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Evolution and health status of Cassava (*Manihot esculenta* Crantz) genetic resources in *ex-situ* conservation in Côte d'Ivoire

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Abstract

Cassava (*Manihot esculenta* Crantz) is a foodstuff that plays a very important role for the world population. In Côte d'Ivoire, its production is estimated at 6.5 million tons after yam. With a view to preserving the genetic diversity of the cassava collection of the National Centre for Agronomic Research, several research projects have been carried out on the characterization (morphological, agronomic) and health status (diseases and pests) of the cassava genetic resources conserved in the station. The present study consisted in analysing the composition and evolution of cassava genetic resources and assessing the incidence of diseases and pests in 727 cassava accessions in the collection of the National Centre for Agronomic Research. After analysis, the collection contained a total of 759 accessions of which 32 had disappeared. Of this total, 603 accessions or 83% of the total were from Côte d'Ivoire, 104 or 14% from the International Institute of Tropical Agriculture and 20 accessions or 3% from various origins. Referring to time and different agronomic research structures, the cassava collection had 106 accessions from 1953 to 1981 for the Office for Scientific and Technical Research Overseas, 101 accessions from 1982 to 1998 for the Savannah Institute and 520 accessions from 1998 to 2019 for the National Centre for Agronomic Research. It was found that the accessions from the International Institute of Tropical Agriculture were more resistant to virus than the accessions from the Côte d'Ivoire farmers' environment. For mites, the attack was strong with 60% of the accessions.

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Introduction

Cassava (*Manihot esculenta* Crantz) is an important root crop cultivated in many countries around the world with 3.03 million tons. Its production is estimated at over 193 million tons in Africa (Faostat, 2020). Africa is the world's largest producer of cassava with 79.5% of the global area and 61.8% of the global production. The main producing countries are Nigeria (with about 33% of the sown area and 25% of African production), the Democratic Republic of Congo, Ghana, Angola and Tanzania (Faostat, 2020). Cassava derives its importance from its tuberous root's rich in starch, its leaves rich in protein and its various energy products (N'Zué *et al.*, 2014; Adjebeng-Danquah *et al.,* 2016). It therefore remains one of the most important food crops in tropical Africa and has an undeniable asset for regions that are increasingly less self-sufficient in food (Gnonloufin *et al.*, 2011).

In Côte d'Ivoire, cassava contains more than 200 cultivars and is the second most important food crop after yam, with an estimated production of 6.5 million tons (Faostat, 2021). It is both a subsistence and cash crop for producers. There are many cassavas by-products (attiéké, foutou, toh, flour, starch, gari, etc.) and they are traded nationally and sub-regionally.

However, in Côte d'Ivoire, due to the action of producers who often abandon accessions that do not meet their requirements, bush fires, diseases and pests, cassava and related wild species are gradually undergoing a degeneration of their genetic heritage (N'Zué, 2007). To limit the extent of the harmful effects, the conservation of genetic resources from collections, introductions and creations is essential, as the conserved genetic heritage forms the basis for all genetic improvement, agronomy and crop protection work. In this context, the National Center for Agronomic Research (CNRA) has enriched its living collection with more than 190 new traditional collections since 2008 thanks to Ivorian-Swiss Fund for Economic and Social Development (FISDES) and West Africa Agriculture Productivity Program (WAAPP) funding (N'Zué, 2007).

Currently, this collection contains several accessions of diverse origins (local and foreign). Nevertheless, there is a need to continue collecting cassava genetic resources in order to further increase the genetic variability in the collection. With a view to preserving the genetic diversity of the cassava collection of the National Center for Agronomic Research (CNRA), several research studies were carried out on the characterization (morphological, agronomic) and health status (diseases and pests) of the cassava genetic resources conserved in the station. This study aims to analyze the composition and evolution of cassava genetic resources and to evaluate the incidence of diseases (virus, anthracnose) and pest attacks (mites, mealybugs) in the collection of the National Center for Agronomic Research (CNRA).

Materials and methods

Study site description

The work of this study was carried out in Bouaké, in central Côte d'Ivoire at Food Crops Research Stations located at 7° 4'N and 5° 2 W. This region is located in the Baoulean climate. This region has a Baoulean climate. The rainfall regime is composed of two rainy seasons from April to July and September to October, and two dry seasons from August and November to March. The average annual rainfall is between 724 and 1423mm (Kouamé, 1992). The soils of Food Crops Research Stations are ferralitic, gravelly, branched and shallow derived from granitic material (N'Cho, 1991).

Plant material

The germplasm contains several accessions all belonging to the cultivated species *M. esculenta*. It consists of local cultivars; local improved varieties and introduced varieties. The collection was renewed in May 2019 in Bouaké.

Technical equipment

The technical materials used for the establishment of the cassava collection are: the tractor, hoes stakes, a notebook, a metric tape, chalk lines and a pencil.

Experimental design

The accessions were arranged in 12 strips of 60 accessions each. The detailed dimensions of the trial are 150 m for the overall length and 50 m for the overall width. The specific spacing is as follows:

- 2 rows / accession with 5 plants /row;
- 0.80 m between lines and 0.80 m between plants per accession;
- 1.2 m between accessions (clones);
- 2 m between strips.

Methods

Observations and measurements

The main observations were made on the following parameters

- Accession counts
- Distribution of accessions according to origin and institution
- Disease incidence (mosaic and anthracnose) at 7 months after planting
- Incidence of pests (mites and mealy bugs) in the dry season
- The incidence (I) is calculated according to the following formula (Hahn *et al.*, 1980):

$$I = \frac{\text{Number of plants affected}}{\text{Total number of plants}} \times 100$$

The level of incidence is based on the rating scale defined by intervals as follows

0% (very resistant)

1-25% (resistant)

26-50% (moderately resistant)

51-75% (susceptible)

76-100% (highly susceptible).

Graphical representations of the data

The graphs used are pie charts and histograms using Excel (2016).

Results

Evolution of cassava genetic resources

Variation in the number of cassava accessions in the collection

The first research institute was ex-ORSTOM from 1953 to 1981 with a collection of 106 cassava accessions. Then, ex-IDESSA which existed from 1982 to 1998 with a collection that had 101 cassava accessions. Since 1998, the cassava collection has been enriched by 658 accessions (Fig. 1), i.e. a total of 759 accessions managed by CNRA. Out of a total of 759 accessions in the CNRA cassava collection, 32 accessions were lost, including 16 accessions that disappeared entirely and 16 very weak accessions, on which agronomic and sanitary observations and measurements could not be made in 2019 (Tab. I). To date, the CNRA's cassava collection includes 727 accessions.

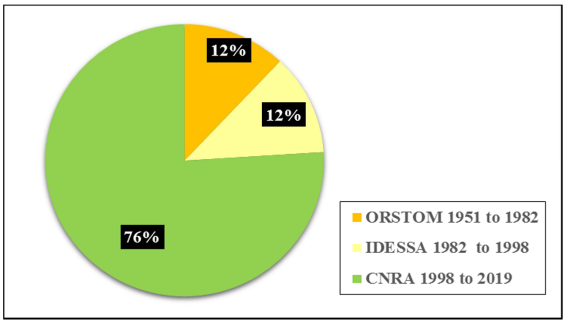

Fig. 1. Distribution of the number of cassava accessions by research institute from 1951 to 2019.

Table 1. Characteristics of lost or stunted cassava accessions in the CNRA collection

Name of accessions	Origin	Date or year of collecte	Observations
Bahiri	Djatokro (CI)	June 30, 2018	Very puny
Dinannin	Ghana	June 29, 2018	Disappeared
Gassia gamé	Togo	1981	Very puny
Attitogon	Togo	1981	Very puny
Kalba akpagin	Togo	1981	Disappeared
Para gbaho	Djidoubaye	August 1, 2009	Disappeared
Abli baha	Djidoubaye	August 1, 2009	Very puny
Baha pli1	Djidoubaye	August 1, 2009	Very puny
Bou fouh4	Djidoubaye	August 1, 2009	Very puny
Bouangaya popo	Inconnu	October 8, 2009	Disappeared
Bouangaya houan	Inconnu	October 8, 2009	Disappeared
Bouangaya nanan	Inconnu	October 8, 2009	Disappeared
I090091	IITA (2014)	November 17, 2016	Disappeared
I090151	IITA (2014)	November 17, 2016	Disappeared
I102429	IITA (2014)	November 17, 2016	Disappeared
Salah n'sianh	Ayamé	June 27, 2018	Disappeared
15(94)5	CNRA	November, 2018	Very puny

Name of accessions	Origin	Date or year of collecte	Observations
15(426)3	CNRA	November, 2018	Very puny
15(436)24	CNRA	November, 2018	Very puny
15(302)1	CNRA	November, 2018	Very puny
15(31)12	CNRA	November, 2018	Very puny
15 (456) 5	CNRA	November, 2018	Very puny
15 (295) 6	CNRA	November, 2018	Very puny
15 (409) 24	CNRA	November, 2018	Very puny
99(149)8	CNRA	2002	Very puny
99(22)1	CNRA	2002	Very puny
Agba wakaoclè1	Sakassou (CI)	2002	Disappeared
Andobo1	CI	Unknown	Disappeared
Ahoulé	Aboisso (CI)	September 24, 2011	Disappeared
Yabroya	Unknown	October 08, 2009	Disappeared
Bassié djélé1	Dougroupalégnoa (CI)	May 28, 2008	Disappeared
Dény cassia	Gouléako 1	August 1,2009	Disappeared

CI : Côte d'Ivoire

Number of accessions in the cassava collection according to their origin

Of the 727 accessions, 104 are from IITA, representing 14% of the total number. Accessions from Côte d'Ivoire numbered 603, representing 83% of the total. A total of 20 accessions, i.e., 3% of the total, were from other origins (Fig. 2).

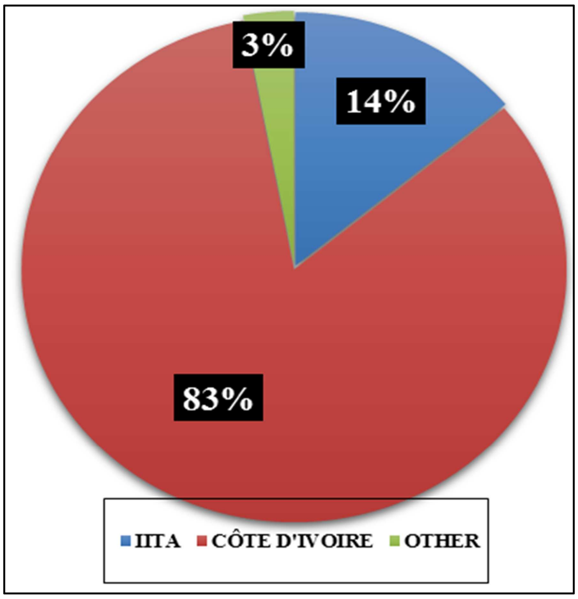

Fig. 2. Distribution of the number of CNRA cassava accessions according to their origins.

Health status of cassava genetic resources with respect to diseases and pests

Cassava mosaic virus disease (CMV)

Out of 727 accessions, 226 accessions showed no visible symptoms of mosaic, i.e. incidences of 0% (Fig. 3). The majority of these 226 unaffected accessions come from IITA and show very good resistance to mosaic. 93 accessions affected by mosaic have incidences between 1 and 25% and 113 accessions with incidences between 26 and 50% show tolerance or even moderate resistance to mosaic, i.e. 28.3% of the accessions in the cassava collection (Fig. 4). On the other hand, 71 accessions show a high susceptibility to mosaic with incidences ranging from 51 to 75%, i.e. 12.8% of the collection. The very high susceptibility of plants to mosaic is observed on the 76 accessions with incidences up to 100%, i.e. 15.5% (Fig. 3 and 4).

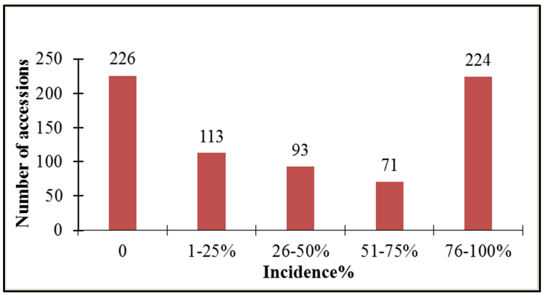

Fig. 3. Number of cassava accessions according to the incidence African mosaic disease.

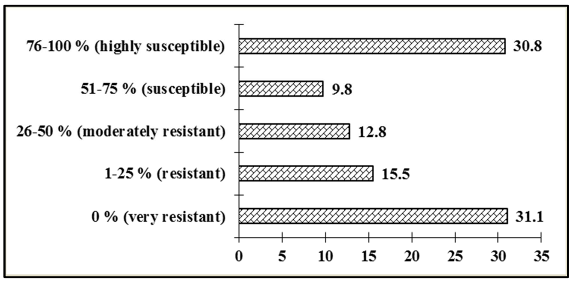

Fig. 4. Proportion of accessions resistant and of susceptible to African mosaic disease in the cassava collection.

Anthracnose caused by Colletotrichum gloeosporioides f. sp. *Manihotis*

With regard to anthracnose, out of a total of 727 accessions in the cassava collection, 129 accessions showed high susceptibility to anthracnose, i.e. 17.7% of the collection, and 598 accessions showed tolerance/resistance, i.e. 82.3% of the collection (Fig. 5 and 6). Of the 598 tolerant/resistant accessions, 157 accessions show incidences between 1 and 25% and 149 accessions with incidences between 26 and 50%. On the contrary, 62 accessions with incidences ranging from 51 to 75% and 67 accessions with incidences ranging from 76 to 100% are heavily affected by anthracnose (Fig. 5 and 6).

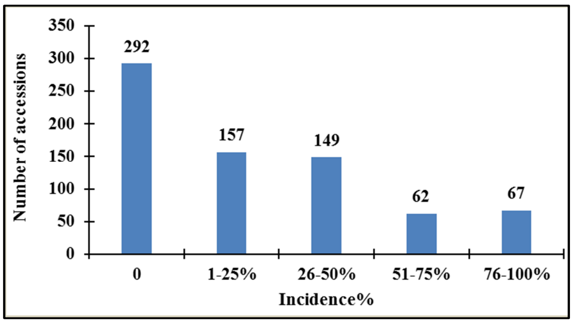

Fig. 5. Number of cassava accessions according to anthracnose incidence.

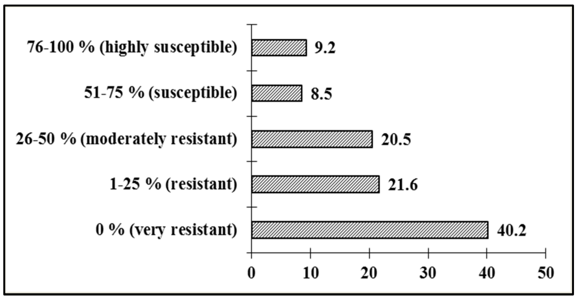

Fig. 6. Proportion of anthracnose resistant and susceptible accessions in the cassava collection.

Green mites (Mononychellus tanajoa)

Out of a total of 727 accessions, 118 accessions were observed to be free of mite attack (Fig. 7). On the other hand, the other accessions suffered from mite attacks in different proportions. Thus, 62 accessions show mite symptoms with incidences ranging from 1 to 25% and 55 accessions with incidences ranging from 26 to 50%. The highest number of mite attacks was observed on 446 accessions with very high incidences ranging from 76 to 100%, i.e., 61.3% of the accessions in the cassava collection were attacked by mites (Fig. 8).

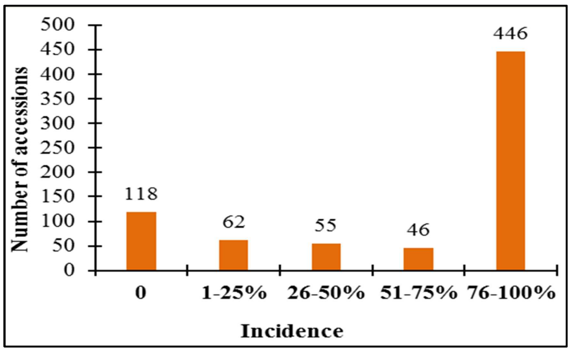

Fig. 7. Number of cassava accessions according to mite incidence.

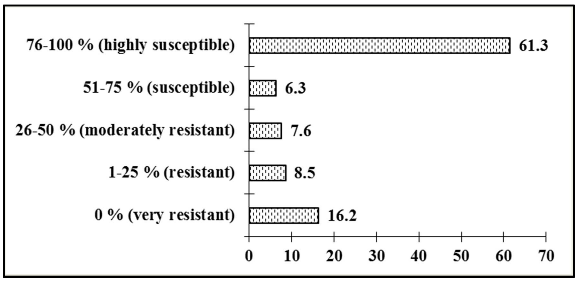

Fig. 8. Proportion of accessions resistant and susceptible to mite attacks.

Mealybugs (Phenacoccus manihoti)

A number of 518 accessions out of 727 are apparently healthy as they did not show any symptoms of the pest (Fig. 9). Of these apparently healthy accessions more than half came from the IITA collection. Of these 209 affected accessions, 96 are in the 1-25% range, 48 are in the 26-50% range and 28 are in the 51-75% range. Finally, those in the 76-100% range numbered 37 accessions (Fig. 9).

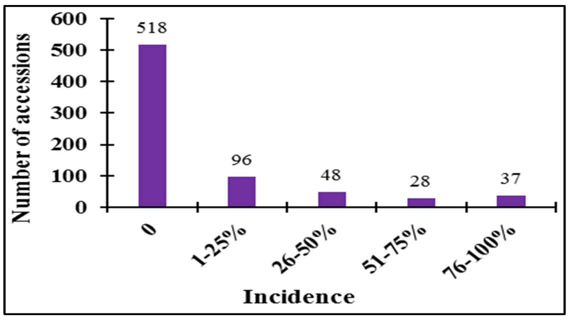

Fig. 9. Number of cassava accessions according to mealy bug incidence.

Fig. 10. Proportion of accessions resistant and susceptible to mealybug attacks.

Discussion

In the framework of the conservation of cassava genetic resources, enrichments or changes in the number of accessions and losses of accessions are observed. For example, the basic cassava collection ex-ORSTOM (Office of Scientific and Technical Research Overseas), consisting of 106 accessions, has been conserved in vivo for 28 years without being enriched. With the arrival of the ex-IDESSA (Institute of Savannahs), this collection went from 106 to 101 accessions, a loss of 6 accessions between 1982 and 1998. This loss would be due to climatic and edaphic changes and to diseases and pests. As indicated by Mabanza *et al.*, in 2001, the method of ex-situ collection of material is penalized by its high maintenance cost and the risks of genetic erosion due to climatic or pathological factors (diseases and pests). Despite these constraints, the CNRA has been able to manage the cassava collection, which has grown by 520 accessions. The evolution of the number of accessions in the collections is essentially due to the various surveys and collections of local cultivars in the western, south-western, central-western, north-western and south-eastern zones of Côte d'Ivoire and to the introduction of clones from the IITA (International Institute of Tropical Agriculture). This is in line with the classic model of collection enrichment, according to Mabanza and Mingui 1998a and b; Mingui *et al.,* 1992, the numerous research programs that have enabled surveys and collections of local cassava cultivars and introductions of varieties from research institutes.

This CNRA collection remains one of the most enriched collections with 727 cassava accessions, compared to those of some institutes such as ITRA (Togolese Institute of Agronomic Research) of Togo with 435 accessions, FAST (Faculty of Sciences and Techniques) of Benin with 600 accessions and FCRI-DA (Field Crops Research Institute Department of Agriculture) of Thailand with 609 accessions (FAO, 2010). A strong presence of African mosaic symptoms was found on a good number of accessions, especially those from the farming environment (traditional varieties). These results are in agreement with those of N'Zué *et al.,* 2004**.** These authors showed that the high susceptibility could be explained by the fact that the resistance of varieties to mosaic is often limited in time, despite the fact that they have benefited from a selection effort against mosaic during varietal creation.

It is therefore necessary to remove cassava plants affected by the disease in the field or to renew planting material from research institutes. The analysis of anthracnose attacks caused by the fungus *Colletrotrichum gloeosporioïdes* f. sp *manihotis* shows that 598 cassava accessions show good tolerance and/or resistance. This can be explained by the fact that the fungus *C. gloeosporioides* f. sp *manihotis*, which is a weak parasite, needs in its parasitic phase to find an easy way of penetration (wound, modification of the epidermis) and tissues weakened by the action of another parasite, an insect or aging. Therefore, if there is no pathway, the symptoms will be relatively on the plants. Some authors have also shown that infection and development of anthracnose require a relative humidity of over 80% (Makambila, 1994).

The CNRA cassava collection is located in the central zone of Côte d'Ivoire, which has an average humidity of 70.9%, an average temperature of 26.1 ^{o}C and 114.4mm of rainfall between 1999 and 2019 **(**Climate-DATA, 2021)**.** Our study shows that mealybug attacks have become more frequent and are on the way out. This disappearance could be explained by the introduction of the natural enemy from South America, the parasitic wasp *Anagyrus lopezi*, which has been introduced in Benin and other African countries (Vernier *et al.,* 2018). The cassava mealybug has thus been successfully suppressed continent-wide. This was also observed in 2009, when the severe mealybug invasion in Thailand was quickly contained by the release of 3 million pairs of *Anagyrus lopezi* in the infested area (FAO, 2013). Mite attacks were strong on several accessions, especially those originating from the farming environment.

This mite outbreak observed on the majority of accessions could be explained by the fact that in breeding programs, the criteria for variety susceptibility to mites seems less reliable. Thus, N'Zué in 2007 indicated that the selection of improved varieties based on the incidence of mites is ineffective. Therefore, control of green mites is necessary using biological agents such as mites belonging to the Phytoseiid family (Cissé, 2015). For each attack (African mosaic, anthracnose, mealybugs and mites) observed, traditional varieties were more susceptible than improved varieties. This could be explained by the fact that when they were created, improved varieties benefited from the main selection criteria such as yield, resistance to diseases (mosaic, bacterial blight) and pests (mites, mealy bugs) (N'Zué *et al.,* 2004).

Conclusion

Our study shows that the genetic resources of cassava (*Manihot esculenta* Crantz) *in ex-situ* conservation in Côte d'Ivoire have been enriched from 106 accessions in 1953 to 727 accessions in 2019. However, these accessions showed heavy mite attacks (83.7% of accessions) and African mosaic symptoms (68.9% of accessions). Overall, accessions from IITA were more resistant to diseases than those from the farmer's environment. This resurgence of the mite outbreak observed on the majority of accessions is of concern to the CNRA, which could draw inspiration from existing biological control programs in certain research institutes such as IITA, CIAT and EMBRAPA for a total sanitation of the cassava collection.

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