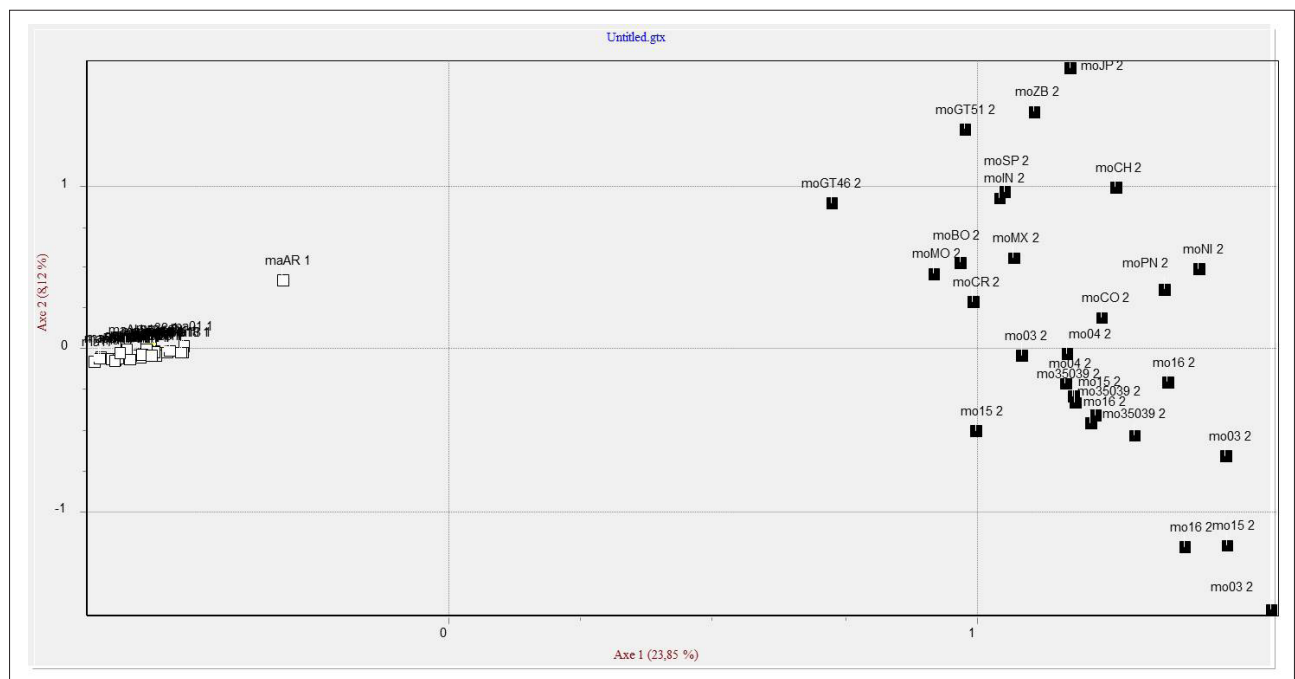
Species	Accession	Number of days to male flowering	Node first male flower	Number of days to female flowering	Node first female flower
<i>C. maxima</i>	ANG01	45.33	10.33	47	23.00
<i>C. maxima</i>	ANG08	59.50	21.00	91	22.00
<i>C. maxima</i>	ANG11	61.67	19.70	No flowering	–
<i>C. maxima</i>	ANG12	58.67	19.70	No flowering	–
<i>C. maxima</i>	ANG13	76.00	20.00	No flowering	–
<i>C. maxima</i>	ANG14	67.67	22.00	No flowering	–
<i>C. maxima</i>	ANG17	155.00	23.50	No flowering	–
<i>C. maxima</i>	ANG18	61.33	16.30	No flowering	–
<i>C. maxima</i>	ANG38	50.67	15.00	59	26.70
<i>C. maxima</i>	ANG44	57.67	23.70	No flowering	–
<i>C. maxima</i>	ANG50	55.50	23.50	No flowering	–
<i>C. maxima</i>	ANG52	54.33	20.00	No flowering	–
<i>C. maxima</i>	ANG53	74.67	19.00	No flowering	–
<i>C. maxima</i>	ANG54	72.50	20.50	No flowering	–
<i>C. maxima</i>	ANG73	55.67	18.70	77	24.00
<i>C. maxima</i>	35040	56.50	23.50	No flowering	–
<i>C. moschata</i>	ANG03	58.50	21.50	No flowering	–
<i>C. moschata</i>	ANG04	115.50	23.00	No flowering	–
<i>C. moschata</i>	ANG15	76.50	23.00	No flowering	–
<i>C. moschata</i>	ANG16	No flowering	–	No flowering	–
<i>C. moschata</i>	35039	87.67	22.00	107	31.00
<i>C. pepo</i>	ANG58	59 <sup>2</sup>	20.00	64	38.00



**Figure 3:** FCA of the Angolan and reference Cucurbita accessions representing the entire Cucurbita genus. Black squares: *C. moschata*, light grey: *C. maxima*, white squares: *C. pepo* and dark grey squares: other species. See tables 1 and 2 for accession codes.

Performing the analysis with *C. maxima* and *C. moschata* separately highlighted the segregation between the two as well as the greater variability of *C. moschata* compared to *C. maxima*, as detected with this set of SSRs (Figure 4). When analysed separately, the accessions of *C. moschata* were highly diverse, to the point of appearing scattered (Figure 5). Both the intra- and inter-accession variabilities were remarkably high in the Angolan germplasm, as can be seen on the right half of the figure. It is separate from the other accessions used as controls, although it does show continuous variation. The most distant accessions from the Angolan ones are

those of eastern origin (China, India and Japan) as well as some from Central America (Mexico and Guatemala). In contrast, the closest accessions are from Africa, mainly Nigeria and Morocco, along with others from South America (Colombia and Bolivia) and the southern part of Central America (Costa Rica and Panama), which may suggest a Central to South American origin of these African accessions. It is interesting to note that the *C. moschata* accessions from Angola were just as diverse as were the reference accessions from many different regions of the world.



**Figure 4:** FCA of the *C. maxima* and *C. moschata* accessions. Black squares: *C. moschata*, white squares: *C. maxima*. See tables 1 and 2 for accession codes.



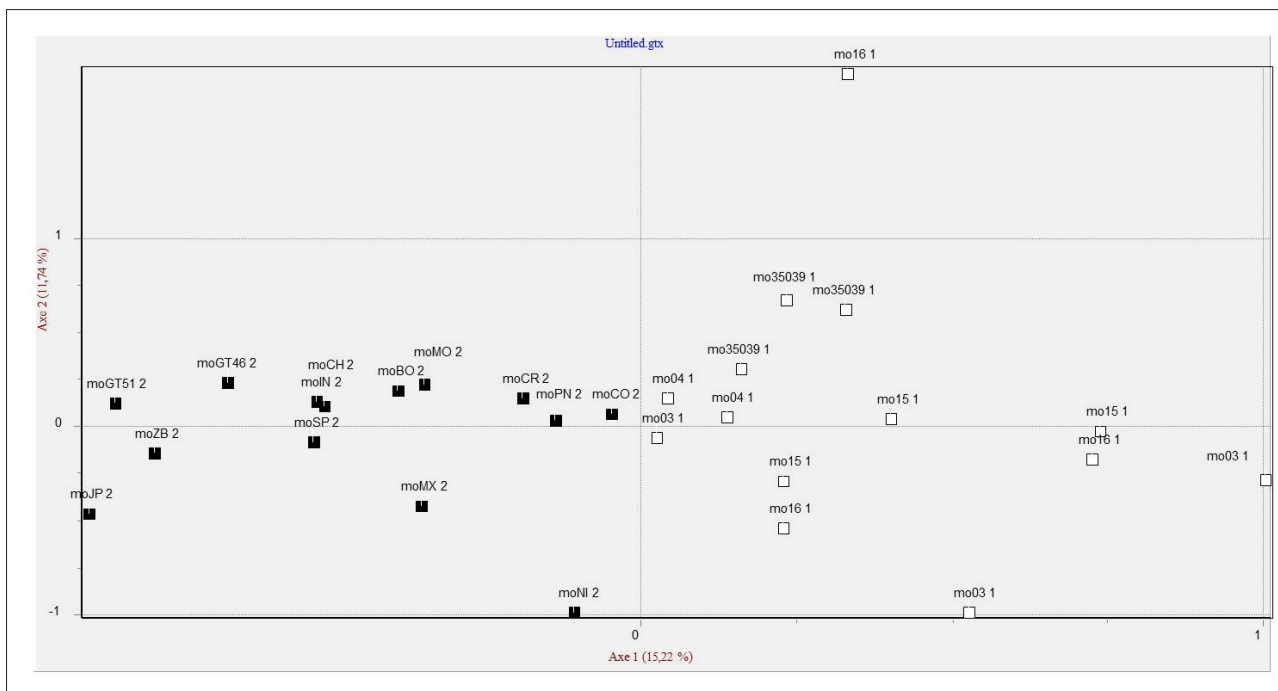


Figure 5: FCA performed with the Angolan and control accessions of *C. moschata*. Black squares: controls, white squares: Angolan accessions. See tables 1 and 2 for accession codes.

The accessions of *C. maxima* seem to be much less diverse, as most of them grouped together except for ANG17, ANG12 and ANG13 (Figure 6). On the other hand, the Angolan accessions were further away from those used as controls (VAV1860 and CATIE9824). The controls SUD-CU-6 and AN-CU-59 were excluded from the analysis because they were located quite distant from the others, which made the complete set of accessions studied too closely grouped. FCA seems to separate the accessions from coastal and northern Kwanza-Sul from those from interior and southern Huila.

The described results of the FCA agree with the polymorphism analysis (Table 6). Therefore, when considering only *C. maxima* and *C. moschata* accessions, the highest polymorphism values, largest number of alleles per locus and highest Polymorphism Information Content (PIC) values of the microsatellites correspond to the Angolan accessions of *C. moschata*, followed by the Angolan *C. maxima*, whereas the *C. moschata* controls from other origins were less variable.

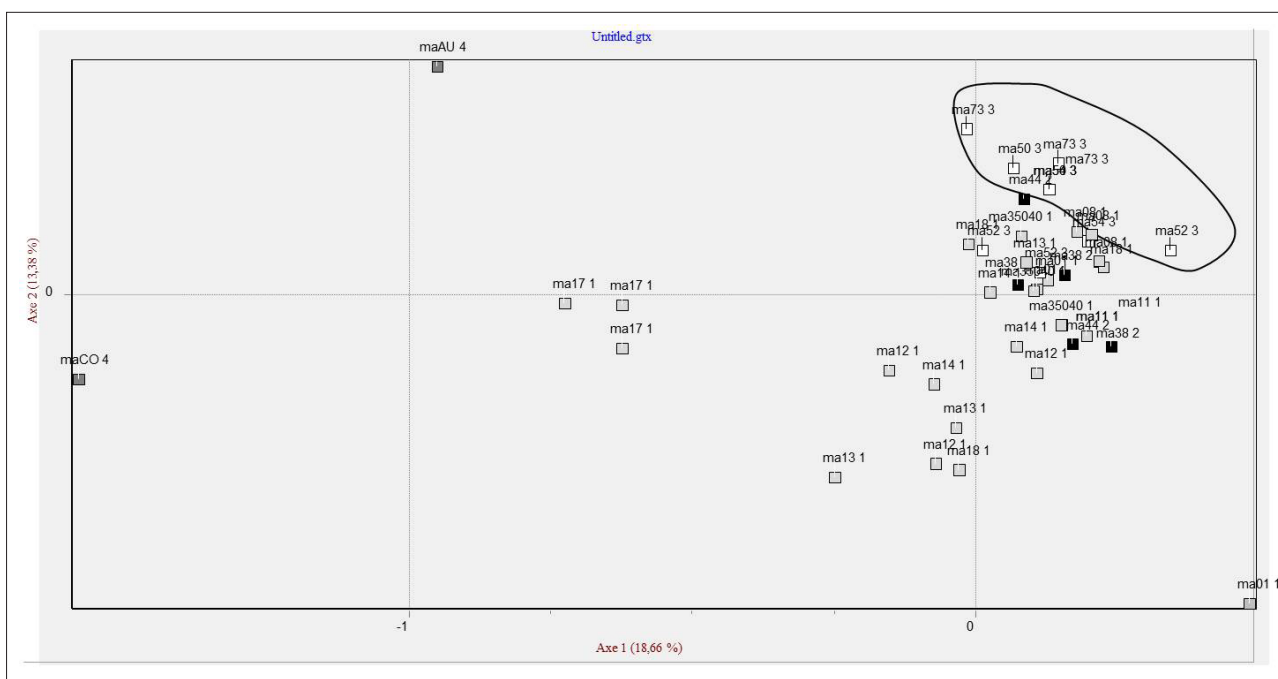


Figure 6: FCA performed with the Angolan and controls accessions of *C. maxima*. Black squares: accessions from Benguela, light grey squares: accessions from Kwanza Sul, white squares: accessions from Huila. Accession from Huila included in the circle. See tables 1 and 2 for accession codes.

**Table 6:** Estimates of molecular polymorphism of the accessions studied by species and origin

Species	Origin (number of plants)	Observed heterozygosity	Polymorphism at 0.95 and 0.99 levels	Number of alleles per locus	Polymorphic Information Content
<i>C. maxima</i>	Angolan (n=43)	0.0578	0.40/0.47	1.47	0.12
<i>C. moschata</i>	Angolan (n=14)	0.0758	0.47/0.60	1.60	0.17
	Controls (n=14)	0.0324	0.29/0.36	1.27	0.15
<i>C. maxima</i> and <i>C. moschata</i>	All accessions including controls of <i>C. maxima</i> (n=75)	0.0578	0.87/0.93	3.00	0.33
All accessions studied	Angolan and controls (n=88)	0.0634	0.93/1.00	4.8	0.43

## Discussion

A collecting expedition was carried out in Angola in 2012 by the Centre for Plant Genetic Resources of Luanda and the Univesitat de València, Spain, with the goal of improving the collection of vegetable crops at the Centre for Plant Genetic Resources of Luanda. Morphological and molecular characterisation of part of the collected accessions was also conducted jointly by the Spanish and Angolan researchers. This expedition was part of a wider project aimed at developing new varieties of vegetable crops that are more productive and better adapted to the agro-climatic conditions of the visited Angolan provinces. The final, long-term goal was to promote the cultivation of the improved varieties of vegetables and introduce them into the Angolan diet, which will ultimately contribute to their diversification and increase their nutritional value.

A total of 80 accessions of vegetable crops were collected, most of which belonged to the *Cucurbita*, *Solanum* and *Capsicum* genera. We were able to confirm the existence of subsistence agriculture and a slow emergence of commercial agricultural production in the visited areas – to a higher extent in the northern provinces and to a lesser degree in Namibe, a more barren province which relies on fishing.

Squashes, pumpkins, peppers and eggplants were found to be the most commonly cultivated crops. Interestingly, pumpkins and eggplants are crops with low farm work needs. Likewise, small-sized peppers need fewer inputs and less care from growers, and set a higher number of fruits much more easily than plants with larger fruits. These types of crops seem to be the ones that have a higher probability of success in unsophisticated agricultural systems like the ones used in the visited areas. Other crops of the same families, such as tomato, melon or cucumber, with more specific cultivation and climatic needs, are much less likely to grow appropriately and give cost-efficient yields to farmers.

Most of the pepper accessions collected belonged to the *C. chinense*, *C. frutescens*, *C. baccatum* and *C. pubescens* species. They were small-sized and sold in local markets as a mix of different types. Given the elevated amount of trade between Angola and Brazil, we speculate that these pepper types may have come from Brazil. A molecular analysis of the collected accessions, including some from South America for reference, could help to elucidate the origin of these materials. In any case, these accessions are of great interest for two reasons: their potential to provide genes resistant to diseases and their adaptation to the specific agricultural conditions of these provinces. In fact, many genes of resistance introgressed in commercial cultivars come from this species.<sup>9</sup>

African eggplants are common in local markets (Figure 2). Most of the twelve eggplant accessions collected belonged to the *Solanum aethiopicum* L. Gilo Group, the so-called ‘scarlet eggplant’. This species, together with *S. macrocarpon* (called gboma), are cultivated African eggplants that are locally important in their region of origin in tropical sub-Saharan Africa.<sup>10-13</sup> *S. aethiopicum* is a hypervariable species characterised by many morphologically different types and

forms, with hundreds of local varieties.<sup>14,15</sup> Both scarlet and gboma eggplants are also important genetic resources for common eggplant (*S. melongena* L.) breeding, as the three species can be intercrossed, thus producing hybrids with intermediate fertility.<sup>16</sup> Two accessions of the very variable *Solanum lichtensteinii* were also collected in Huíla province. This species grows as a weed in disturbed and overgrazed areas, along roadsides and is used as a traditional medicine. The most outstanding value of this eggplant collection is its uniqueness and specific adaptation to the climatic conditions of Angola.

A total of 5 accessions of the wild form of watermelon *Citrullus lanatus* var. *citroides* were collected. This old variety is perfectly adapted to arid and desert conditions and plays a major role in the economy of the San of the Kalahari Desert. It accumulates a large amount of water in its fruits and is commonly used both to feed livestock as well as for human consumption during the dry season, especially the non-bitter form. In fact, it has been identified as a species with potential for commercial exploitation in arid areas of Botswana.<sup>17</sup> Additionally, *C. lanatus* var. *citroides* is readily crossable with the cultivated watermelon, which makes it of great interest for breeding, as it has been found to be resistant to different species of nematodes.<sup>18,19</sup>

The morphological characterisation conducted with the *Cucurbita* accessions was our first contact with the Angolan materials and allowed us to classify most of them as *C. maxima* and *C. moschata* species. Although the genus is native to the northern parts of South America,<sup>20</sup> after their domestication, most species spread to the rest of the world, and secondary centres of diversification exist with landraces adapted to different conditions.<sup>2</sup> The centre of diversity of *C. maxima* lies in the South American temperate zones, but many landraces of this species from other regions, including many countries in tropical Africa (the countries neighbouring Angola, such as the Democratic Republic of Congo, Zambia, Zimbabwe, Nigeria and Cameroon), are also maintained in international germplasm collections. In addition, the greatest diversity occurs among the Central and South America landraces of *C. moschata*,<sup>21</sup> but there also exists a significant diversity in landraces from the warm regions of other continents, including Africa and Asia.<sup>22,23</sup> In fact, tropical pumpkin (*C. moschata*) is one of the most important vegetable crops in some African countries.<sup>24</sup> In general, *C. maxima* is more important in the cooler parts of southern Africa and the Sahel region, and less important in west and east Africa, where it is more humid, and where *C. moschata* is more common. Our results fit with this information. The explored area of Angola is located between the latitudes 10° and 15° south, where the humid climate and tropical forest give way to different types of savannah, the predominant ecosystem of Angola, which is more welcoming to *C. maxima* species. The fruits, leaves and seeds of the *Cucurbita* species are consumed extensively in Angola. They are locally called ‘abóbora’, ‘cabaça’, ‘diangua’, ‘dinhungo’, ‘matira’ and ‘malengue’. Croquettes of crushed seeds are used to cook a meat or fish stew called ‘muteta’. Leaves (locally called miengueleka) are also stewed together with the fruit of the palm (*Elaeis guineense*) or groundnut (*Arachis hypogaea*) and salt.

The fruits are used to cook soup, puree and cakes. Other *Cucurbita* species also exist as landraces for farmers' own consumption and local markets. These crops contribute to nutrition through their abundant supply of minerals.

The agronomic characterisation and molecular analysis conducted have unveiled certain aspects of the cultivation requirements of these plants and also the genetic variability of the Angolan accessions. The collected materials are strongly adapted to the specific agro-climatic conditions of the collection areas. This has been revealed by the lack of flowering when cultivated over a long period (from February to December) at the latitude of Valencia, Spain. Many *Cucurbita* landraces from tropical areas are not adapted to temperate regions, especially the many cultigens of *C. moschata* that are short-day plants which require long growing seasons.<sup>25,26</sup> Fewer adaptation problems arose with *C. maxima*.<sup>27,28</sup> The behaviour observed in our assay agrees with this differential of specific requirements.

The molecular analysis conducted demonstrated high molecular variability and uniqueness in the collected accessions, as shown by the segregation of the collection from the analysed set of controls from all over the world. *C. moschata* is the most diverse species, even more so, in terms of number of alleles per locus and PIC value, than the set of controls included in the assay from a wide range of origins. Also, the collected accessions of *C. maxima* show high levels of variability, and seem to group according to their origin, suggesting an adaptation of the different types to different agro-climatic areas. There are only a few papers prior to this one that describe the molecular variation in the African germplasm of these species, but none describes the diversity in Angola. For example, Gwanama et al.<sup>29</sup> analysed a collection of *C. moschata* accessions from Zambia and Malawi using RAPDs, and also found differences related to the origin of the accessions.

Our molecular results showed a high intra-accession variability, suggesting that cross-pollination probably occurs in their natural habitat. Also, the proximity of different fields in their traditional mode of cultivation most likely favours the existence of cross-pollination between different types.

The variability and singularity of the accessions makes this material highly valuable for use in breeding and even more so seeing as previous analyses of African accessions of *C. moschata* indicate resistance to viruses and fungi.<sup>30</sup> For example, Nigerian Local, an accession from Nigeria, has been reported as resistant to potyvirus and powdery mildew and has already been used for *Cucurbita* breeding. The accessions from Angola seem to be genetically close to this material, so they must be further analysed to determine their potential for pest and disease resistance. Also, some of the collected accessions have yellow-to-dark-orange flesh. Intensity of flesh colour is known to be related to the carotenoid composition of the fruit, mainly  $\alpha$  and  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lutein and zeaxanthin,<sup>31</sup> so this material could be a promising source of vitamins in this country. Screening of accessions has been conducted in order to identify accessions with high carotenoid and ascorbic acid contents.<sup>32</sup> Some screenings have used landraces from secondary centres of diversity, such as Brazil and Malaysia, and have found high levels of variation for these traits in landraces from different areas.<sup>33-35</sup> Further studies will be conducted with the set of collected accessions to evaluate their carotenoid content. Accessions with high carotenoid contents may be utilised for further quality improvement of pumpkin during the development of new varieties, which will contribute to minimising nutritional problems in the population, especially those arising from vitamin A deficiency in children and women.

In summary, the material collected in this expedition is quite valuable given its uniqueness and its desirable breeding characteristics. Accessions from Angola are scarce in germplasm collections all over the world. As an example, none of the 72 accessions of *Solanum aethiopicum* included in the European Cooperative Programme's eggplant database come from this country. The same is true for the 67 accession of this species included in the USDA germplasm databases. In fact, Angola has been avoided by extensive collecting expeditions for many years, probably because of security reasons. These collected

accessions can be used in breeding for many purposes, including resistance to diseases, adaptation to abiotic stresses, such as arid conditions, and to improve fruit quality. In the case of the *Cucurbita* accessions, the high molecular variability found makes the use of this adapted material in breeding quite promising. Breeding programmes aimed at increasing yield, resistance to diseases and quality may be successful using this starting material. However, complementary to breeding programmes, other initiatives focused on improving cultivation techniques should be undertaken to take advantage of all the genetic potential of the improved varieties.

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## Authors' contributions

MJD, JPD, PM and MBP conceived and designed the study; AMF, JP, FS and MJD participated in the collecting expedition; AMF, AS, IHD, JS and NBP performed the molecular analysis, MBP designed and performed the phenotyping trial; and MJD and MBP wrote the manuscript. All authors critically reviewed and approved the final manuscript.

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