Vol. 17(9), pp. 76-84, October 2023
DOI: 10.5897/AJPS2023.2319
Article Number: 86FDF2671482
ISSN 1996-0824
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African Journal of Plant Science

Full Length Research Paper

Prospecting, survey and plant material collection for *Hibiscus sabdariffa* L. (Malvaceae) genebank establishment in Côte d'Ivoire

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Received 16 July, 2023; Accepted 10 October, 2023

***Hibiscus sabdariffa* L. (Malvaceae) is a plant whose leaves and calyxes are integral to the local diet. The leaves are commonly used in sauce preparations, while the calyxes are utilized in making the local drink known as "bissap," which is highly appreciated by the local population. Despite its socio-economic significance, the production of this species remains relatively low in Côte d'Ivoire. This decline in yield may be attributed to the lack of available information regarding the identification of different *H. sabdariffa* cultivars. To establish a comprehensive database, a survey and collection of various local cultivars of *H. sabdariffa* were conducted in 12 locations across Côte d'Ivoire. In total, 80 accessions were recorded, with 53.75% of them located in the North. Among these collected accessions, two primary cultivar types were identified based on calyx coloration: the red type (Hibiscus sabdariffa var sabdariffa) and the green type (*Hibiscus sabdariffa var altissima*). Within these two types, the presence of other morphotypes has been observed. Cultivation and trade of these cultivars are primarily carried out by women, with over 90% of them having no formal education. The price of calyxes of the red type varies from 3000 to 4700 FCFA per kg, providing an opportunity for farmers to diversify their sources of income. The various accessions collected thus constitute a substantial genetic reservoir necessary for the development of new *H. sabdariffa* varieties.**

Key words: *Hibiscus sabdariffa*, *ex-situ,* Collection, Seed genebank, Côte d'Ivoire.

INTRODUCTION

Countries need genetic resources to sustainably increase their agricultural production. This is particularly important for countries which are south of the Sahara, where agriculture is the main currency sources. Also, in the face

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of rapid population growth, crop diversification is essential to adapt to the adverse effects of climate change and to meet the challenges of the food crisis. The need and urgency to ensure food security and economic prosperity for the populations of developing countries today necessarily involves the enhancement and promotion of crops of local interest (Sourabie et al., 2020).

Indeed, local crop plants abound in traits of interest, notably adaptation and resistance to biotic and abiotic pressures (Garcia et al., 2021). Such plant species are generally found in developing countries, where the introduction of cash crops to improve farmers' incomes and living standards has led to their abandonment. As a result, local plant species of less immediate economic interest are rapidly declining in traditional production systems.

One example is Roselle, *Hibiscus sabdariffa* L., a traditional leafy vegetable whose flower calyx is also used to prepare tea and the widely consumed local beverage (bissap) (Ross, 2003). *H. sabdariffa* is also used to regulate blood pressure and to treat liver ailments and fever (Wang et al., 2000). *H. sabdariffa* is an antioxidant, anticancer agent and contains organic acids and other vitamin compounds (Salah et al., 2002). It is rich in polyphenols and vitamin C (Fall, 2001). Anthocyanins extracted from Roselle callus may have a protective effect against atherogenesis through their antioxidant capacity (Abeda et al., 2015).

However, despite its socio-economic importance, *H. sabdariffa* production remains relatively low (Cissé et al., 2008, Alassi et al., 2017). Improving *H. sabdariffa* production, which is well adopted by farmers, could be beneficial for the rural communities. To obtain improved varieties that are both productive and stable, the breeders must have at his disposal the greatest possible genetic diversity of the species studied (Baudoin, 2001). Prospecting missions, including surveys and collections of *H. sabdariffa* accessions in seed form, have therefore been carried out in Senegal (Diouf et al., 2004), Benin (Alassi et al., 2017) and Burkina Faso (Ouangraoua et al., 2021). The aim was to establish a genebank and obtain genetic data on local cultivars of interest for the direct development of the species at national level.

In Côte d'Ivoire, little information is available on the morphological, agronomic, biochemical and molecular characteristics of *H. sabdariffa* cultivars found in village land. The research activities about regeneration from cuttings (Sié et al., 2008) and agromorphological characterization (Sié et al., 2009) have been carried out using accessions obtained in only two localities. Although the species is grown in several Côte d'Ivoire agro-climates, only *H. sabdariffa* seeds were collected from various markets in Abidjan (South) and Korhogo (North) (Sié et al., 2008,2009). Previous research was also undertaken on the two forms *Hisbiscus sabdariffa* var *sabdarrifa* and *Hisbiscus sabdariffa* var *altissima* from Korhogo locality alone, to demonstrate the effect of explant genotype and culture medium composition on *in vitro* regeneration via somatic embryogenesis (Sié et al., 2010). In 2011, seeds collected in the Korhogo locality were used to reveal the *in vitro* resistance of the sabdariffa form and the sensitivity of the altissima form to one of the species' major pests, the fungus *Fusarium oxysporum* (Boulanger et al., 1984).

A collection representative of the diversity of *H. sabdariffa* in Côte d'Ivoire would be essential to guarantee the sufficiency of genetic data and to establish a breeding programme to improve this species for the benefit of rural communities. Knowledge about the various local cultivars of *H. sabdariffa* first requires an inventory of existing cultivars, to provide the breeder with a varied range of genotypes within the two forms already reported. This study was carried out in order to establishing an ex situ genebank representative of the diversity of *H. sabdariffa* from existing local diversity in Côte d'Ivoire.

MATERIALS AND METHODS

Plant material and study area

Surveys and collections of *Hibiscus sabdariffa* seeds were carried in 12 localities in Côte d'Ivoire from September 2022 to March 2023. Of these, 05 were in the North (Ferkessédougou, Boundiali, Korhogo, Kanawolo and Niaka), 03 in the West (Bangolo, Daloa and Duekoué), 02 in the East (Bondoukou and Agnibilékro), 01 in the center (Bouaké) and 01 in the South (Abidjan) of Côte d'Ivoire. These locations were selected to cover all the country's agro-ecological zones. The localities surveyed are shown in Figure 1.

METHODS

In each locality, the participatory method exploiting the endogenous knowledges of the famers (producers and traders) both men and women as described by Adoukonou-Sagbadja et al. (2006) and Dansi et al. (2010) was used to obtain information (Figure 2). The data collection method consisted of direct interviews with *H. sabdariffa* farmers and traders randomly selected in the localities surveyed. This survey method was based on a list of questions drawn up in advance. The questionnaire was designed with the survey's objectives in mind. It concerns the identification of farmers and traders, the origin of cultivated seeds, farming practice, method of obtaining and preserving seeds, criteria for identifying local cultivars and economic data on *H. sabdariffa*.

Samples of *H. sabdariffa* seeds were collected both from markets in the various localities visited, in the field and directly from the seed stocks of the famers surveyed, where available. The seeds of the various cultivars collected were then placed in labelled envelopes to be entered as accessions in the collection under a unique 7-digit alphanumeric code. Accessions are coded using the first two letters of the species' scientific name (HS), followed by two letters indicating the abbreviated name of the locality and three arabic numerals indicating the chronological order of introduction into the genebank. For example, the coded accession HSDA001 represents the first accession (001) of *H. sabdariffa* (HS) introduced

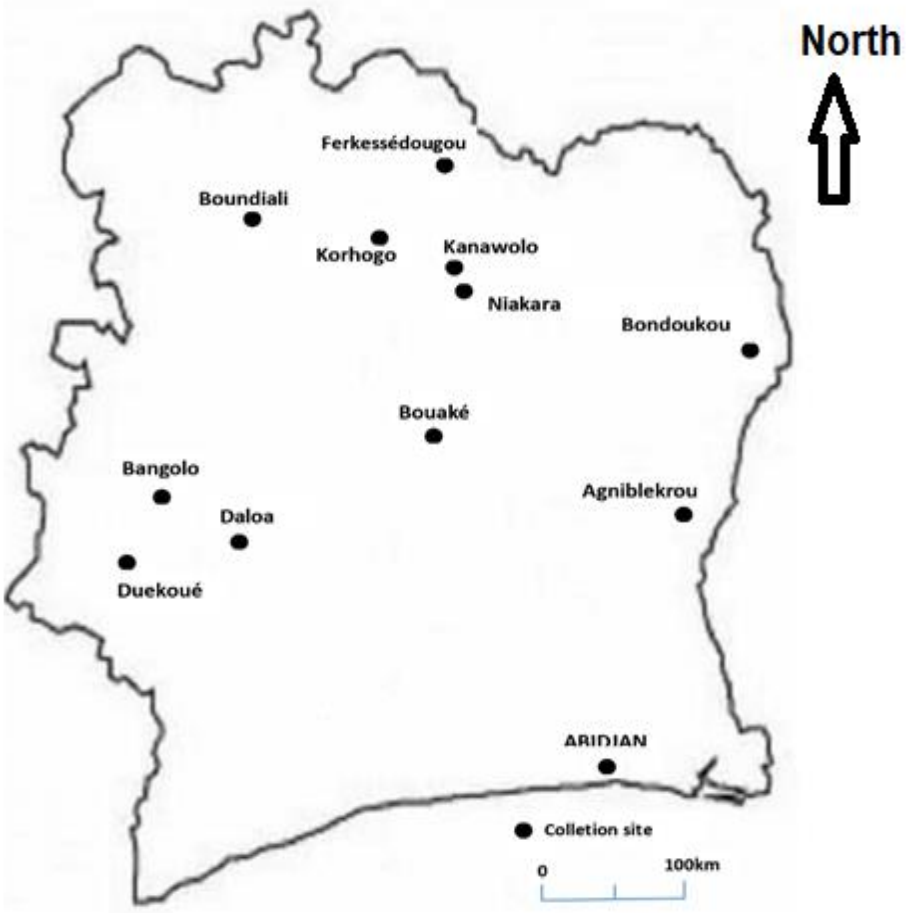

Figure 1. Map of Côte d'Ivoire with surveyed localities.

Figure 2. Interview by the research team (a) directly with the producer; (b) in a group with women producing *Hibiscus sabdariffa* in the village of Tchepke in the Ferkessédougou locality.

Table 1. Number of accessions collected according to localities and introduced into three genebanks in Côte d'Ivoire.

Zone	Localities	Accessions numbers	Gene Bank of UPGC	Gene Bank of UJLoG	Gene Bank of UNA
North	Korhogo	20	x	x	x
	Ferkéssedougou	07	x	x	x
	Boundiali	04	x	x	x
	Niakara	09	x	x	x
	Kanawolo	03	x	x	x
Centre	Bouaké	07	x	x	x
West	Duékoué	02	x	x	x
	Bangolo	03	x	x	x
	Daloa	01	x	x	x
Est	Bondoukou	01	x	x	x
	Agnibilékrou	03	x	x	x
South	Abidjan	20	x	x	x
Total	12	80	x	x	x

UPGC: University of Peleforo GON COULIBALY; UJLoG: University of Jean Lorougnon Guédé; UNA: University of Nangui Abrogoua; x: Presence of the accession in the genebank of the research institution.

into the seed genebank from Daloa (DA) locality.

Statistical analyses

Prospecting, survey and collection data were subjected to descriptive analysis (frequency, mean, percentage) using R software version 4.1.2 (R Core Team, 2021). The results are presented in tabular and graphical form.

RESULTS

Valuation of accessions sampled at locations visited

Eighty (80) *Hibiscus sabdariffa* accessions were collected in all the localities visited. Of these, 43 (53.75%) were found in the North. Twenty accessions (25%) came from in the South. In the Centre, 7 (8.75% of accessions) were counted. Six (7.5%) and 4 (5%) accessions came from Western and Eastern Côte d'Ivoire respectively (Table 1). Of all the accessions collected, two types can be distinguished: the red type was abundantly reported (67.50%) compared with the green type (33.50%).

To optimize the strategy of conserving *H. sabdariffa* accessions in Côte d'Ivoire, the genebank was duplicated in three replicate and hosted by the University of Peleforo GON COULIBALY (UPGC) at Korhogo, the University of Jean Lorougnon Guedé (UJLoG) at Daloa and the University of Nangui Abrogoua (UNA) at Abidjan (Table 1). The seed genebank for *H. sabdariffa* at the UPGC (Korhogo) is hosted by the National Shea Research Program, which aims to promote crops associated with shea parklands in order to enable rural communities to adapt to the adverse effects of climate change by diversifying their crops. All accessions were surveyed from 205 persons (men and women), including 151 traders and 54 farmers. More than half of those surveyed, 58.20%, were from the North. Next come the West and South with 19.40 and 11.95% respectively. The two other zones, the Centre and the East, had the lowest numbers of person surveyed, with 6.47 and 3.98% respectively.

Characteristics of the people surveyed

The people surveyed comprised 15 men (7.31%) and 190 women (92.69%). In terms of traders, *Hibiscus sabdariffa* is sold mainly by women (90.72%). The age of the traders surveyed ranged from 20-30 years to 50-60 years. The 30-40 age group is in the majority with 51.65%, followed by the 40-50 age group with 26.50%. The other two age groups, 20-30 and 50-60, account for 13.90 and 7.95% respectively. Cultivation is also practiced almost exclusively by women, with 97.15%. Among these farmers, 83.3% have no formal education. This population is subdivided into three groups according to age. The 20-30 age group, comprise 92.3% of farmers

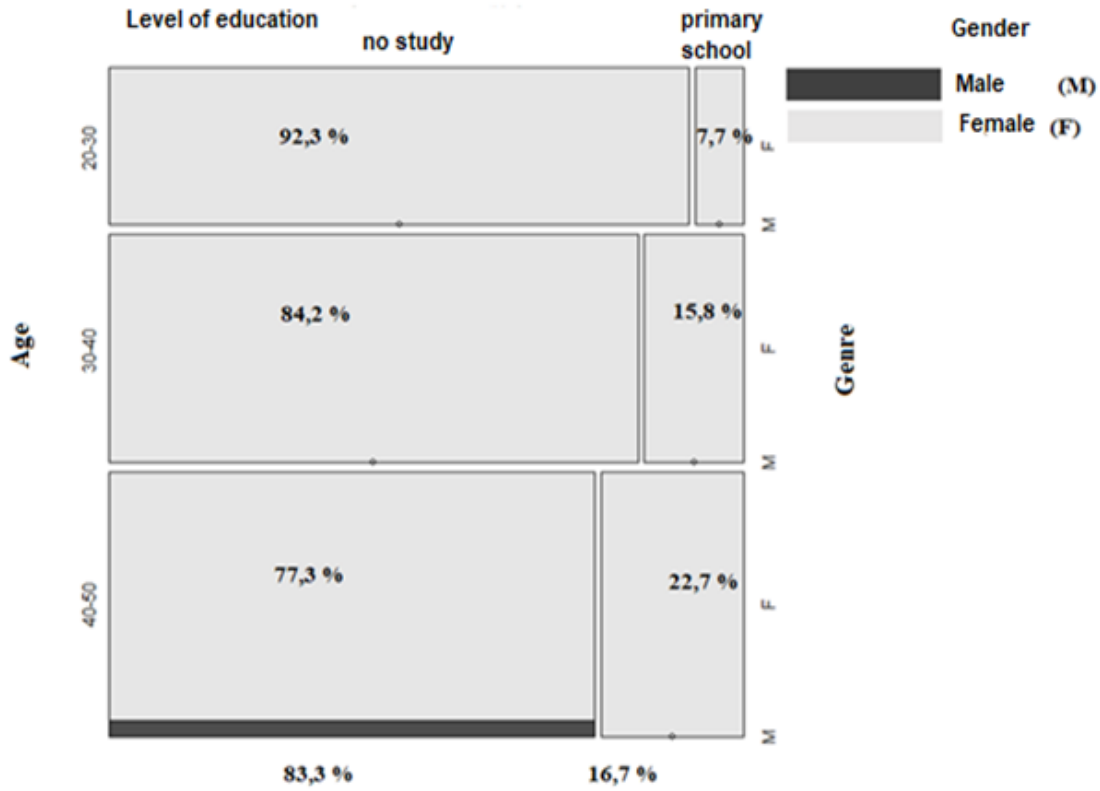

Figure 3. Characteristics of farmers by age, level of education and gender.

with no schooling. The 30-40 and 40-50 age groups have 84.2% and 77.3% respectively of farmers with no schooling (Figure 3).

Methods of supply and origins of *Hibiscus sabdariffa* seeds grown or sold

A proportion of 90.74% of grower surveyed reported that they acquired seeds through previous cultivation, while 9.26% acquired seeds by purchase. In the case of traders, 13.90% acquire seed through their own production and 86.10% through purchase. The origin of seeds comes from Burkina with 49%. 41.07% of seeds come from Korhogo, Ferkessédougou and Boundiali, (with 17.22%, 16.55% and 7.30% respectively). Duekoué and Mali account for 3.97 and 3.30% respectively. The other towns, notably Séguela, Bonoufla, Katiola and Napié, represent 0.66% each.

Description and use of *Hibiscus sabdariffa*

People surveyed in Northern, Central and Eastern Côte d'Ivoire recognized the existence of two cultivars based on calyx color within *Hibiscus sabdariffa*: the green cultivar (*Hibiscus sabdariffa* var. *altissima*) and the red cultivar (*Hibiscus sabdariffa* var. *sabdariffa*). Proportions of 33.33% and 17.50% of People surveyed in the South and West respectively recognize only one cultivar, the red one. According to information received from traders in the Centre and North, the leaves of both cultivars are eaten in sauces. In other areas, the red-calyx cultivar is used exclusively to produce the fresh juice widely known as "bissap", while the leaves of the green-calyx cultivar are used in sauces. Accessions collected from traders and growers show that it is difficult to distinguish between the seeds of the two cultivars (Figure 4a and b). On the other hand, the calyxes of the red cultivar sold on the various markets are of two types: light red and dark red. The dark-red calyx comes from Burkina Faso and is more popular with traders because of its high demand. Within the green type, two morphotypes according to leaf size have been reported: the large-leaf morphotype and the small-leaf morphotype (Figure 4c and d).

Survey work revealed that in the Senoufo language, the red morphotype is called *Tangnin* and the green type, *Tanga*. In the Malinké language, *Dawoueni* is the name of the red morphotype and *Dah*, the green morphotype. In almost all ethnic groups, *Hibiscus sabdariffa* is

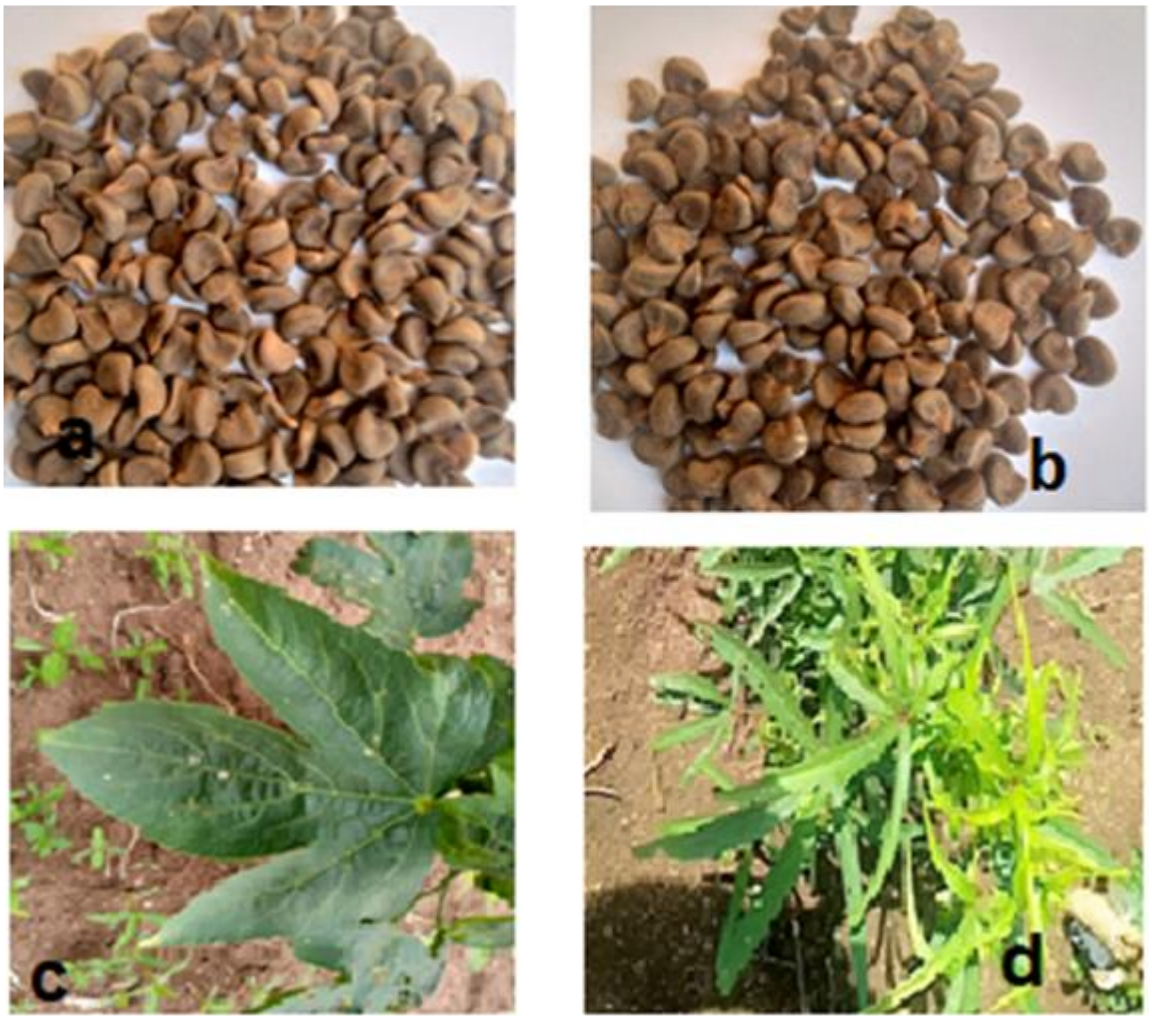

Figure 4. *Hibiscus sabdariffa* seeds and leaves; a: Seeds of the red calyx cultivar; b: Seeds of the green calyx cultivar; c: big leaf of the green calyx cultivar; d: small leaf of the green calyx cultivar.

commonly known as bissap. As a result, its vernacular names are ignored or unknown.

Constraints to *Hibiscus sabdariffa* production in Côte d'Ivoire

Constraints to *Hibiscus sabdariffa* production are generally linked to disease and pest damage to leaves and flowers. According to traders, in 2022, the plants were severely attacked by jassids (Homoptera: Cicadellidae), resulting in considerable production losses. Control methods need to be deployed against these pests, which pose a serious threat to the survival of *H. sabdariffa* cultivation in Côte d'Ivoire. Growers do not resort to the use of phytosanitary products for lack of financial means. Of all the growers surveyed, only 11.11% use pesticides chemical products, especially on plots used for market gardening. All the people surveyed had no knowledge of improved *H. sabdariffa* varieties, and polyculture was the only cultivation technique applied in all the plots visited. In central Côte d'Ivoire, *H. sabdariffa* is grown with cabbage, lettuce, tomato, okra and other crops. In the north, on the other hand, *H. sabdariffa* is grown with rice, groundnuts, cotton, cashew, maize, often in shea parks, home gardens, etc. Sowing begins at the start of the rainy season, practically in June. *H. sabdariffa* leaves are harvested two months after sowing for consumption or sale. The calyxes are also harvested during flowering, i.e. 3 to 4 months after sowing. Once harvested, the seeds are dried in the sun and stored in a canary until the next season. At the market, traders store seeds in bags (54.30%), plastic sachets (22.52%), cans (17.22%) and basins (5.96%),

Figure 5. Measuring instrument for sale in the market of *Hibiscus sabdariffa. (*a) Fresh leaves; (b) Tomato box for sale.

without the use of phytosanitary products against pests.

Some economic aspects of *Hibiscus sabdariffa* cultivation in Côte d'Ivoire

The price of *Hibiscus sabdariffa* seeds, leaves and calyxes varies according to the sales period and year, as well as availability. Fresh leaves are sold in heaps of 25 to 100 FCFA in the markets (Figure 5a). Dried calyxes of the red calyx cultivar are sold in large tomato cans (around 0.5kg of dried calyxes) (Figure 5b) and the selling price varies from 3,000 to 4,700 FCFA per Kg, depending on availability. In the dry season, the quantity of dried calyxes on the market drops, so the price is high. The price of seeds of both types is relatively identical, ranging from 750 to 1000 FCFA per Kg.

DISCUSSION

The overall aim of this study is to improve the production of *Hibiscus sabdariffa* and socio-economic situation of rural populations in Côte d'Ivoire. Survey of local cultivars is a prerequisite for any genetic improvement strategy for this species. A total of 80 accessions were collected in the main agro-ecological zones of Côte d'Ivoire. Of these, 53.75% were found in the North. In this zone, *H. sabdariffa* leaves are widely consumed in sauces, and have long been an integral part of the population's dietary habits. Cultivation of this species is more suited to the North, which remains the epicenter of *H. sabdariffa* cultivation in Côte d'Ivoire. Nevertheless, almost half of all *H. sabdariffa* seeds grown during this survey originated in Burkina Faso. Indeed, the Western part of Burkina Faso, which borders northern Côte d'Ivoire, is the leading area for *Hibiscus sabdariffa* production (Ouangraoua et al., 2021). Northern Côte d'Ivoire is therefore a zone of massive and regular importation of this species.

Over 90% of *H. sabdariffa* cultivation and trade is carried out by women. Indeed, in Africa, the cultivation of traditional leafy vegetables is an activity essentially reserved for women (Diouf et al., 1999). In our studies, we found that over 80% of women interested in growing *H. sabdariffa* had no formal education. The schooling of young girls should be encouraged. This will enable them to easily master the cultivation techniques for this species, with a view to improving production.

Two types of *H. sabdariffa* have been reported. These are the red type and the green type. Within the red type, two cultivars, dark red and light red, were recorded during the surveys. These numbers are lower than those recorded in Senegal by Cissé et al. (2008), who counted seven cultivars, only three of which originated in Senegal. The low diversity found within this species may be due to its predominantly autogamous mode of reproduction, which is not conducive to genetic cross-fertilization. According to the testimonies gathered, the dark red cultivar is the most appreciated for its high acidity and the quantity of bissap juice it yields. Leaves of the red variety are also eaten in sauces, as are leaves of the green variety. This latter was found in almost all the markets we visited, but the large-leaf cultivar is more in demand than the small-leaf variety. It has also been reported that women leaf growers in Senegal prefer the large-leaf green cultivars (Diouf et al., 2004). From a nutritional point of view, *H. sabdariffa* leaves are very rich and could make a significant contribution to improving human nutrition (Soro et al., 2022). While the seeds of *H. sabdariffa* are much sought-after and used in food in Mali for their richness in amino acids and fatty acids (Fane et al., 2021), most growers we met in Côte d'Ivoire do not harvest the seeds. Leaves and calyxes are harvested at the vegetative and flowering stages respectively. The pods are left in the field. They burst at some point under the effect of the sun, releasing the seeds which germinate in the next rainy season.

Various field visits have shown that farmers grow *H.*

sabdariffa in association with other crops. This is because it is not considered a main crop. This combination of crops is thought to be one of the reasons for the low production of *H. sabdariffa*. This is confirmed by the research work of Faye et al. (2001), which revealed that *H. sabdariffa* yields are lower in association with cereals, notably millet and sorghum. In addition, insect pests destroy the leaves and flowers. During our surveys, only 11.11% of farmers used phytosanitary products. Lack of financial means is one of the reasons cited. However, for soil fertility, some farmers in Benin have had the merit of using animal excrement, notably cattle and poultry, to increase *H. sabdariffa* production (Alassi et al., 2017). At market level, the storage of seeds in makeshift objects such as cans, bags and plastic sachets allows pests to affect seed quality. Although *H. sabdariffa* is considered a minor crop according to Cissé et al. (2008), it is currently a significant source of income for farmers. Farmers grow it for their own consumption, and the surplus is sold on markets. The relatively more profitable price of red morphotype calyxes, ranging from 3,000 to 4,700 FCFA per kg, would be an opportunity for farmers to diversify their sources of income.

Conclusion

The aim of the present study was to establish an ex-situ genebank representative of the diversity of *H. sabdariffa* from existing local diversity in Côte d'Ivoire. Accessions were collected in the main agro-ecological zones of Côte d'Ivoire, resulting in the identification of 80 accessions, over 50% of which come from the north. Two cultivars were reported: the green cultivar and the red cultivar. The existence of morphotypes within these two types was also reported by the people surveyed. These cultivars are mainly grown and traded by women. The plant is grown in association with other crops, notably rice, millet and groundnuts. Morphological and molecular characterization studies should be carried out to gain a better understanding of the performance of *H sabdariffa* accessions and facilitate their use in varietal selection programs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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