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ASSESSMENT OF GENETIC DIVERSITY IN WEST AFRICA ROSELLE (*Hibiscus sabdariffa* L.) ACCESSIONS BY MORPHOLOGICAL EVALUATION AND RAPD GENOTYPING

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BSc. Molecular Biology and Biotechnology

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ASSESSMENT OF GENETIC DIVERSITY IN WEST AFRICA ROSELLE (*Hibiscus sabdariffa* L.) ACCESSIONS BY MORPHOLOGICAL EVALUATION AND RAPD GENOTYPING

A THESIS SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF

MASTER OF PHILOSOPHY IN BIOTECHNOLOGY

NANCY COFFIE

NOVEMBER 2016

DECLARATION

I hereby declare that except for references cited in relation to other works, which have duly been acknowledged, this work is the outcome of my own research and that this thesis has neither in whole or part been submitted for any other degree at any other University.

Nancy Coffie …….. ……

(PG3888909) Signature Date

Student „s Name & ID

We declare that we have supervised the above student to undertake the study submitted herein and confirm that she has our permission to submit.

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Dr. H. D. Zakpaa …….. ……

Head of Department Signature Date

DEDICATION

I dedicate this thesis to my dear husband, Mr. Rockson Coffie, and our divine gifts, Samuella Bennie Coffie and Daniel Doi Coffie.

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ABSTRACT

Roselle (*Hibiscus sabdariffa* L.) is an important crop purported to originate from West Africa where its economic importance as source of red anthocyanin-rich pigments, bast fiber, and leafy vegetable is ignored and little is done to collect, conserve and assess its genetic diversity. Genetic diversity studies reveal both useful genotypes and allele diversity for trait improvement. Little genetic variability exists in exotic roselle genotypes making trait improvement difficult. In this work, 39 roselle landraces collected from eight regions in West Africa, Bawku, Upper West, Ouagadougou, Lome, Senegal, Mali, Nigeria, and Cote D"Ivoire were evaluated for agromorphological and RAPD genotyping. A large variability was found in calyx pigmentation, stem colour, and leaf lamina colour, all of which recorded 4 to 5 variants with substantial and fairly equal number of genotypes in each class. The large variability in quantitative traits estimated by coefficient of variability and mean square decreased in the order, leaf area 43.58% and 66,386.24, plant height 28.72% and 7,489.05, height at first branching 36.71% and 25.02, number of internodes 18.44% and 52.95, stem width 19.87% and 44.47, to branch number 27.66% and 5.48, respectively. Useful accessions identified were HS09, HS14, HS19, HS32 and HS50 from Ouagadougou, HS02 from Cote D"Ivoire, HS69 from Bawku, and HS68 and HS86 from Mali for leafy vegetable production; HS20 and HS69 from Bawku, HS83 from Cote D"Ivoire, HS04 from Lome, HS16, HS68, HS86 from Mali, HS09, HS14, HS19, HS24, HS50, and HS77 from Ouagadougou for high calyx productivity. For high fiber productivity, HS16 and HS68 from Mali, HS20 and HS69 from Bawku, HS13 from Nigeria, HS02 from Cote

D‟Ivoire, and HS09, HS14, HS19, HS25, HS32, and HS50 from Ouagadougou were delineated on the basis of large values of plant height, stem width, and number of internodes. The predominantly low heritability estimates of 0 to 62% except for plant height represented dominance genetic effects that will achieve slow progress in breeding. All traits exhibited inconsistent genotype×environment effect across the populations. Low to moderate positive significant correlation coefficients of r =0.11 to 0.41 in plant height and number of internodes with all other traits was present. Genetic similarity based on morphological structuring ranged from 0.00 to 0.94 with mean of 0.27±0.26. UPGMA clustering split the accessions into three main clusters independent of their origin suggesting seed flow in the region. The first two principal components which accounted for 100% of the variance identified four uncorrelated groups and showed all traits to be correlated except height at first branching. Statistical analysis of 12 random decamer oligonucleotide genotyping revealed 80 to 100% rate of polymorphism, 1,297 alleles across genotypes, 63 alleles across loci, and average of 5.25 alleles per locus. Intralocus gene diversity range of 0.15 to 0.32 and mean of 0.21±0.05 denoted higher allele diversity than exotic genotypes. Jaccard‟s similarity coefficient of 0.00 to 0.79 with average of 0.29±0.14 for the entire population uncovered substantial genetic diversity. UPGMA cluster analysis based on RAPDs grouped accessions into four major classes on a fair basis of their origin confirming the morphological PCA biplot analysis. Cluster I contained two heterogeneous subclusters IA and IB with average genetic similarities of 0.42±0.12 and 0.43±0.10 delineated a „Ouagadougou‟ and a „West African Mix‟ clusters, respectively. Cluster II was with similarity coefficient of 0.60±0.04 defined a „Ghana group‟. Cluster III, a single Senegal genotype, separated entirely from all accessions. Finally, cluster IV, having

genetic similarity of 0.10 to 0.40 and average of 0.29± 0.11 represented a substantially large genetic diversity for a „Ouagadougou-Togo" mix. The region from Mali through Ouagadougou, Bawku to Lome was reckoned as the plausible center of roselle diversity. The large variability, polymorphism and allele diversity confirmed the Roselle landraces as rich reserve of allele diversity yet to be harnessed for crop improvement.

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CHAPTER ONE

1.0 INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) is an annual shrub belonging to the mallow family Malvaceae, which includes vegetable crops such as okra (*Abelmoschus*), the three world"s most important fiber-producing crops, cotton (*GossypSium*), jute (*Corchorus*), and kenaf (*Hibiscus cannabinus*), as well as some ornamentals such as the Japanese lantern (*Hibiscus schizopetalus*) and Chinese hibiscus (*Hibiscus rosasinensis*). Hibiscus is the largest genus in this family, having about 300 species (Hinsely, 2008). Besides cotton, the most important fiber-producing species of the mallow family are roselle and kenaf, collectively known as „mesta". Within the genus *Hibiscus*, the chromosome number varies from *n* =12 to ~144, with 14 or 18 chromosomes being predominant (Fryxell, 1988, 1968). However, *Hibiscus sabdariffa* is a tetraploid species with 2n=4x=72 (Akpan, 2000).

Roselle is known by different local names in many countries, such as *soobolo* and *suure* in Ghana; *zobo* in Nigeria; *karkadé* in Sudan and Egypt; *sour tea, bissap*, or *da* in Senegal, Mali, Burkina Faso, Togo and Cote D"Ivoire; and *sorrel* in Guinea (Diouf *et al*., 2007a; McClintock, 2004;). *Hibiscus sabdariffa* is believed to have arisen from a domestication event from the wild progenitor *H. machowii* found in Uganda, to which it has close resemblance while the wild fiber types are products of introgression between *H. asper* and *H. machowii* (Singh, n.d., para. 15). The origin of roselle is believed to be tropical Africa or Asia (Tounkara *et al*., 2011; Cheng *et al*. 2004; Gautam 2004; Rhodin and Panchoo, 1990; Akobundu 1987; Morton, 1987; Wilson and Menzel, 1964) where it was first found in the wild and later domesticated for its leaves and seeds. The evidence of its origin is seen in many wild varieties found in Senegal, Mali and Ghana (McClintock and El Tahir, 2004; McClintock, 2004).

Two types of roselle occur, the wild edible and the cultivated fiber types. Webster (1914) described an unusually tall fiber yielding type (4.8 m) from Gold Coast (Ghana) which had been accidentally mixed with seeds imported into Thailand. Khan (1930) named this fiber type *altissima* and has since been a cash crop for its jute-like bast fiber in India, Indonesia, Jamaica, Nigeria, and Kenya from where they are principally exported into Europe and the U.S.A. (Mwasiagi *et al*., 2014; McClintock and El Tahir, 2004). Currently, the two types are described as *H. sabdariffa* var. *sabdariffa,* the true wild, somewhat short and bushy type with edible calyxes, and *H. sabdariffa* var. *altissima*, the tall sparsely-branched fiber type with fibrous calyxes.

Besides being a source of soft and flexible fiber, specifically bast or phloem fiber which finds uses in manufacture of carpets, automobile and airplane upholstery, yarn, burlap, rope and paper, roselle edible calyxes are consumed for their red acidic refreshing drink and as a therapeutic concoction in folk medicine. The leaves and fleshy calyxes are used as vegetables in Ghana, Nigeria, Senegal, Burkina Faso, Mali, and Cote D"Ivoire (Falusi *et al*., 2014; Diouf *et al*., 2007a).

Despite the economic value of roselle fiber and the fact that the first fiber type originated from Ghana, by and large, the crop was neglected and used only for food (leafy or dried and powdered vegetable) but to a limited extent for fiber. Nevertheless, in the period from 1960 to 1991, plantations of the fiber producing roselle (*worgta* in Builsa language), kenaf (*kazaasa*), and jute (*ayoyo*) were cultivated in the Upper East region, the Afram plains of the Eastern Region, Brong-Ahafo and Ashanti Regions of Ghana for supply of bast fiber to the then Kumasi Fiber and Bag Factory for manufacture of jute sacks and fiber bags.

Customarily, roselle types may be identified by their morphological characteristics such as growth habit, floral morphology, and pigmentation pattern of the whole plant.

Regarding growth habit, accessions vary from edible, dwarf and bushy types (Howard and Howard (1911) to nonedible, tall and nonbushy types (Webster 1914); floral morphology variation covers yellow to pink flowers, with brown or yellow anthers, including long, succulent and glanded calyx to short, fibrous and nonglanded calyxes; the capsule may be ovoid or rounded. In addition to these features, the leaves may vary from entire to deep-lobed forms to manifest trilobed and palmate types. Pigmentation patterns comprise a whole array of green to dark red.

Both *Hibiscus sabdariffa* var. *sabdariffa* (hereinafter referred to as *sabdariffa*) and *Hibiscus sabdariffa var. altissima* (hereinafter referred to as *altissima*) occur in variable types which differ in colour variations of different parts of the plant. On the basis of texture and colour of the calyxes, variety s*abdariffa* consists of four races: *bhagalpuriensis* with green, red-streaked, nonedible calyxes; *intermedius* and *albus* fiber types with yellow-green edible calyxes; and finally, *ruber* with edible calyxes. Similarly, variety *altissima* possesses green or red stems, and essentially, entire green or green with red veined leaves, yellow flowers, and red or green, non-fleshy spiny nonedible calyxes. According to Crane (1949) and Wilson and Menzel (1964), the presence of nectar on the mid ridge of the sepal is characteristic feature of both the edible *sabdariffa* and inedible types *altissima*.

Basu and Chakravarty (1972) observed that yellow anthers are characteristic of completely green types, while brown anthers are a characteristic of all red and intermediate types. However, a new red-type roselle, HS 8413, which had yellow anthers with no nectary on the mid ridge of the sepal, was discovered by Chakravarty *et al*. (1981).

In view of the demand for roselle for its red calyxes as source of natural anthocyanin pigments in food and pharmaceutical preparations, its use as alternative source of hardy

leafy vegetable for adaptive food security measure, and for their economically important bast fiber, there is urgent need to collect and characterize accessions in West Africa. Moreover, there are reports among the indigenous populace in Ghana about the gradual disappearance of roselle fiber types as these genotypes are no longer cultivated to feed the defunct Kumasi Fiber and Bag Factory.

In 1962, the Kumasi Fiber and Bag Factory was established to process fiber into burlap using jute, kenaf and roselle. From the time of its establishment to 1991 roselle fiber was supplied from the Afram Plains in the Eastern region, Mampong, Konongo, and Yankyenase, in the Ashanti region, some parts of Brong-Ahafo region, and from the Kassena-Nankana district of the Upper East region of Ghana (www.sydneyabugri.com). From 1991 to 2015 the Kumasi Fiber and Bag Factory went defunct until a recent attempt by the government to restore production.

Roselle is used variously for food and fiber. As food, roselle is exploited for its brilliant deep red pigment and tart flavor of the calyx in red tea, sherbets, jams and jellies (Tsai and Ou, 1996; Sato *et al*., 1991). In folk medicine, it is the anthocyanins and other antioxidants including flavonoids, polyphenols, β-carotene and α-tocopherol of the red calyxes that elicit physiological activity such as reduction in serum cholesterol and sugar levels (Mozaffari-Khosravi *et al*., 2009; Lin *et al*., 2007; Frank *et al*., 2005; Prenesti *et al*., 2005). In West Africa, roselle drinks and sherbets are popularly named „sobooro" in Ghana, „drink of the pharaohs in Egypt", „zoborodo" in Nigeria, „bissap" in Burkina Faso and Senegal, and „da bilenni" in Mali (Padmaja *et al*., 2014; Mohammed and El-Gabri, 2013; Falusi, 2007; Schippers, 2000; Bricage, 1984). Nonetheless, in Ghana there has been a shift in use of roselle from fiber to primarily a source of food and drink.

The use of roselle as source of bast fiber is known in Kenya, Nigeria, Sudan, Italy, and Cote D"Ivoire (Mwasiagi *et al*., 2011; Schippers, 2000; Babalola, 2000; Sie *et al*., 2009; Siepe *et al*., 1997)) while knowledge and use of same in Ghana has regrettably diminished (www.sydneyabugri.com). Substantial wealth is though derived from the bast fiber of roselle in India, Southeast Asia, and Russia (Singh, 1991).

Despite the importance of roselle as a commercial crop in many countries including Mexico, Kenya, and Niger (Bakasso *et al*., 2013; Mwasiagi *et al*., 2011; TorresMoran *et al*., 2011) there are few reports on genetic improvement of roselle through breeding, apparently due to its cleistogamous mating system. Osman *et al*. (2011) employed mutation breeding on some Malaysian genotypes. There is limited information on studies which targets a systematic collection, characterization, and evaluation of genetic diversity of roselle in Africa. Such a study is required to reveal information on genotypic variability, estimate genetic relationships, identify useful genotypes as source of alleles for crop improvement, as well as identify groupings within the germplasm for an efficient breeding program. In addition, determination of genetic diversity in roselle would contribute to parental selection for genotype improvement, systematic conservation and management, and interventions in preventing germplasm loss.

Morphoagronomic and molecular characterization studies have the capacity to reveal genetic diversity within and among genotype collections and refine varietal registration (Torres-Moran *et al*., 2011; Diouf *et al*., 2007a). Effective crop improvement on a wide genetic base is prerequisite for crop resilience to biotic and abiotic stresses, as well as crop conservation both of which rely on genetic diversity assessments. Narrow genetic diversity estimates arise from recycling of few genotypes in breeding programs, whereas large genetic diversity estimates is a product of abundant allelic variation often stored in the landraces and wild relatives of modern crops (Dwivedi, *et al*., 2008).

Until recently, there have been few reports of genetic diversity studies on *Hibiscus sabdariffa* (Sie *et al.*, 2009; Siepe *et al*., 1997), however lately, worldwide interest in the nutritional, medicinal, and fiber properties of the crop has led to advances in assessment of genetic diversity among *H. sabdariffa* accessions (Medagam *et al*., 2015; Abou El-Nasr *et al*., 2014; Falusi *et al*., 2014; Omalsaad., 2014; Sabiel *et al*., 2014; Alarcón Cruz *et al*., 2013; Bakasso *et al*., 2013; Mohammed and El Gabri, 2013; Osman *et al*., 2011; Torres-Moran *et al*., 2011; Sie *et al*., 2009; Yusof and Saud, 2009; Diouf *et al*., 2007a; Alam *et al*., 2006; Barik *et al*., 2006; Ibrahim and Hussein, 2006; Cheng *et al*., 2002)). Many of these studies revealed narrow genetic diversity in the roselle accessions considered.

Interestingly, majority of these studies employed only agro-morphological markers to estimate genetic diversity in roselle (Medagam *et al*., 2015; Abou-El-Nasr *et al*., 2014; Falusi *et al*., 2014; Omalsaad *et al*., 2014; Sabiel *et al*., 2014; Alarcón Cruz *et al*., 2013; Mohammed and El Gabri, 2013; Osman *et al*., 2011; Sie *et al*., 2009; Diouf *et al*., 2007a; Alam *et al*., 2006; Siepe *et al*., 1997). Being more robust than morphological markers, molecular markers with a genome-wide coverage and environmental insensitivity reveal polymorphisms arising from mutations down to the

DNA level (Barik *et al*., 2006; Melchinger *et al*., 1991; Paterson *et al*., 1991; Williams *et al*., 1990).

Random amplified polymorphic DNA (RAPD) marker technique has proved to be useful in many plant species for varietal analysis, population studies, and genetic linkage mapping, marker-assisted breeding, genome fingerprinting, and study of genetic variation and relationships among plant species (Prasad, 2014; Rout *et al*., 2003; Rafalski *et al*., 1996; Vogel *et al*., 1996; Yu *et al.*, 1993; Williams *et al*., 1990)). Application of RAPD markers to the genetic characterization of roselle includes the

works of Omalsaad *et al*., 2014, Yusof and Saud (2009), Barik *et al*. (2006), and Cheng *et al.* (2004).

The advantages of RAPD markers that make them applicable for efficient detection of DNA polymorphisms include requirement of low technical input, fast and cost effective method, and requirement of small quantity of DNA (Rafalski and Tingey, 1993). Moreover, the RAPD technique can be carried out successfully without knowledge of the sequence of the genome (Grattapaglia and Sederoff, 1994).

Although the RAPD technology employs dominant markers, demonstrates low resolving power and are less reproducible compared to other molecular markers, the method is simple, low cost and requires low technical input. More importantly, because the use of RAPD assay led to the detection of relatively higher percentage of polymorphism in a number of *Hibiscus* species than that for AFLP (Prasad, 2014; Bakasso *et al*., 2013, Yusof and Saud, 2009; Barik *et al*., 2006; Cheng *et al*., 2004) its use remains popular in molecular marker work in the Malvaceae family. Moreover, there are ways to overcome most of the drawbacks encountered with RAPDs. For example, reproducibility may be improved by the use of highly purified DNA, optimization of the operative parameters including temperature, pH, ionic concentration, type of gel (Skroch and Nienhuis, 1995; Micheli *et al*., 1994; Ellsworth *et al*., 1993), and the use of appropriate DNA polymerase brand, and scoring only the reproducible DNA fragments (Kresovich *et al*., 1992). More so, screening several oligonucleotides should be done in order to select only primers that provide useful amplification products. Also the use of appropriate DNA polymerase brand, and scoring only the reproducible DNA fragments (Barik *et al*., 2006; Yang and Quiros, 1993) could be helpful. In addition, the poor reliability arising from presence of both false negatives and co-migrating bands which may be mistaken for homology may be

corrected by resolution of fragment size using polyacrylamide gels and silver nitrate staining (Huff *et al*., 1993).

In sum, the available records on genetic diversity in roselle populations studied so far have either been undefined in origin, limited to local geographic area and do not include accessions from West Africa, despite the general belief that West Africa is the origin of diversity in roselle. In addition, genetic diversity studies in roselle by molecular tools are limited. For a better understanding of the existing genetic diversity and relationships among the genotypes of West Africa, there is urgent need for an assessment that employs both morphological characterization and molecular tools. Moreover, a genetic diversity study by application of these methods can reveal the history of roselle in West Africa. Such information is expected to impact roselle improvement in West Africa.

The overall objective of the present research was to determine genetic diversity in *H. sabdariffa* germplasm collected from West Africa.

The specific objectives include:

(i) To determine genetic relationships in *H. sabdariffa* germplasm at morphoagronomic level

(ii) To determine heritability and genotypic correlation among agronomic and fiber traits

(iii) To evaluate allelic diversity among the collection of *H. sabdariffa* accessions by means of RAPD genotyping

(iv) To assign genotypes into groups for breeding purposes

(v) To reveal the evolutionary history of roselle in West Africa

RESEARCH HYPOTHESIS

The centre of diversity of a species is endowed with genetically diverse genotypes, hence, roselle accessions of West Africa are expected to display large diversity in the crop. The research hypothesis dwells on two thematic areas: variability can be identified within the West Africa roselle population and that evolutionary factors such as mutation, recombination, gene flow, and genetic drift have contributed to the current variability in roselle.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 ORIGIN AND GEOGRAPHIC DISTRIBUTION OF *Hibiscus sabdariffa*

Hibiscus sabdariffa L., commonly known as roselle, is an annual and/or perennial herbaceous shrub used for its pergamentaceous and swollen fleshy green or red calyx and fibrous stem. It belongs to the family Malvaceae of the section Furcaria, a group of about 100 species which have in common a prominent calyx with ten prominent veins, of which five each run to the apices of the segments and into the sinuses and bear a nectary (Plate 2.1).

Roselle is native to West Africa and some parts of East and Central Africa (David and Adam, 1988) specifically Angola and Sudan (Gautam, 2004), where it was domesticated about 6000 years ago. It was later introduced to India and the Americas in the 17th century (David and Adam, 1988). Currently, roselle is widely cultivated in the tropics and subtropics (Appell, 2003) including many areas of the West Indies, Central America, Malaysia Indonesia, Thailand, Australia, Iran, and China (Morton, 1974).

Despite the availability of wide array of roselle types in West Africa, top world producers of roselle are not situated in any of the countries in this region but rather a traditional subsistence cropping for domestic use and for local markets dominate its production. Major producers of roselle are China and Thailand which contribute about 15,000 metric tons to international trade (McClintock, 2004 and El-Tahir., 2014). Minor producers of roselle include Egypt, Senegal, Sudan, Mali, Mexico and Nigeria. Major importers of roselle are the U.S.A. and Germany where the calyxes are processed into jams, jellies, perfumes, and herbal tea (Plotto, 2004). In recent years, the expanded use of roselle for its medicinal properties and pigment in both food and pharmaceutical applications, in addition to its silky soft and light-coloured bast fiber substitutive to jute (*Corchorus capsularis*) (Crane, 1949) have led to a renewed interest and increase in demand of roselle.

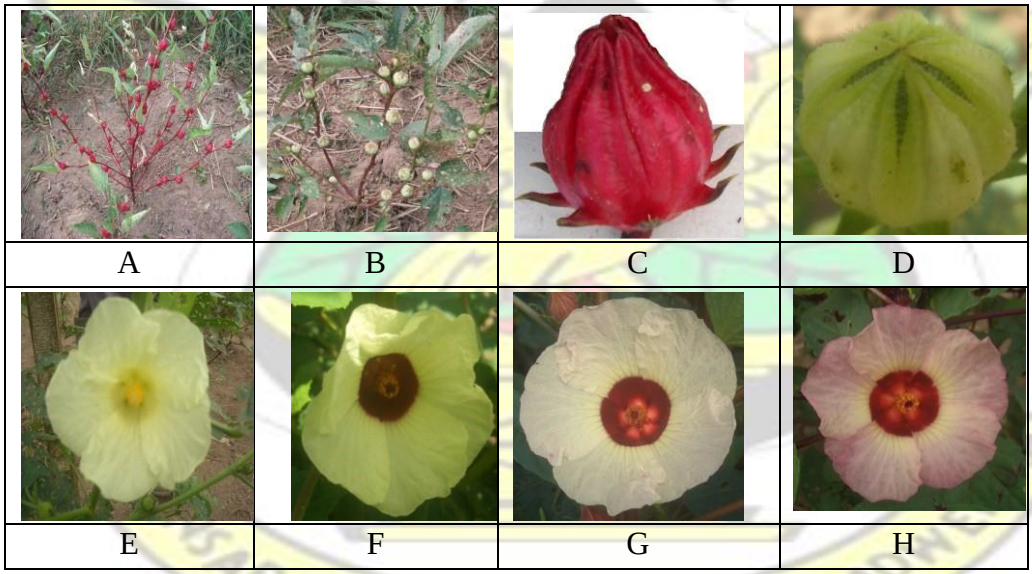


Plate 2.1. Image of *H. sabdariffa* L. full plant and plant parts. (A) Mature *H. sabdariffa* var. *sabdariffa* bearing red fruits. (B) Mature *H. sabdariffa* var. *altissima* bearing green fruits. (C) Fruit of *H. sabdariffa* var. *sabdariffa* enclosed in expanded and elongated smooth red calyx easily detached from the capsule. (D) Round fruit of *H. sabdariffa* var. *altissima* enclosed in fibrous and hairy green calyx difficult to detach from the embedded capsule. (E) Flower of green roselle on a mature plant. (F) Flower of green

pigmented roselle types on a mature plant. (G) Flower of some green pigmented and some red roselle on a mature plant. (H) Flower of full red type.
Source: Coffie *et al*., (unpublished).

2.2 CLASSIFICATION AND SUBDIVISIONS

Hibiscus sabdariffa is tetraploid with 2n=4x=72 (Bruna *et al*., 2009; Akpan, 2000) and is subdivided into two major botanical forms on the basis of growth habit and pigmentation of the calyx, sepal, leaf, capsule, anther, and flower.

2.2.1. Growth habit

Roselle demonstrates three main variations in growth habit, namely, dwarf bushy, tall nonbushy, and bushy-nonbushy types. The plant can grow up to a height of 2.4 m for edible calyx-producing types and up to 4.8 m high for fiber types (McClintock, 2004; Morton, 1987; Communication) and has a girth which varies between 1.2 cm and 3.5 cm (Duke, 1983). There are two main botanical varieties of *H. sabdariffa*, namely, variety *altissima* and variety *sabdariffa*. Variety *sabdariffa* is cultivated as a vegetable and mostly consists of dwarf and bushy forms having a glanded sepal, long and succulent sharply tapering calyxes, covering an entire capsule which is normal and ovoid in shape (Basu and Charkravarty, 1972). Morton (1974) reported of a variant of variety *sabdariffa* grown in the Bahamas that had a dark red, plump but stubby calyx that did not completely cover the entire capsule. In contrast, the variety *altissima*, grown for its fiber comprises tall non-bushy fiber types with short, thin, fibrous and inedible calyxes, and a glanded sepal (Webster, 1914). According to Basu and Charkravarty (1972), the bushy-nonbushy habit and the edible-inedible calyx are under the control of a single gene. Charkravarty *et al*. (1981) described an unusual type of *H. sabdariffa* which exhibited a dwarf growth habit, succulent calyxes, with nonglanded short sepals which do not entirely cover a round capsule.

In a recent study on characterization of West African *H. sabdariffa* collection, two unusually dwarf, nonbushy and vinelike genotypesdesignated HS03 and HS15, having red and succulent calyxes (Plate 2.2) were identified (Coffie et al., unpublished).

A	B
	

Plate 2.2. Unusual roselle types. (A) HS03: unusually dwarf nonbushy genotype. (B) HS07: brown pigmented dwarf genotype. Source: Coffie *et al*., (unpublished).

2.2.2. Pigmentation pattern

Various parts of *H. sabdariffa* plant including the stem, leaf blade, leaf venation, calyxes, flower, flower throat, and anthers exhibit varying pigmentation patterns. On the basis of pigmentation pattern, variety *sabdariffa* is divided into four races, viz., race *bhagalpuriensis* (inedible, green or red petiole, green calyx with red splashes), race *intermedius* (edible, green and some red stem and leaf, yellowish green calyx and fiber), race *albus* (edible, stem and petiole green, yellow calyx, yellow flowers, and fiber) and race *ruber* (edible, entirely red stem and petiole, red calyx, pink flowers, no fiber), all breeding true from seed (Morton, 1974; Howard and Howard, 1924, 1911). Table 2.1 shows the main morphological characteristics of the 4 races of variety *sabdariffa*.

The variety *altissima* comprises four classes namely, full green, green pigmented, green to light red and dark red (Sanyal *et al*., 1960). The entire roselle plant may be uniformly green with respect to the stem, leaves, calyx, epicalyx, and petioles, or may be uniformly red/dark red, or exhibit combination of green and red. In Ghana, many roselle genotypes including red, green and fiber types are grown by traditional farmers

in the Northern, Ashanti, Brong-Ahafo and Western regions, mainly for its leaf and calyx as vegetable and to a limited extent, for its bast fiber (Obodai, 2007; www.sydneyabugri.com; Personal communication).

Although roselle is commonly called *suure* or *sooboro*, they are known predominantly in indigenous Ghanaian languages as *tingyanbam* in Kokomba *digbemne*/*injamgbam* in Dagbani; *riaripari* in Guan; *evema* in Ewe; *sakpa* in Ga; *nangana* in Frafra and Moshi (Frimpong, 2014), *nyanngban* (Baasare), *denyanngba* in Chamba; *worgta* in Builsa; *vio* in Grushi and Kasem; *haasa*/*pisa* (Busanga), *biito* (Nankana and Frafra) *ber* in Dagaba, *guanwana/tuguaan* (Gruma), *bra* (Dagbani and Mampruli); *vio* in Grushi and Kasem and*haasa*/*pisa* (Busanga) Accessions of the botanical variety, var. *sabdariffa* have been designated names such as *branemdi* (Dagbani) and *berbiila* (Dagaba), *wogkola* (Builsa), *bekpabe nyanngban* (Baasare) due to their use as a source of condiment.

The nonbushy and unusually tall fiber types of the *altissima* variety are known locally as *biuur* among the Dagabas, *beto* or *berzohoko* among the Frafras, *bramihi* by the Dagombas, *worgta* (Bulisa), *nyanngban mmete* (Baasare) (Personal communication). The myriad of names and the existence of wild accessions identified as *saa bra* (Dagbani) and *berkanyanse* (Frafra), *koowol* (Baasare), *wogyiesa* (Builsa) which sprout naturally on virgin or semi-virgin lands at the onset of the rains provide some form of historical evidence of roselle domestication in Ghana (www.sydneyabugri.com; Personal communication), yet, there are no reports on their characterization and genetic diversity.

2.2.2.1. Composition of roselle pigments

The characteristic bright red to dark red pigments in roselle stems, leaves, calyxes, and pinkish petals commonly used for making jams, jellies and beverages (Wong *et al*.,

2002; Clydesdale *et al*., 1979) arise from accumulation of anthocyanins (Tsai and Ou, 1996). The major anthocyanin in roselle is delphinidin-3-sambubioside, which imparts red-violet colour to the calyx and occurs at a concentration of 43 g/L (Wong *et al*., 2003; Sato *et al*., 1991; Du and Francis, 1973). The second most abundant anthocyanin is cyanidin-3-sambubioside which occurs with two other minor anthocyanins, namely, cyanidin-3-glucoside and delphinidin-3-glucoside (Wong *et al*., 2002; Pouget *et al*., 1990; Du and Francis, 1973).

Table 2.1. Pigmentation patterns of the two botanical varieties of *H. sabdariffa*

Botanical var. *sabdariffa*				
Race Type	**Stem colour**	**Petiole colour**	**Calyx colour**	**Flower colour on withering**
Ruber	Entirely red	Entirely red	Red	Pink
Intermedius	Green	Green, with red pulvinus	Yellowish green	Yellow
Albus	Green	Green	Green/greenish white	Yellow
Bhagalpuriensis	Green	Green/ red	Green with red streaks	pink
Botanical var. *altissima*				
Class type	**Stem colour**	**Petiole colour**	**Calyx colour**	**Flower colour on withering**
Green to light red	Green pigmented	Green pigmented	Green-light red	pink
Dark Red	red	Red	Pink to red	Pink
Green pigmented	Green, high intensity pigmentation at nodes	Green pigmented	Green with red pigmentation	pink
Full green 1	Light green	Light green	Light green	Yellow
Full green 2	Deep green	Deep green	Deep green	Yellow

Source: Chakravarty *et al*., 1972; (Sanyal *et al*., 1960); Sanyal, 1959; Howard and Howard (1911, 1924).

Total anthocyanin content in roselle is reported to be 1.5 g/100 g on dry weight basis when expressed in terms of delphinidin-3-glucoside (Du and Francis, 1973). These

heat-stable anthocyanins are responsible for the natural red color, serve as antioxidant-rich factors, and are proven to be non-carcinogenic (Chang *et al*., 2005).

Roselle extracts also possess anti-microbial activity (Nizar *et al*., 2013). The desirable qualities of the natural pigment from roselle make it a suitable substitute for the carcinogenic amaranth pigment (Frimpong, 2007) employed in pharmaceuticals. The red pigment from roselle calyx has therefore been recommended to be used in place of the natural colour obtained from amaranth. In addition to pigmentation pattern and growth habit, roselle demonstrates wide variation in many plant parts including sepal, calyxes, capsule, and leaf morphology.

2.2.2.2. Calyxes, epicalyxes, and capsule

The sepals of roselle are accrescent and give rise to calyxes which occur in two forms. Essentially, these forms, upon close examination of their calyxes, as well as interviews with the indigenes of Northern Ghana reveal subtle differences between variety *sabdariffa* and variety *altissima*. Variety *sabdariffa* possesses long or short succulent, edible calyxes that may be open, converge with or without overlapping with a small opening at the apex which descends to the receptacle. This space facilitates easy detachment of the calyxes from the capsule. In contrast, variety *altissima* calyxes are inedible, short, fibrous, dry or fleshy, round, and, firmly attached to and completely cover a round capsule without an overlap. Unlike in variety *sabdariffa*, detachment of the calyx from the capsule is difficult and this is the main distinguishing feature. Epicalyxes of roselle range from seven to ten in number in both varieties and are often small and dry, but may be prominent and succulent in some variety *sabdariffa* genotypes.

The mid-ridge of each sepal may be glanded or nonglanded. Capsule may vary between normal ovoid, in which the long and sharply tapering calyx covers the entire capsule

having a narrow base, or a round capsule with a broad base with short sepals which do not cover the entire capsule. The capsule may reach a maximum size of 5 cm long and 5.3 cm wide (Brink and Escobin, 2003).

2.2.2.3 Leaf morphology

The most variable feature of roselle is the leaf shape and venation colour, whereas a serrated leaf margin is consistent throughout the species. Leaf shape may be entire or lobed. The entire leaves may be ovate, linear, lanceolate, or elliptical, while the lobing pattern may be tri-, penta- or hepta-lobed to various depths (Morton, 1974). The diversity in morphology of roselle may be governed primarily by mutation and environmental influence over many generations. Plate 2.3 presents images of types of roselle plants in West Africa.

Features		Red	Intermediate	Green
A	Entire broad			
B	Pentalobed broad			
C	Pentalobed slender			
D	Partial trilobed and broad			

Plate 2.3. Roselle plants at four months after planting, demonstrating diversity in leaf form and plant colour. (A) Entire broad leaves; (B) Pentalobed broad leaves; (C) Pentalobed slender leaves; (D) Partial trilobed and broad leaves; Source: Coffie *et al*., (unpublished).

2.2.2.4. Flower and anther description

The colour of roselle flower depends on the plant type. Usually pigmented plant types have pink, yellow or buff petals with crimson throat, while green types have yellow flowers and yellow throat. Anther and pollen may either be brown in red types and all other pigmented varieties or may be yellow in green varieties (Basu and Chakravarty,

1972). However, some red varieties with yellow anthers have been identified (Basu and Chakravarty, 1981; Webster, 1914).

2.3.3 Roselle varieties

On the basis of general structural appearance, three edible roselle varieties, grown in the Philippines are described in the literature, namely, *Rico*, *Victor* and *Archer* **(**Paull *et al*., 2008; Morton, 1974). *Rico* is a dwarf type which spreads at low height above the ground. The leaves are tri-lobed and are borne for a long period of time. Flower has crimson throat and golden-yellow anthers. Mature calyx size can be up to 5 cm long and 3.2 cm wide. The bracts are plump and stiffly horizontal. It has the highest yield of calyx per plant. The juice and preserves of the red calyx and herbage is richred.

The „Victor" variety is tall reaching about 2.13 m, and is erect and robust. It bears (pink) flower with crimson throat and golden-brown pollen and long, slender calyxes which are pointy at the apex. It blooms somewhat earlier than *Rico*. The bracts are longer, slenderer and curved upward. The juice and preserves of *Victor* calyxes and herbage are rich-red. „Archer" is a roselle variety also known as *White Sorrel*. The plant is as tall and robust as *Victor* but has green stems, yellow flower with deep yellow throat and pale-brown pollen. The calyx is green or greenish-white and smaller in size than those of *Rico* and *Victor*, but the yield per plant is much greater. Juice and other products of *Victor* are nearly colourless to amber. It is believed to be of the race *albus*. Variety RT768 is a red dwarf bushy type with long succulent, edible calyxes, whereas HS4288 is a tall green pigmented high fiber *altissima* type having less branching and inedible thin calyxes both of which grow in India (Basu and Chakravarty, 1971).

In Senegal, there are about 9 varieties with over 51 accessions that are cultivated. The most common among the nine are three green types, namely *Mame Diarra*, *Bambara* or *Five Fingers*, *Ordinaire* and four red types, commonly called *Vimto*, *Koor*, L28, and

VF (Diouf *et al*., 2007b). Four of these varieties are described in Table 2.2. McClintock (2004) also reported of an unnamed tall and woody variety cultivated for fiber in Senegal. The major characteristics of the red varieties over the green ones include their good vegetative performance, high anthocyanin and nutrient contents, and their acidic taste (McClintock and El Tahir, 2004).

Table 2.2. Local taxonomy and morphology of *H. sabdariffa* produced in Senegal.

Variety	**Description**
„Mame Diarra“	Leaves: edible, green and entire Calyx: edible and green
„Bambara“ or „Five Fingers“	Leaves: edible green and deep lobed Calyx: edible and green
„Koor“	Leaves: edible and red veined Calyx: edible and red
„Vimto	Leaves: edible and red veined Calyx: edible and red

Source: Diouf *et al*. (2007b).

2.3 CULTIVATION

2.3.1 Propagation and culture

Roselle is a photoperiodic plant that thrives at elevation of up to 900 m.a.s.l. at temperatures ranging between 18 ºC and 35 ºC and annual rainfall of about 182 mm (Mizukami *et al*., 1989, 1988), though it can also do well with minimum rainfall of 100 -150 mm/month during vegetative growth. Roselle requires 13 hours daylight and relatively drier periods during vegetative stage but less than 12 hours daylight for flowering (Morton, 1974). Roselle thrives in a wide range of soils including deep, fairly fertile and sandy loam (Mizukami *et al*., 1989,1988) but the best soil for optimum productivity is retentive friable loams (Morton, 1974).

Roselle is usually propagated by seed but also grows readily from stem cuttings, which rather produce short stems and low yield (Morton, 1974). An essential aspect of

planting roselle is the achievement of optimum intra-row spacing for vegetable or fiber types. As a leafy vegetable, seeds are either, broadcast or sown directly with 3–5 seeds per hole, 2–3 cm deep, at an average spacing of 40–60 cm in rows and 60–90 cm between rows, whereas for calyx production, intra-row spacing should be wider, up to 100 cm apart. There are reports of contrasting though significant effect of plant population on pheno-morphological characters of roselle. At intra-row spacing of 10 and 20 cm, vegetable roselle, produced short plants, fewer branches, and fewer number of calyces per plant compared to 30 and 40 cm spacing (El Naim *et al*., 2012). In contrast, at a much wider spacing of 70 cm compared to 30 and 50 cm spacing, roselle plants were shorter with more branches and larger number of fruits per plant, while lower plant spacing of 30 cm promoted higher fruit weight and overall yield (Shalaby and Razin, 1989). An intra-row spacing variation of 10, 20, 30 and 40 cm did not have significant differences in number of days to flowering and number of days to physiological maturity (El Naim *et al*., 2012; 2010), although plants would typically flower and reach physiological maturity earlier with increased planting density (Alessi *et al*., 1977). In contrast, El Naim and Ahmed (2010) stated that spacing had no effect on number of days to flowering and days to physiological maturity.

The variations in flowering and physiological maturity responses of roselle to changes in plant spacing appear to arise from genotype differences. Reduced intra-row spacing up to 10 and 20 cm favoured increased calyx yield per plant (El Naim *et al*., 2012). For best calyx yield, spacing of 50 cm is required while for increased plant height in fiber types, spacing of less than 50 cm is recommended. Planting fiber types closer together results in tall plants of 3 to 5 m high, with few branching (ref) . Roselle may also be nursed and transplanted at about the fourth week after germination. Seeds of roselle usually germinate within 2-3 days after planting.

2.3.2 Growth and development

Roselle is an annual/perennial self-pollinating (Vaidya, 2000) shrub which has two growth phases: vegetative phase and flowering and fruiting phase. The vegetative growth period lasts from 4 months to 6 months. Flowering requires a short day length and can begin at 2 months after planting or latest by 7 months (McClintock and El Tahir, 2004). Depending on day length, some varieties may flower between 20 to 24 days after planting (Coffie *et al.*, unpublished). Following pollination, flowers turn pink and wither (compare to Table 2.1 last column) at the end of the day. The calyx then begins to enlarge, becomes fleshy, crisp but juicy around the capsule and fruits mature in two or three months after pollination (McClintock and El Tahir, 2004; Morton, 1974).

2.4 Nutritional information

The edible parts of roselle consist of varying nutritional composition with the seeds having the highest protein, carbohydrate, fat, and B vitamin content, whereas the calyxes are rich in vitamin C and the leaves possess abundant vitamin A, calcium and iron (Leung *et al.*, 1968). Nutritional composition of roselle calyxes, seeds and leaves are presented in Table 2.3. The dried red calyxes contain organic acids, sugars and anthocyanin pigments. They are high in citric, malic and ascorbic acids. Roselle seed oil has properties similar to cotton seed oil and contains linoleic, oleic, palmitic and stearic acids as major fatty acids. Also present in the seed oil are some unusual fatty acids, such as epoxy oleic acid and the cyclopropene acids, sterculic and malvalic acid. The seed proteins consist predominantly of globulins (McClintock and El Tahir, 2004).

Table 2.3. Nutritional composition of the edible parts of *H. sabdariffa* L. in 100 g of plant part

Nutritional value	**Leaves**	**Red calyx**	**Green calyx**	**Seed**
Ash Content (g)	7.50	12.24	6.83	6.89
Fat Content (g)	6.30	2.01	2.17	21.60

Crude Fiber (g)	12.04	4.69	6.75	4.12
Protein Content (g)	46.56	4.71	6.45	31.02
Moisture content (g)	12.08	7.60	6.24	9.25
Carbohydrate (g)	15.79	68.75	71.56	36.37
Sodium (mg)	46.98	96.66	48.1	ND
Potassium (mg)	84.11	49.35	49.59	ND
Calcium (mg)	110.16	12.65	21.58	6.6
Magnesium (mg)	120.09	38.65	47.54	ND
Iron (mg)	21.84	3.22	3.37	ND
Zinc(mg)	15.43	12.22	16.28	ND
Manganese (mg)	6.14	2.39	5.61	ND
Nickel (mg)	10.35	1.78	3.57	ND
Phosphorus (mg)	36.91	36.30	15.05	6.8
Ascorbic acid (mg)	-	16.67	12.50	ND

ND = Not determined; Source: Mehdi *et al*. (2013); Asaolu *et al*. (2012)

2.5 Economic importance

Roselle finds uses as food, fiber, source of pigment, and a preventive and therapeutic agent in folk medicine. Throughout West Africa, the tender stem, leaves and calyx of the green varieties of both *sabdariffa* and *altissima* are used as vegetable, especially in Senegal, Cape Verde, Ghana and Nigeria (McClintock *et al*., 2004; Obodai, 2007). The red types are often used in manufacture of sherbets and drinks under a brand name, *sooboro* (Ghana) or z*oborodo* (Nigeria), „*drink of the pharaohs*" (Egypt) and *da bilenni* or *bissap* (Mali, Senegal, Burkina Faso, Cote D"Ivoire and Togo) (McClintock, 2004; Diouf *et al*., 2007a; Personal Communication). Worldwide, roselle"s tart flavour and dyes of the red types, as well as its rich pectin content (Khatun, 2004) are exploited for preparation of beverages, jams and jellies, ice cream, cakes, confectioneries, and exotic teas (Plotto, 2004; Akindahunsi and Olaleye, 2003). In Nigeria, a condiment by name „mungza ntunsa" and „daddawa" is made from fermented roselle seeds (Falusi, 2007; Balaami, 1998). The red-pigmented anthocyanin-rich fraction of the calyx is used as food dye (Wong *et al*., 2002) and a colourant for alcoholic beverages and wine

(Plotto, 2004). Studies are underway for its use as a safe colourant in paediatric cough syrup and expectorant preparations (Frimpong, 2014).

2.5.1 Medicinal uses of roselle

Wong *et al*. (2009) reported of high antioxidant properties of roselle leaves in terms of total phenolic content expressed as 523 mg gallic acid equivalent/100 g and an overall radical-scavenging activity of 351 mg ascorbic acid/100g. Roselle has marked physiological activity (Wong *et al*., 2002) due to its high content of the anti-oxidative anthocyanin principles of the red calyx such as delphinidine-3-sambutanol, cyanidine3-sambubioside and other phenolic compounds. Prenesti *et al*. (2007) reported high phenolic content of roselle petals which were directly related to the antioxidant activity (Tsai and Ou, 1996). Roselle calyx extracts are consequently used effectively in folk medicine for the treatment of high blood pressure (Wahabi *et al*., 2010; Mozaffari-Khosravi *et al*., 2009; Faraji and Tarkhani, 1999; Haji and Haji, 1999; Onyenekwe *et al*., 1999) The mechanism of effectiveness of the calyx extract in hypertensive conditions as being the inhibition of calcium-influx (Hopkins *et al*., 2013).

Other medicinal properties of roselle include lowering the risk of cardiovascular and liver diseases (Ali *et al*., 2003), inflammatory diseases (Dafallah and al-Mustafa, 1996), reduction in serum cholesterol level and protection from atherosclerosis (Gurrola-Díaz *et al*., 2010; Tsai *et al*., 2002), reduction in hyperlipidemia, and its efficacy in reduction of low-density lipoprotein oxidation (Alarcón-Aguilar *et al*., 2007; Lin *et al*., 2007; Chang-Che *et al*., 2004). The antioxidant principles of roselle anthocyanins offer protection through their effect on low-density lipoprotein and lecithin-liposome systems (Meyer *et al*., 1997; Satué-Gracia, 1997). The anticarcinogenic activity and other beneficial effects of anthocyanins in the treatment of diseases has been demonstrated (Tamura and Yamagami, 1994; Igarashi *et al*., 1989). Chen *et al*. (2003)

showed that sour tea extracts reduce serum triglycerides, cholesterol, low-density lipoprotein cholesterol (LDLc) and LDLc/HDLc in
hyperlipidaemic rats.

Roselle extract is inhibitory against growth of microorganisms such as *Candida glabrata*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis* and *C. tropicalis* (Rukayadi *et al*., 2008; Oboh and Elusiyan, 2004), demonstrates uricosuric effect and could be effective in the treatment of hyper-uricemia in gout disease (Vitoon *et al.*, 2008).

The flower and fleshy fruits of roselle are used to relieve symptoms of bronchitis and coughs (Omobulajo *et al*., 2000). Other physiological activities of the calyx extracts include antiscorbutic, emollient, diuretic, sedative and anti-pyretic effects (Akindahunsi and Olaleye, 2003). The flowers contain substantial quantities of flavonoids and proanthocyanidins, plus analgesic and spasmolytic activities (Salah *et al*., 2002; Dafallah and al-Mustafa, 1996).

2.5.2 Bast fiber of roselle

In addition to leaf and calyx, the stem of roselle produces a type of fiber known as bast fiber, which include the fibers of sisal (*Agave sisalana*), jute (*Corchorus oliotorus* and *C. capsularis*), kenaf (*Hibiscus cannabinus*), hemp (the high growing industrial variety of *Cannabis*), and flax (*Linum usitatissimum*) (Chhabilendra, 2009). Bast fiber is obtained from the phloem or skin of the stem of these plants, hence also named phloem or skin fiber. Both botanical varieties of roselle are reported to yield fiber, however, variety *altissima* is preferred in Ghana, India, Nigeria, and South America (Eltayeib and Elaziz, 2014) due to its high main stem which yields longer length fibers (personal communication with Alangeya).

Roselle fibers are strong cellulose bundles of fibers which form about a third of the weight of the plant. In the stem, the bundles of fiber are layered with pectin and calcium

hence the need to process after harvest to remove the pectin, the xylem, and epidermis, in a process known as retting. Retting involves steeping roselle stems for about 14 days (Mwasiagi *et al*., 2014; Thiruchitrambalam *et al*., 2010; personal communication with Alangeya) to promote growth of microorganisms with

pectinolytic activity, after which the fibers are peeled off from the stem and dried in the shade. The dried fibers which measure about 1 - 1.5 m are soft, lustrous, cream or silvery-white in colour. Roselle bast fibers find uses in carpets, automobile and airplane upholstery, baskets, bags, cocoa bean sacs, floor mat and ropes.

Demand for bast fibers has increased in recent years owing to stringent environmental regulations and restrictions on non-biodegradable synthetic fibers (Padmaja *et al*., 2014). The regulations advocate for total replacement or at least a natural-synthetic fiber composite of which roselle fiber is being explored as reinforcement material for fiber composites (Razali *et al*., 2015; Reem *et al*., 2012; Nirmal *et al*., 2011; Favaro, 2010). Bast fiber offers advantages over synthetic fibers by being inexpensive, renewable, biodegradable and non-abrasive (Ishak *et al*., 2013; Jawaid and Abdul Khalil, 2011). The direct advantages of roselle fibers compared to other bast fiber sources are specific strength, availability, light weight, ease of separation, high toughness, low density, good thermal properties (Mwasiagi *et al*., 2014; Singha and Thakur, 2007). The availability of roselle in northern Ghana is abundant and the plant sprouts spontaneously after the first rains (Personal Communication with Alangeya).

A recent study among Kenyan roselle revealed that the strength of roselle fiber is comparable to the lignocellulolytic fiber obtained from kenaf (Mwasiagi *et al*., 2014), but the cellulosic content and tensile strength decreases with age (Razali *et al*., 2015). Although African countries like Nigeria, Kenya, Cote D"Ivoire, Sudan, Uganda and Ghana grow roselle for variety of reasons (Falusi *et al*., 2014; Mwasiagi *et al*., 2014;

Sie *et al*., 2009; Obodai, 2007), the commercial value of the crop as bast fiber source has either dwindled or received little attention (Mwasiagi *et al*., 2014; www.sydneyabugri.com; Personal communication with Alangeya). Currently, roselle is underutilized as after harvesting the edible calyxes and leaves the remaining plant biomass, has no use other than firewood for cooking. In the past, a composite of roselle, kenaf and jute fiber cultivated by a number of state farms and local farmers was processed into cocoa sacs and other fiber articles by the defunct Kumasi Bast Fiber Factory of Ghana at a capacity of 6,000 metric tons/year (www.sydneyabugri.com; Personal Communication with Alangeya).

Cultivation and processing of roselle in Ghana was an important economic activity from 1960 to 1991. Irrespective of the enormous local benefit obtained from roselle as a fiber commodity and its high industrial and export potential, Ghana inexplicably failed to maintain and keep this all important factory (www.sydneyabugri.com). Ghana currently imports bast fiber sacs from Bangladesh, Niger and Cote D"Ivoire for packaging of cocoa beans for export (www.sydneyabugri.com; Personal communication with Alangeya). In 2015, the twenty-six year old dormant Kumasi Fiber Bag Factory was reopened under the name Jute Mills Ghana limited (gbcghana.com; ghheadlines.com; myjoyonline.com) to partly meet the present demand for fiber bags.

2.6 International trade

In market terms, the most important parts of the plant are the deep red dried calyxes and the bast fiber. In Mexico, Egypt, Sudan, Senegal, Mali, Chad, China and Malaysia, large quantities of calyxes are produced for export to U.S.A. and Europe (McClintock, 2004; McClintock and El Tahir, 2004) for industrial and domestic

purposes. However, the most popular variety of dried roselle is „El Rahad‟ of Sudan, which is considered of superior quality worldwide (McClintock and El Tahir, 2004). Beside the quantities processed for domestic use, Sudan exported a total of about 18,531 metric tons of dried roselle calyx, with total income of USD17.59 million in 2011 alone (Ibrahim *et al*., 2013).

The sale of roselle for vegetable is also an important source of income for women and girls in Ghana, Senegal, and Mali (Obodai, 2007; Diouf *et al*., 2007b; McClintock, 2004). Roselle fiber has long been commercially exploited in Asian countries like China, Thailand, Philippines, and India (Mohammed *et al*., 2012). Currently, world leading producers and exporters of roselle are India (1,567,000 metric tons of jute and Mesta per year), Bangladesh (800,000 metric tons in 2003), China (300 million metric tons in 2005) and Thailand (McClintock and El Tahir, 2004). Interestingly, Nigeria, Egypt, Cote D‟Ivoire, Kenya, Sudan, Burkina Faso, Senegal, Niger and Mali are also on the world list of bast fiber producers (The World Fact Book. Retrieved on 06 January 2016).

2.7 Genetic resources and germplasm collection

Crop conservation, improvement and effective utilization are fundamental to the survival of species, economic growth and assurance of food and job security of any nation. Effective conservation relies on germplasm collection and characterization, just as crop improvement depends heavily on availability of large variability among accessions. The various forms of characterization, including genetic, physicochemical, and functionality contributes to effective germplasm identification and utilization.

Records of H. *sabdariffa* collections and characterization include 126 accessions in Sudan, (El-Gabri and El-Tahir, 2013), 95 accessions in the United States and 75

accessions in India (McClintock and El Tahir, 2004). In West Africa, genetic characterization of roselle includes evaluation of 4 accessions in Senegal (Diouf *et al*., 2007a), assessment of 124 accessions from Niger (Bakasso *et al*., 2013), and collection and documentation of 60 accessions in Nigeria (Daudu *et al*., 2015). Regrettably, no documentation on roselle germplasm collection, conservation, characterization and genetic diversity estimation in Ghana is available. Considering that roselle has become a cash crop for its leaves, calyxes and natural fiber with a potential of creating jobs and becoming an adaptive leafy vegetable in current era of climate anomalies, there is urgent need to embark on collection and characterization to provide information for its conservation management, for crop improvement via breeding. In the same vein, its hardiness in recent climate anomalous conditions of Africa necessitates characterization to reveal potential leafy vegetable genotypes for adoption. Most importantly, reports of little variability among the evaluated accessions imply challenges in breeding for crop improvement; hence researchers have resorted to mutation breeding. However, the drawbacks of mutation breeding in roselle, such as serious physiological damage to seedling height upon treatment with gamma radiation distorted shapes of leaves and flowers and compacted internodes on treatment with colchicines limits the application of mutation breeding in roselle (Osman *et al*., 2011; Harding and Mohammad, 2009; Mohammad *et al*., 2005). For these reasons, collection and characterization of the West African accessions of roselle with the aim of identifying variability has become more important than ever.

2.8. Genetic diversity studies in *H. sabdariffa*

Among the *H. sabdariffa* species, genetic variation within and among the fiber types (var. *altissima*) is known (Haque *et al*., 2007; Cheng *et al*., 2004; Menzel and Wilson, 1964). Studies on characterization and/or assessment of genetic diversity in roselle include evaluation of 16 accessions from Russia, Italy, Thailand and France (Siepe *et*

al., 1997), evaluation of 16 Egyptian genotypes (Ibrahim and Hussein, 2006), examination of both variety *sabdariffa* and variety *altissima* from Burkina Faso and Korhogo, Cote D"Ivoire (Sie *et al*., 2009). Other important works include determination of relationships among 12 roselle genotypes in Mexico (Torres-Morán *et al*., 2011), characterization of 45 accessions cultivated in the Guerrero State of Mexico (Alarcón Cruz *et al*., 2013), determination of population structure of 124 accessions from Sudan (Mohammed and El-Gabri, 2013) and characterization of six and 124 roselle genotypes from Niger State of Nigeria (Bakasso *et al*., 2013; Falusi *et al*., 2014). Notable is the work done by Louis *et al*. (2013) on genetic variability and the combining ability of agronomic traits among six Nigerian roselle cultivars.

The basis of characterization of edible roselle included stem colour, leaf shape and calyx characteristics, plant height, branch number, leaf length, leaf width, number of balls/fruits per plant, calyx yield, seed weight. It is common to find information on the genetic variability of *altissima* as part of a structuring involving kenaf and its allied crops (Sie *et al.* 2009; Cheng *et al*., 2002; Siepe *et al*. 1997). Studies on diversity in roselle fiber types in India was carried out by Satyanarayana and Sai (1995) yet, the only report on genetic diversity in the var. *altissima* alone in Africa is that of Mwasiagi *et al*. (2014) in which the quality of bast fiber extracted from the stems a Kenyan roselle accession (*L24*) was evaluated. Parameters considered in characterization of roselle fiber types include plant height, basal diameter, height at first branching, number of branches, number of internodes, bark thickness, dry weight per plant, dry stick weight per plant, and fiber yield and weight of stem.

Besides the use of pheno-morphological characterization for crop identification and genetic diversity estimation, application of molecular methods to genotyping and determination of relationships among roselle accessions have been conducted, such as studies on six Malaysian roselle genotypes by random amplified polymorphic DNA

(RAPD) analysis (Yusof and Saud, 2009) and characterization of twelve roselle genotypes by means of Inverse Sequence-tagged Repeat (ISTR) markers in Mexico (Torres-Morán *et al*., 2011). In addition, Bakasso *et al*. (2014) assessed genetic diversity in 124 *Hibiscus sabdariffa* using amplified fragment length polymorphism (AFLP) fingerprinting while Abou El-Nasr *et al*. (2014, studied 3 selected Sudanese roselle lines using inter-sequence simple repeat (ISSR) markers.

2.9 Methods of estimation of genetic diversity

The primary method of characterization of species was by agromorphological means (Farooq and Azam, 2002; Stadler, 1929) in which plants were described and grouped by their differences in seed, plant architecture, yield components, and some compositional or functional property. The advantage of agro-morphological evaluation cannot be underestimated, in that it uncovers the differences that exist phenotypically in the nature of leaf, calyx, flower, stem, branching habit, growth habit, and plant height. This information is a prerequisite for any meaningful genetic structuring work, since it will serve as observable markers for which accurate and reliable information on the differences between the accessions or the species under consideration can be ascertained via molecular means. However, morphological assessments alone are non-reliable due to their late expression and low heritability

(Beyene *et al*., 2005), and influence of environmental factors and natural selection (Hartings *et al*., 2008). Morphological parameters also exhibit low rate of polymorphism.

Estimation of genetic variability by isozyme analysis has proven to be a better method for evaluation of genetic diversity applicable to many crops (Tanksley and Orton, 1983; Brown, 1979; Markert and Moller, 1959). Isozyme analysis is rapid, simple, and inexpensive. The isozyme proteins are codominant markers; hence offer the ability to

directly compare the magnitude and distribution of genetic diversity between different populations and species (Lu *et al*., 2001). Isozyme assay has been used successfully to investigate variation in *Hibiscus* species.

Alam *et al*. (2006) investigated the sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE) pattern in *H. cannabinus* and *H. sabdariffa* and showed that the pattern of acid phosphatase and peroxidase were no different while esterase banding differed between the two species, an indication of descent from a common ancestor. Rizk and Soliman (2014) performed electrophoresis analysis of seed protein isozymes to identify genetic relationship among six taxa of Malvaceae crops including *H. sabdariffa*. Their study revealed that among the taxa, *H. sabdariffa* had the highest banding, characterized by four, three, and two loci of peroxidase, acid phosphatase, and esterase, respectively.

Protein and isozyme markers have a disadvantage of requirement of different protocol for each isozyme system, as well as difficulty in automating the method hence it is limited in evaluation of genetic diversity (Farooq and Azam, 2002). Molecular markers have been extensively used to identify genetic and phylogenetic relationships among taxa because they are automatable, relatively simple, rapid and easy to use. The availability of co-dominant markers offers accurate genotyping of accessions over dominant markers. Additionally, molecular markers offer the ability to compare data from different populations separated by time and space. Advantages of molecular markers include their immunity to environmental influence, high heritability (100 %), and their ability to distinguish differences in genotypes at all stages of plant growth and development; inexpensive and automatable compared to morphological and isozyme analysis (Farooq and Azam, 2002; Rafalski *et al*., 1996; Winter and Kahl, 1995).

The DNA-based molecular markers are designed to detect genetic polymorphisms, hence variation in species. The polymorphisms, detected by the polymerase chain reaction (PCR) consist of two types, detection based on DNA hybridization as found in restriction fragment length polymorphisms (RFLPs) (Botstein *et al*., 1980) or simply PCR-based DNA markers, such as Random Amplified Polymorphic DNA (RAPD) (Williams *et al*., 1990), AFLP (Vos *et al*., 1995), and microsatellites or simple sequence repeats (SSRs) (Pejic *et al*., 1998). Other molecular markers include sequence characterized amplified regions (SCARs) (Xu *et al*., 2001; Naqvi and Chattoo, 1996), sequence tagged sites (STS) (Perry and Bousquet, 1998; Olson *et al*., 1989) and single nucleotide polymorphism (SNPs) (Guerra and Yu, 2005; Collins *et al*., 1998).

2.10 RAPDs as molecular markers

The RAPD technique is a PCR-based method which employs DNA sequences of single, short and arbitrary oligonucleotide primers to detect polymorphisms in anonymous amplification products. The technique does not require prior knowledge of a DNA sequence, is less expensive and requires less sophisticated equipment. Application of RAPD in structuring and/or identifying roselle genotypes include the work of Barik *et al*. (2006), who included two varieties of roselle in characterization of some *Hibiscus* species. Yusof and Saud (2009) also worked on six genotypes of roselle as part of an investigation on some members of the *Hibiscus* family, while Omalsaad *et al*. (2014) worked on nine genotypes of roselle using RAPDs.

2.11. Measures of genetic diversity

Measures of genetic diversity determine variation and relationships within and among populations and/or individuals on the basis of some metric traits, allele frequencies or presence or absence data. Its application permits reliable identification of genotypes,

their classification, and determination of relationships among sets of genotypes (Mohammadi and Prasanna, 2003). Typically, a combination of data sets from pedigree information (Bernardo, 1993; Messmer *et al*., 1993), passport data, morphological data (Smith and Smith, 1992; Bar-Hen *et al*., 1995), biochemical data from seed storage proteins and isozyme analysis (Lu *et al*., 2002; Hamrick and Godt, 1997; Smith *et al*., 1987), as well as DNA-based marker data are employed.

The type of population, that is, inbred lines, hybrids, pure lines, or germplasm accessions and whether they are in Hardy-Weinberg equilibrium also determine the kind of genetic diversity measure to use. Moreover, it is important to apply statistical theory to the genetic data analysis to compensate for the unknown sampling distribution and reduce the sampling error associated with the data (Weir, 1990; Brown and Weir, 1983).

The key elements of measures of genetic diversity are, (i) allelic richness, which expresses polymorphism in distinct genotypes, and (ii) frequency of genotypes or alleles (Frankel *et al*., 1995). The richness of alleles is sensitive to sampling error but can be improved by evaluating a large number of loci (Brown and Weir, 1983). A polymorphic locus is one for which the most common allele has a frequency of less than 0.99. A monomorphic gene is one with a frequency of 1.00. An allele is rare if its frequency is less than 0.005 (Hartl and Clark, 1997). Measure of evenness, which is less affected by sampling error associated with rare alleles, constitutes average observed heterozygosity, expected heterozygosity, and effective number of alleles (Mohammadi and Prasanna, 2003). Heterozygosity refers to the proportion of heterozygous loci in a population and is directly related to the degree of polymorphism as a variety of alleles there are, the larger the fraction of heterozygous individuals. In a

quantitative sense, the degree of polymorphism is measured by two distinct parameters, heterozygosity and its variance (Nei and Rouchoudhury, 1974).

2.12 Genetic distance

The quantitative measure of genetic divergence at the allelic level between species or populations within a species which have descended from a common ancestor is referred to as genetic distance (Beaumont *et al*., 1998). Populations with small genetic distance have close genetic relationship while those with large genetic distance are more distant. Various distance measures are available depending on the kind of data, viz., interval or metric data obtained from morphological evaluations, allele frequency data from isozyme or DNA amplification products, and presence or absence data. The distance coefficient may be expressed in similarity or dissimilarity terms, where dissimilarity is 1.0 minus similarity. An extensive review of genetic distance measures is provided by Sokal and Sneath (1963), Sneath and Sokal (1973), and Mohammadi and Prasanna, 2003).

Common distance measures employed in interval data are Euclidean, Gower‟s, and correlation coefficient. Euclidean distance between two individuals is given by the square root of the sum of all squares of pairwise differences between two individuals, X and Y, having morphological measures (i) where i = 1....., p. The distance d_{xy} between X and Y is shown in Equation 2.1.

$$d_{xy} = [(x_1 - y_1)^2 + (x_2 - y_2)^2 + \ldots + (x_p - y_p)^2]^{1/2} \quad \ldots\ldots\ldots 2.1$$

With multivariate data, the correlation coefficient distance measure provides a powerful estimation of genetic distance as standardization removes the adverse size effect (Rohlf, 2009). Unlike Euclidean distance which is based on a metric character, Gower‟s genetic distance (Gower, 1971) coefficient estimates distance on both qualitative traits,

where a match between two individuals is scored as 0 and a mismatch is assigned 1, and quantitative traits which are calculated as the difference in trait value divided by the overall range of trait.

Distance measures associated with allele frequency data include Nei‟s (1972) distance, Roger‟s distance, Cavalli-Sforza and Edward‟s (1967) arc and chord distances. Mohammadi and Prasanna (2003) give a detailed account of the various distance measures for frequency data. For binary data derived from molecular markers, four measures of genetic distance are often employed, viz., Nei and Li‟s (1979) coefficient GD_{NL}, also known as DICE coefficient, the Modified Roger‟s distance GD_{MR} (Wright, 1978), Jaccard‟s (1908) coefficient GD_J, and simple matching coefficient GD_{SM} of Sokal and Michener (1958) of Equations 2.3, 2.4, 2.5, and 2.6, respectively. In these equations, X_{00} expresses the number of bands absent in both individuals; X_{01} represents the number of bands present in individual *j* only; X_{10} is the number of bands present in individual *i* only; and X_{11} represents the number of bands present in both individuals *i* and *j*. The Simple Matching and Modified Roger‟s are examples of Euclidean distance measures. The formulae for estimation of the genetic distances of a binary matrix data are presented in equations 2.3, 2.4, 2.5, and 2.6.

$$GD_{NL} = 1 - \left(\frac{2X_{11}}{2X_{11} + X_{10} + X_{01}}\right) \quad \text{....2.3}$$

$$GD_{MR} = \sqrt{\frac{X_{10} + X_{01}}{2l}} \quad \text{....2.4}$$

$$GD_J = 1 - \left(\frac{X_{11}}{X_{11} + X_{10} + X_{01}}\right) \quad \text{....2.5}$$

$$GD_{SM} = 1 - \left(\frac{X_{11} + X_{00}}{X_{11} + X_{10} + X_{01} + X_{00}} \right) \quad \ldots.2.6$$

The choice of a genetic distance measure for RAPD data in current study was based on presence-absence scoring data.

2.13 Multivariate techniques for interpretation of genetic distance

Regardless of population size, genetic distance among accessions is better visualized by application of various multivariate statistical techniques that analyse relationships among accessions and traits and group them into clusters on the basis of their genetic distance from multiple measurements on individual operative taxonomic units (genotypes, accessions, etc.). The most common multivariate techniques include cluster analysis, principal component analysis or principal coordinate analysis and multidimensional scaling (Brown-Guedira *et al*., 2000; Thompson *et al*., 1998; Johns *et al*., 1997; Melchinger, 1993).

2.13.1 Cluster analysis

Cluster analysis, a multivariate technique which groups individuals on the basis of some similarity in their characteristics, was developed by Hair *et al*. (1995). Members within a cluster exhibit internal homogeneity while members in different clusters would show heterogeneity. A plot of the individuals would usually reveal that similar members are closer in space while different members would be far apart.

Two methods of cluster analysis are often employed in genetic data analysis, namely, the distance-based cluster algorithm which employs either hierarchical or nonhierarchical approach to display a tree or dendrogram (Johnson and Wichern, 1992). Other methods are the maximum likelihood estimation and Bayesian methods of Pritchard *et al*. (2000). Mohammadi and Prasanna (2003) compared the most used

hierarchical tree-producing cluster method to the less commonly used nontreegenerating non-hierarchical methods. The agglomerative hierarchical algorithm proceeds by sequentially combining genotypes on the basis of some similarity into various groups.

Of the most commonly used clustering methods, viz., minimum (single linkage), maximum (complete linkage), average distances (Unweighted Pair Group with Arithmetic Means, UPGMA (Sneath and Sokal, 1973; Panchen, 1992), and Ward‟s minimum variance (Ward, 1979), the UPGMA method is preferred due to its accuracy and consistency with heterotic and pedigree data (Kantety *et al*., 1995; Peeters and Martinelli, 1989; Rohlf and Wooten, 1988; Sokal, 1986).

A major weakness of the UPGMA clustering method is its sensitivity to unequal evolutionary rates. The UPGMA cluster method was employed to group 18 populations of *H. sabdariffa*, *H. rosa sinensis* and *H. schizopetalus* into two main clusters (Barik *et al*., 2006), 23 accessions of kenaf and 2 roselle into 3 main clusters (Cheng *et al*., 2004), and 16 accessions of roselle and kenaf into 2 main clusters (Omalsaad *et al*., in 2014). Other important works on UPGMA analysis include clustering of 126 Sudanese roselle accessions into two major groups (El-Gabri and El-Tahir, 2013), 47 Mexican roselle accessions into two main groups (Alarcón Cruz *et al*., 2013) and grouping of 12 varieties of *Hibiscus sabdariffa*, *Hibiscus cannabinus*, *Hibiscus radiatus*, *Hibiscus acetosela* into 2 main clusters (Alam *et al*., 2006).

2.13.2 Principal Components Analysis

The quantitative or binary data sets of agromorphological and molecular marker evaluations are often multivariate. The multi-dimensional property of multivariate data often presents difficulty in multivariate data analysis. Various methods of analysis have been developed to analyze such data including principal component analysis (PCA),

cluster analysis, factor analysis, discriminant analysis, canonical correlation analysis, logistic regression, and others. Johnson and Wichern (2007) and Wilks (2006) give an extensive review of various analysis techniques for multivariate data. In this study, the principal component analysis (PCA) was the primary technique employed in analysis of the agromorphological and binary multivariate data.

The concept of PCA was developed by Pearson (1901) in the field of Social Sciences and later developed independently by Hotelling (1933). PCA was applied in the field of genetics in the classical text of Menozzi *et al*. (1978) and Cavalli-Sforza *et al*. (1993, 1994) on human population genetics. PCA is now one of the major tools used in genetic diversity studies. Application of PCA to study the genetics of *H. sabdariffa* includes works of Bakasso *et al*. (2014), Alarcón-Cruz *et al*. (2013), Torres-Morán *et al*. (2011) and Sie *et al*., 2009.

In PC analysis the typically large standardized variance-covariance data matrix (also the correlation matrix) of $n \times p$ dimensions (where n is number of individuals, p is the number of response variables) undergoes a vector-space linear transformation. The transformation converts the data set into a new uncorrelated coordinate system of few linear combinations, known as principal components (PC), which carry a very large proportion of the variance contained in the original data set (Johnson and Wichern, 2007; Wilks, 2006; Jolliffe, 2002; Richardson, 2009). In effect, PCA reduces dimensionality in a data set, hence is a data reduction technique which conserves both the key characteristics of the original and the transformed data in terms of the proportion of the variance they carried. The output of the analysis expresses a sequential arrangement of the PCs in terms of the proportion of variance carried into first or highest proportion of the variance as PC1, followed by the second as PC2, third PC3, and so on.

Processing of PCA data produces three important parameters, viz., eigenvalues, eigenvectors, and scores, whose magnitudes express the dominant characteristics embedded in the original data. Eigenvalues represent the variance, while eigenvectors (positive or negative) stand for the spatial loadings that are important in each PC.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant material and collection

A well planned collection of *Hibiscus sabdariffa* accessions has so far not been carried out in Ghana; hence seeds were not readily available at the Plant Genetic Resource Centre, Bunso. In this work, *H. sabdariffa* collection was carried out in 2010 and 2011. To obtain a representative collection, thirty-nine accessions were collected from 8 regions belonging to 7 countries in West Africa including Bawku and Upper West (Ghana), Ouagadougou (Burkina Faso), Lome (Togo), Cote D"Ivoire,

Munga (Nigeria), Mali, and Senegal, where many roselle types are commonly grown. The collection was facilitated through a chain of local traders from the individual regions who assembled roselle seeds from local farmers as well as open markets. The seeds though presented as mixtures were then sorted on the basis of origin and morphological similarities in a preliminary field trial and stored in brown paper bags and transported to the Kwame Nkrumah University of Science and Technology,

Kumasi. The accessions were then evaluated in field trials in 2011 and 2012 in Kumasi, Ghana, to determine phenotypic diversity and classify the landraces into groups for further evaluation. Table 3.1 shows the origins of the accessions.

3.2 Location of experimental site

All accessions were grown in field trials at two locations in Kumasi Metropolis in the Ashanti region of Ghana, namely, the Kwame Nkrumah University of Science and

Technology

Table 3.1. The origins of the 39 *Hibiscus sabdariffa* accessions used in current study showing the assigned collection codes and country of origin.

	Accession	Collection Region	Country	Longitude	Latitude
1	HS01	Munga	Nigeria	8.66°E	11.75°N

2	HS02	Cote D"Ivoire	Cote D"Ivoire	4.03ºW	5.31ºN
3	HS04	Lome	Togo	1.22ºE	6.13ºN
4	HS08	Mali	Mali	8.15ºE	12º00'N
5	HS09	Ouagadougou	Burkina Faso	1.53ºW	12.35ºN
6	HS11	Upper West	Ghana	0.85ºW	10.78ºN
7	HS13	Munga	Nigeria	8.66ºE	11.75ºN
8	HS14	Ouagadougou	Burkina Faso	1.53ºW	12.35ºN
9	HS16	Mali	Mali	8.15ºE	12º00'N
10	HS19	Ouagadougou	Burkina Faso	1.53ºW	12.35ºN
11	HS20	Bawku	Ghana	0.23ºW	11.05ºN
12	HS22	Ouagadougou	Burkina Faso	1.53ºW	12.35ºN
13	HS24	Ouagadougou	Burkina Faso	1.53ºW	12.35ºN
14	HS25	Ouagadougou	Burkina Faso	1.53ºW	12.35ºN
15	HS27	Bawku	Ghana	0.23ºW	11.05ºN
16	HS29	Ouagadougou	Burkina Faso	1.53ºW	12.35ºN
17	HS30	Ouagadougou	Burkina Faso	1.53ºW	12.35ºN
18	HS32	Ouagadougou	Burkina Faso	1.53ºW	12.35ºN
19	HS41	Ouagadougou	Burkina Faso	1.53ºW	12.35ºN
20	HS48	Mali	Mali	8.15ºE	12º00'N
21	HS50	Ouagadougou	Burkina Faso	1.53ºW	12.35ºN
22	HS56	Ouagadougou	Burkina Faso	1.53ºW	12.35ºN
23	HS58	Lome	Togo	1.22ºE	6.13ºN
24	HS59	Cote D"Ivoire	Cote D"Ivoire	4.03ºW	5.31ºN
25	HS65	Ouagadougou	Burkina Faso	1.53ºW	12.35ºN
26	HS68	Mali	Mali	8.15ºE	12º00'N
27	HS69	Bawku	Ghana	0.23ºW	11.05ºN
28	HS70	Ouagadougou	Burkina Faso	1.53ºW	12.35ºN
29	HS75	Munga	Nigeria	8.66ºE	11.75ºN
30	HS77	Ouagadougou	Burkina Faso	1.53ºE	12.35ºN
31	HS78	Upper West	Ghana	0.85ºW	10.78ºN
32	HS81	Senegal	Senegal	17.44ºW	14.69ºN
33	HS82	Ouagadougou	Burkina Faso	1.53ºW	12.35ºN
34	HS83	Cote D"Ivoire	Cote D"Ivoire	4.03ºW	5.31ºN
35	HS84	Ouagadougou	Burkina Faso	1.53ºW	12.35ºN
36	HS85	Senegal	Senegal	17.44ºW	14.69ºN
37	HS86	Mali	Mali	8.15ºE	12º00'N
38	HS87	Upper West	Ghana	0.85ºW	10.78ºN
39	HS88	Lome	Togo	1.22ºE	6.13ºN

(KNUST) Agricultural Experimental Station, Anwomaso, from April to August 2011 and from March to July 2012 and also at the Horticultural Science Research field of

KNUST from September 2011 to January 2012. Anwomaso station is located at latitude 6º 41´ 28.4″N and longitude 1º 30´ 58.8″ W at 300 m.a.s.l. Soil texture is oxisol well-drained sandy loam with pH 5.2 and organic matter content of 1.8 % (Experimental site survey, 2010, CSIR, 2010).

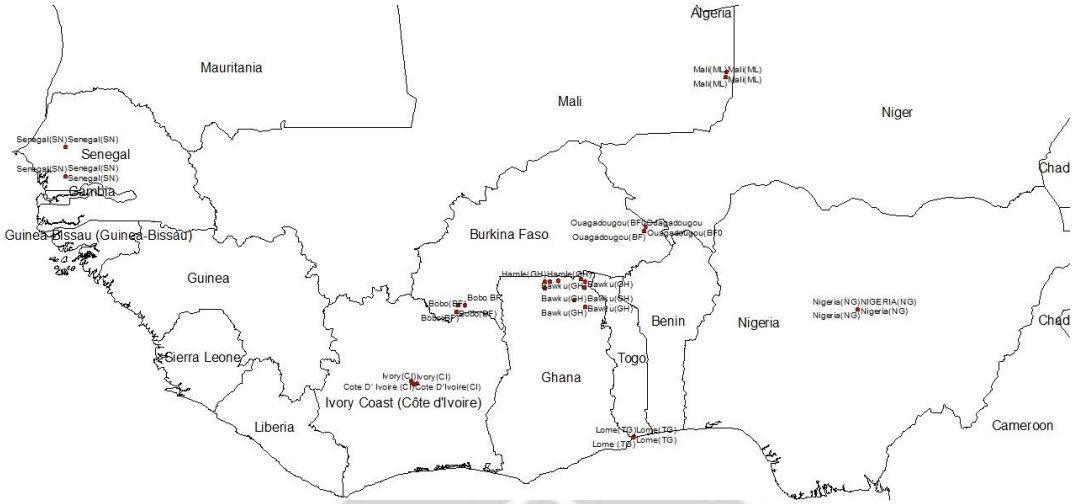


Figure 3.1. Schematic map of West Africa. The position of capital cities is indicated with open circles. Collection sites are indicated with closed circles.

Environmental conditions in Anwomaso, a semi-deciduous forest zone, include heavy rains beginning in March with a short dry period in August, followed by minor rains which start in September and end in November. Mean annual rainfall is 1500 mm and average temperature is 25 ºC throughout the year.

The Department of Horticulture field site lies on latitude 6º 43´ and 6º 45´N and longitude 1º 25‟ and 1º 36‟W. It is within the moist semi-deciduous belt. The soil is sandy loam with slight acidic reaction. It is of the series of Auroso Orchrosols. The land was previously cropped with vegetables such as okra and cabbage. This site is characterized by a bi-modal rainfall distribution with peaks in June and September. The first and second growing seasons typically last from late March to mid-July and from mid-August to the end of November, respectively, separated by a short dry spell of about four weeks in July. The major dry season starts mid-November and lasts till the

end of February or mid-March. The vegetation cover is characterized by nut grass (*Cyperus rotundus*) and Guinea grass (*Panicum maximum*).

3.3 Land preparation, planting and experimental design

Land preparation involved ploughing and harrowing, followed by application of Round Up Ready (glyphosate, 360 g/L) applied at 5.0 L/ha and Gramoxone (Paraquat) applied at 3.5 L/ha for pre-emergence weed control. All entries were planted in a randomized complete block design with three replications. Experimental plots consisted of 6 m × 0.6 m row containing 8 to 12 plants per plot. Plots were separated by 1.0 m alley and blocks were separated by 2 m. Planting density was 20,000 plants/ha. Recommended crop management techniques were applied. Irrigation was applied regularly as needed. Fertilizer equivalent to 120:60:40 kg ha^{-1} of N-P_2O_5-K_2O was applied at 14 days after planting. Post-emergence weeds were controlled with Atrazine (4.5 L ha^{-1}) and hand weeding with a hoe. The pests, cabbage fly (*Delia radium*) and cotton stainer (*Dysdercus superstitious and Dysdercus parasiticum*) were controlled using Conpyrifos 48 % (1-1.5 L ha^{-1}) and Cymethoate Super (1-1.5 L ha^{-1}) and 100 g/L alpha-cypermethrin (1 L ha^{-1}). Irrigation was applied regularly as needed.

3.4 Data collection

Data collection was started at physiological maturity (90 to 120 days after planting) Accessions were evaluated for twenty-six qualitative traits on 5 competitive plants and for six quantitative traits on 8 to 12 competitive plants per row. The qualitative traits included stem characteristics (pigmentation, pubescence, branching, and growth habit), leaf characteristics (shape and pigmentation of petiole, leaf blade, as well as leaf margin and pubescence), calyx characteristics (pigmentation, shape, length, pubescence), epicalyx (size and pubescence, stipule, flower characteristics (bud, petal and throat pigmentation, pollen colour and capsule shape). The quantitative traits consisted of

plant height, height at the first branch from soil level, number of branches, leaf area derived from leaf length and width, basal diameter, and number of internodes. Measurements were taken with meter rule, micrometre screw gauge, and Vernier calliper, as appropriate. Table 3.2 shows the various traits, their definition, and the method of scoring. In all, 1,020 plants were evaluated.

3.5 Morphological data analysis

Morphological variability in the qualitative data was evaluated by calculating frequencies and percentages of plants exhibiting each trait by means of PROC FREQ option. On the quantitative data, means, standard deviation, minimum and maximum values were calculated using PROC MEANS. Analysis of variance was computed with PROC GLM in which random effects were the accessions and fixed effects were replications and blocks within replications. Table 3.3 shows the analysis of variance and expected mean squares (EMS) for extracting the variance components. All computations were carried out using SAS 9.3 (SAS Institute, Cary, NC, 2011).

Table 3.2. List of 32 morphological descriptors used in characterization of *H. sabdariffa* accessions evaluated in July to January 2011 and August to January 2012 in Ghana

	Phenotypic data	**Definition (units)**
1	Stem colour	Predominant colour of stem (uniformly green=0; intermediate green=1; uniformly brown=2; uniformly red= 3 dark red=4)
2	Stem pubescence	Characteristics of the surface (glabrous/absent =0; pubescent/present=1)
3	Stem pubescence type	Evenness of surface of stem (smooth=1; rough=3; prickly=5)
4	Branching habit	Branching habit (Weak=1; intermediate=3; strong=5)
5	Plant height	Height of plant from ground level (short=1; intermediate=3; tall=5)
6	Growth habit	Shape, appearance, or growth form (nonbushy=1; bushy=5;
7	Plant type	Predominant colour of plant (green=1; intermediate=3; red=5
8	Leaf lamina colour	Colour of leaf blade and veins (green=1; dark green=3; green pigmented=5; red=7)
9	Leaf margin colour	Colour of leaf margin (green=0, red=1, dark red=3; others=4
10	Leaf size	Size of leaf (slender=1; broad=5)

11	Leaf shape	The shape of leaves (entire=1; partially-lobed=3; deeplylobed=5)
12	Leaf lobe type	Number of lobes (no lobe=1; trilobed =3; pentalobed=5)
13	Leaf pubescence	Absent=0; present=1
14	Petiole colour	Predominant colour of petiole (green=1; intermediate=2; brown=3; red=4; dark red=5)
15	Stipule colour	The pigmentation of the stipule (green=0; green pigmented=1; brown=2; red=3; dark red=4)
16	Calyx pigmentation	Predominant colour of calyx (green=1, intermediate=3; red=5, dark red=7)
17	Calyx pubescence type	Nature of the surface of calyx (smooth=1; hairy=3; Rough=5; prickly=7)
18	Calyx texture	Texture of calyx (succulent=0 dry=1)
19	Epicalyx colour	Pigmentation of the epicalyx (green=1; green pigmented=3; red=5; dark red=7)
20	Epicalyx texture	Texture of epicalyx (succulent=0; dry=1)
21	Bud pigmentation	Colour of bud (green=1; green pigmented=3; red=5; dark red=7)
22	Petal colour	Colour of the petiole (pale yellow or straw=1, yellow=3, pink=5; magenta=7)
23	Throat colour	Colour of throat (yellow=1; crimson=3)
24	Pollen colour	Colour of pollen (yellow=1; brown=3)
25	Capsule shape	Shape of capsule (ovoid=0; intermediate=1; round=2)
26	Fruit size	Size of fruit (small=1; intermediate=3; large=5)
27	Plant height (PH)	From soil level to growing point on main stem (cm)
28	Height at first branch (HA)	Measured from soil level to first branch (cm)
29	Branch number (BN)	Number of branches determined by counting
30	Leaf area (LA)	Area of leaf calculated from product of leaf length and leaf width of the 4th matured leaf from the apex (cm^2)
31	Stem width (SW)	Diameter of stem below first node (cm), also described as basal diameter
32	Number of internodes (NI)	Number of internodes

Table 3.3. Analysis of variance for obtaining variance components

Source	df	Mean square	Expected Mean Square
Year	y-1	M_y	$\sigma^2_e + r\sigma^2_{gy} + g\sigma^2_{r(y)} + rg\sigma^2_y$
Rep (year)	$y(r$-1)	M_{ry}	$\sigma^2_e + g\sigma^2_{r(y)}$
Genotype	g-1	M_g	$\sigma^2_e + r\sigma^2_{gy} + ry\sigma^2_g$
Gen*Year	(y-1)(g-1)	M_{gy}	$\sigma^2_e + r\sigma^2_{gy}$
Error	$y(g$-1)(r-1)	M_e	σ^2_e

Genotypic and phenotypic variance components were calculated from the linear functions of the mean squares, where *g*, *y* and *r* are numbers of genotypes, year, and replicates, respectively.

$\sigma^2_e = M_e$ = error variance component $\sigma^2_g = M_g = (M_g - M_{gy})/ry$ = genotypic variance component $\sigma^2_y = \{(M_y + M_e)-(M_{ry}+M_{gy})\}/rg$ = variance component associated with year $\sigma^2_{gy} = (M_{gy} - M_e)/r$ = variance component associated with $g \times y$ $\sigma^2_{r(y)} = (M_{ry} - M_e)/g$ = variance component associated with replication within year

The standard errors of the variance components were calculated from the method of Hallauer and Miranda (1981). The approximate variance, *V*, of a variance component, $\hat{\sigma}^2_i$, is determined as

$$V(\hat{\sigma}^2_i) = \frac{2}{f^2} \frac{(f M_i)^2}{df_i + 2} \quad \ldots\ldots (1)$$ (Snedecor, 1956).

where, *V* = variance Snedecor (1956) demonstrated that if the variance component was computed from a linear function of independent mean squares, *f* = is the coefficient of the component of variance f_i = is the degrees of freedom of the respective mean squares

$\sigma_i = \sigma 1$

M_i = are the mean squares used to determine the component of variance

Broad sense heritability (H^2), the proportion of the total variance due to genetic effects was calculated as

$$H^2 = \frac{\sigma^2_g}{\sigma^2_p} = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_e} \quad \ldots\ldots (2)$$ (Doolittle, 1987).

$$\sigma^2_{Pg} + \sigma^2_e / r$$

where σ_g^2 is the genotypic variance,σ_e^2 is the error variance, and *r* is the number of replications.

The standard error of this heritability was approximated with the equation of Hallauer and Miranda (1981) as:

$$SE\,H^2 = \frac{SE(\sigma^2_g)}{\sigma^2_P} \quad \ldots\ldots (3)$$

where $SE(\sigma^2_g)$ is the square root of the variance of (σ^2_g) and the denominator is the phenotypic variance (Knapp *et al*., 1985; Knapp, 1986). All computations for heritability were carried out by means of PROC MIXED option of SAS by means of the Restricted Maximum Likelihood Estimation method for generation of variance components. The genotypic and phenotypic coefficients of variation were estimated as

$$GCV = \frac{100(\sigma_g)}{\bar{X}} \quad \ldots\ldots (4)$$

$$PCV = \frac{100(\sigma_P)}{\bar{X}} \quad \ldots\ldots (5)$$

where σ_G and σ_P are the genotypic and phenotypic standard deviations, respectively, and $\bar{X}$ is the population mean of the trait under consideration.

The means for each trait were then standardized to avoid the influence of different scale of measurements in different traits on the data and from this a trait by means data matrix was constructed for multivariate data analyses.

3.6 Pearson correlation coefficients and their standard error

Genotypic and phenotypic correlations were calculated between traits by considering accessions as random effects. Using the genotypic variance and covariance component estimates, the genotypic correlation between any two traits, *i* and *j* was estimated as:

$$r_{Gij} = \frac{\sigma_{Gij}}{\sigma_{GiGj}} \quad \ldots\ldots (6)$$

$$r_{Pij} = \frac{\sigma_{Pij}}{\sigma_{PiPj}} \quad \ldots\ldots (7)$$

where r_{Gij} and r_{Pij} are the genotypic and phenotypic correlation coefficients between traits *i* and *j*, respectively; σ_{Gij} and σ_{Pij} are the estimated genotypic and phenotypic covariances between traits *i* and *j*, respectively; σ_{Gi}, σ_{Gj}, σ_{Pi}, and σ_{Pj} are the genotypic and phenotypic standard deviations for traits *i* and *j*, respectively (Holland, 2006).

3.7 Assessment of relationships between genotypes

3.7.1 Distance measurements

Relationships between genotypes were assessed by calculating correlation coefficients on the trait by means data matrix. Correlation coefficients were employed because it standardized the data. The square of the correlation coefficient gave an estimate of genetic distance between accessions while the sign indicated the direction of the association. The squared value was used to calculate overall average genetic distance, as well as average distances within individual clusters.

3.7.2 Cluster analysis

Using the correlation distance matrix, cluster analysis was carried out to identify groups with similar morpho-agronomic and phenotypic characters. Cluster analysis identifies groups that are homogeneous as possible and heterogeneous among groups (Franco *et al*., 2001). The hierarchical cluster method of grouping employed was the Unweighted Pair Group Method with Arithmetic Average (UPGMA) to generate a dendrogram based on the distance coefficients. The adjustment between the distance matrix and the dendrogram was estimated by the cophenetic correlation coefficient, *r* (Sokal and Rolf, 1962).

3.7.3 Principal components analysis

A principal component analysis (PCA) was performed on the standardized variancecovariance matrix of the accession by trait data in order to depict non-hierarchical relationships among the genotypes and determine the traits that are most effective in discriminating between accessions. Through singular value decomposition, the eigenvectors (principal component coefficients), correlation coefficients, and eigenvalues (the loadings which explain relative proportions of the total variance) as well as cumulative proportions expressed sequentially by single traits were determined. Relationships between traits were investigated by means of graphing the principal components and generating 2D plots. All computations and plots were performed using the appropriate options of NTSYSpc 2.2 (Rolf, 2009).

3.8 Genetic diversity in *H. sabdariffa* by means of RAPD analysis

3.8.1 DNA isolation

Thirty-two of the thirty-nine West African accessions were investigated at the molecular level. About one centimeter square of young leaves from 10 plants of each field-grown *H. sabdariffa* accession were harvested under sterile conditions, bulked

and placed on ice and transported to the lab for storage at -80 ^{o}C until ready for use. Genomic DNA was extracted from *H. sabdariffa* leaf tissue using the CTAB procedure (Dellaporta *et al*., 1983) with minor modifications by the Cocoa Research Institute of Ghana.

Each bulked sample was ground into powder in liquid nitrogen. To 20 mg of the bulked sample was added 0.8 ml 2 % CTAB buffer (Appendix A1) incubated for 30 min at 65 ^{o}C in sand bath with intermittent vortexing. The mixture was centrifuged at 14,000 r.p.m. for 15 min and supernatant transferred into clean microfuge tubes. A 0.4 ml of ice cold isopropanol was added and centrifuged to pellet nucleic acids at 14,000 r.p.m. for 5 min. Pellets were washed twice with chloroform isoamyl alcohol (Appendix A2). The DNA pellet was washed with washing buffer (Appendix A3) and then with 80 % ethanol, air-dried and resuspended in 100 µl of 1× TE buffer with 100 mg/µl RNAse (Appendix A4). Gel electrophoresis was run on 1 % agarose gel to assess the quality and the quantity of the DNA obtained. The DNA was stored at -20 ^{o}C until required for primer amplification.

3.8.2 RAPD primer selection and amplification

A set of 12 random decamer oligonucleotides belonging to series A, B, and G were purchased from Operon Technologies Inc., Austria. Table 3.4 shows the names of primers and their sequence repeats. These were used as primers for the amplification of RAPD fragments. Amplification reactions were performed in 15 µl mix constituted from 0.6 µl of 1.25 mM each of dNTPs, 10 ng of the RAPD primer, 1× Taq polymerase buffer, 0.5 U

Table 3.4: Names of the 12 RAPD primers and their sequences.

	Name of primer	Primer sequence
1	OPA-03	AGTCAGCCAC

2	OPA-07	GAAACGGGTG
3	OPA-11	CAATCGCCGT
4	OPA-12	TCGGCGATAG
5	OPA-16	AGCCAGCGAA
6	OPA-20	GTTGCGATCC
7	OPG-05	CTGAGAGGGA
8	OPB-08	GTCCACACGG
9	OPB-09	TGGGGGACTC
10	OPH-13	GACGCCACAC
11	OPG-03	GAGCCCTCCA
12	OPAB-03	TGGCGCACAC

Taq polymerase and 10 ng genomic DNA. Amplification reagents were sourced from Bioneer, South Korea. Amplification was carried out in a thermocycler (Applied Biosystems, Singapore) programmed for a first cycle at 94 ºC for 5 min, 38 ºC for 1 min and 72 ºC for 2 min, followed by 44 cycles each at 94 ºC for 1 min, 38 ºC for 1 min and 72 ºC for 2 min, then one final extension cycle of 5 min at 72 ºC. Amplified products were loaded on 2.0 % agarose gel (Seakem, Cambrex BioScience Rockland Inc, U.S.A.) with 1× TAE buffer together with a 100 bp DNA ladder and stained with ethidium bromide. Electrophoresis was run at 120 volts for 120 min and gels were photographed under UV light with a UV Transilluminator (Uvitec, UK). The amplification product sizes were estimated from the 100 bp DNA ladder (Bioneer, South Korea).

3.8.3 Allele scoring and data analysis

Gel photographs were examined for presence (1) or absence (0) of bands. On the binary data the intra locus gene diversity was computed for each primer locus and similarity indexes were estimated by the Jaccard‟s coefficient (Jaccard, 1908). Cluster analysis was performed on the similarity coefficients using the UPGMA procedure

All computations were conducted with NTSYS 2.21c (Rohlf, 2009).

CHAPTER FOUR

4.0 RESULTS

This study seeks to evaluate the genetic diversity existing in Roselle (*Hibiscus sabdariffa* L.) by means of morphological variation and molecular genotyping. Thirtynine accessions originating from were eight regions in West Africa were evaluated. The largest number of accessions (17) was obtained from Burkina Faso (43.6%). Five accessions originated from Mali (13%), six from Ghana (15.4%), three each (7.7%) from Nigeria, Cote D‟Ivoire and Togo (total of 23.08%), and two from Senegal (5%). Morphological characterization based on qualitative and quantitative traits was carried out on 35 accessions whereas molecular characterization by means of RAPD fingerprinting was determined on 32 accessions.

4.1 Morphological analysis

4.1.1 Morphological description of qualitative traits

Substantial morphological variability was identified in the West African roselle accessions. Traits that were most variable were petiole colour, stipule colour and stem colour. Each of these traits was represented by 5 variants of colour shades with variable frequencies. Similarly, large variations in calyx shape and pigmentation, epicalyx, capsule, and leaf forms were identified. Plates 4.1A and B show images of a range of roselle genotypes in current study having varying fruit shape, epicalyx shape, leaf form, calyx shape and extent of coverage of capsule, calyx pigmentation, leaf and stem pigmentation. For stem colour, the distribution of frequencies ranged from 1.2% for intermediate green to 46.3% uniformly green. Frequencies in petiole colour were in the range of 5.0% (red) to 42.5% dark red, and for stipule colour 11.3% red to 41.3% dark red (Table 4.1). The least variabilities were detected in leaf pubescence, pollen colour and throat colour in which only two variants were present with one class being

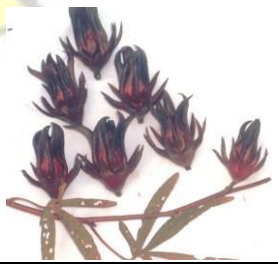
preponderant. Brown pollen, yellow throat, and absence of leaf pubescence were represented by 85%, 91.3%, and 90%, respectively, while the alternative traits were 15% for yellow pollen, 8.7% for crimson throat, and 10% pubescent plants (Table 4.1).

Among the 1,020 plants studied the predominant features were uniformly green and smooth stems with bushy growth habits and broad, trilobed leaves, lacking pubescence. The principal colours of the petals were yellow and pink. These had succulent, dark red calyxes with ovoid capsules. Plants which occurred in minor frequencies were intermediate green with prickly stems, extensive branching and intermediate plant height. These were also found to be nonbushy with red leaf lamina, slender, entire and pubescent leaves. Their calyxes were red, hairy and dry and possessed round capsules. El Tahir and El Gabri (2013) reported of predominantly green, entire leaves, pink petals and red calyxes among Sudanese roselle accessions

A			
	HS25	HS68	HS69
B			
	HS04	HS27	HS08
C			
	HS50	HS59	HS65

D			
	HS83	HS30	HS86
E			
	HS11	HS16	HS01

Plate 4.1. Pigmentation pattern of the calyx, stem and leaf of 15 out of the 39 roselle genotypes collected from seven countries in West Africa and evaluated in 2011 and 2012 in Ghana. Genotypes of panel A (*H. sabdariffa* var. *altissima*); Panels B to J are genotypes of *H. sabdariffa* var. *sabdariffa*. B, C and D (HS83) belong to race *bhagalpuriensis*; D (HS30 and HS86) and E, genotypes of the race *ruber*.

F			
	HS20	HS75	HS32
G			
	HS56	HS22	HS87
H			
	HS41	HS58	HS84

I			
	HS85	HS48	HS02
J			
	HS77	HS19	HS70

Plate 4.2 B. Pigmentation pattern of the calyx, stem and leaf of 15 out of the 39 roselle genotypes collected from seven countries in West Africa and evaluated in 2011 and 2012 in Ghana. Genotypes of panels F, G, H and I are race *ruber*; J (HS77 and HS19) belongs to race *albus* and HS70, a genotype of the race *intermedius*

4.1.1.1 Stem characteristics

In this investigation, five categories of stem colours were identified among the West African roselle accessions. Stem colour showed the widest variability with uniformly green stems accounting for 46.25%, dark red 37.5%, and intermediate green, uniformly brown and uniformly red together accounting for 16.3%. In contrast to this observation, the work of El Tahir and El Gabri (2013) displayed a relatively narrow variation in stem colour since red colour alone was responsible for 86.3% and all other colours accounted for only 13.7%. Three types of stem texture were identified. The predominant texture was smooth (56%) while the others were rough (34%) and very few plants (10%) were prickly (Table 4.1). El Tahir and El Gabri (2013) also observed a similar percentage distribution among 126 Sudanese accessions in which about 90% of the plants had smooth stem and about 10% were prickly. The trend in stem texture distribution may be attributed to the wider geographical origins of the accessions in the current study.

Alam *et al. (*2006) reported similar qualitative traits among four Hibiscus species (*H. sabdariffa, H. cannabinus, H. acetosella* and *H. radiatus*) from Bangaldesh.

Table 4.1. Qualitative description of *Hibiscus sabdariffa* accessions collected from eight countries in West Africa and evaluated in 2011 and 2012 in Ghana

No.	Trait		Description	Class	No. of plants	Percentage (%)
1	Stem	Colour	Uniformly green	0	37	46.3
			Intermediate green	1	1	1.2
			Uniformly brown	2	5	6.3
			Uniformly red	3	7	8.8
			Dark red	4	30	37.5
3		Stem texture	Smooth	1	45	56.3
			Rough	3	27	33.7
			Prickly	5	8	10.0
4	Plant	Branching habit	Few	1	1	1.25
			Intermediate	3	67	83.75
			Extensive	5	12	15.00
5		Height	Short	1	44	55.0
			Intermediate	3	14	17.5
			Tall	5	22	27.5
6		Growth habit	Bushy	1	62	77.5
			Non bushy	5	18	22.5
7		Plant type	Green	1	7	8.8
			Intermediate	3	35	43.8
			Red	5	38	47.5
8	Leaf	Leaf lamina colour	Green	1	43	53.8
			Dark green	3	14	17.5
			Green pigmented	5	22	27.5
			Red	7	1	1.3
9		Leaf margin colour	Green	0	22	27.5
			Red	1	12	15.0
			Dark red	3	28	35.0
			Others	4	18	22.5
10		Leaf size	Slender	1	26	32.5
			Broad	5	54	67.5
11		Leaf shape	Entire	1	12	15.0
			Partially lobed	3	29	36.3
			Deeply lobed	5	39	48.8
12		Leaf lobe type	No lobe	1	12	15.0
			Trilobed	3	29	48.8
			Pentalobed	5	39	36.3

13		Leaf pubescence	Absent	0	72	90.0
			Present	1	8	10.0
14		Petiole colour	Green	1	9	11.3
			Intermediate	2	21	26.3
			Brown	3	12	15.0
			Red	4	4	5.0
			Dark red	5	34	42.5

Table 4.1 cont"d

No.	Trait		Description	Class	No. of plants	Percentage (%)
15	Flower	Stipule colour	Green	0	11	13.8
			Pigmented	1	15	18.8
			Brown	2	12	15.0
			Red	3	9	11.3
			Dark red	4	33	41.3
16		Calyx pigmentation	Green	1	12	15.0
			Intermediate	3	27	33.8
			Red	5	10	12.5
			Dark red	7	31	38.8
17		Calyx pubescence type	Smooth	1	46	57.5
			Hairy	3	13	16.3
			Rough	5	19	23.8
			Prickly	7	2	2.5
18		Calyx texture	Succulent	0	59	73.8
			Dry	1	21	26.3
19		Epicalyx colour	Green	1	33	41.3
			Pigmented	3	12	15.0
			Red	5	8	10.0
			Dark red	7	27	33.8
20		Epicalyx texture	Succulent	0	31	38.8
			Dry	1	49	61.3
21		Bud pigmentation	Green	1	13	16.3
			Green pigmented	3	30	37.5
			Red	5	8	10.0
			Dark red	7	29	36.3
22		Petal colour	Pale yellow or straw	1	11	13.8
			Yellow	3	31	38.8
			Pink	5	27	33.8
			Magenta	7	11	13.8
23		Throat colour	Yellow	1	73	91.2
			Crimson	3	7	8.8
24		Pollen colour	Yellow	1	12	15.0
			Brown	3	68	85.0
25	Fruit	Capsule shape	Ovoid	0	56	70.0
			Intermediate	1	13	16.3
			Round	2	11	13.8

26		Fruit size	Small	1	26	32.5
			Intermediate	3	18	22.5
			Large	5	36	45.0

4.1.1.2 Plant characteristics

Variation in branching habit among the population was weak. About 83.75% of the total plant population showed intermediate branching, while fewer plants (1.25% and 15%) demonstrated few and extensive branching, respectively (Table 4.1). On the contrary, variability in plant height was fairly high as 55% of the population was found to be short, 17.5% intermediate and 22.5% were tall. Sufficient variability in growth habit was detected where 77.5% were non-bushy and 22.5% bushy. The results of El Tahir and El Gabri (2013) showed quite a different frequency pattern in a study of 126 roselle accessions from Sudan in which 10.7% of the plants were nonbushy erect, 48.9% were nonbushy compact, while 40.4% were prostrate habit. On plant type, a fairly wide variability was observed with 8.75% being green, 43.8% intermediate and 47.5% red.

4.1.1.3 Leaf characteristics

Generally, a wide variation was observed in all leaf traits except leaf pubescence in which 90% of the accessions lacked this trait (Table 4.1). Ample variation was observed in leaf margin colour for which 22.7% were green, 15% red, 35% dark red and 22.5% for others. Majority of the green leaves also had green margins, while all other leaf lamina colours had margins which bore the colour of the predominant pigmentation. El Tahir and El Gabri (2013) reported distribution of roselle leaf colours as 93.9% green and 6.1% reddish green, red and other colours. Majority of the leaves were broad (67.5%) and nonpubescent (90%). On leaf lobe type, 15% had no lobe, 48.75% were trilobed and 36.25% were pentalobed (Table 4.1).

4.1.1.4 Flower characteristics

Besides stipule colour, petal features, throat colour and bud pigmentation were described under flower characteristics of roselle. Dark red stipule colour was dominant (41.3%) followed by pigmented stipules (18%), brown colours (15%), green (13.75%), and red (11.3%). Four types of bud pigmentation were identified, namely, green, green pigmented red and dark red at 16.3%, 37.5%, 10%, and 36.3%, respectively. For petal colour, yellow and pink petals dominated with 38.8% and 33.8% respectively whereas straw and magenta petals were represented by 13.8% each. Throat colour had 91.3% yellow and 8.8% crimson, while pollen colour had 15% yellow and 85% brown.

4.1.1.5 Calyx characteristics

A wide diversity in calyx characteristics was detected with dark red pigmentation being the predominant calyx colour (38.8%). Many of the plants had intermediate calyx colours characterized by green background with a tinge of dark red or bright red. These types formed 33.8% of the entire population. Other calyx colours were uniformly red (10%) and uniformly green (15.0%) (Table 4.1). El Tahir and El Gabri (2013) recorded 43.8% red, 8.5% light red, 3.1% each for dark red and green (also referred to as white) and 41.5% for other colours.

On calyx pubescence type, 57.5% of the population was smooth, 23.8% were rough, and 16.3% were hairy, while 2.5% were prickly. However, for epicalyx colour, 41.3% of the accessions studied were green in colour, 33.8% were dark red, 15.0%, were pigmented and 10% of the total population were red. Calyx and epicalyx texture recorded 73.8% and 38.8%, respectively for the dry texture, and 26.3% and 61.3%, respectively for those with succulent texture.

4.1.1.6 Fruit characteristics

Substantial variation was recorded for both fruit size and capsule shape. However, fruit size showed a significantly wider variability than capsule shape. While for fruit size 32%, 22.50% and 45% were small, intermediate and large fruit size, respectively, the percentage scores for capsule shape were 70.0%, 16.3% and 13.8% for ovoid, intermediate and round, respectively.

4.1.2 Range, mean, standard error, standard deviation and coefficient of variation and variance of agro-morphological traits of *H. sabdariffa*

Table 4.2 shows the results of descriptive analysis of evaluation of the entire roselle accessions. A high phenotypic variability was observed for all six traits with a coefficient of variation ranging between 18.84 and 43.58%. The largest variabilities were observed for leaf area (43.58%) and height at first branching (36.71%) while the least variabilities were number of internodes (18.84%) and stem width (19.87%). These values represent substantial variability available in the accessions for exploitation for trait improvement via selection. Variations on plant to plant basis were recorded for plant height 26 to 144 cm with a mean of 76.52±21.98, leaf area 24.50 to 500.00 mm^2 with a mean of 178.14±77.64 mm^2, height at first branching 1.5 to 28 cm and mean of 8.90± 3.27 cm, branch number of 2 to 26 and mean of 12.72±3.52, stem width 5.95 to 25.98 mm with mean 15.05±2.99 mm, and number of internodes 9 to 34 with mean of 19.51±2.99 (Table 4.2).

Table 4.2. Range, mean, standard error, standard deviation and coefficient of variation of agro-morphological and phenotypic variations evaluated in 35 *Hibiscus sabdariffa* accessions collected from eight regions in West Africa and evaluated in Ghana in 2011 and 2012

No.	**Trait**	**Mean**	**SD**	**Min**	**Max**	**CV (%)**	**SE**	**Mean Square (Acc)**
1	PH (cm)	76.52	21.98	26.00	144	28.72	0.74	7,498.05***

2	LA (mm^2)	178.14	77.64	24.50	500.00	43.58	2.62	66,386.24***
3	HA (cm)	8.90	3.27	1.50	28.00	36.71	0.11	25.02***
4	BN	12.72	3.52	2.00	26.00	27.66	0.12	35.48***
5	SW (mm)	15.05	2.99	5.95	25.98	19.87	0.10	44.47***
6	NI	19.51	3.67	9.00	34.00	18.84	0.12	52.95***

PH = plant height; LA = leaf area; HA = height at first branching; HB = height at second branch; BN = branch number; SW = stem width; NI = number of internodes; ***P<0.001

4.1.3 Variability in plant height, leaf area, and fiber characteristics

On the basis of population, the mean square results for evaluation of the six traits (Table 4.3) were significantly different (P≤0.05) but not for all populations and all traits. Main effects of variety and environment were the most important sources of variation. Analysis of variance showed significant differences in variety mean squares in all traits but the differences varied across the regions. The most variable trait was leaf area, after which variability decreased in the order, plant height, number of internodes, stem diameter, and branch number. The least variable trait was height at first branching which was not significantly different (P>0.05) in any of the regions, except Bawku. All populations showed highly significant differences in plant height and leaf area except Cote D"Ivoire. Branch number, stem width and number of internodes were highly significantly different (P<0.01) only in Bawku, Mali, and Ouagadougou, but only significantly different (P<0.05) for Lome and Cote D"Ivoire populations. On the basis of mean squares, the most variable populations which demonstrated variability in all six traits were Bawku, followed by Mali, then

Ouagadougou. The population-specific variability revealed that besides Nigeria and Cote D"Ivoire populations, the Upper West, Senegal, Ouagadougou, Mali, Lome, and least of all, Bawku populations (mean

Table 4.3. Mean squares for within population differences in six traits measured on *H. sabdariffa* collected from West Africa

	Source	df	PH	LA	HA	BN	SW	NI
Bawku	Env	1	1,366.61***	3,083.43*	5.02	26.78***	5.72	104.79***
	Rep	2	0.45	346.57	0.53	0.00	0.98	5.06*
	Rep(Env)	2	0.46	2024.14*	0.89	1.70	7.78	3.07*
	Accession	2	3,438.52***	5,763.59**	17.72**	11.70**	17.39**	22.30***
	Env*Acc	2	319.22***	979.04	9.56*	11.13**	1.29	0.94
	Error	8	11.82	353.95	1.78	0.84	1.79	0.64
	R^2		0.99	0.88	0.81	0.92	0.81	0.97
	CV (%)		4.19	10.35	14.07	7.1	8.58	3.93
Upper West	**Source**	**df**	**PH**	**LA**	**HA**	**BN**	**SW**	**NI**
	Env	1	560.79**	1,529.85	11.86*	6.73	3.62	40.82*
	Rep	2	31.26	59.33	2.416	2.195	0.764	2.637
	Rep(Env)	2	30.37	576.66	3.53	3.04	0.26	0.56
	Accession	1	302.00*	25,341.52***	2.38	2.39	1.99	9.24
	Env*Acc	1	272.65*	10.59	1.52	5.09	14.57	6.70
	Error	4	19.42	554.22	1.28	4.34	2.06	1.94
	R^2		0.94	0.92	0.84	0.59	0.73	0.89
	CV (%)		6.41	13.40	12.37	16.9	9.69	7.26
Lome	**Source**	**df**	**PH**	**LA**	**HA**	**BN**	**SW**	**NI**
	Env	1	1,105.92***	2,788.14	16.87*	8.74	1.39	13.78
	Rep	2	4.33ns	317.38	4.18	1.29	2.14	1.23
	Rep(Env)	2	1.39ns	103.28	2.68	0.83	0.63	1.98
	Accession	1	1,243.05***	13,986.13**	1.07	5.148	8.39	29.79*
	Env*Acc	1	260.09**	19726.22**	7.38	2.49	6.75	12.87
	Error	4	9.39	35.66	1.12	1.8	1.68	3.59
	R^2		0.99	0.93	0.89	0.74	0.77	0.81
	CV (%)		4.78	15.65	11.99	10.29	8.88	9.76
Mali	**Source**	**df**	**PH**	**LA**	**HA**	**BN**	**SW**	**NI**
	Env	1	4,240.82***	4,476.27	0.29	28.60**	1.79	115.62***
	Rep	2	3.99	40.28	1.59	0.09	0.13	2.48
	Rep(Env)	2	0.85	528.90	2.27ns	8.39*	0.18	0.35
	Accession	4	2,709.78***	15,191.65***	5.28	30.55***	18.27***	28.79***
	Env*Acc	4	35.29*	143.95	3.28	2.22	8.48***	4.86
	Error	16	8.99	678.87	2	1.93	0.61	1.64
	R^2		0.99	0.87	0.57	0.85	0.92	0.91
	CV (%)		3.91	17.60	15.56	10.43	5.29	1.28
Nigeria	**Source**	**df**	**PH**	**LA**	**HA**	**BN**	**SW**	**NI**
	Env	1	61.50	122.62	0.26	0.84	0.003	5.11
	Rep	2	64.77	467.74	6.20	0.60	2.68	16.53*
	Rep(Env)	2	3.09	1,267.54	5.66	1.96	0.95	1.74
	Accession	1	1,105.28**	1095.38	11.51	1.95	1.59	6.50
	Env*Acc	1	60.00	3459.50	14.63	3.17	2.65	5.11
	Error	4	24.5	761.21	2.79	1.18	1.37	1.33

	R^2		0.93	0.73	0.82	0.7	0.68	0.91
	CV (%)		7.11	16.02	18.68	9.2	7.62	5.94
Ouagadougou	**Source**	**df**	**PH**	**LA**	**HA**	**BN**	**SW**	**NI**
	Env	1	4,530.19***	5,3569.59***	22.84**	110.64***	8.57*	386.53***
	Rep	2	14.04	1,223.80	1.21	3.21	3.26	4.457
	Rep(Env)	2	14.58	536.19	2.64	4.03	1.05	4.85
	Accession	16	1,746.35***	18,095.16***	2.56	6.90*	12.15***	15.55***
	Env*Acc	16	274.27***	5,631.77***	4.09*	6.72*	3.57*	5.00***
	Error	64	13.27	554.73	2.21	3.19	1.66	1.84
	R^2		0.98	0.93	0.49	0.63	0.72	0.86
	CV (%)		4.91	13.47	16.58	13.99	8.41	6.86

Table 4.3 cont"d

Senegal	**Source**	**df**	**PH**	**LA**	**HA**	**BN**	**SW**	**NI**
	Env	1	139.97*	3,971.80	6.33	0.65	12.15	38.34*
	Rep	2	4.57	280.43	3.74	1.00	1.38	0.76
	Rep(Env)	2	6.44	303.26	4.81	1.00	6.03	6.57
	Accession	1	594.79**	21,307.27**	5.40	5.86	1.54	12.10
	Env*Acc	1	62.94	7,554.73	0.12	7.34	0.19	0.08
	Error	4	13.79	1,588.78	6.41	2.65	6.29	2.2
	R^2		0.94	0.84	0.53	0.63	0.53	0.88
	CV (%)		6.32	23.05	30.76	13.8	17.83	7.94
Cote D"Ivoire	**Source**	**df**	**PH**	**LA**	**HA**	**BN**	**SW**	**NI**
	Env	1	781.24**	8,846.72*	4.33	26.19**	24.93**	106.83**
	Rep	2	15.44	1,048.27	0.78	2.03	0.56	0.04
	Rep(Env)	2	1.049	668.74	4.71	1.32	0.49	1.29
	Accession	1	1,937.05***	1,115.33	4.07	0.29	12.69*	0.611
	Env*Acc	1	424.77**	7.78	26.67*	14.95*	17.47*	14.28
	Error	4	12.45	187.89	1.39	1.18	1.01	2.11
	R^2		0.98	0.94	0.89	0.91	0.93	0.94
	CV (%)		4.25	6.61	13.76	7.71	6.72	6.86

Acc= Accession; df = degree of freedom; *P<0.05; **P<0.01; ***P<0.001; PH = plant height; LA = leaf area; HA = height at first branching; BN = branch number; SW = stem width; NI = number of internodes.

Squares of 25,341.52 to 5,763.59; P<0.01) were good for leaf area. Furthermore, Bawku, Mali and Ouagadougou populations were good for fiber characteristics in that all three traits, branch number, stem width, and number of internodes were highly variable (P<0.01), whereas Lome, Nigeria and Upper West accessions were not outstanding for fiber characteristics (Table 4.3).

The genotype×environment interaction effect was inconsistent; it was important for many of the traits in Bawku, Ouagadougou, Lome, and Cote D"Ivoire populations but

not important for any of the traits in Nigeria and Senegal populations (Table 4.3). The results were consistent with the findings of Abou El-Nasr *et al.* (2014) who recorded significant main effects of genotypes (P≤0.05), environment (P≤0.01), and genotype×environment interaction (P≤0.01) for fifteen Sudan roselle genotypes.

4.1.4 Comparison of means of agromorphological traits on accession basis

On accession mean basis, plant height varied from 50.92 ± 13.16 cm in HS30 to 105.63 ± 14.18 cm in HS09, both of Ouagadougou (Table 4.4). The height of the tallest plant was not significantly different from that of HS69 (105.60 ±17.55 cm) of Bawku. Sixteen (45%) accessions were taller than the overall mean height of 76.52 cm (Table 4.2) and were considered as potentially tall genotypes. Important members of the tall plants, besides HS09 and HS69 with height exceeding 90 cm, included HS02 (99.32 ± 21.75 cm), HS16 (95.91 ± 16.12 cm), HS14 (94.38 ± 20.88 cm), HS86 (93.84 ± 15.14 cm), HS32 (93.04 ± 18.44 cm), HS25 (91.80 ± 15.77 cm), HS20 (91.72 ± 14.12 cm), HS19 (91.09 ± 11.94 cm), and HS50 (91.04 ± 23.52 cm). Two dwarf types were identified, HS08 (53.00 ± 13.02 cm) of Mali and HS81 (51.67 ± 6.81 cm) of Senegal.

Mean leaf area varied from 58.08 ± 3.43 mm^2 to 279.37 ± 3.43 mm^2 in HS19 and HS30 of Ouagadougou, respectively (Table 4.4). With a mean of 178.14 mm^2, all accessions having leaf area below this value were considered to have narrow leaves. Six categories of leaf sizes were identified, very large (>270 mm^2, HS14 and HS19 of Ouagadougou), large (184 to 230 mm^2), intermediate (139-173 mm^2), small (103 -130 mm^2), and very small (<76 mm^2, HS08 and HS30). Besides HS30 and HS08, HS29 and HS48 also had very narrow leaves. Height at first branching (HA) ranged from 7.48 cm for HS22 of Ouagadougou to 11.42 cm for HS69 of Bawku (Table 4.4). On accession mean basis,

HA ranged from 7.48 cm for HS22 of Ouagadougou to 11.42 cm for HS69 of Bawku (Table 4.4).

Mean branch number ranged from 9.79 ± 2.02 in HS08 to 15.34 ± 3.73 in HS68 both of Mali. In general, three groups of branching types were identified, highly branched (13-15 branches), moderately branched (11 or 12 branches), and few branching types (9 to 10 branches). Important members of the highly branched group were HS68 (15.34 ± 3.73), HS86 (14.34 ± 3.82), HS09 (14.44 ± 2.68), HS16 (14.60 ± 3.37), HS19 (14.35 ± 4.72), and HS14 (13.74 ± 3.26). Of these, HS09, HS19, and HS14 were both highly branched and tall genotypes.

Roselle stem width ranged from 11.98 ± 2.82 in HS08 to 17.75 ± 4.49 mm in HS09. Besides HS09, eight other accessions had large stem width, exceeding 16.00 mm. These include HS50 (17.26 ± 2.65 mm), HS14 (16.63 ± 2.35 mm), HS19 (16.74 ± 2.56 mm), HS32 (16.56 ± 2.61 mm), HS20 (16.54 ± 2.69 mm), HS69 (16.61 ± 2.82 mm), HS68 (16.16 ± 2.80 mm), and HS25 (16.20 ± 2.56 mm). A large stem width is indicative of a thick phloem for high fiber yield. Among the 35 accessions, these eight genotypes could be exploited for fiber production on the basis of their large stem width.

Two broad categories of number of internodes were identified, the first group having 19-22 internodes and a second group with 16 to 18 internodes. Number of internodes ranged from 16.83 ± 2.51 in HS30 to 22.18 ± 2.96 in HS68. Other equally important accessions with large number of internodes are HS69 (22.09 ± 3.55), HS86 (22.03 ± 3.97), HS32 (21.96 ± 4.77), HS25 (21.74 ± 4.05), and HS14 (21.82 ± 4.95) and would be useful for fiber production.

Table 4.

4 Means of agromorphological traits evaluated on 35 *H. sabdariffa* accessions collected from eight regions of West Africa and grown in Ghana in 2010 and 2011

	Acc	**Origin**	**PH**	**LA**	**HA**	**BN**	**SW**	**NI**
1	HS20	Bawku	88.53^{de} 14.12	173.97^{efghij} 50.61	8.92^{bcd} 2.79	13.71^{abcde} 3.16	16.54^{abcd} 2.69	20.79^{abcdef} 3.58
2	HS27		55.50^{mnop} 11.62	159.26^{hijkl} 64.00	8.12^{bcd} 1.87	11.26^{ghi} 1.96	13.63^{hij} 2.68	18.30^{ghijk} 3.44
3	HS69		105.60^{a} 17.55	220.36^{bcd} 40.79	11.42^{a} 4.04	13.64^{abcdef} 3.67	16.61^{abc} 2.82	22.09^{ab} 3.55
4	HS02	Cote D"Ivoire	95.64^{b} 21.75	227.30^{bc} 85.28	9.16^{bcd} 3.84	14.23^{abc} 4.02	16.01^{abcde} 2.80	21.39^{abcd} 4.86
5	HS83		70.23^{i} 6.75	208.45^{bcdef} 62.59	7.99^{cd} 3.29	13.92^{abcd} 3.13	13.95^{fghij} 3.71	20.94^{abcde} 3.64
6	HS04	Lome	74.33^{ghi} 6.44	212.04^{bcde} 84.43	8.53^{bcd} 2.66	13.69^{abcde} 2.96	15.45^{cdefg} 3.24	21.00^{abcde} 3.24
7	HS58		53.98^{nop} 16.17	120.07^{nlm} 29.05	9.13^{bcd} 3.12	12.38^{bcdef} 3.54	13.78^{ghij} 1.82	17.85^{hijk} 3.32
8	HS08	Mali	51.99^{op} 13.02	75.30^{op} 12.92	8.12^{bcd} 2.54	9.76^{i} 2.02	11.98^{k} 2.82	18.55^{ghijk} 2.55
9	HS16		91.68^{bcd} 16.12	184.21^{defghi} 33.57	8.22^{bcd} 2.14	14.60^{ab} 3.37	15.47^{cdefg} 2.74	21.35^{abcd} 3.77
10	HS48		54.87^{nop} 13.69	115.11^{nm} 37.12	9.08^{bcd} 2.77	12.50^{bcdefg} 2.56	13.92^{ghij} 2.20	17.38^{jk} 2.58
11	HS68		89.89^{cde} 13.87	194.70^{cdefgh} 51.99	10.26^{ab} 4.34	15.40^{a} 3.73	16.16^{abcde} 2.80	22.18^{a} 2.96
12	HS86		94.61^{b} 15.14	168.89^{fghijk} 71.84	9.76^{bcd} 2.15	14.32^{abc} 3.82	15.91^{bcde} 2.87	22.03^{ab} 3.97
13	HS01	Nigeria	59.97^{klm} 2.45	178.35^{efghij} 53.68	9.92^{abc} 2.95	12.22^{cdefg} 2.23	15.76^{bcdef} 2.24	20.14^{abcdefg} 3.93
14	HS13		79.17^{f} 10.25	164.99^{ghijk} 25.36	7.96^{cd} 2.65	11.42^{fghi} 2.77	15.03^{cdefgh} 2.35	18.67^{ghijk} 1.94
15	HS09	Ouagadougou	105.63^{a} 14.18	161.36^{ghijk} 51.33	9.90^{abc} 4.22	14.44^{abc} 2.68	17.75^{a} 4.49	21.09^{abcde} 4.34
16	HS14		92.44^{bcd} 20.88	274.71^{a} 122.12	9.13^{bcd} 4.69	13.74^{abcde} 3.26	16.63^{abc} 2.35	21.82^{abc} 4.95
17	HS19		91.46^{bcd} 11.94	279.37^{a} 88.05	8.50^{bcd} 3.67	14.35^{abc} 4.72	16.74^{abc} 2.56	21.39^{abcd} 3.99
18	HS22		75.53^{fgh} 5.08	221.21^{bcd} 69.58	7.48^{d} 2.41	12.90^{bcdefg} 4.28	14.93^{cdefghi} 3.10	19.81^{cdefgh} 3.35
19	HS24		55.73^{mno} 6.25	139.31^{jklmn} 51.79	9.27^{bcd} 3.34	13.17^{bcdefg} 2.74	14.52^{efghi} 2.01	19.09^{efghij} 3.23
20	HS25		89.62^{cde} 15.77	150.21^{jklmn} 36.67	9.52^{abcd} 4.04	12.93^{bcdefg} 4.60	16.20^{abcde} 2.56	21.74^{abc} 4.05

21	HS29		55.56^{mnop} 12.13	120.65$_{lmn}$ 40.82	8.90$_{bcd}$ 2.86	12.42$_{bcdefg}$ 1.87	14.09$_{fghij}$ 2.24	17.92$_{hijk}$ 3.24
22	HS30		50.92^{p} 13.16	58.08^{p} 26.52	7.79$_{cd}$ 2.10	10.08$_{hi}$ 2.64	12.49$_{jk}$ 1.76	16.83^{k} 2.51
23	HS32		93.98$_{bc}$ 18.44	246.92^{ab} 87.19	8.13$_{bcd}$ 3.84	12.24$_{cdefg}$ 5.51	16.56$_{abcd}$ 2.61	21.96$_{ab}$ 4.77
24	HS41		61.93$_{jk}$ 18.55	165.47$_{ghijk}$ 64.96	9.49$_{abcd}$ 2.98	12.50$_{bcdefg}$ 2.68	15.38$_{cdefgh}$ 2.54	18.89$_{fghij}$ 2.94
25	HS50		86.65^{e} 23.52	229.97^{bc} 56.18	9.45$_{abcd}$ 3.02	13.53$_{abcdef}$ 3.44	17.26$_{ab}$ 2.65	21.06$_{abcde}$ 2.66
26	HS56		53.99^{h} 10.37	127.96$_{klmn}$ 38.16	9.23$_{bcd}$ 2.61	11.62$_{efghi}$ 3.90	14.59$_{efghi}$ 2.22	17.96$_{hijk}$ 3.20
27	HS65		57.06$_{lmn}$ 21.57	103.94^{no} 20.27	9.34$_{bcd}$ 2.65	11.61^{efghi}3 .77	13.98$_{fghij}$ 2.92	18.29$_{ghijk}$ 2.22

Table 4.4 cont"d

	Acc	**Origin**	**PH**	**LA**	**HA**	**BN**	**SW**	**NI**
28	HS70	Ouagadougou	74.23$_{ghi}$ 12.79	201.40$_{cdefg}$ 61.08	9.34$_{bcd}$ 2.20	12.91$_{bcdefg}$ 2.79	15.52$_{bcdefg}$ 2.23	19.16$_{efghij}$ 2.95
29	HS77		60.98$_{kl}$ 9.15	158.60$_{hijkl}$ 44.18	9.03$_{bcd}$ 3.47	13.06$_{bcdefg}$ 2.03	13.44$_{ijk}$ 2.32	19.45$_{defghi}$ 3.24
30	HS82		77.63$_{fgh}$ 17.49	190.70$_{cdefghi}$ 79.13	9.10$_{bcd}$ 3.62	12.19$_{bcdefg}$ 3.78	15.74$_{bcdef}$ 3.22	18.35$_{ghijk}$ 2.67
31	HS84		78.59$_{gf}$ 7.96	189.48$_{cdefghi}$ 48.12	8.59$_{bcd}$ 2.62	13.57$_{abcdef}$ 2.96	14.76$_{defgh}$ 1.87	20.89$_{abcde}$ 3.10
32	HS81	Senegal	51.74$_{op}$ 6.81	131.78$_{klmn}$ 49.89	8.90$_{bcd}$ 3.16	11.10$_{ghi}$ 2.50	13.72$_{ghij}$ 3.17	17.68$_{ijk}$ 2.69
33	HS85		65.82^{j} 9.03	230.13^{bc} 62.58	7.56^{d} 2.39	12.50$_{bcdefg}$ 3.59	14.43$_{efghi}$ 2.93	19.69$_{defghi}$ 3.57
34	HS11	Upper West	73.80$_{hi}$ 13.52	222.93$_{bcd}$ 61.18	8.71$_{bcd}$ 2.17	12.77$_{bcdefg}$ 3.41	15.21$_{cdefghi}$ 3.28	20.08$_{bcdefg}$ 3.60
35	HS78		63.76$_{jk}$ 8.32	131.32$_{klmn}$ 34.15	9.61$_{abcd}$ 2.95	11.88$_{defgh}$ 3.44	14.39$_{efghi}$ 2.26	18.32$_{ghijk}$ 2.33

Figures below the means represent standard deviations; PH = plant height; LA leaf area; HA= height at first branching; BN=branch number; SW=stem width; NI=number of internodes; *P<0.05; **P<0.01; ***P<0.001.

4.1.5. Comparison of means, standard deviation and mean squares of *Hibiscus sabdariffa* by origin

Analysis of variance was computed for trait differences by origin. The results of the mean squares of roselle populations from the eight regions showed significant differences (P≤0.05) except in plant height, leaf area, and branch number. Differences

Table 4.

in plant height were identified in only Cote D"Ivoire, Lome, and Mali genotypes. Similarly, differences in leaf area occurred in Cote D"Ivoire and Mali genotypes, whereas for branch number, differences were identified among Cote D"Ivoire, Nigeria, and Senegal genotypes. These results indicate en masse accession homogeneity in height at first branching, stem width, and number of internodes in roselle collection. An outstanding observation made was consistently highest values for plant height, leaf area, and branch number among the Cote D"Ivoire and Bawku accessions, while the Senegal accessions had least values for plant height and branch number (Table 4.5).

5 Means, standard deviation, minimum and maximum values of *H. sabdariffa* accessions

	N	PH	LA	HA	BN	SW	NI
Bawku	87	86.11±25.08^{a} (45-134)	185.74±57.45^{ab} (31.50-306.0)	9.74±3.46 (4-22.50)	12.64±3.17^{ab} (3.00-22.00)	15.60±2.99 (7.78-23.19)	19.95±3.79 (12.00-29.00)
Upper West	45	69.91±12.87^{abc} (51-100)	181.28±68.17^{ab} (68.80-342)	8.92±2.57 (5.00-15.00)	12.11±3.43^{ab} (3.00-19.00)	14.65±2.83 (8.40-22.21)	18.81±3.15 (14.00-17.00)
Cote D"Ivoire	60	85.60±21.61^{a} (45-135)	218.35±75.34^{a} (92-420.00)	8.43±3.70 (3.00-17.00)	13.61±3.60^{a} (5.00-24.00)	14.58±3.55 (7.95-21.85)	20.17±4.28 (13.00-30)
Lome	52	68.25±14.63^{bc} (26.00-89.00)	173.13±80.66^{ab} (84.00-345.80)	8.35±2.89 (3.00-15.00)	12.89±3.22^{ab} (5.00-20.00)	14.50±2.78 (8.62-21.23)	19.21±3.46 (9.00-28.00)
Mali	139	80.30±23.01^{ab} (28.00-141)	155.27±63.58^{b} (56-311.4)	9.22±3.2 (3.00-28.00)	13.44±3.74^{ab} (7.00-23.00)	14.88±3.05 (5.95-24.31)	20.20±3.66 (11.00-30.00)
Nigeria	24	69.38±12.54^{abc} (58.00-92.00)	172.23±42.77^{ab} (93.6-284.5)	8.96±2.92 (3.00-16.00)	11.79±2.47^{b} (8.00-17.00)	15.34±2.28 (10.68-21.21)	19.42±3.16(14.00-28.00)
Ouagadougou	436	75.92±22.12^{abc} (30.00-144)	178.25±85.32^{ab} (24.5-500.0)	8.86±3.32 (1.50-21.00)	12.55±3.59^{ab} (2.00-26.00)	15.28±2.92 (8.04-25.28)	19.35±3.67^{a}(12.00-34.00)
Senegal	46	60.37±11.36^{c} (41.00-93.00)	184.21±75.12^{ab} (32.5-311.6)	7.93±2.85 (3.00-16.00)	12.00±3.19^{b} (6.00-21.00)	13.85±3.01 (9.08-20.56)	18.27±3.22 (12-29)

PH = plant height; LA=leaf area; HA=height at first branch; BN=branch number; SW=stem width; NI=number of internodes; Means with common superscripts are not significantly different at 0.05 level.

4.2 Genetic parameters for predicting gain in roselle improvement

For the purpose of devising an efficient scheme for estimating genetic gain in breeding for roselle improvement, the genetic parameters, heritability and genetic correlation were estimated.

4.2.1 Heritability

Two main characteristics about heritability estimates of roselle traits were identified. First, heritability estimates covered a wide range from 0 to 84% (Table 4.6) and some traits showed consistently low heritabilities across populations. Except for plant height (3% to 84%) leaf area (0% to 62%) which showed some moderate to high heritability estimates, all the fiber traits demonstrated low estimates (0 to 31%). The heritability estimates in fiber characteristics decreased in the order, number of internodes (38%, 0%, 18%, 35%, 2%, 16%, 21%, 8%), to stem width (27%, 0%, 6%, 18%, 0%, 16%, 0%, 0%), then branch number (10%, 0%, 0%, 1%, 31%, 0%, 0.49%, 3%, 0%), with the least of all being height at first branching (15%, 0%, 0%, 5%, 6%, 0%, 10%, 0.53%) for the populations, Bawku, Cote D"Ivoire, Mali, Nigeria, Ouagadougou, Senegal, and Upper West, respectively. In terms of rank, the highest estimates were found in Mali (84%, 56%,5%, 31%, 18%, 35%), followed by Bawku (72%, 24%, 15%, 10%, 27%, 38%), then Lome (57%, 3%, 0%, 1%, 66%, 18%) populations in plant height, leaf area, height at first branching, branch number, stem width, and number of internodes, respectively. Heritability estimates of all traits were low in the populations Ouagadougou, Senegal, Nigeria and Upper West. Cote D"Ivoire population consistently demonstrated least heritability estimates for all traits (Table 4.6).

Table 4.

6. Genotypic variance, phenotypic variance, and heritability estimates of plant height, leaf area, and fiber characteristics in eight populations of *H. sabdariffa*.

Population	**Trait**						
	Variance	**PH**	**LA**	**HA**	**BN**	**SW**	**NI**
Bawku	V_G	525.47	800.56	1.88	0.01	2.47	3.80
	V_P	731.79	3354.77	12.46	9.98	9.28	9.91
	% H^2	**72** (0.26)	**24** (0.22)	**15** (0.24)	**10** (0.25)	**27** (0.21)	**38** (0.25)
Cote D"Ivoire	V_G	240.75	25.32	0.00	0.00	0.00	0.00
	V_P	550.66	5260.57	14.30	11.52	12.33	10.87
	% H^2	**44** (0.54)	**0.48** (0.00)	**0** (0.05)	**0** (0.00)	**0** (0.00)	**0** (0.00)
Lome	V_G	164.49	215.56	0.00	0.12	0.50	2.55
	V_P	286.99	6632.08	7.33	9.82	8.25	14.17
	% H^2	**57** (0.54)	**3** (0.56)	**0** (0.00)	**1** (0.00)	**6** (0.18)	**18** (0.40)
Mali	V_G	448.41	2507.18	0.53	4.28	1.87	3.83
	V_P	532.09	4507.77	10.31	13.83	10.19	10.97
	% H^2	**84** (0.09)	**56** (0.18)	**5** (0.06)	**31** (0.16)	**18** (0.19)	**35** (0.20)
Nigeria	V_G	202.05	0.00	0.52	0.00	0.00	0.18
	V_P	254.29	1839.07	8.32	6.09	5.19	8.25
	% H^2	**79** (0.24)	**0** (0.00)	**6** (0.35)	**0** (0.00)	**0** (0.00)	**2** (0.15)
Ouagadougou	V_G	244.91	2206.43	0.00	0.06	1.41	1.66
	V_P	470.03	6670.45	10.89	12.13	8.66	10.47
	% H^2	**52** (0.11)	**33** (0.13)	**0** (0.00)	**0.49** (0.04)	**16** (0.07)	**16** (0.07)
Senegal	V_G	94.40	1632.40	0.75	0.35	0.00	1.73
	V_P	163.32	5622.16	7.48	10.45	8.34	8.35
	% H^2	**58** (0.43)	**29** (0.08)	**10** (0.18)	**3** (0.16)	**0** (0.00)	**21** (0.27)
Upper West	V_G	5.17	4050.35	0.03	0.00	0.00	0.71
	V_P	156.10	6574.32	5.63	11.97	8.45	8.49
	% H^2	**3** (0.67)	**62** (0.34)	**0.53** (0.16)	**0** (0.00)	**0** (0.00)	**8** (0.39)

Values in parenthesis are standard errors

4.2.2 Correlation among plant height, leaf area, and fiber characteristics

In the current study, Pearson correlation coefficients were calculated to examine association among traits. In general, low positive significant correlation coefficients ranging from 0.01 to 0.41 were observed. Correlation of plant height and number of

internodes with all other traits were low to moderate and positively significant (r= 0.11 to r=0.35; P<0.01) and (r= 0.07 to r=0.35; P≤0.05), respectively. The highest correlation of r=0.41 was observed between branch number and number of internodes. A low negative significant correlation between height at first branching and leaf area was identified. Contrary to the results of Ibrahim and Hussein (2006) which showed strong and positive correlation between PH and BN, the association between these two traits in this study was very weak.

Table 4.7. Pearson correlation coefficients among plant height, leaf area, and fiber characteristics in roselle

	PH	**LA**	**HA**	**BN**	**SW**
LA	0.32 (<0.01)				
HA	0.18 (<.0.01)	-0.08 (0.01)			
BN	0.11 (<0.01)	0.10 (0.01)	-0.04 (0.23)		
SW	0.35 (<0.01)	0.20 (<0.01)	0.07 (0.04)	0.32 (<0.01)	
NI	0.16 (<0.01)	0.05 (0.16)	0.09 (<0.01)	0.41 (<0.01)	0.29 (<0.01)

Numbers in parenthesis are probabilities

4.3 Genetic distance and cluster analysis

A data matrix of traits and accessions was used to compute genetic distances by means of Pearson correlation coefficient to generate a distance matrix (Appendix B). Of the 595 accession pairs, 305 (51.26%) had negative correlation coefficients. The coefficients span a range of -0.99 in HS32/HS58 (Ouagadougou and Lome) the most diverse pair to 0.97 in both HS29/HS58 (Ouagadougou and Lome) and HS14/HS19 (Ouagadougou). The large number of negative coefficients represents widespread divergent genotypes possibly resulting from a repertoire of multiple alleles which may differ in nucleotide sequence to give divergent heterozygous genotypes. This observation gives an indication of both historical and novel evolutionary mechanisms

Table 4.

operating in roselle. The evolutionary mechanism that may account for this pattern of divergence and wide diversity is possibly the generation and maintenance of polymorphism by some form of balancing selection, mutation, recombination, as well as some speciation event. Implication of the widespread divergence is conferment of

fitness to the germplasm. Because of the limitation of the morphological data set which has a low power of detection of allele diversity, a molecular approach to determine sequence or loci alleles to substantiate this observation was needed.

However, because roselle genome is not yet sequenced, investigation by means of RAPDs was appropriate to further dissect the diversity.

Genetic distance, expressed as similarity between pairs of accessions was estimated by computing the square of the correlation matrix. The similarity coefficients which ranged from 0.00 to 0.98 with an average of 0.27±0.26, representing only 27% similarity among the genotypes, indicated substantial variability. A total of 40 pairs (6.72%) with similarity coefficients of 0.0 accounted for distinct and unrelated pairs that did not share any common units. Among these were 10 accession pairs of Bawku genotypes with those of other origins: HS20/HS22 HS20/HS30 HS20/HS14 HS20/HS84, HS27/HS77 HS27/HS78, HS69/HS09 HS69/HS27, HS69/HS82, HS69/HS86; and 5 accession pairs of Upper West with other genotypes, viz., HS11/HS16, HS11/HS70, HS78/HS50, HS78/HS68, and HS78/HS77. The accessions of Mali with other genotypes which separated by a genetic distance of zero were eight, viz., HS08/HS32, HS08/HS58, HS08/HS68, HS08/HS77, HS08/HS84, HS04/HS68, HS04/HS86, and HS48/HS86. The remaining accession pairs with genetic distance of zero include 15 of Ouagadougou genotypes with others:

HS30/HS02, HS30/HS13, HS30/HS68, HS30/HS70 HS30/HS82, HS24/HS08 HS24/HS09 HS24/HS19, HS70/HS14, HS70/HS19, HS70/HS09, HS25/HS56, HS77/HS01, HS77/HS41, HS82/HS85 and 2 pairs of Nigeria with others: HS01/HS68 and HS01/HS77.

On the distance data, a total of 468 (78.66%) accession pairs had similarity coefficients of less than 0.5, which further supports the widespread genetic diversity in roselle. The

UPGMA analysis revealed a roselle diversity comprising three divergent clusters (at insertion point 0.024), namely, cluster I, II and III (Figure 4.1). Cluster I was heterogeneous and had the lowest membership of six (17%) accessions with a range and mean genetic distance of 0.02 - 0.81 0.29±0.24, respectively. The most similar accessions in cluster I had a genetic distance of 0.81 (HS09/HS20) (Figure 4.1). Grouping of cluster I was on the basis of tall plant height, large values of height at first branching, large leaf area, many branch number, long internodes, wide stem width, but small leaf area (Table 4.8).

Main cluster II had a membership of sixteen accessions with range of genetic distance of 0.00 to 0.94, and average of 0.33±0.28. The most similar accession pair in cluster II (HS29/HS58) was separated by a genetic distance of 0.94 (Figure 4.1). Two subclusters, IIA and IIB were delineated. Cluster IIA consisted of thirteen members HS69, HS30, HS08, HS01, HS24, HS29, HS58, HS48, HS41, HS56, HS81, HS65 and HS78 with range and average genetic distance of 0.00 to 0.94, and 0.32±0.29, respectively. Cluster IIB had three members, HS70, HS82, and HS27 and covered a range and average genetic distance of 0.08 to 0.46 and 0.26±0.19, respectively. Cluster II was grouped on the basis of low values of plant height, leaf area, branch number, stem width, number of internodes, but high values of height at first branching (Table 4.8). Examples of cluster II genotypes with few branch number and short heights were HS08 (9.76±3.52 and 51.99±21.98), HS30 (10.08±3.52 and 50.92±21.98), and HS56 (11.62±3.52 and 53.99 ±21.98).

For main cluster III two subclusters, namely subcluster IIIA and 1IIB. Four accessions (HS32, HS13, HS50 and HS02) grouped in subcluster IIIA. The range of genetic distance among members of subcluster IIIA was 0.02 for HS50/HS02 pair to 0.62 (HS32/HS13), with a mean of 0.35±0.25.

Subcluster IIIB had nine members, HS14, HS19, HS22, HS11, HS85, HS04, HS84, HS83, HS16) with a mean genetic distance of 0.49±0.31. Accessions in this subcluster were Subcluster by high to highest values of all traits but height at first branching and stem width (Table 4.9).

Except for accession pairs HS24/HS29 of cluster II and HS14/HS19 of cluster III all of which originated from Ouagadougou, which were substantially similar and related by genetic distances of 0.97 and 0.96, respectively, all other accessions clustered somewhat independent of their origin. For instance, the seventeen accessions from Ouagadougou were distributed among all subclusters, such that three accessions (HS77, HS29 and HS09) grouped under cluster I, five (HS30, HS24, HS29, HS41, HS56) under cluster IIA, three (HS70, HS80, HS27) under IIB, two (HS32, HS50) under IIIA and four (HS14, HS19, HS22, HS84) under IIIB.

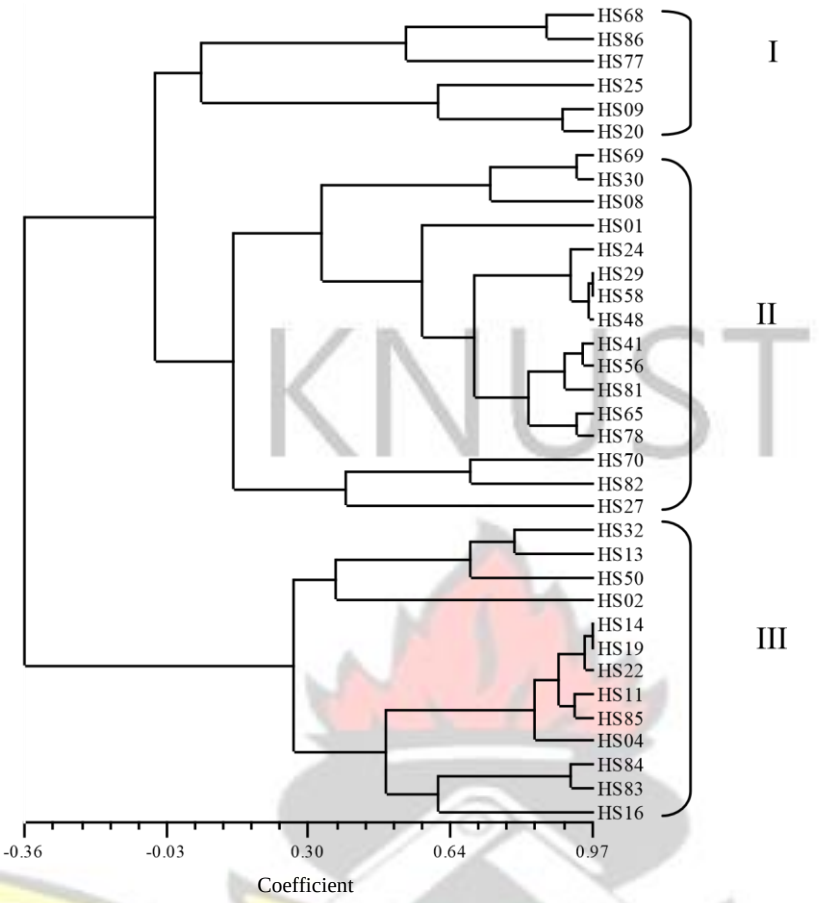


Figure 4.1. A dendrogram showing two divergent clusters in 35 *H. sabdariffa* accessions collected from West Africa using UPGMA cluster analysis on morphological data. Clusters are shown in Roman numerals.

Similarly, two each of the Mali accessions clustered under 1 (HS86, HS68) and 1IB (HS08, HS48), and one in 11B (HS16). The multiple distributions of the accessions over all six subclusters is suggestive of separate origins, as well as transfer of *H. sabdariffa* germplasm across many countries such that there exists a close genetic link between the genotypes currently found in the various regions.

Table 4.8. Cluster means of 35 *H. sabdariffa* accessions based on plant height, leaf area, and fiber characteristics

Trait	**Overall Mean**		**Cluster I**			**Cluster II**			**Cluster III**		
		SD	**Mean**	**SD**	**Var[1]**	**Mean**	**SD**	**Var[1]**	**Mean**	**SD**	**Var[1]**
PH	76.52	21.98	89.21	18.36	**12.69**	63.88	20.21	**-12.64**	82.14	19.69	**5.62**
LA	178.14	77.64	170.28	52.44	**-7.86**	141.82	64.79	**-3632**	214.15	87.83	**36.01**

HA	8.90	3.27	9.55	3.68	**0.65**	9.16	3.04	**0.26**	8.24	3.14	**-0.66**
BN	12.72	3.52	13.89	3.58	**1.17**	11.86	3.07	**-0.86**	12.94	3.75	**0.22**
SW	15.05	2.99	15.99	3.16	**0.94**	14.39	2.72	**-0.66**	15.12	3.06	**0.07**
NI	19.51	3.67	20.83	3.72	**1.32**	18.31	3.12	**-1.2**	20.01	3.88	**0.5**

[1] Difference between a cluster mean and overall mean

Table 4.9. Subcluster means and differences from overall mean of 35 *H. sabdariffa* accessions based on plant height, leaf area and fiber characteristics

Trait	**Overall Mean**	**IIA**	**Var**	**IIB**	**Var**	**IIIA**	**Var**	**IIIB**	**Var**
PH	76.52	61.87	**-14.65**	71.51	**-5.01**	84.71	**8.19**	81.06	**4.54**
LA	178.14	130.68	**-47.46**	184.00	**5.86**	193.06	**14.92**	223.24	**45.1**
HA	8.90	9.27	**0.37**	8.75	**-0.15**	8.72	**-0.18**	8.03	**-0.87**
BN	12.72	11.79	**-0.93**	12.10	**-0.62**	12.19	**-0.53**	13.26	**0.54**
SW	15.05	14.22	**-0.83**	15.04	**-0.01**	15.53	**0.48**	14.94	**-0.11**
NI	19.51	18.31	**-1.2**	18.29	**-1.22**	19.81	**0.30**	20.10	**0.59**

This observation is similar to the findings of Bakasso *et al*. (2013) who reported of no significant relationships between genetic and geographic isolation among the 124 roselle accessions in his study. Also, El Tahir and El Gabri (2013) detected an unexpected grouping of 126 roselle accessions under different clusters regardless of their origin. Cruz *et al*. (2013) also reported of 47 Mexican accessions which clustered independently of the edaphic-climatic characteristics of their collection sites.

4.4 Principal components analysis

The first two principal components which had eigenvalues greater than 1.0 explained 100% of the total variance (Table 4.10). In the first PC which accounted for 78.72% of the total variance the predominant traits were plant height, leaf area, branch number, stem width, and number of internodes. The second principal component (PC2) explained 21.28% of the total variance, with height at first branching predominating. On the basis of the eigenvectors of the PC1 and PC2, all six traits were important in determining genetic diversity in *H. sabdariffa* accessions. An earlier work report of plant height and number of internodes being the central traits for structuring roselle

accessions in Cote D"Ivoire and Burkina Faso accessions (Sie *et al*., 2009). Although flowering time, budding time, seed weight, plant height, height at first branching and branch number have been observed to be very important in characterizing roselle (Abou El-Nasr *et al.,* 2014; Sabiel et *al*., 2014; Bakasso *et al*., 2013; Torres- Moran *et al.,* 2011; Siepe *et al.,* 1997), this study has confirmed usefulness of the morphological markers, plant height and number of internodes in characterizing roselle genotypes. In addition, the current study has also identified stem width (branch number, height at first branching, and leaf area, as four equally important traits to be considered in the assessment of genetic diversity in roselle populations. All the traits examined correlated with the extracted components.

Table 4.10. Eigenvalues, eigenvectors, and cumulative percentage of variance explained by the first two principal components (PC) from evaluation of plant height, leaf area, and fiber characteristics on 35 genotypes of *H. sabdariffa*

Variables	PC1	PC2
PH	**0.93**	0.03
LA	**0.78**	0.49
HA	0.42	**-0.88**
BN	**0.83**	0.03
SW	**0.90**	-0.18
NI	**0.90**	0.11
Eigenvalue	3.944	1.066
Percentage	78.72	21.28
Cumulative percentage	78.72	100.00

4.4.1 Biplot analysis

To better visualize the relationships among accessions and among traits, biplots (Figure 4.2) were constructed from the principal components. A plot of PC1 and PC2 accounted for 100% of the total variance. All traits, except height at first branching grouped on the right side of the PCA biplot indicating their positive contribution to the variance while height at first branching contributed to reduction in the total variance (Figure 4.2A). One dominant correlation grouping consisting of plant height and fiber

characteristics was delineated based on the tight angles (0 to 90^{o}) between their vectors. Leaf area showed marginal relationship whereas height at first branching showed no relationship with the other traits.

A biplot analysis on the two PCs of the entire 35 accessions is shown in Figure 4.2B. Four major correlation groups were identified. One group on the basis of least values of height at first branching (HS22, HS83, HS85), a second group separated according to large leaf area and medium values of height after first branching (HS32, HS19, HS14), a third group scattered along both PCs by being separated according to branch number, stem width, and number of internodes. Accessions HS69, HS68, HS09, and HS27 did not group with the other accessions.

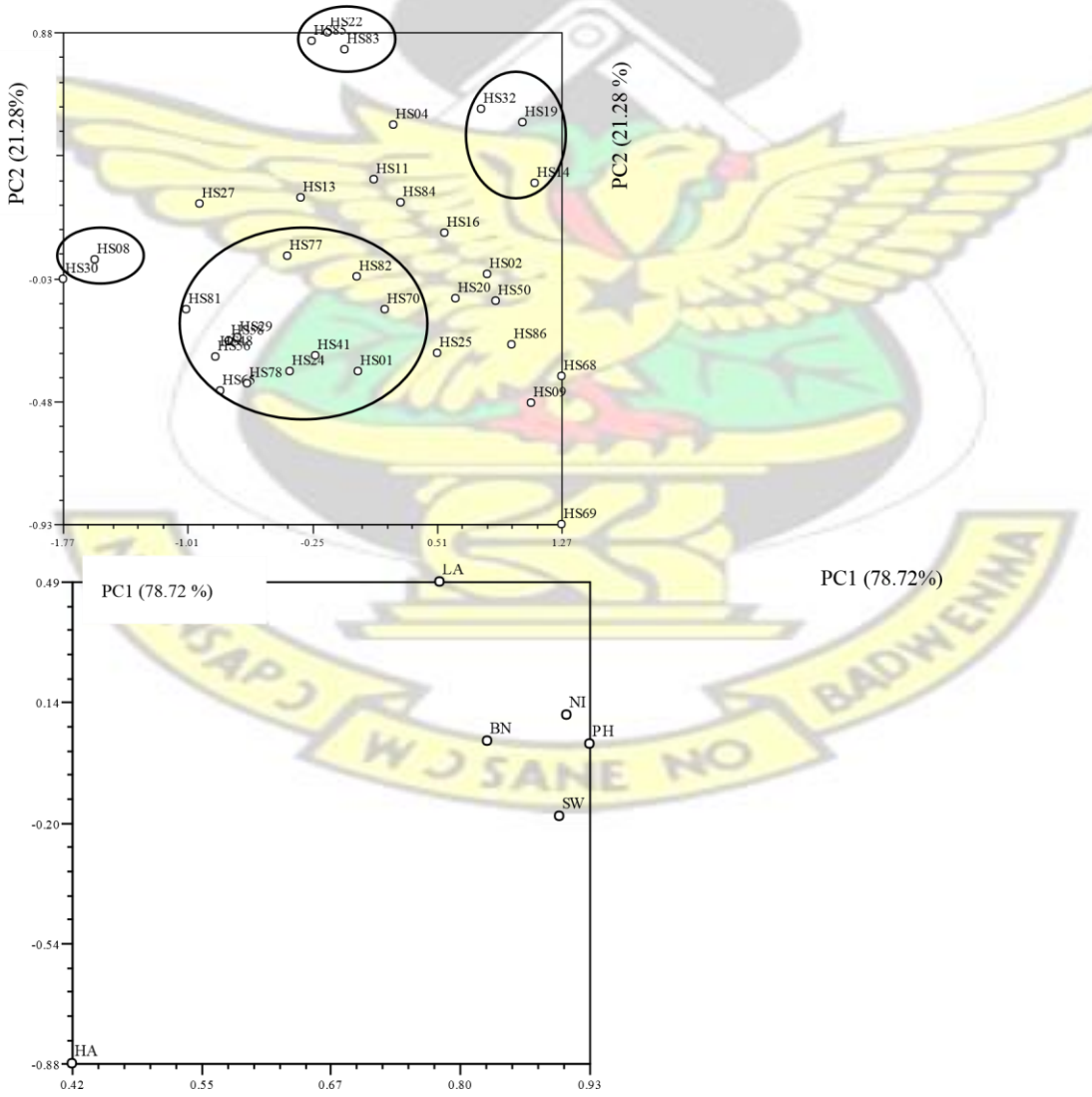


Figure 4.2. PCA biplots showing correlations between the 35 accessions of *H. sabdariffa* in A and the six morphological traits in B.

4.5 Molecular analysis

4.5.1 Statistical analysis of RAPD data

A total of 32 accessions of *H. sabdariffa* were studied using RAPD analysis. All accessions yielded both good quality DNA and good amplification products. All twelve primers produced clear and sharp bands. DNA bands were scored for present (1) or absent (0) (Appendix D). Plate 4.2 shows images of gels portraying DNA bands. Table 4.11 shows the statistics of RAPD loci amplification products, sequences, number of alleles at each locus, and the intra locus gene diversity. In all, a total of 1,329 amplified fragments were obtained for 12 loci.

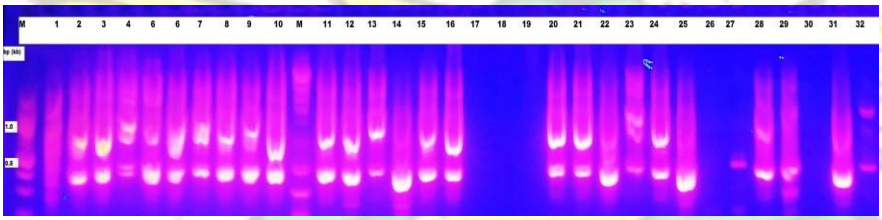


Plate 4.3. PCR amplification of 32 *H. sabdariffa* accessions by RAPD primers.OPA12 (B) OPA-09 with 100 bp DNA ladder. 1=HS77; 2=HS22; 3=HS08; 4=HS30; 5=HS70; 6=HS41; 7=HS75; 8=HS65; 9=HS84; 10=HS43; 11=HS32; 12=HS13; 13=HS29, 14=HS04; 15=HS16; 16=HS48; 17=HS50; 18=HS02; 19=HS25; 20=HS20; 21=HS56; 22=HS59; 23=HS11; 24=HS85; 25=HS24; 26=HS58; 27=HS14; 28=HS86; 29=HS78; 30=HS88; 31=HS83 and 32=HS87.

Table 4.11. Statistics of RAPD loci, sequences, number of alleles at each locus, and expected heterozygosity for the 32 *H. sabdariffa* accessions collected from the eight regions in West Africa and evaluated in 2010 and 2011 in Ghana.

	RAPD ID	**Primer sequence**	**Total no. of amplified fragments**	**Fragment size (bp)**	**No of alleles**	**No. of polymorphic alleles**	**Intra locus gene diversity**

1	OPA-03	AGTCAGCCAC	124	150-750	5	4	0.20
2	OPA-07	GAAACGGGTG	96	200-500	5	5	0.15
3	OPA-11	CAATCGCCGT	108	50-1000	6	6	0.22
4	OPA-12	TCGGCGATAG	120	150-800	5	5	0.32
5	OPA-16	AGCCAGCGAA	112	100-1000	6	6	0.26
6	OPA-20	GTTGCGATCC	80	200-700	4	4	0.22
7	OPG-05	CTGAGAGGGA	124	200-500	5	5	0.17
8	OPB-08	GTCCACACGG	104	350-900	7	7	0.24
9	OPB-09	TGGGGGACTC	108	200-600	5	5	0.18
10	OPH-13	GACGCCACAC	100	200-500	5	5	0.19
11	OPG-03	GAGCCCTCCA	128	150-450	5	5	0.24
12	OPAB-03	TGGCGCACAC	125	200-650	5	5	0.18
	Total	-	1,329	-	63	62	-
	Min	-	80	50	4	4	0.15
	Max	-	128	1000	7	7	0.32
	Mean	-	110.8	-	5.25	5.16	0.21
	SD		14.39		0.75	0.83	0.05

Amplified fragment size varied between 50 and 1000 bp. The rate of polymorphism among the loci was 80% to 100%. No rare allele was found. The range of polymorphism was a minimum of 4 alleles for marker OPA-20 to 7 alleles for OPB08. One allele in OPA-07 was monomorphic. The twelve primers produced a total of 1,329 bands of which 1,297 were polymorphic. Majority of the RAPD loci (83.3%) had 5 or more alleles. A total of 63 alleles were detected across loci with an average of 5.25 alleles per locus (Table 4.11) which represents ample genetic diversity within the roselle genotypes. The intra locus gene diversity for the 12 loci ranged from ranged 0.15 (OPA-07) to 0.32 (OPA-12) with an overall mean of 0.21±0.05 (Table 4.11).

4.5.2 Relationships among roselle accessions

The relationship among roselle accessions and traits was determined by estimation of genetic distances between pairs of accessions on the accession by marker binary data

matrix using the Jaccard similarity coefficient. The similarity matrix is presented in Appendix C.

Genetic distance ranged from the most distantly related pair having a the lowest value of 0.00 for six accession pairs (HS50/HS11, HS50/HS29, HS50/HS41, HS50/HS65, HS50/HS75, and HS50/HS84) to the most similar pair of accessions HS13/HS32 with a similarity coefficient of 0.79. Average genetic distance for the entire accessions was 0.29±0.14, which translates into a genetic diversity of 29%. Of the six dissimilar accession pairs with HS50, five originated from Ouagadougou (HS41, HS65, HS84, and HS29, one (HS11) from Ghana, and one (HS75) from Nigeria. This makes Ouagadougou the only origin having the most diverse accessions, hence, probably the center of diversity of roselle.

Overall genetic distance among roselle accessions for each country was calculated from the similarity matrix (Appendix C). Within country average similarity coefficients were fairly low having range and mean values increasing from Lome (0.10 to 0.14, 0.11), to Mali (0.10 to 0.35, 0.24), to Senegal (0.26), to Ghana (0.15 to 0.64, 0.33), to Ouagadougou (0.0 to 0.64, 0.34), to Cote D"Ivoire (0.30 to 0.53, 0.42), then Nigeria (0.43). The rather low similarity coefficients of three accessions from Lome (HS04, HS58, and HS88) denote largest diversity though three accessions were too few to draw conclusions on the estimates of diversity. On the contrary, Yusof and Saud (2009) who worked on six Malaysian *H. sabdariffa* genotypes using RAPD analysis reported low dissimilarity coefficients and concluded that genetic diversity among Malaysian roselle was very narrow.

4.5.3 Hierarchical cluster analysis

A UPGMA cluster analysis of the similarity matrix produced four major clusters, I, II, III, and IV (Figure 4.3). Main cluster I was heterogeneous and consisted of twentyfour

accessions which differentiated into two divisions, subcluster IA with 13 members of which nine accessions originated from Ouagadougou (HS22, HS29,

HS30, HS32, HS41, HS65, HS70, HS77, and HS84), two from Nigeria (HS13 and HS75), one from Mali (HS08) and one from Lome (HS04). The range of genetic similarity in subcluster IA was 0.17 to 0.79 and with a mean of 0.42±0.12 which represents substantial similarity. Subcluster IA was termed the „Ouagadougou group". Members of subcluster IA had succulent calyx.

Subcluster IB consisted of 11 members with vastly diverse origins spanning five countries, yet considerably similar with a range of similarity coefficient of 0.19 to

0.69 and mean of 0.43±0.10. Three accessions each originated from Ouagadougou (HS24, HS25, HS56) and Mali (HS16, HS48, HS86), two each from Cote D"Ivoire (HS02, HS59) and Ghana (HS11, HS20), and finally one from Senegal (HS85). It is interesting to note that, the only semi wild type, HS86 from Mali, was identified with this group. On the basis of their varied origins, subcluster IB was designated the „West Africa Mix group". The principal morphological basis for their grouping was the crimson throat and brown pollen colour of the flower.

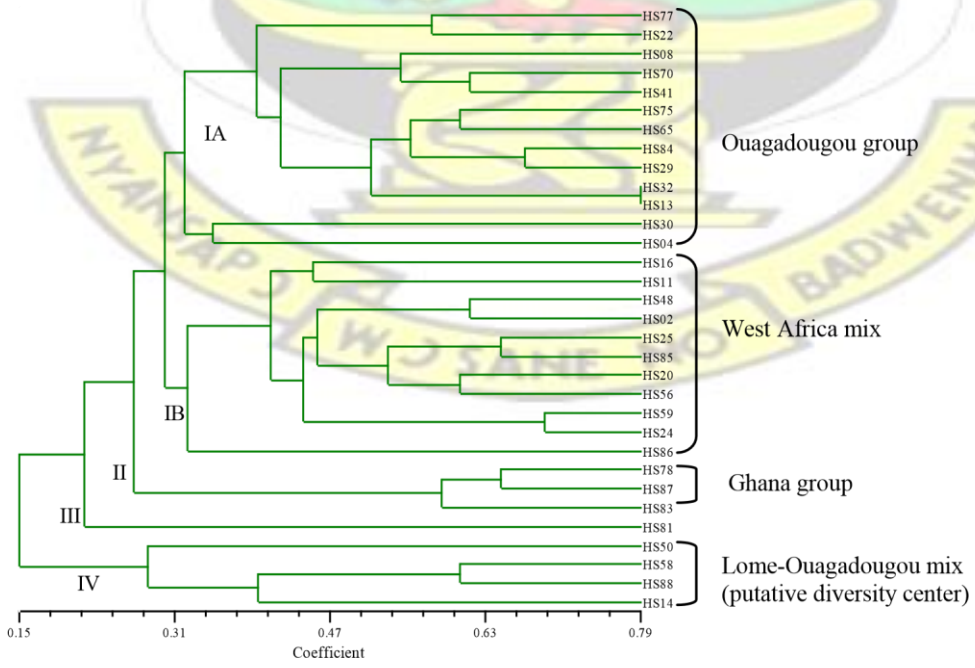


Figure 4.3. A UPGMA cluster on Jaccard‟s similarity matrix based on RAPD analysis on 32 *H. sabdariffa* accessions

Cluster II was fairly uniform with three accessions which demonstrated a high similarity coefficient of 0.60±0.04 covering a range of 0.56 to 0.64, such that the accessions HS78 and HS87 of Ghana and HS83 of Cote D‟Ivoire were almost indistinguishable. Based on their high level of similarity in the morphological traits of predominantly nonbushy growth habit, absent leaf pubescence or glabrous, brown pollen and crimson throat colour, cluster II was termed „the Ghana group‟.

Clusters III had a single member, the wild type roselle HS81 of Senegal. The accuracy of the cluster analysis was evident in precisely distinguishing the wild and semi wild types from the cultivated genotypes. In addition, separation of the two wild types (HS50 and HS81) from one another suggests their marginal relatedness.

Finally, cluster IV had four members, viz., HS58 and HS88 from Lome, and HS14 and HS50 of from Ouagadougou having genetic similarity range of 0.10 to 0.40 in a range of 0.29± 0.11 representing a substantially low similarity, hence a wide variability. Although members of this cluster differed at the molecular level, they were the group that shared the most number of morphological traits, such as absence of stem, leaf and calyx pubescence, intermediate branching habit, nonbushy growth habit, crimson throat colour, brown pollen colour, and ovoid capsule shape. Interestingly HS50 is one of the three wild accessions in the current study. The other wild accession, HS30, also from Burkina Faso, clustered with the Burkina Faso group.

The overall genetic similarity range of 0.00 to 0.79 and an average of 0.29 represent sufficient genetic diversity within the 32 roselle accessions. This range is wider than the genetic similarity coefficient range of 0.53 to 0.87 with an average of 0.71 recorded

among nine roselle accessions in Malaysia (Omalsaad *et al*., 2014) by means of RAPD, and 0.91 to 0.98 similarity coefficient among 94 roselle accessions in Thailand (Handboosong *et al*., 2000). The difference in variability range may be attributed to not only the wide geographic scope of the genotypes, but also, the fact that West Africa may be the center of genetic diversity of roselle.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Morphological analysis

Hibiscus sabdariffa is popularly known for its uses for food, medicinal and fiber purposes. In recent years uses of the plant has rapidly expanded to almost all regions of the world. The center of diversity is reported to be West Africa. Genetic improvement in various traits of roselle is essential to derive maximum benefits. Information on genetic diversity and relationship among roselle genotypes is important to expose useful genotypes that can be sources of alleles for trait improvement, genotypes for efficient conservation, and reveal the history of roselle. Agromorphological evaluation uncovers the differences that exist phenotypically in the leaf, calyx, flower, stem, branching habit, growth habit, plant height and fiber characteristics while molecular markers reveal reliable and accurate information about subtle differences among genotypes.

In the current study 39 *H. sabdariffa* accessions belonging to four races, namely, *ruber*, *albus*, *intermedus* and *bhagalpuriensis* of the botanical variety *sabdariffa* and *altissima* were collected from seven West African countries and subjected to agromorphological field evaluation and examination with twelve RAPD primers to estimate the genetic diversity within the germplasm.

5.1.1 Morphological variation within and among the seven roselle populations

Besides the low variability identified in calyx texture, throat colour and leaf pubescence, considerable variability was identified among accessions expressed by a broad range of two to five variants of shape, size, pigmentation, pubescence and texture of leaf, stem, stipule, corolla throat, and pollen, in addition to plant type, branching and other calyx presentations. Being the most important commercial characteristic of roselle, when calyx pigmentation was examined at all levels of other traits, it was revealed that 90% of red and dark red calyxes (race *ruber*) were borne on red stems and had succulent calyx, while 50 to 60% green (race *albus*) and intermediate (race *intermedus* and *bhagalpuriensis*) calyxes had uniformly green stem (χ^2 = 73.50, P<0.001) and dry calyxes ((χ^2 = 12.18, P=0.0068). All intermediate, red and dark red plants had yellow flower throat ((χ^2 =43.47, P<0.001), and 58% of green types had crimson throat ((χ^2 = P<0.001). No other definite pattern among the traits was evident.

The variability recovered in roselle of West Africa was similar to the report of El Tahir and El Gabri (2013) where among 126 roselle genotypes from Sudan 10.7% nonbushy erect, 48.9% nonbushy compact, and 40.4% prostrate habit were observed. Fifty-five percent of the population was found to be short, 17.5% intermediate and 22.5% were tall with predominantly green leaves (93.9%), red stem colour (86.3%), and predominantly smooth stem (90%). Being qualitative traits, the differences in traits are purely genotypic. Accessions HS20, HS41, HS01, HS09, HS13, HS14, HS56, HS30, and HS22 would find uses in food, confectionery, cosmetic, pharmaceutical, and naturoceutical applications for their intense red calyx pigmentation. The wide variation in plant characteristics is an indication of the existence of a rich genetic diversity among West African roselle cultivars.

On the metric traits, the substantial variability (CV of 18.84 to 43.58%) observed among the West African roselle collection was in close agreement with the phenotypic variability values of 15 to 30% for plant height, leaf length, leaf width, and stem width in Mexican roselle accessions (Cruz and Solano, 2013; Osman *et al*., 2011; Torres-Morán *et al*., 2011; Diouf *et al.*, 2007) and 34.07% and 28.7% for plant height and stem width among Asia and Europe genotypes (Siepe *et al*., 1997). The differences in variabilities may be attributed to variations in environmental conditions for growth.

The strong variety main effect for plant height, leaf area, branch number, and stem width in Mali, Bawku and Ouagadougou populations indicates the possibility of improving roselle with accessions from these sources rather than those of Senegal, Nigeria, Lome, and Cote D"Ivoire which demonstrated homogeneity in these traits. Similar results of strong genotype effect was reported for plant height, leaf area, branch number, and stem width and number of internodes among 12 genotypes in Mexico (Torres-Morán *et al*., 2011) and five roselle genotypes (Osman *et al*., 2011), among 16 Egyptian roselle genotypes (Ibrahim and Hussein, 2006) and 32 Senegal accessions (Diouf *et al.*, 2007). The cleistogamous nature of roselle makes the high variability observed in the current investigation an unexpected one. However, such high variability can occur among landraces from a wide geographic area. Secondly, variability was contributed by environmental effects. The accessions HS20, HS69, HS83, HS04, HS16, HS68, HS86, HS09, HS14, HS19, HS24, HS50 and HS77 having significant mean squares for plant height and branch number, and for, HS09, HS69, HS02, HS86, HS68, HS14, HS19, HS32 and HS50 represent ample variability that could be exploited for fiber and vegetable leaf production. The strong environmental effect on roselle morphological traits was corroborated by Koorsa (1987), Thirthamallappa and Sherif (1991), and Zayed *et al*. (1996).

The inconsistent G×E interaction effects for all other traits confirm the heterogeneity of the accessions. The significant G×E interaction term and environment main effect in all populations except Nigeria and Senegal indicate that in cultivar development the populations have to be evaluated in multiple locations to select best performers for each population will be required. The results were consistent with the findings of Abou El-Nasr *et al.* (2014) who recorded strong main effects ($P \leq 0.05$) for genotype, environment, and genotype×environment interaction effect among 15 lines of Sudanese roselle in Egypt for plant height and number of branches.

However, owing to the inconsistent environment and interaction effects, achievement in selection gains would require evaluation of stability and adaptation of the genotypes in a range of environments.

Being tetraploid and cleistogamous, the large variability in accessions was unexpected as cleistogamous flowers generally reduce gene flow and restrict diversity leading to little genetic variation. With the low heritability of the major traits and an inconsistent environment and G×E effect, conventional breeding through hybridization should be difficult (Osman, 2011; Vaidya, 2000). Roselle would therefore benefit from mutation breeding as has been carried out in three varieties from Australia with success (Osman *et al*., 2011). In this study, genetic variation was increased via mutation and the mutants performed better in nine traits including branch number and plant height than the control variety.

The identification of a large variability in West African roselle may be the result of mutations over many generations or speciation. In West Africa where roselle originates, landraces form the sole genotype of the production system with no record of formal breeding for genetic improvement although many studies have been carried out on genetic diversity and genetic variability for calyx production in India and

Senegal, Sudan and Egypt (El Tahir and El Gabri, 2013; Thirthamallappa and Sherif, 1991; Gasim, 1994, Zayed *et al*., 1996; Ibrahim and Hussein, 2006; Ahmed *et al*., 2009, Atta *et al*., 2011; Sabiel *et al*., 2014) as well as leaf production (Thirupathi *et al*., 2015).

5.1.1.1 Plant characteristics

Tall roselle plants are more desirable for fiber production than short types as long and uninterrupted fiber strands exhibit more strength than short fibers which need to be joined to provide the desired length. Comparing to plant heights of 75.91 cm to 141.83 cm among 6 genotypes from Nigeria (Falusi *et al*., 2014), 65.35 cm to 168 cm in 9 accessions from Cameroon, Cuba, Sudan, and Nigeria (Omalsaad and Islam, 2012) roselle heights obtained in current study were relatively short. Maximum heights were not realized owing to the large plant spacing applied. Plants with intra row spacing of 30cm and below have been found to grow relatively higher in height than those planted with spacing between 30cm and 60cm (Okosun *et al*., 2006). Furthermore, the time of planting was found to have strong effect on height, whereby planting in March promoted an extended vegetative phase in which plants grew tall and flowered in November, whereas planting in August produced plants that failed to achieve appreciable height due to their early flowering response to the short day length. Once flower was produced, plants failed to grow tall.

5.1.1.2 Leaf area

Accessions with large to very large leaf sizes may be recommended for use as leafy vegetables as the potentially large leaf yield would be more economical to grow than those with intermediate or small leaf area. For this reason, HS19, HS14, HS32, HS85, HS50, HS02, HS11, HS22, HS69, HS04, HS83, and HS70 which had leaf area exceeding 200 mm^2 would be good for leafy vegetable production.

For fiber processing, the preferred long and uninterrupted fibers are produced from tall plants with few branching. In current study, tall accessions with few branches were not found, however, tall with intermediate branching genotypes were identified as HS25 (89.62±15.77 cm, 12.93±3.67) and HS32 (93.98±18.44 cm, 12.24±5.51), respectively. Further studies are required to understand spacing effect on plant height and branching. All *altissima* roselle exhibited tall plant heights, large stem width, and large number of internodes. Unexpectedly, some *sabdariffa* accessions exhibited same traits and may be recommended as good sources of fiber. However, more work is required to ascertain differences in fiber from *altissima* and *sabdariffa* types, if any.

5.1.2 Comparison of heritability

The populations exhibited large differences in heritability estimates confirming that heritability is specific to population and environment. The low heritability estimates for all traits except plant height in many of the populations (0 to 84%) is indicative of large environmental influence on the expression of the traits. The relatively moderate to high heritability estimates for plant height (44% to 84%) in all populations except Upper West was also reported among 16 roselle accessions in Egypt (Ibrahim and Hussein, 2006) to be 92% and 92 to 99% in some Sudanese roselle genotypes (Abou El-Nasr *et al.*, 2014).

The high heritability estimates in plant height could be attributed to presence of alleles possibly in additive gene action with some non-heritable effects arising from nonadditive gene action arising from dominance and epistasis (Nyquist, 1991; Falconer and Mackay, 1996) as well as genotype by environment effects. The low to moderate heritability estimates of leaf area (0 to 62%) signify large contribution of nongenetic effects and a rather strong influence of environment on the traits. The almost 0% heritability estimates for height at first branching and branch number across all the

populations indicate that alleles for these traits are fixed, hence, no genetic variance, or that the expression of these traits is under strong environmental influence rather than genetic control.

In contrast, alleles for stem width and number of internodes are fixed in some populations, such as Lome, Cote D"Ivoire, Nigeria and Senegal while in the Bawku, Upper West, Ouagadougou, and Mali populations, these traits are undergoing segregation. The low values of standard errors may have been caused by small sample sizes, and/or the inherent demerits of the determination of standard errors of estimation of variance components leading to biases in the standard error (Rendel, 1989).

The moderate to high heritability estimates in plant height and leaf area is an indication that these traits can be successfully improved via selection. Variation in the heritability estimates for each of the six traits across the populations confirms that heritability is population specific. Moreover, height at first branching, branch number and stem width are not selectable.

The large phenotypic variance relative to the genotypic variance is in consonance with the results of Sabiel *et al*. (2014) and Bakasso *et al*. (2013, 2009) who observed large environmental influence on calyx yield. Similarly, Islam *et al*. (2008) observed a high environmental influence on plant height, stem width, number of branches, leaf and calyx yield, as well as the number of capsules per plant among some roselle accession in Bangladesh.

Other traits in roselle that have low heritability is calyx yield (Sabiel *et al*., 2014; Bakasso *et al*., 2009; 2013) and yield components (Islam *et al*., 2008). Islam *et al*. (2008) concluded that the strong environmental effect in roselle results from its photoperiodic nature. They observed that, the yield of roselle cultivated in different

seasons were remarkably different, the most important factor being the day length such that some genotypes may perform poorly depending on the season of planting. Hence to achieve the desired results in a particular breeding program the day length of that geographic area should be taken into account.

5.1.3 Correlation among traits

The generally low positive significant correlation coefficients (r = 0.07 to 0.41; P<0.05) between plant height and all other traits except between height at first branching and leaf area, height at first branching and branch number, and between leaf area and number of internodes indicate that only 0.5% to 17% {R^2= $(0.07)^2$ to (0.41^2)} of the variation in plant height, branch number, stem width are explained by variations in leaf area, height at first branching and number of internodes. A low negative significant correlation between height at first branching and leaf area indicates that increase in height at first branch was associated with decrease in leaf area and that only 0.6% {R^2= $(-0.08)^2$} of the variation in height at first branching is explained by leaf area. Contrary to the results of Ibrahim and Hussein (2006) which showed strong and positive correlation between plant height and branch number, the association between these two traits among the West African roselle accessions was weak.

Chang *et al*. (2006) observed that tall roselle plants are more stable in yield characteristics than short plants. Since plant height is found to correlate with branch number (Ibrahim and Hussein (2006) and branch number with calyx yield per plant (Ibrahim and Hussein, 2006; Sabiel *et al*., 2014), improvement in calyx yield per plant could be achieved on the basis of selection for plant height, and/or branch number. Similarly, selection on the basis of plant height and stem width is expected to lead to high yield in stem biomass, a trait useful for fiber production but only to a limited extent as only 10 to 12% of variation in plant height is explained by stem width. The current

roselle collection could therefore be used as a germplasm resource in breeding programs aimed at improving calyx and fiber yield.

5.1.4 Genetic distance and cluster analysis

The genetic diversity based on morphological and molecular evaluations were fairly similar. In the molecular assessment, the RAPD markers produced a diversity of 4 to 7 distinct alleles, a total of 63 alleles across the accessions with a high level of average polymorphism of 98.4% and an intra-locus gene diversity of 0.21±0.05. Compared to the work of Satya *et al*. (2013) on roselle and kenaf of Indian origin, in which 113 alleles were identified yet the polymorphism was 35 to 57%, the polymorphism in the West African accessions represented higher diversity. Similarly, Bakasso *et al*. (2014) reported a low rate of polymorphism and polymorphic information content of 18.4% and of 0.166 for *H. sabdariffa* and 10.33% and 0.106 in *H. cannabinus,* respectively.

The polymorphic loci adequately resolved the accessions on the basis of their geographical origin, in which accessions with common geographical origins clustered together. The polymorphism further revealed a mixed group that has arisen from gene flow events, termed in current study as the „West African mix". Being an inbreeding species, the intra locus gene diversity in *H. sabdariffa* of 0.21±0.05 was not unexpected, though it was low compared to that of outbreeding species. This result agrees with the wide survey and comparison of genetic diversity in many crops provided by Hamrick and Godt (1990). Cheng *et al*. (2002) obtained similar results of

3 to 7 polymorphic alleles using six of the RAPD markers in current study (OPA-03, OPA-07, OPA-11, OPA-12, OPA16 and OPA-20) in structuring 14 varieties of kenaf (*H. cannabinus*).

On the basis of morphological evaluation, the range of similarity coefficients of 0.00 to 0.98 with an average of 0.27±0.26 represented a wide genetic diversity among the West African roselle accessions. This was confirmed in the molecular assessment which produced a Jaccard‟s index range of similarities of 0.00 to 0.79 and average of 0.29±0.14. This observation is not consistent with the RAPD results of Yusof *et al*. (2009) who observed a close genetic relationship among a highly morphologically varied six Malaysian roselle cultivars. Bakasso *et al*. (2014) reported a close relationship among 124 roselle accessions from Niger. Similar wide range in genetic distance of 0.280 to 0.878 among nine roselle accessions in Malaysia was reported by Omalsaad *et al*. (2014). In contrast, 94 roselle accessions in Thailand were found to be highly similar with a narrow genetic similarity range of 0.91 to 0.98 (Handboosong *et al*., 2000). The large genetic distance based on morphological evaluation among the West African roselle indicates the wide variation both within and among the populations. It is generally believed that *H. sabdariffa* originated from West Africa, the region of maximum diversity of the species hence, these results confirm that roselle is native to West Africa. However, a more extensive study incorporating larger number of accessions representative of each region may be required to substantiate the findings of this current research. Genetic distance is a measure of genetic divergence between populations in a species arising from mutation or fluctuations in allele frequencies that is genetic drift, as well as speciation.

Accession pairs which showed similarity coefficients of 0.00 represent genotypes that are diverse and not correlated in any of their traits. Despite these dissimilarities, the qualitative traits demonstrated some similarities. For instance, HS48/HS58 (J=0.0) were similar in plant type (red), calyx texture (succulent), leaf shape (entire), flower colour (pink), leaf pubescence (absent), growth habit (nonbushy), plant height (dwarf) and pollen colour (brown). In contrast, HS11/HS30 (J=0.0) were similar in plant type

(red), calyx texture (succulent), flower colour (pink), and plant height (dwarf) but dissimilar in leaf shape (penta/entire), leaf pubescence (present/absent), growth habit (bushy/nonbushy) and pollen colour (brown/yellow). The differences in both qualitative and quantitative traits may be accounted for by differences in alleles for the individual traits. The two main clusters identified in the accessions corroborated with the findings of Bakasso *et al*. (2014) who also identified two clusters among 124 accessions collected from southern Niger on the basis of ten agrophenological traits including flowering to maturity characteristics, plant height, branch number, basal diameter or stem width, capsule number, seed weight, calyx dry weight, and 100-seed weight.

In their study, the two clusters were discriminated by two key traits, flowering time and 100-seed weight, with calyx diameter, a popular parameter for classifying roselle among farmers not being important. Similarly, Satyanarayana and Sai (1995) who worked on 60 genotypes of roselle from India on eleven traits including plant height, fiber and fiber yield components reported classification into seven clusters independent of their geographical origin and identified that fiber yield per plant and dry stick weight contributed most to the variance, while plant height, petiole length and basal diameter contributed little to the variance.

As with other works the isolation of the mixed group from other accessions is in agreement with Bakasso *et al*. (2013), El Tahir and El Gabri (2013), Cruz *et al*. (2013) who reported of accessions grouping independently of their origin. This observation confirms the hypothesis of movement of roselle germplasm from one part of the West African region to the other (Diouf *et al*., 2007). The large genetic diversity confirms West Africa as the center of diversity of *H. sabdariffa*. In addition, the diversity is an

indication that the accessions are a rich source of genes that could be incorporated into breeding programmes.

Traditionally, less tall, highly branched, and large leaf area genotypes are preferred as leafy vegetables while tall, few branching, and wide basal diameter plants are chosen for fiber production Though as few as three *altissima* accessions were included in current study, all three grouped in cluster I demonstrating the accuracy of the cluster analysis based on morphological evaluation. Cluster IA (HS68, HS86, HS77, HS25, HS09, HS20), IC (HS70, HS82, HS27), and IIB accessions (HS14, HS19, HS22, HS11, HS85, HS04, HS84, HS83, HS16) would be suitable for leafy vegetable and calyx production roselle owing to their large leaf area and tall plant height and large number of branches. On the basis of high values of plant height, height at first branching, stem width, and number of internodes, subcluster IA members would be valuable for improvement in fiber yield.

Although large values of plant height, branch number, stem width, and number of internodes are expected to discriminate the true fiber types from edible ones, other *sabdariffa* types could equally be classified with the fiber types as they exhibited similarities to the fiber types, HS25, HS68 and HS69. Twelve accessions were found to possess good fiber characteristics. These include the true *altissima* types HS25, HS68, and HS69, and the *sabdariffa* accessions HS09, HS20, HS32, HS13, HS50, HS02, HS14, HS19, and HS16. Accessions with large number of internodes which may be exploited for improving fiber characteristics include HS04, HS84, HS83, and HS86. Likewise accessions having large branch numbers, such as HS68, HS86, HS09, HS19, HS16, HS83, HS14, 20, and HS02 would be important for calyx production. As the economic value of roselle fiber increases owing to discovery of its unique fiber characteristics, as well as increasing global demand for natural fibers to replace

synthetic ones, further work will be required to identify more accessions which can equally be exploited for fiber production.

5.1.5 Principal Components and biplot analysis

According to the first two components which explained 100% of the total variance, all six traits were critical for structuring roselle accessions, the most important being plant height, number of internodes, and stem width. In addition to flowering time, budding time, seed weight and plant height employed by (Bakasso *et al*., 2013; Torres- Moran *et al.* 2011; Siepe *et al. (*1997; Abou El-Nasr *et al.* 2014; Sabiel *et al*., 2014), the number of internodes, height at first branching branch number and leaf area have proved to be very important in characterizing roselle.

The significance of the delineation of the genotypes into the uncorrelated groups is for parent selection aimed at maximization of genetic variance in breeding programs. Crossing of members belonging to different groups is expected to produce beneficial improvements in trait performance. However due to the tetraploid nature and the predominantly nonadditive gene effects, progress in breeding for trait improvement will be realized with accessions that are correlated rather than uncorrelated.

Examination of the PCA trait biplot revealed an array of angles among the traits indicating diverse relationships between plant architecture (plant height), fiber characteristics (height at first branching, stem width, and number of internodes), branching number and leaf characteristics (LA). Besides HS69, HS85, HS22, HS68, and HS09, which demonstrated large projections from the origin, signifying substantially large contributions to the variance, all other accessions contributed fairly good proportions to the variance in having appreciable and almost equal distances from the origin. The directions to which the accession vectors point covered the entire circumference of the orthogonal axes revealing the large biodiversity within the roselle

accessions. On the basis of the large diversity it may be possible to enhance the traits through selection. Improvement in number of internodes would also lead to better performance in plant height and branch number which would also influence calyx yield (Ibrahim and Hussein (2006; Sabiel *et al*., 2014; Chang *et al*., 2006). Similarly, the tight correlations between branch number and number of internodes, plant height and stem width and plant height and number of internodes indicate that improvement in plant height and number of internodes would lead to improvement in stem width. Besides plant height, branch number, number of internodes and stem width, the biplot analysis for traits has identified leaf area as one of the useful traits to consider in characterizing *H. sabdariffa* germplasm.

5.2 RAPD molecular diversity

The wide range of genetic distance of 0.00 to 0.79 with an average of 0.29 based on Jaccard‟s similarity represented a large variability among the 35 roselle accessions. The fact that the accessions investigated in the current study was higher in number and sourced from wider geographic zone in comparison to the limited number and narrow scope of that of Yusof *et al*. (2009), shows how efficient and reliable the RAPD technique is in identifying and characterizing roselle germplasm. This efficiency was further confirmed by the ability of the RAPD markers to discriminate among accession sets which were difficult to differentiate by morphological characteristics such as HS11, HS84 and HS85 (all red types, with pentalobed leaves), HS29 and HS78 (red types), HS50 and HS82 (intermediate types with partial trilobed leaves), HS16 andHS86 (red types with partial trilobed leaves). In addition, RAPDs provided clear information on genetic relationships in estimating polymorphism which could be useful in laying the foundation for constructing genetic map for the species.

5.2.1 Diversity, relationships and center of diversity of West African roselle

Comparison of genetic distances for the accessions studied revealed that the highest within population dissimilarity occurred among the Bawku, Ouagadougou and Lome accessions (Figure 4.3). This observation suggests that possibly these three regions are the centers of West African roselle diversity. This is not surprising since the Northern part of Burkina Faso shares common border with Togo (Figure 3.1).

Unexpectedly, two accessions each from Lome (HS58 and HS88) and Ouagadougou (HS14 and HS50) clustered together in cluster IV (Figure 4.3), further reinforcing the assertion of Ouagadougou and Lome (Togo) as center of diversity for roselle. Although these four accessions were very similar in many of the morphological traits, the mean genetic distance of 0.29, among cluster IV members shows the members are disparate at the molecular level. From the quantitative analysis (Figure 4.3.), although accessions were very similar in height at first branching and branching number they were disparate in plant height, number of internodes, leaf area, and stem width.. The fact that the accessions in this cluster were found to be diverse regardless of their clustering together is further evidence supporting two origins as the center of diversity of West African roselle accessions.

In contrast to the report of Heliyanto (1992) that bast fibre crops (*H. cannabinus* and *H. sabdariffa* L.) in the major fibre growing countries of Asia possess narrow genetic base, and exhibit major constraints including low yield potential, low adaptability to various agro-climatic situations and susceptibility to biotic and abiotic stresses (Edmonds 1991), the findings of this study have revealed that the well adapted roselle of West Africa is rich in genetic variability and exhibits large genetic diversity.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

In summary, the goal of current study was to determine the level of genetic diversity and relationships among 39 *Hibiscus sabdariffa* accession collections in West Africa by means of agromorphological trait evaluation and RAPD marker genotyping. Motivation for the study derived from the fact that, there had not been a single study to characterize, evaluate the, estimate genetic diversity, and determine variability and relationships among roselle in West Africa. Furthermore, there has not been any breeding activities in roselle in this region, neither has there been identification of genotypes that can be useful in roselle trait improvement. In Ghana, a new interest in the bast fiber of roselle has been generated due to its increasing economic value worldwide for the manufacture of automobile including airplane upholstery, carpets, shoes and bags, with the aim to create jobs, conserve the crop, and derive from it economic benefits as other countries such as Malaysia, Thailand, and India are doing. Unfortunately, commercial cultivation of roselle in Ghana is woefully limited although a current trend of increase in calyx consumption is evident. Additionally, the recent activities of the Jute Mills Ghana Limited rely on roselle fiber imports from India.

Regrettably, reports on breeding activities of roselle in other countries report on the existence of low variability in roselle genotypes at hand. Mutation breeding, the alternative to introducing variability in roselle is fraught with many drawbacks including serious physiological damage to the plant. Additionally, in Ghana, there is absence of information on systematic roselle collection and conservation. In this era of climate change anomalies, alternative sources of leafy vegetables from hardy plants are being explored, of which roselle leaves are good options. Moreover, due to the stringent

environmental regulations on synthetic fibers, natural sources of fiber are being explored around the world, and roselle is a potential candidate. The increase in popularity of the medicinal properties of the calyx anthocyanins is driving search for more genotypes with high yield of calyx. Moreover, the assertion that West Africa is the center of diversity of roselle requires validation, as this information is fundamental to the success of roselle improvement and conservation.

Although studies on roselle have been conducted in other regions, information on the West African landraces is absent. This research was therefore carried out to investigate the nature and magnitude of the existing genetic diversity among roselle in West Africa, to identify genotypes worth incorporating into breeding programmes, to determine groupings and the relationships among the genotypes for effective breeding system. The approach used in carrying out this work was a systematic collection of 39 roselle accessions from eight regions of West Africa, followed by morphological evaluation of the genotypes in field trials over two seasons to determine leaf characteristics, calyx yield components, and fiber yield components. Finally, molecular profiling of the genotypes to estimate genetic diversity and reveal the evolutionary history and interrelationships was carried out by means of variation in RAPD polymorphism.

The geographical area of collection spanned longitude 17.44^{o}W and latitude 14.69^{o}N in Senegal to longitude 5.25^{o}E and latitude 13.08^{o}N in Nigeria, involving indigenous women traders and subsistence farmers in roselle from each of seven countries. Traditionally, these traders have conserved a wide diversity of roselle germplasm through passing on of seeds along family lines generation after generation chiefly for the medicinal value of the calyx and the bast fiber. In the absence of a systematic collection and conservation roselle preservation has nevertheless been protected to some extent, while some genotypes may have been lost over the years to stressful

environmental factors. It was therefore expected that the thirty-nine accessions in current research was a good representation of the genetic diversity in roselle available in West Africa.

The study revealed large genetic variability in both qualitative and quantitative traits. On the basis of the qualitative evaluation, the least variable traits were throat colour of flower, leaf pubescence, pollen colour, and branch habit. Traits which presented many discrete classes include leaf lamina and margin colour, leaf lobe type, petiole and stipule colour, petal colour and calyx pubescence. In terms of the economies of roselle production, the most important qualitative trait is calyx pigmentation, texture, and yield. The wide range of red to deep red calyxes offers to the food and pharmaceutical industry a wide scope of shades of red to select from. Variation in these discrete classes is expected to be controlled by single or few genes, be less influenced by environment, and a simple selection method on the basis of the phenotypic expression would achieve the desired improvement in the trait.

In the quantitative traits, the large variabilities identified in plant height, leaf area, branch number, stem width, and number of internodes on the basis of highly significant mean squares and large coefficient of variability would be important from the breeding standpoint. The occurrence of good fiber yield components in some *sabdariffa* accessions indicates the opportunity to utilize *sabdariffa* types for fiber production too and not only *altissima* types. Studies are required to determine the inheritance and heritability of calyx pigmentation and texture, branch number, stem width and number of internodes in roselle. A major distinguishing feature between the *altissima* and *sabdariffa* varieties was found to be a firm attachment of the calyx onto the capsule in *altissima* types which was not present in *sabdariffa* types, where the calyx could easily be detached.

The substantial variability as well as the existence of low to medium heritability estimates among most of the traits studied suggests that improvement in roselle traits via selection of agro-morphologically beneficial genes could be viable, although difficult. The medium to high heritabilities of plant height in all populations except Upper West is an indication of high genetic variance arising from genetic components possibly additive genetic variance with some contributions from non-additive effects. In contrast, the low genetic variance for the fiber yield components, branch number, stem width, and number of internodes indicates that the principal gene effect is nonadditive effects of dominance and epistatsis involving dominant and recessive alleles in intralocus and interlocus regions of the genome. Although dominant effects are not heritable due to segregation into haploid (n) gametes in diploid organisms, for tetraploids such as roselle, the gamete is diploid (4n→2n) and so retains some level of interactions which are passed on to progeny making nonadditive genetic effects important in tetraploids. The same phenomenon maintains heterozygosity in tetraploid populations. These phenomena may account for the substantial variability in roselle, a cleistogamous crop.

Implications of the above genetic effects on breeding strategy are that, evaluation of a large number of genotypes is required to determine accurately the inheritance of the economically important traits; that parent selection must maximize the nonadditive dominance and epistatic interactions and so those that are genetically related must be intercrossed; additionally, it is important to examine stability of cultivars by evaluation of advanced selections in multiple environments and years as the genotype by environment interaction was high, especially the high sensitivity to day length.

Accessions with large leaf area and large number of branches are productive for leaf and calyx production which are good sources of nutrients such as vitamin A, B, and C,

the minerals, iron and phosphorus, as well as antioxidants, and offer good protein source for both human and animal feed.

Tall accessions with large number of internodes on the main stem and large stem widths were identified as good sources of fiber. Some var. *sabdariffa* accessions unexpectedly were tall, sparsely branched, with large number of internodes, within the limitations of the large plant spacing used in this study. These genotypes may be improved for fiber production as alternatives to *altissima* types. For improved cultivar, roselle growers aim at tall plants with high main stem for high fiber yield whereas a thick stem favours paper and pulp yield.

The similarity coefficients which ranged from 0.00 to 0.98 with an average of 0.27±0.26 and representing only 27% similarity among the genotypes, is an indication of substantial variability. Although clustering into two main clusters were independent of the origin of the accessions, the basis on which the grouping was done shows a high differentiation for fiber, calyx and leaf yield to delineate vegetable types as cluster II and fiber types as cluster I. Within cluster I, subcluster IA clearly separated accessions that demonstrated fiber characteristics as HS09, HS20 HS25, and
HS68. Cluster IIA separated genotypes that were good for both leaf and fiber yield. Important genotypes of this class are HS02, HS13 HS32, and HS50. In general, cluster II genotypes were found to be good for leafy vegetable production as in HS02,
HS14, HS19, HS32 and HS85 and good for calyx production based on their extensive branching in HS02, HS16, HS68, HS86, HS09, and HS19. Although HS68, a fiber type, demonstrated ideal fiber traits of tall plant, large stem diameter, large number of internodes, it also had extensive branching for calyx production. Besides its capacity as a source of seeds, its inedible calyxes represented a sink for biomass, which may be considered for redirection for taller plants instead.

The principal component analysis revealed that in addition to plant height, branch number, stem width, height at first branching and, number of internodes which are previously reported to be useful in structuring roselle, the current research revealed that leaf area is an additional trait that must be considered for characterization of roselle because of their large weights among the eigenvectors.

Similarly, accessions that correlated in their principal components are expected to produce much progress in breeding owing to the tetraploid nature and the predominantly nonadditive gene effects in roselle. Such highly correlated accessions include HS69, HS68, HS25, HS02, HS20, HS86 and HS02 for fiber yield gene pool, and HS22, HS32, HS19, HS14, HS84, HS16, HS13, HS11, HS16, HS83 and HS04 as gene pool for calyx and leaf yield.

On the molecular data, the 80 to 100% rate of polymorphism with a majority of the loci (83.3%) having five or more alleles suggests considerable genetic diversity within the 32 roselle genotypes. The mean value of 5.25±0.75 alleles and 5.16±0.83 polymorphic fragments per locus and a mean intra locus gene diversity of 0.21±0.05 across the 12 RAPD primers confirm the wide genetic diversity in roselle.

Similarly, the similarity coefficient 0.00 to 0.79 and the mean of 0.29±0.14 recorded for the RAPD molecular structuring is an indication of the existence of large intraspecific variability within the West African roselle germplasm. On average, accessions were only 29% similar. Indeed the variability was unexpected owing to earlier reports of lack of variability in other genotypes. However, this low similarity was not unexpected as large geographic range influences genetic diversity. Cleistogamy gives a small room for recombination which when it persists for a long period of time can lead to increase in variability. Hence the wide variability observed

within West African collection, may have been introduced, gradually over time. The mixed cluster that was identified accounts for this variability.

Contrary to the trend observed by earlier research works based on the genetic similarity values, the diversity observed within roselle by means of the morphological tool was more spectacular than the one revealed by the RAPD. This notwithstanding, the ability of RAPD to partition the genotypes on the basis of their origin, and to differentiate between accessions with very subtle differences such as HS29 and HS78 (red types) is a proof of the efficiency of this molecular tool in structuring genotypes both at the intraspecific and interspecific levels. The close value of mean genetic distance for both morphological (27%) and molecular 29%), indicates the reliability of morphological evaluation in genetic diversity estimation, though this reliability would depend on the life history traits of the germplasm, especially its breeding system, Although both morphological and molecular tools have proved useful in structuring roselle, the superiority of molecular tools over morphological ones was reemphasized in the current investigation by the inefficiency of the latter to cluster the accessions on the basis of origin, and separate the accessions with very subtle differences in morphology as in the case of the pair HS11 and HS84 with slender and pentalobed leaf red types. In this respect, the RAPD genotyping has proved to be superior to the morphological evaluation by its ability to unearth true differences irrespective of environmental influences on the observed traits. This investigation has emphasized the need to couple morphological and molecular structuring tools for reliable assessment of genetic diversity.

Comparison of genetic distances within clusters and among the countries point to the region from Mali, Ouagadougou, Bawku, and Lome as the center of West African

roselle diversity. Future roselle collection expeditions must of necessity target this geographic location for a representative collection.

In order to benefit from this rich genetic resource, a registry, germplasm collection, proper identification, and characterization study covering a wider collection must be undertaken. Results of this study have revealed that substantial variability exists in roselle and the application of appropriate breeding strategy may be considered besides mutation breeding.

Few drawbacks were encountered during the research period. One of such was the unequal accessional representation of country accessions. An investigation which includes more accessions and use of more discriminatory marker such as inter simple sequence repeats could give an improved estimation of the existing variability in roselle. Another shortcoming was the inability to include calyx shape habit in the qualitative descriptor list used currently. The spectacular variability observed in this trait implies that possibly its absence may have led to the inability to bring out some subtle differences between the accessions. A setback was with the use of agarose gel for separating the bands owing to its inability to separate DNA fragments which overlap only by a single base pair.

Today, improvement in roselle traits has become more urgent than ever due to its multipurpose need for fibers to augment the world supply of environmentally friendly fibers and the need for its natural red pigment in the beverage, cosmetic, confectionery and pharmaceutical industries., the need for drought-tolerant and hardy crops as alternative sources of leafy vegetables makes roselle a good candidate for such purpose. The information on the magnitude of the existing genetic diversity, the relationships in roselle and the nature of polymorphism could be useful in laying the foundation for constructing genetic map for *Hibiscus sabdariffa* L. The large diversity observed in the

West African roselle genotypes suggests ample opportunity for development of improved lines via conventional breeding.

The current research has emphasized the efficiency and reliability of the RAPD molecular tool over moprhological evaluation, though more information is derived by coupling the two methods.

The current study has identified desirable genotypes for incorporating into breeding programs, and that there is a wide array of pigmentation pattern in the genotypes to select from. Moreover, in addition to *altissima*, some *sabdariffa* genotypes had equally good fiber yield potential.

6.2 Recommendations

On the basis of the findings and shortcomings of the current research, the following recommendations for further work are proposed:

1. A wider collection of both *altissima* and *sabdariffa* ecotypes in quantities representative of the various geographical regions in West Africa must be done..
2. That genotyping is conducted by means of more robust molecular marker such as intersimple sequence repeats (ISSR) or amplified fragment length polymorphism (AFLP) as information on roselle genome sequence is not yet available.
3. Determination of inheritance of the commercially important roselle traits including fiber and clayx yield characterstics
4. A descriptor list for morphological classification of roselle must be created with the help of the traits that have been found to be important in structuring roselle.

5. Any future study of this nature should include calyx shape around the capsule to help bring out other important differences between the accessions.

6. Further studies should concentrate on intrapopulation gene diversity.

7. Application of a more powerful separating media such as polyacrylamide gel with silver staining.

REFERENCES

Abou El-Nasr, T. S. H., Magda, A. M., El-Enany, A. M. and Ibrahim, M. M. (2014). Genetic parameters evaluation among some selected lines of Sudanese Roselle variety in Egypt, using morpho-agronomic traits and ISSR markers. Middle East Journal of Applied Science (4) 42:181-190.

Adam, W. C. (1960). Inheritance of leaf shape in roselle, *H. sabdariffa* L. Journal of Heredity 74:485-486.

Akindahunsi, A. A. and Olaleye, M. T. (2003). Toxicological investigation of aqueous-methanolic extract of the calyces of *Hibiscus sabdariffa* L. Journal of Ethnopharmacology 89:161-164.

Akobundu, I. O. (1987). Weed Science in the Tropics: Principles and Practices. New York, Wiley.

Akpan, G. A. (2000). Cytogenetic characteristics and the breeding system of six *Hibiscus* species. Theoretical and Applied Genetics 100:315-318.

Alam, N., Ahmed, F. A., Begum, R. and Alam, S. S. (2006). Differential fluorescent banding and isozyme assay of *Hibiscus cannabinus* L. and *H. sabdariffa* L. (Malvaceae). Cytologia 71:175-180.

Alam, N., Pasha, M. K. and Ahmed, S. (2006). Identification of some *Hibiscus* germplasm through numerical analysis. Bangladesh Journal of Plant Taxonomy 13:49-54.

Alarcón-Aguilar, F. J., Zamilpa, A., Pérez-García, D., Almanza-Pérez, J. C., Romero-Nuñez, E., Campos-Sepúlveda, E. A., Vázquez-Carrillo, L. I. and RománRamos, R. (2007). Effect of *Hibiscus sabdariffa* on obesity in MSG mice. Journal of Ethnopharmacology 114:66–71.

Alarcón Cruz, N., Solano, L. and Porfirio, J. (2013). Morphological characterization of an ethnographic sample of roselle (*Hibiscus sabdariffa* L.) Revista Chapingo Serie Horticultura 19: 85-98.

Alessi, J., Power, J. F. and Zimmerman, D. C. (1977). Sunflower yield and water use as influenced by planting date, population and row spacing. Agronomy Journal 69:465-469.

Ali, B. H., Al, W. N and Blunden, G. (2005). Phytochemical, pharmacological and toxicological as aspects of *Hibiscus sabdariffa* L.: a review. Phytotherapy Research 19:369-375.

Ali, B. H., Mousa, H. M. and El-Moughy, S. (2003). The effect of a water extract and anthocyanins of *Hibiscus sabdariffa* L. on paracetamol induced hepatotoxicity in rats. Phytotherapy Research 17:56-59.

Appell, S. D. (2003). Red sorrel *Hibiscus sabdariffa* - the other „cranberry". Plants and Gardens News 18:1-2.

Asaolu, S. S., Adefemi, O. S., Oyakilome, I. G., Adjibulu, K. E. and Asaolu, M. E. (2012). Proximate and mineral composition of Nigerian leafy vegetables. Journal of Food Research 1:214-218.

Atta, S., Seyni, H. H., Bakasso, Y., Sarr, B., Lona, I. and Saadou, M. (2011). Yield character variability in a roselle (*Hibiscus sabdariffa* L.). African Journal of Agricultural Research 6: 1371-1377.

Babalola, O. (2000). Chemical composition of roselle (*Hibiscus sabdariffa* L.) leaf. Proceedings of the 24th Annual Conference of the Nigerian Institute of Food Science and Technology. Pp. 119-121.

Bakasso, Y., Saadou, M., Zongo, J-D., Baidou-Forson, J. and Toujani, A. (2009). Preliminary assessment of the influence of environment on growth parameters and yield in some genotypes of *Hibiscus sabdariffa* L. from Niger. International Journal of Biological and Chemical Sciences 3:1133-1140.

Bakasso,Y., Zaman-Allah, M., Mariac, C., Billot, C., Vigouroux, Y., Zongo, J. D. and Saadou, M. (2013). Genetic diversity and population structure in a collection of Roselle (*Hibiscus sabdariffa* L.) from Niger. Plant Genetic Resources: Characterization and Utilization. Pp. 1-8.

Balaami, A. (1998). The effects of pressing conditions, packaging and storage on selected quality attributes of *Munga ntusa*. MSc. Thesis, University of Ibadan, Nigeria.

Bar-Hen, A. A., Charcosset, M. B. and Cuiard, J. (1995). Relationships between genetic markers and morphological traits in a maize inbred lines collection. Euphytica 84:145-154.

Barik S., Senapaki, S. K., Aparajita, S., Mohapatra, A. and Rout, G. R. (2006). Identification and genetic variation among *Hibiscus* species (Malvaceae) using RAPD markers. Verlag der Zeitschrift fur Naturfoschung 1:123-128.

Basu, N. C. and Chakravarty, K. (1972). Inheritance of certain morphological characters in roselle (*H. sabdariffa* L.). Current Science 41:189-190.

Basu, N. C. and Chakravarty, K. (1972). Inheritance of certain morphological characters in roselle (*H. sabdariffa* L.). Jute Agricultural Research Institute, Barrackpur, West Bengal, India.

Beaumont, M. A., Ibrahim, K. M., Boursot, P. and Bruford, M. W. (1998). Measuring Genetic Distance. Pp. 315-325. In Karp, A., Isaac, P. G., and Ingram, D. S. (eds.). Molecular tools for Screening Biodiversity. London, Chapman and Hall.

Bernardo, R. (1993). Estimation of coefficient of co ancestry using molecular markers in maize. Theoretical and Applied Genetics 85:1055-1062.

Bernardo, R. (1993). Estimation of coefficient of coancestry using molecular markers in maize. Theoretical and Applied Genetics 85:1055-1062.

Beyene, Y., Botha, A. and Myburg, A. A. (2005). A comparative study of molecular and morphological methods of describing genetic relationships in traditional Ethiopian highland maize. African Journal of Biotechnology 4:586-595.

Botstein, D., White, R. L., Skolnick, M. and Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics 32:314-331.

Bricage, P. (1984). Influence of the seed-bed density, the parental phenotype and the surrounding on the efficiency of seeding *Hibiscus sabdariffa* L. seeds. Bulletin the Fundamental Institute of Black Africa Series A. Natural Sciences 40:728730.

Brink, M. and Escobin, R. P. (2003). Fibre Plants. Plant Resources of South-East Asia, No 17. Leiden. The Netherlands. Backhuys Publishers. Pp. 456.

Brown, A. H. D. (1979). Enzyme polymorphisms in plant populations. Theoretical Population Biology 15:1-42.

Brown, A. H. D. and Weir, B. A. and (1983). Measuring genetic variability in plant populations. In: Tanksley, S. D. and Orton, T. J. (eds.) Isozymes in Plant Genetics and Breeding, Elsevier, Amsterdam, Pp. 219–239.

Brown-Guedira, G. L., Thompson J. A., Nelson, R. L., Warburton, M. L. (2000). Evaluation of genetic diversity of soybean introductions and North American ancestors using RAPD and SSR markers. Crop Science 40:815-823.

Bruna, S., Portis, E., Braglia, L., De Benedetti, L., Comino, C., Acquadro, A. and Mercuri, A. (2009). Isolation and characterization of microsatellite markers from *Hibiscus rosa-sinensis* (Malvaceae) and cross-species amplifications. Conservation Genetics 10: 771-774.

Cary, N. C. (2011). SAS Institute, Inc., Base SAS 9.1.3 User‟s Guide, statistical procedures.

Cavalla-Sforza, L. L. and Edwards, A. W. F. (1967). Phylogenetic analysis-models and estimation procedures. The American Journal of Human Genetics 19:1-3.

Cavalli-Sforza, L. L., Menozzi, P. and Piazza, A. (1993). Demic expansions and human evolution. Science 259(5095):639-646.

Cavalli-Sforza, L. L., Menozzi, P. and Piazza, A. (1994). The history and geography of human genes. Princeton University Press.

Chakravarty, K., Banerjee, S. P. and Shome, A. (1981). A new plant type in roselle (*H. sabdariffa*) and inheritance of two of its main distinguishing characteristics. Genetica 55:181-183.

Chakravarty, K., Banerjee, S. P. and Shome, A. (1981). A new plant type in roselle (*H. sabdariffa*) and inheritance of two of its main distinguishing characteristics. Genetica 55:181-183.

Chang, Y. C., Huang, K. X., Huang, A. C., Ho, Y. C. and Wang, C. J. (2006). Hibiscus anthocyanins-rich extract inhibited LDL oxidation and oxLDLmediated macrophages apoptosis. Food and Chemical Toxicity 44:1015-1023.

Chang, Y.C., Huang, H.P., Hsu, J.D., Yang, S.F and Wang, C.J., (2005). Hibiscus anthocyanins rich extracts induced apoptotic cell death in human promyelocytic leukemia cells, Toxicology and Applied Pharmacology 205:205-212.

Chang-Che, C., Chou, F-P., Ho, Y-C., Lin, W-L., Kao, E. S., Huang, A-C. and Wang, C-J. (2004). Inhibitory effects of *Hibiscus sabdariffa* L. extract on low-density lipoprotein oxidation and anti-hyperlipidemia in fructose-fed and cholesterol-fed rats. Journal of the Science of Food and Agriculture 84:19891996.

Chen, C. C., Hsu, J. D., Wang, S. F., Chiang, H. C., Yang, M. Y., Kao, E. S, Ho, Y.C. and Wang, C. J. (2003). *Hibiscus sabdariffa* extract inhibits the development of atherosclerosis in cholesterol fed rabbits. Journal of Agriculture and Food Chemistry 51:5472-5477.

Cheng, Z., Lu, B.-R., Baldwin, B. S., Sameshima, K. and Chen, J. K. (2002). Comparative studies of genetic diversity in kenaf (*Hibiscus cannabinus* L.) varieties based on analysis of agronomic and RAPD data. Hereditas 136:231-239.

Cheng, Z., Lu, B-R., Sameshima, K., Fu, D-X. and Chen, J-K. (2004). Identification and genetic relationships of kenaf (*Hibiscus cannabinus L.*) germplasm revealed by AFLP analysis. Genetic Resources and Crop Evolution 51:393401.

Cheng, Z., Sameshima, K. and Chen, J. K. (2002). Genetic variability and relationship of kenaf germplasm based on RAPD analysis (in Chinese). Plant fibre and products 1:1-12.

Chhalbilendra, R. (2009). The International Jute Commodity System- The World of Natural Fibers. Northern Book Centre New Delhi, Pp 7.

Clydesdale, F. M., Main, J. H. and Francis, F. J. (1979). Roselle (*Hibiscus sabdariffa* L.) anthocyanins as colorant beverage and gelatin deserts. Journal of Food Protection 42: 204-267.

Coffie, N., Tetteh, A. Y. and Twumasi, P. (unpublished). Characterization of roselle (*Hibiscus sabdariffa* L.) accessions by agromorphological traits.

Collins, F. S., Brooks, L. D. and Chakravarty, A. (1998). A DNA polymorphism discovery resource for research on human genetic variation. Genome Research 8:1229-1231.

composition, physical, tensile, morphological , and thermal properties of roselle fibre : Effect of fibre maturity. Bioresources 10:1803-1824.

composition, physical, tensile, morphological , and thermal properties of roselle fibre : Effect of fibre maturity. Bioresources 10:1803-1824.).

Crane, J. C. (1949). Roselle - a potentially important plant fiber. Economic Botany 3:89-103.

Cruz, N. A. and Solano, J. P. L. (2013). Morphological characterization of an ethnographic sample of roselle (*Hibiscus sabdariffa* L.). Revista Chapingo Serie Horticultura 19:85-98.

Dafallah, A. A. and al-Mustafa, Z. (1996). Investigation of the anti-inflammatory activity of *Acacia nilotica* and *Hibiscus sabdariffa*. American Journal of Chinese Medicine 24:263-269.

Daudu, O. A. Y., Falusi, O. A., Dangana, M. C., Abubakar, A.,Yahaya, S. A. and Abejide, D. R. (2015). Collection and evaluation of roselle (*Hibiscus sabdariffa* L.) germplasm in Nigeria. African Journal of Science 9:92-96.

David, G. and Adam, P. (1988). Crops of the Drier Regions of the Tropics. ELBS Edition.

Dellaporta, S. L., Wood, J. and Hicks, J. B. (1983). A plant DNA minipreparation: Version 11. Plant Molecular Biology Reporter 1:19-21.

Diouf, M., Gueye, M., Faye, B., Dieme, O. and Lo, C. (2007b). Commodity systems of four indigenous leafy vegetables in Senegal. Water SA Volume 33, No., 3 (Special edition), Pp. 343-348.

Diouf, M., Mbengue, N. B. and Kante, A. (2007a). Characterization of four accessions and species of traditional leafy vegetables (*Hibiscus sabdariffa* L., *Vigna unguiculata* (L.) Walp. *Amaranthus* spp. L. and *Moringa oleifera* AML.) in Senegal. African Journal of Food, Nutrition and Development 7:1-14.

Doolittle, D.P. 1987. Population genetics: Basic Principles. Advanced Series in Agricultural Sciences. vol. 16. Springer-Verlag, Berlin. Pp.183-186.

Du, C. T. and Francis, F. J. (1973). Anthocyanins of Roselle (*Hibiscus sabdariffa* L.). Journal of Food Science 38:810-812.

Duke, J. A. (1983). *Hibiscus sabdariffa*. from http://www.hort.purdue.edu/newcrop/duke_energy/Gliricidia_sepium.html. Verified 26th February, 2016.

Dwivedi, S., Perottiand, E. and Ortiz, R. (2008). Towards molecular breeding of reproductive traits in cereal crops. Plant Biotechnology Journal 6:529-559.

Edmonds, J. M. (1991). The distribution of *Hibiscus* L. section *Fucaria* in Tropical East Africa. Systematic and Ecogeographic studies on Crop gene pools. IBPGR. 6.

El Naim, A. M. and Ahmed, S. E. (2010). Effect of weeding frequencies on growth and yield of two roselle (*Hibiscus sabdariffa* L.) varieties under rain fed. Australian Journal of Basic and Applied Sciences 4:4250- 4255.El-Tahir, M. I. and El Gabri, M. A. M. (2013). Morpho-agronomic variation within local genetic resources of roselle (*Hibiscus sabdariffa* var. *sabdariffa* L.) in Sudan. Scholarly Journal of Agricultural Science 3:317-324.

El Naim, A. M., Khaliefa, E. H., Ibrahim, K. A., Ismaeil, F. M. and Zaied, M. M. B. (2012). Growth and yield of Roselle (*Hibiscus sabdariffa* L.) as influenced by plant population in arid tropic of Sudan under Rain-fed. International Journal of Agriculture and Forestry 2: 88-91.

Ellsworth, D. L., Rittenhouse, D. and Honeycutt, R. L. (1993). Artifactual variation in random amplified polymorphic DNA banding patterns. Biotechniques 14:214217.

Eltayeib, A. A. and Elaziz, A. A. (2014). Physicochemical properties of roselle (*Hibiscus sabdariffa* L.) seed oil (Elrahad-1) in North Kordofan, Sudan. Journal of Scientific and Innovative Research 3:578-582.

Experimental Site Survey, (2010). Soil sample analysis results for Anwomaso Experimental Research Station . Council for Scientific and Industrial Research (CSIR) Kwadaso, Kumasi.

Falconer, D. S. and Mackay, T. F. C. (1996). Introduction to Quantitative Genetics (4th Ed). Longman Group Ltd. Pp.128 -131.

Falusi, A. O. (2007). Cultivation and use of roselle (*Hibiscus sabdariffa* L.) in Nigeria. Production Agriculture and Technology Journal 3:129-134.

Falusi, A. O., Dangama, M. C., Daudu, O. A. Y., Oluwajobi, A.O., Abejide, D. R. and Abubakar, A. (2014). Evaluation of some roselle (*Hibiscus sabdariffa* L.) germplasm in Nigeria. International Journal of Biotechnology 2:16-20.

Falusi, A. O., Dangama, M. C., Daudu, O. Y. and Teixeira da Silva, J. A. (2012). Studies on some morphological and yield parameters of three varieties of Nigeria okro (*Abelmoschus esculentus*). Journal of Horticulture 4:126-128.

Faraji, M. H. and Tarkhani, H. H. (1999). The effect of sour tea (*Hibiscus sabdariffa*) on essential hypertension. Journal of Ethnopharmacology 65:231-236.

Farooq, S. and Azam, F. (2002). Molecular markers in plant breeding -I: concepts and characterization. Pakistan Journal of Biological Sciences 5:1135-1140.

Favaro, S. L. (2010). Chemical, morphological and mechanical analysis of sisal fiberreinforced recycled high-density polyethylene composites. Express Polymer Letters 4(8):465-473.

Franco, J., Crossa, J., Ribaut, J. M., Bertran, J., Warburton, M. L. and Khairallah, M. (2001). A method for combining molecular markers and phenotypic attributes for classifying plant genotypes. Theoretical and Applied Genetics 103:944952.

Franco, J., Crossa, J., Villasenor, J., Taba, S., and Eberhart. S.A. (1998). Classifying genetic resources by categorical and continuous variables. Crop Science 38:1688-1696.

Frank, T. Janβen, M., Netzel, M., Straβ, G., Ler, A., Kriesi, E. and Bitsch, I. (2005). Pharmacokinetics of anthocyanidin-3-glycosides following consumption of *Hibiscus sabdariffa* L. extract. Journal of Clinical Pharmacology 45:203-210.

Frankel, O. H., Brown, A. H. D. and Burdon, J. J. (1995). The Conservation of Plant Biodiversity. Cambridge University Press, Cambridge, England.

Frimpong, G., Adotey, J., Ofori-Kwakye, K., Kipo, S. L. and Dwomo-Fokuo, Y. (2014). Potential of aqueous extract of *Hibiscus sabdariffa* calyces as coloring agent in three pediatric oral pharmaceutical formulations. Journal of Applied Pharmaceutical Science 4:001-007.

Fryxell, P. A. (1968). A redefinition of the tribe Gossypiaceae. Botanical Gazette 129:296-308.

Fryxell, P. A. (1988). Malvaceae of Mexico. Systematic Botany Monographs 25:1522.

Gasim, S. M. (1994). Genetic variability of some agronomic characters on Roselle (*Hibiscus sabdariffa* L.) MSc. Thesis, Faculty of Agriculture, University of Khartoum, Sudan.

Gautam, R. D. (2004). Roselle: a lesser-known source of medicinal and soft drink in India. Natural Product Radiance 3:338-342.

Gower, J. C. (1971). A general coefficient of similarity and some of its properties. Biometrics 27:857-874.

Grattapalglia, D. and Sederoff, R. (1994). Genetic linkage map of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo test cross mapping strategy and RAPD markers. Genetics 137:1121-1137.

Guerra, R. and Yu, Z. (2005). Single nucleotide polymorphism and their applications. Computational and statistical approach to genomics pp 311-349. Department of Statistics, Rice University, Houston, Texas, U.S.A.

Gurrola-Díaz, C. M., García-López, P. M., Sánchez-Enríquez, S., Troyo-Sanromán, Haji, F. M. and Haji, T. A. (1999). The effect of sour tea (*Hibiscus sabdariffa*) on essential hypertension. Journal of Ethnopharmacology 65:231-236.

Gurrolla-Diaz, C. M., Garcia-Lopez, P. M., Sanchez-Enriquez, S., Troyo-Sanroman, R., Andrade-Gonzalez, I. and Gómez-Leyva, J. F. (2010). Effect of *Hibiscus sabdariffa* extract powder and preventive treatment (diet) on the lipid profiles of patients with metabolic syndrome (MeSy). Phytomedicine 17:500-505.

Hair, J. R., Anderson, R. E., Tatham, R. L. and Black, W. C. (1995). Multivariate Data Analysis with Readings. 4th edition. Prentice-Hall, Englewood Cliffs, New Jersey.

Haji, F. M. and Haji, T. A. (1999). The effect of sour tea (*Hibiscus sabdariffa*) on essential hypertension. Journal of Ethnopharmacology 65:231-236.

Hallauer, A. R. and Miranda, J. B. (1981). Quantitative Genetics in Maize Breeding. Iowa State University Press, Ames.

Hamrick, J. L. and Godt, M. J. W. (1990). Allozyme diversity in Plant Species. In Plant Population Genetics, Breeding and Genetic Resources. Brown, A. D.H., Clegg, M. T., Kahler, A. L. and Weir, B. S. Sinauer, (Eds.). Sinauer, Sunderland, MA. Pp.42-63.

Hanboonsong, Y., Vinijsanun, T. and Ponragdee, W. (2000). Molecular Characterization and Genetic Relationships of Roselle Germplasm in Thailand. Proceedings of the Final Workshop on "Application of Biotechnology in the Improvement of Jute, Kenaf and Allied Fibres-Phase II," (IJO/AGR/10). Beijing, China. Pp. 95-106.

Haque, S., Begum S., Sarker, R. H. and Khan, H. (2007). Determining genetic diversity of some jute varieties and accessions using RAPD markers. Plant Tissue Culture Biotechnology 17:183-191.

Harding, S. S. and Mohammad, O. (2009). Radiosensitivity test on two varieties of Terrengganu and Arab used in mutation breeding of roselle (*H. sabdariffa* L.). African Journal of Plant Science 3:181-183.

Hartings, H., Berardo, N., Mazzinelli, G. F., Valoti, P., Verderio, A. and Motto, M. (2008). Assessment of genetic diversity and relationships among maize (*Zea mays* L.) Italian landraces by morphological traits and AFLP profiling. Theoretical and Applied Genetics 117: 831-842.

Hartl, D. L. and Clark, A. C. (1997). Principles of Population Genetics. Sinauer Associates. Inc. Sunderland, MA. Pp.542.

Heliyanto, B. (1992). Evaluation of kenaf (*Hibiscus cannabinus* L.) germplasm through breeding test. M.Sc. (Agric.) thesis. Department of Genetics and Plant Breeding. Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India.

Hinsley, R. S. (2008). Economic uses of Malvaceae - Overview.

http://www.malvaceae.info/Economic/Overview.html. Verified 09 January 2016.

Holland, J. B. (2006). Estimating genotypic correlation and their standard errors using multivariate restricted maximum likelihood estimation with SAS proc mixed. Crop Science Society of America 46:642-654.

Hopkins, A. L., Lamm, M. G., Funk, J. and Ritenbaugh, C. (2013). *Hibiscus sabdariffa* L. in the treatment of hypertension and hyperlipidemia: a comparative review of animal and human studies. Fitoterapia 85:84-89.

Hotelling (1933). Analysis of a Complex of Statistical Variables into Principal Components. Journal of Educational Psychology, 24:417-441,498-520.

Hotelling, H. (1933). Analysis of a complex of statistical variables into principal components. Journal of Educational Psychology 24**:**417-441 and 498-520.

Howard, A. and Howard, G. L. C. (1911). Studies in Indian fibre plants, No. 2. On some new varieties of *Hibiscus cannabinus* L. and *Hibiscus sabdariffa* L. Memoirs of the Department of Agriculture in India Botany Series 4.

Howard, A. and Howard, G. L. C. (1924).). Studies in Indian fibre plants, No. 3. On the inheritance of characters in *Hibiscus sabdariffa* L. Memoirs of the Department of Agriculture in India Series 13:47-87.

Huff, D. T., Peakall, T, and Smouse, P. E. (1993). RAPD variation within and among natural population of outcrossing buffalo grass (*Buchlodactyloides* (Nutt.) Engelm). Theoretical and Applied Genetics 86: 927-934.

Ibrahim, E. B., Abdalla, A. H. W., Ibrahim, E. A. and El Naim, A. M. (2013). Interrelationships between yield and its components in some roselle (*Hibiscus sabdariffa* L.) genotypes. World Journal of Agricultural research 1:114-119.

Ibrahim, M. M. and Hussein, R. M. (2006).Variability, heritability and genetic advancement in some genotypes of roselle (*Hibiscus sabdariffa* L.).World Journal of Agricultural Science 2:340-345.

Igarashi, K., Takanashi, K., Makino, M. and Yasui, T. (1989). Antioxidative activity of major anthocyanin isolated from wild grapes (*Vitis coignetiae*). Nippon Shokuhin Kogyo Gakkaishi, 36:852-856.

Ishak, M. R., Sapuan, S. M., Leman, Z., Rahman, M. Z., Anwar, U. M. K., and Siregar, J. P. (2013). Sugar palm (*Arenga pinnata*): Its fibres, polymers and composites. Carbohydrate Polymers 91:699-710.

Islam, M. M., Islam, F., Islam, M. S. and Fakir, A. (2008). Effect of planting date on canopy characters and capsule production in *Hibiscus sabdariffa* (var. *sabdariffa*) grown in the boundaries of rice field. Journal of Agroforestry and Environment 2:1-6.

Jaccard, P. (1908). Nouvelle researches sur la distribution florale. Bulletin de la Soci t Vaudoise des Sciences Naturelles 44:223-270.

Jawaid, M. and Abdul Khalil, H. P. S. (2011). Cellulosic/synthetic fibre reinforced polymer hybrid. Carbohydrate Polymers Composites: A review 86(1): 1-18.

Johns, M. A., Scrotch, P. W., Nieinhuis, J., Hinricsen, P., Bascur, G. and MunozSchick, C. (1997). Gene pool classification of common bean landraces from Chile based on RAPD and morphological data. Crop Science 37:605-613.

Johnson, R. A. and Wichern, D. W. (1992). Applied Multivariate Statistical Analysis. Prentice Hall.

Joliffe, I. T. (2002). Principal component analysis. 2nd ed. Springer Series in statistics. New York: Springer-Verlag New York.

Kantetey, R. V., Zeng, X., Jeffrey, L. B. and Zehr, B. E. (1995). Assessment of genetic diversity in dent popcorn (Zea mays L.) inbred lines using inter-simple sequence repeat (ISSR) amplification. Molecular Breeding 1:365-373.

Khalil, H. P. S., Jawaid, M., Hassan, A., Paridah, M.T., and Zaidon, A. (2012). Oil palm biomass fibres and recent advancement in oil palm biomass fibre-based hybrid biocomposites. In: Composites and their Applications. In Tech open, Croatia.

Khan, A. R. (1930). A new type of Roselle Hemp. Journal of India 25:210.

Khatun, A. (2004). Recent agricultural developments in jute, kenaf and MESTA through traditional and Biotechnological approaches. Genetic Resource and seed Division, Bangladesh Jute Research Institute (BJRI), Manik Mia Avenue, Dhaka 1207, Bangladesh. Pp 6.

Knapp, S. J. (1986). Confidence intervals for heritability for two-factor mating design single environment linear models. Theoretical and Applied Genetics 72:587591.

Knapp, S. J., Stroup, W. W. and Ross, W. M. (1985). Exact confidence intervals for heritability on a progeny mean basis. Crop Science 25:192-194.

Koorsa, K. V. (1987). Genetic variability studies in roselle (*Hibiscus sabdariffa* L.). Journal of Agricultural Science 21:90-91.

Kresovich, S., William, J. G. K., McFerson, J. R., Routman, E. J. and Schaal, B. A. (1992). Characterization of genetic identities and relationships of *Brassica oleracea* L. via a random amplified polymorphic DNA assay. Theoretical and Applied Genetics 85:190-196.

Leung, W. T. W., Busson, F. and Jardin, C. (1968). Physical and chemical properties of leafy vegetables. Plant Resources of Tropical Africa 2:522-527.

Lin, T-L., Lin, H-H., Chen, C-C., Lin, M-C., Chou, M-C. and Wang, C-J. (2007). *Hibiscus sabdariffa* extract reduces serum cholesterol in men and women. Nutrition Research 27: 140-145.

Louis, S. J., Kadams, A. M., Simon, S. Y. and Mohammed, S. G. (2013). Combing ability of roselle cultivars for agronomic traits in Yola Nigeria. Greener Journal of Agriculture 3:145-149.

Markert, C. L. and Moller, F. (1959). Multiple forms of enzymes: tissue, ontogenetic and species specific patterns. Proceedings of the National Academy of Sciences, U.S.A 45:753-763.

McClintock, N. C. (2004). Roselle in Senegal and Mali. LEISA Magazine 20.1, North Carolina State University, Department of Crop Science, Box 7620, 2409 Williams Hall, Raleigh, NC 27695, U.S.A.

McClintock, N. C. and El Tahir, I. M. (2004). *Hibiscus sabdariffa* L. In: Plant Resources of Tropical Africa: Vegetables/Légumes, Grubben, G. J. H. and Denton, O. A. (Eds.). Wageningen, Netherland, PROTA 2.

Medagam, T. R., Begum, H., Rao, N. H., Neelam, S., Pandravada, S. R. and Natarajan, S. (2015). Genetic diversity and variability in landraces for key agroeconomic traits in vegetable roselle (*Hibiscus sabdariffa* var. sabdariffa L.). Jordan Journal of Biological Sciences 8:113-125.

Mehdi, A., Touba, E., Zarim, S. and Tahereh, E. (2013). An overview of roselle plant with particular reference to its cultivation, diseases and usage. European journal of medicinal plants 3:135-145.

Melchinger, A. E. (1993). Use of RFLP markers for analysis of genetic relationships among breeding materials and prediction of hybrid performance. In: D.R Buxton (Ed.). Proceedings of the International Crop Science Congress. 1st, Ames, IA, July, 1992. CSSA, Madison, WI. Pp. 621-628.

Melchinger, A. E., Messmer, M. M., Lee, M., Woodman, W.L. and Lampkey, K. R. (1991). Diversity and relationship among U.S. maize inbred revealed by restriction fragment length polymorphism. Crop Science 31:669-678.

Menozzi, P., Piazza, A. and Cavalli-Sforza, L. (1978). Synthetic maps of human gene frequencies in Europeans. Science. September 1, 201(4358):786-792

Menzel, F. D. and Wilson, M. Y. (1964). Kenaf (*Hibiscus cannabinus*), Roselle (*Hibiscus sabdariffa*) Economic Botany 18:80-91.

Messmer, M. M., Melchinger, A. E., Reinhold, G., Hermann, R. G. and Boppenmaier, J. (1993). Relationships among early European maize inbreds. II. Comparison of pedigree and RFLP data. Crop Science 33:944-950.

Meyer, A. S., Yi, O. S., Pearson, D. A., Waterhouse, A. L. and Frankel, E. N. (1997). Inhibition of human low-density lipoprotein oxidation in relation to

composition of phenolic antioxidants in grapes (*Vitis vinifera*). Journal of Agriculture and Food Chemistry 45:1638-1643.

Micheli, M. R., Bova, R., Pascale, E. and D‟Ambrosio, E. (1994). Reproducible DNA fingerprinting with the random amplified polymorphic DNA (RAPD) method. Nucleic Acids Research 22:1921-1922.

Mizukami, H., Tomita, K. and Ohashi, H. (1989). Anthocyanin accumulation and changes in activities of phenylalanine ammonia-lyase and chalcone synthase in roselle (*Hibiscus sabdariffa* L.) callus cultures. Plant Cell Report 8:467-470.

Mizukami, H., Tomita, K., Ohashi, H. and Hiraoka, N. (1988). Anthocyanin production in callus cultures of roselle (*Hibiscus sabdariffa* L.). Plant Cell Report 7:553-556.

Mohamad, O., Herman, S., Modh Nazir, B., Aminah, A., Mamot, S., Jalifah, T., Bakhendri, S. and Abdul R, M. (2002). Mutation breeding of roselle in Malysia. Paper presented at the FNCA 2005 Workshop on Mutation Breeding. 5-9 Dec., Kuala Lunpur. Pp.1-7

Mohamed, B. B, Suleman, A. A. and Dahab, A. A. (2012). Roselle (*Hibiscus sabdariffa* L.) in Sudan, cultivation and their uses. Bulletin of Environmental, Pharmacology and Life Science 1:48-54.

Mohammadi, S. A. and Prasanna, B. M. (2003). Analysis of genetic diversity in crop plant- salient statistical tool and considerations. Crop Science 43:1235-1248.

Morton, J. F. (1974). Renewed interest in roselle (*Hibiscus sabdariffa* L.), the longforgotten „Florida cranberry‟. Proceedings of the Florida State Horticultural Society 87:415-425.

Morton, J. F. (1987). Roselle. In: Morton, J. F. (Ed.). Fruits of Warm Climates. Greensboro, NC: Media, Inc., Pp. 281-286

Mozaffari-Khosravi, H., Jalali-Khanabadi, B-A., Afkhami-Ardekani, M., Fatehi, F and Noori-Shadkam, M. (2009).The effects of sour tea (*Hibiscus sabdariffa*) on hypertension in patients with type II diabetes. Journal of Human Hypertension 23:48-54.

Mwasiagi, I. J., Yu, C. W., Phologolo, T., Waithaka, A., Kamalha, E. and Ochola, J. R. (2014). Characterization of Kenyan *Hibiscus sabdariffa* L. (roselle) bast fibre. Fibre and Textiles in Eastern Europe 22, 3(105):31-34.

Naqvi, N. I. and Chattoo, B. B. (1996). Development of sequence characterized amplified regions (SCAR) based on indirect selection method for a dominant blast resistant gene in rice. Genome 39:26-30.

Nei, M. (1972). Genetic distance between populations. American Naturalist. 106:283292.

Nei, M. and Li, W. H. (1979). Mathematical methods for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences, USA. 76:5269-5273.

Nei, M. and Li, W. H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences, U. S. A. 76: 5269-5273.

Nei, M. and Rouchoudhury, A. K. (1974). Genetic variation within and between the three major races of man, Caucasoids, Negroids and Mongoloids. American Journal of Human Genetics 26:421-443.

Nirmal, U., Singh , N., Hashim, J., Lau, S. T. W., and Jamil, N. (2011). On the effect of different polymer matrix and fibre treatment on single fibre pullout test using betelnut fibres. Materials and Design 32:717-2726.

Nizar, S., Elhadi, A. M., Algaili, A. M. and Hozeifa, H. M. (2013). Antimicrobial activity of roselle (*Hibiscus sabdariffa* L.) calyx extract. International Journal of Indigenous Medicinal Plants 46:1487-1491.

of non-conventional hybrids in maize. Mysore Journal of Agricultural Sciences 29: 213:218.

Nyquist, W. E. (1991). Estimation of heritability and predictions of selection response in plant populations. Critical Review of Plant Sciences 10:232-235.

Obodai, G. (2007). A survey and effect of methods of establishment and spacing on the growth and leaf yield of Roselle (*Hibiscus sabdariffa*). MSc. Thesis, Department of Horticulture, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Oboh, G. and Elusiyan, C. A. (2004). Nutrient composition and antimicrobial activity of

Okosun, L. A., Magaji, M.D. and Yakubu, A.I. (2006). Effect of sowing date and planting distance on growth and yield of two cultivars of roselle (*Hibiscus sabdariffa var. sabdariffa*). Journal of Plant Sciences 1: 297-305.

Olaleye, M. T. (2007). Cytotoxicity and antibacterial activity of methanolic extract of *Hibiscus sabdariffa.* Journal of Medicinal Plants Research 1:009-013.

Olson, M., Hood, L., Cantor, C. H. and Botstein, D. (1989). A common language for physical mapping of the human genome. Science 24:1434-1435.

Omalsaad, A. K. M., Islam, A., Juhan, M. A., Yaakob, Z. and Osman, M. (2014). Genetic relationship between roselle (*Hibiscus sabdariffa* L.) and kenaf (*Hibiscus cannabinus* L.) accessions through optimization of PCR based RAPD method. Emirates Journal of Food Agriculture 26:274-258.

Omobulajo, T. O., Sanni, L. A and Balaami, Y. A. (2000). Physical properties of sorrel (*H. sabdariffa*) seeds. Journal of Food Engineering 45:37-41.

Onyenekwe, P. C., Ajani, E. O., Ameh, D. A. and Gamaniel, K. S. (1999). Antihypertensive effect of roselle (*Hibiscus sabdariffa*) calyx infusion in spontaneously hypertensive rats and a comparison of its toxicity with that in Wistar rats. Cell Biochemistry and Function 17:199-206.

Osman, M., Golam, F., Saberi, S., Abdul Majid, N., Nagoor, N. H. and Zulqarnain, M. (2011). Morpho-agronomic analysis of three Roselle (*Hibiscus sabdariffa* L.) mutants in tropical Malaysia. Australian Journal of Crop Science 5:11501156.

Padmaja, H., Sruthi, S. and Vangalapati, M. (2014). Review on Hibiscus sabdariffa-A valuable Herb, International Journal of Pharmacy and. Life Science 5:37473752.

Panchen, A. L. (1992). Classification, Evolution and Nature Biology Diversities. Cambridge University Press, Cambridge, England.

Paterson, A. H., Tanksley S. D. and Sorreis, M. E. (1991).DNA markers in plant improvement. Advances in Agronomy 46:39-90.

Paull, R. E., Janick, J. and Troop, J. (2008). Malvaceae. In: The Encyclopaedia for Fruits and Nuts. Pp. 466-468.

Pearson, K. (1901). On lines and planes of closest fit to systems of points is space. Philosophical Magazine (6), 23:559-572.

Peeters, J. P. and Martinelli, J. A. (1989). Hierarchical cluster analysis as a tool to manage vegetation in germplasm collection. Theoretical and Applied Genetics 78:42-48.

Pejic, I., Ajmon-Marson, P., Morgante, M., Kozumplick, V., Castiglioni, P., Tarmino, G. and Motto, M. (1998). Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs and AFLPs. Theoretical Applied Genetics 97:1248-1255.

Perry, D. J. and Bousquet, J. (1998). Sequence tagged sites (STS) markers of arbitrary genes: development, characterization and analysis of linkage in black spruce. Genetics 149:1089-1098.

Plotto, A. (2004). Hibiscus: Post-Production Management for Improved Market Access. Mazaud, F., Röttger, A. and Steffel, K. (Eds.). Hibiscus Postharvest Operations. Inpho Postharvest Compendium. Food and Agriculture Organization of the United Nations (FAO), AGST. P1-19.

Pouget, M. P., Vennat, B., Lejeune, B. and Pourrat, A. (1990). Identification of anthocyanins of (*Hibiscus sabdariffa* L.). Lebens-mittel Wissenchaft und Technologie 23: 101-102.

Prasad, M. P. (2014). Molecular characterization and genetic diversity determination of *Hibiscus* species using RAPD molecular markers. Asian Journal of Plant Science and Research 4:50-56.

Prenesti, E., Berto, S., Daniele, P. G. and Toso, S. (2005). Antioxidant power quantification of decoction and cold infusions of *Hibiscus sabdariffa* flowers. Food Chemistry 100:433-438.

Pritchard, J. K., Stephens, M and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Rafalski J. A. and Tingey S. V. (1993). Genetic diagnostics in plant breeding: RAPD‟s microsatellites and machines. Trends in Genetics 9: 375-380.

Rafalski, J. A., Morgante, M., Powell, W., Vogel, J. M. and Tingey, S,V. (1996). Generating and Using DNA Markers in Plants. In: Analysis of Nonmammalian genomes: A Practical Guide (B. Birren and E. Lai, Eds.). Academic Press, Boca Raton, FL.

Rajasekar, S., Fei, S. and Nick, E. C. (2009). Analysis of genetic Diversity in rough bluegrass determined by RAPD markers. Crop Science 46:162-167.

Razali, N., Salit, M.S., Jawaid, M., Ishak, M.R. and Lazim, Y. (2015). A study on chemical

Reem, S. M., Ansari, M., and Saleh, M. (2012). A study on mechanical, thermal and morphological properties of natural fibre/epoxy composite. Journal of Purity, Utility Reaction and Environment 1:267-296.

Rendel, J. M. (1989). A study of variability estimates of heritability and their standard errors derived by parental-sib techniques using simulated data. PhD Thesis, Massay University.

Rhodin, E. G. and Panchoo, L. (1990). Effect of transplant timing and terminal bud removal on Sorrel. Caribbean Food Crop Society Proceedings 26: 245-254.

Richardson, M. (2009): “Principal Component Analysis“, URL: http://people.maths.ox.ac.uk/richardsonm/ SignalProcPCA.pdf Verified 02 April 2016.

Rizk, R. M. and Soliman, M. I. (2014). Biochemical and molecular genetic characterization of some species of family Malvaceae, Egypt. Egyptian European Journal of Basic and Applied Science 1:167-176.

Rohlf, F. J. (1974). Methods of comparing classification. Annual Review of Ecology, Evolution and Systematics 5:101-113.

Rohlf, F. J. (2009). NTSYSpc: Numerical Taxonomy System. Version. 2.21c. Exeter Software: Setauket, New York.

Rohlf, F. J. and Wooten, M. C. (1988). Evaluation of restricted maximum likelihood method for estimating polygenic allele- frequency data. Evolution, 42:581595.

Rout, G. R., Bhatacharya D., Nanda, R. M., Nayak, S. and Das, P. (2003). Evaluation of genetic relationship in Dalbergia species using RAPD markers. Biodiversity Conservation 12: 197-206.

Rukayadi, Y., Jae-Seok Shim, J-S. and Hwang, J-K. (2008). Screening of Thai medicinal plants for anticandidal activity. Mycoses 51:308-312.

Sabiel, S. I. A., Ishmail, M. I., Omam, K. A. and Sun, D. (2014).Genetic variability for yield and related attributes of Roselle (*Hibiscus sabdariffa* L.) genotypes under rain-fed conditions in semi-arid zone of Sudan. Persian Gulf Crop Protection 3:33-40.

Salah, A. M., Gathumbi, J., Vierling, W. (2002). Inhibition of intestinal motility by methanol extracts of *Hibiscus sabdariffa* L. (Malvaceae) in rats. Phytotherapy Research 16:283-285.

Sanyal, P. (1959). Studies on pollen tube growth in six species of *Hibiscus* and their crosses

Sanyal. P., Ghosh. K. L. and Kundu, B. C. (1960). Inheritance of anthocyanin pigmentation patterns in *Hibiscus sabdariffa* L. Journal of Genetics 57:313326.

SAS Institute Inc. 2011. Base SAS® 9.3 Procedures Guide: Statistical Procedures. Cary, NC: SAS Institute Inc.

Sato, K., Goda, Y., Yoshihira, K. and Nogucji, H. (1991). Structure and contents of main coloring constituents in the calyces of *Hibiscus sabdariffa* and commercial Roselle color. Journal of the Food Hygienic Society of Japan 32:301-307.

Satué-Gracia, M. T., Heinonen, M., and Frankel, E. N. (1997). Anthocyanins as antioxidants on human low-density lipoprotein and lecithin-liposome systems. Journal of Agriculture and Food Chemistry 45:3362-3367.

Satya, P., Karan, M., Kar, C.S. and Mahapatra, A. K. and Mahapatra, B.S. ., (2013). Assessment of molecular diversity and evolutionary relationships of kenaf (*Hibiscus cannabinus* L.), roselle (*Hibiscus sabdariffa* L.) and their wild relatives. Plant Systematics and Evolution 299:619-629

Satyanarayana, E. and Sai Kumar, R. (1995). Genetic variability and *Per se* performance of non-conventional hybrids in maize. Mysore Journal of Agricultural Sciences 29: 213:218.

Schippers, R. R. (2000). African Indigenous Vegetables, An Overview of the Cultivated Species. Chatham, UK. Natural Resources Institute/ACP-EU Technical Centre for Agricultural and Rural Cooperation Pp. 122-133.

Shalaby A. S. and Razin A. M. (1989). Effect of Plant Spacing on the Productivity of Roselle (*Hibiscus sabdariffa* L.) Grown in Newly Reclamed Land. Journal of Agronomy and Crop Science 162:256-260.

Sie, R. S., Akaffou, D. S., Konan, K. J. L., Toueix, Y., Charles, G., Je, Y and Branchard, M. (2009). Characterization of diversity and agronomical assessment of a collection of *H. sabdariffa* in Ivory Coast. Science Africa 5:65-76.

Siepe, T., Ventrella, D. and Lapenta, E. (1997). Evaluation of genetic variability in a collection of *Hibiscus cannabinus* (L.) and *Hibiscus* spp. (L.). Industrial Crops and Products 6:343-352.

Singh, D.P. (n.d.). Mesta (*Hibiscus cannabinus and Hibiscus sabdariffa*). Central Research Institute for Jute and Allied Fibers. BARRACKPORE, 743: 24-parganas (North), West Bengal, India, pp:28. Retrieved from http://assamagribusiness.nic.in/Mesta.pdf

Singha, A. S. and Thakur, V. K. (2008). Mechanical properties of natural fibrereinforced polymer composites. Bulletin of Material Science 31:791-799. © Indian Academy of Sciences.

Sinha, R. P and Chowdhury, S. K. (1991.) Induced codominant mutation for dwarfism in lentil (*Lens culinaris* Med.). Indian Journal of Genetics 51:370-371.

Smith, J. S. C. and Smith, O. S. (1992). Fingerprinting crop varieties. Advances in Agronomy 47:85-140.

Smith, J. S. C., Paszkiewics, S., Smith, O. S. and Schaeffer, J. (1987). Electrophoretic, chromatographic and genetic techniques for identifying Associations and measuring genetic diversity among corn hybrids. Proceedings, 42nd Annual Corn Sorghum Research Conference, Chicago, Seed Trade Association, Washington, DC. Pp. 187–203.

Sneath, P. H. A. and Sokal, R. R. (1973). Numerical taxonomy. Freeman, San Francisco.

Snedecor, G. W. (1956). Statistical Methods. 5th Ed. Iowa State University Press, Ames, Iowa.

Sokal, R. R. (1986) Spatial Data Analysis and Historical Processes, In: Data Analysis and Informatics, IV. Diday, E. Escoffier, Y. Lebart, L. Pages, J. Schektman, Y. and Tomassone, R. (Eds.). North-Holland, Amsterdam. Pp. 29-43.

Sokal, R. R. and Michener, C. D. (1958). A statistical method for evaluating systematic relationships. University of Kansas Science Bulletin 38:1409-1438.

Sokal, R. R. and Rohlf, F. J. (1962). The comparison of dendrograms by objective methods. Taxon 11:33-40.

Sokal, R. R. and Sneath, P. H. A. (1963). Principles of Numerical Taxonomy, San Francisco: W.H. Freeman and company.

sorrel drink (*soborodo*). Journal of Medicinal Food 7:340-342.

Stadler, L. J. (1929).Chromosome number and mutation rate in *Avena* and *Triticum*. Proceedings of the National Academy of Sciences of U.SA 15:876-881.

Tamura, H. and Yamagami, A. (1994). Antioxidative activity of monoacylated anthocyanins isolated from Muscat Bailey A grape. Journal of Agriculture and Food Chemistry 42:1612-1615.

Tanksley, S. D. and Orton, T. J. (1983). Isozymes in plant genetics and breeding. Elsevier, Amsterdam.

Thirthamallappa, G. and Sherif, R. A. (1991). Genetic variability studies in roselle. Current Research, University Agricultural Science, Bangalore, 20:257-258.

Thiruchitrambalam, M., Athijayamani, A. and Sathiyamurthy, S. (2010). A review on the natural fiber-reinforced polymer composites for the development of roselle fiber-reinforced polyester composite. Journal of Natural Fibers 7:307-323.

Thompson, J. A., Nelson, R. L., and Vodkin, L. O. (1998). Identification of diverse soybean germplasm using RAPD markers. Crop Science 38:1348-1355.

Tingey, S. V. and Del Tufo, J. P. (1993). Genetic analysis with random amplified polymorphic DNA markers. Plant Physiology 101:349-352.

Torres-Morán, M. I., Escoto-Delgadillo, M., Ron-Parra, J., Parra-Tover, G., Mena-Munguia, S., Rodriguez-Garcia, A., Rodriguez-Sahagun, A. and CastellanosHermandez, O. (2011). Relationships among twelve genotypes of Roselle (*Hibiscus sabdariffa* L.) cultivated in western Mexico. Industrial Crops and Products 34:1079-1083.

Tounkara, F., Amadou, I., Guo-Wei, L. and Yong-hui, S. (2011). Effect of boiling on the physicochemical properties of Roselle seeds (*Hibiscus sabdariffa* L.) cultivated in Mali. African Journal of Biotechnology 10:18,160-18,166.

Tsai, P. J. and Ou, A. S. M. (1996). Colour degradation of dried roselle during storage. Food Science 23:629-640.

Tsai, P. J., Mclintosh, J., Pearce, P., Camden, B. and Jordan, B. R. (2002). Anthocyanin and antioxidant capacity in roselle (*Hibiscus sabdariffa* L.) extract. Food Research International 35:351-356.

Vaidya, K. R. (2000). Natural cross-pollination in roselle, *Hibiscus sabdariffa* L. (Malvaceae). Genetics and Molecular Biology 23:667-669.

Vitoon, P., Woottisin, S., Sriboonlue, P. and Kukongviriyapan, V. (2008). Uricosuric effect of Roselle (*Hibiscus sabdariffa*) in normal and renal-stone former subjects. Journal of Ethnopharmacology 117:491-495.

Vogel, J. M., Rafalski A., Powell, W., Morgante, M., Andre, C., Hanafey, M. and Tingey, S. V. (1996). Application of genetic diagnostics to plant genome analysis and plant breeding. HortScience 31:1107-1108.

Vos, P., Hogers, R., Sleeker, M., Reijans, M., Lee, T. and Homes, M. (1995). AFLP: a new concept for DNA fingerprinting. Nucleic Acids Research 23:4407-4414.

Wahabi, H. A., Alansary, L. A., Al-Sabban, A. H. and Glasziuo, P. (2010). The effectiveness of *Hibiscus sabdariffa* in the treatment of hypertension: a systematic review. Phytomedicine 17:83-86.

Ward, J. H., (1963). Hierarchical grouping to optimize an objective function. Journal of the American Statistical Association 58:236-244.

Webster, P. J. (1914). New varieties of roselle. Philippine Agricultural Review 7: 266-269.

Weir, B. S. (1990). Genetic data analysis. Methods for discrete genetic data. Sinauer Associates, Sunderland, MA.

Wilks, D. S. (2006). Statistical Methods in the Atmospheric Sciences, 2nd ed. International Geophysics Series, Vol. 59, Academic Press: New York, 627.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18:6531-6535.

Wilson, F. D. and Menzel, M. Y. (1964). Kenaf (*Hibiscus cannabinus*), Roselle (*Hibiscus sabdariffa*). Economic Botany 18:80-91.

Winter, P. and Kahl, G. (1995). Molecular marker technologies for plant improvement. World Journal of Microbiology and Biotechnology 1:438-448.

Wong, P-K., Salmah, Y., Ghazali, Y. M. and Yaakob, C. M. (2002). Physicochemical characteristics of roselle (*Hibiscus sabdariffa* L.). Nutrition and Food Science 32:68-73.

Wong, P-K., Salmah, Y., Hasanah, M. G. and Yaakob, C. M. (2003). Optimization of hot water extraction of roselle juice using response surface methodology: a comparative study with other extraction methods. Journal of the Science of Food and Agriculture 83:1273-1278.

Wong, S. K., Lim, Y. Y. and Chan, E.W.C. (2009). Antioxidant properties of *Hibiscus*: species variation, altitudinal change, coastal influence and floral color change. Journal of Tropical Forest Science 21:307-215.

Wright, S. (1978). Evolution and the Genetics of Population. Vol. 4. Variability Within and Among Natural Population. University of Chicago Press. Chicago.

www.ghheadlines.com www.myjoyonline.com
www.sydneyabugri.com

Xu, M., Huaracha, E. and Korban, S. S. (2001). Development of sequencecharacterized amplified regions (SCARs) from amplified fragment length polymorphism (AFLP) markers tightly linked to the *Vf* gene in apple. Genome 44: 63-70.

Yang, X. and Quiros, C. (1993). Identification and classification of celery cultivars with RAPD markers. Theoretical and Applied Genetics 86:205-212.

Yu, K., Van Deynze, A. and Pauls, K. P. (1993). Random Amplified Polymorphic DNA (RAPD) Analysis. In: Methods in Plant Molecular Biology and Biotechnology (Glick B. R. and Thompson J. E., Eds.). CRC Press, Boca Raton. U.S.A.

Yusof, M. M. and Saud, H. M. (2009). Cultivars and variety identification and their genetic relationships by randomly amplified polymorphic DNA (RAPD) markers in roselle (*Hibiscus sabdariffa* L). Proceedings of the 8th Congress on Genetics 4-6 August. Genting Highlands, Malaysia.

Zayed, A. A., Abo Elfadle, I. A. and Hussein, E. H. (1996). Estimate of variability, heritability and correlation coefficient among some characters of roselle (*Hibiscus sabdariffa* L.). Egyptian Journal of Applied Science 11:26-33.

LIST OF APPENDICES

APPENDIX A. Preparation of reagents

1. **2 % CTAB**

Six hundred ml of 2 % CTAB (Hexadecyltrimethyl-ammonium bromide) was prepared by transferring 0.5 g CTAB into an Erlenmeyer flask. To this was added 60 ml 1 M TRIS (pH 7.5 at 65 ^{o}C), 84.0 ml of 5 M NaCl, and 60 ml 0.5 M EDTA (pH 8.0). The volume was made up to 600 ml with 390.0 ml of deionized water. Just before use, 1.0 ml of 14 M β- mercaptoethanol was added.

2. **Chloroform: isoamyl alcohol (24:1)**

A volume of 960 ml Chloroform and 40 ml isoamyl alcohol in 1 L

3. **Washing Buffer (500 ml)**

This was prepared by adding 380 ml of absolute ethanol, 5 ml of 1 M ammonium acetate and 115 ml of deionized water.

4. **RNase (10 mg/ml)**

A quantity of 10mg/ml of RNAse was dissolved in 0.01 M Sodium acetate. The mixture was heated in boiling water for 15 min and allowed to cool slowly to room temperature.

Aliquots were dispensed into 1 ml and store at -20 °C. Working stock may be stored at 4 °C.

5. 1 % agarose gel.

A quantity of 1 g agarose gel was dissolved in 100 ml TBE.

6. 2 % agarose gel.

Two grams agarose gel was dissolved in 100 ml TBE.

7. 1 L 70 % ethanol

Distilled water was added to 700 ml absolute ethanol to make a 1000 ml solution.

8. 1 L 80 % ethanol

Distilled water was added to 800 ml absolute ethanol to make a 1000 ml solution.

9. 0.5 M EDTA

A quantity of 186.1 g of Na_2EDTA dihydrate (MW=372.24) was dissolved in approximately 750 ml of deionized water. Pellets of NaOH were added to adjust pH to 8.0. Deionized water was then added to make a 1000 ml solution and autoclaved.

10. 50× TAE Buffer stock

Two hundred and fourty-two grams (242 g) of TRIS base was weighed and transferred to a 2 L conical flask. 100 ml of 0.5 M EDTA and 57.1 ml of glacial acetic were added. deionized water was then added to the 1 L mark. The pH of the resulting solution was then adjusted to 8.0.

11. **5× TBE Buffer stock**

One thousand milliliters of 5× TAB buffer was prepared by weighing 54 g TRIS base and 27.5 g Boric acid into a 2 L conical flask. Eight hundred milliliters (800 ml) of deionized water and then 20 ml 0.5 M EDTA (pH 8.0) were added to make the solution.

12. 10 mg/mL Ethidium bromide solution

One hundred milligram (100 mg) of ethidium bromide was dissolved in sterile water (ddH_2O) in a tube. The tube and its content was wrapped in aluminium foil and stored in the refrigerator at 4 °C.

13. 250 µM dNTP

The dNTP mix was composed of 2.5 mM each of dCTP, dGTP, dATP, and dTTP. Each set of the dNTP mix came with 4 individual tubes containing dCTP, dGTP, dATP, and dTTP at 100 mM concentration. To mix, 250 µl of each nucleotide was placed in a 10 ml tube, 9000 µl of sterile water (ddH2O) was added to obtain a 2.5 mM concentration of each nucleotide.

APPENDIX B: Morphological distance matrix

	HS68	HS86	HS32	HS69	HS25	HS14	HS50	HS84	HS19	HS83	HS09	HS04	HS16	HS20	HS02	HS01	HS11	HS22	HS77	HS85	HS70	HS13	HS24	HS08	HS41	HS82	HS65	HS78	HS29	HS27	HS58	HS56	HS81	HS48
HS68																																		
HS86	0.74																																	
HS32	0.24	0.03																																
HS69	0.01	0.00	0.16																															
HS25	0.02	0.29	0.04	0.19																														
HS14	0.33	0.25	0.67	0.47	0.13																													
HS50	0.57	0.26	0.40	0.02	0.14	0.18																												
HS84	0.23	0.28	0.14	0.32	0.01	0.10	0.21																											
HS19	0.16	0.15	0.50	0.72	0.20	0.93	0.06	0.18																										
HS83	0.09	0.05	0.08	0.46	0.22	0.22	0.28	0.84	0.34																									
HS09	0.04	0.20	0.04	0.00	0.39	0.14	0.01	0.09	0.09	0.23																								
HS04	0.00	0.00	0.41	0.78	0.12	0.62	0.01	0.64	0.77	0.73	0.12																							
HS16	0.09	0.29	0.03	0.22	0.01	0.03	0.19	0.41	0.09	0.34	0.13	0.25																						
HS20	0.02	0.21	0.04	0.08	0.35	0.00	0.03	0.00	0.01	0.03	0.81	0.01	0.31																					
HS02	0.35	0.12	0.07	0.02	0.01	0.18	0.02	0.01	0.15	0.01	0.03	0.02	0.26	0.06																				

HS01	0.00	0.03	0.06	0.24	0.01	0.15	0.09	0.24	0.22	0.32	0.04	0.27	0.85	0.15	0.60																					
HS11	0.29	0.35	0.41	0.31	0.37	0.83	0.05	0.13	0.75	0.34	0.49	0.54	0.00	0.16	0.12	0.09																				
HS22	0.18	0.09	0.65	0.51	0.11	0.91	0.05	0.29	0.89	0.43	0.14	0.77	0.17	0.00	0.25	0.35	0.79																			
HS77	0.49	0.13	0.28	0.01	0.25	0.11	0.83	0.25	0.04	0.35	0.19	0.02	0.02	0.25	0.15	0.00	0.00	0.03																		
HS85	0.07	0.12	0.28	0.61	0.44	0.74	0.01	0.33	0.83	0.64	0.32	0.79	0.11	0.05	0.12	0.27	0.85	0.83	0.03																	
HS70	0.19	0.54	0.20	0.02	0.28	0.00	0.02	0.62	0.00	0.24	0.00	0.18	0.33	0.08	0.01	0.11	0.00	0.07	0.02	0.01																
HS13	0.54	0.10	0.62	0.01	0.13	0.37	0.53	0.00	0.21	0.01	0.03	0.05	0.06	0.15	0.47	0.16	0.14	0.34	0.67	0.05	0.03															
HS24	0.33	0.04	0.77	0.10	0.04	0.58	0.24	0.10	0.43	0.09	0.00	0.30	0.21	0.08	0.46	0.36	0.35	0.67	0.30	0.28	0.17	0.84														
HS08	0.00	0.08	0.00	0.57	0.33	0.17	0.01	0.00	0.33	0.03	0.03	0.16	0.03	0.07	0.04	0.08	0.08	0.10	0.00	0.21	0.24	0.01	0.00													
HS41	0.01	0.15	0.43	0.17	0.04	0.22	0.01	0.71	0.23	0.52	0.01	0.51	0.55	0.05	0.11	0.47	0.16	0.48	0.00	0.28	0.72	0.20	0.54	0.04												
HS82	0.62	0.50	0.01	0.00	0.01	0.10	0.38	0.52	0.06	0.30	0.10	0.06	0.04	0.06	0.36	0.01	0.02	0.02	0.56	0.00	0.46	0.31	0.08	0.19	0.16											
HS65	0.07	0.03	0.57	0.73	0.10	0.86	0.02	0.36	0.94	0.49	0.07	0.89	0.23	0.03	0.15	0.35	0.66	0.94	0.02	0.82	0.08	0.23	0.53	0.21	0.46	0.01										
HS78	0.00	0.03	0.48	0.71	0.02	0.55	0.00	0.68	0.68	0.65	0.03	0.94	0.41	0.07	0.05	0.39	0.38	0.74	0.00	0.63	0.33	0.11	0.42	0.10	0.68	0.05	0.87									
HS29	0.29	0.04	0.88	0.08	0.03	0.60	0.25	0.16	0.42	0.13	0.06	0.35	0.10	0.01	0.24	0.20	0.44	0.69	0.20	0.31	0.24	0.71	0.93	0.03	0.59	0.01	0.53	0.44								
HS27	0.42	0.76	0.04	0.00	0.47	0.25	0.07	0.04	0.15	0.01	0.68	0.02	0.29	0.62	0.02	0.05	0.56	0.14	0.00	0.27	0.25	0.01	0.01	0.01	0.04	0.08	0.05	0.00	0.05							
HS58	0.25	0.03	0.98	0.18	0.03	0.69	0.33	0.16	0.54	0.11	0.03	0.44	0.08	0.05	0.14	0.14	0.43	0.71	0.27	0.32	0.22	0.67	0.86	0.00	0.51	0.02	0.63	0.53	0.94	0.03						

HS56	0.02	0.12	0.34	0.45	0.00	0.33	0.05	0.79	0.42	0.73	0.02	0.76	0.65	0.06	0.11	0.59	0.25	0.61	0.03	0.49	0.50	0.11	0.44	0.01	0.89	0.12	0.66	0.88	0.44	0.03	0.42					
HS81	0.02	0.19	0.37	0.45	0.04	0.27	0.01	0.56	0.35	0.40	0.05	0.59	0.73	0.34	0.14	0.59	0.09	0.47	0.01	0.26	0.49	0.21	0.48	0.03	0.77	0.03	0.58	0.81	0.40	0.17	0.45	0.86				
HS48	0.15	0.00	0.92	0.07	0.07	0.49	0.26	0.23	0.33	0.13	0.08	0.36	0.05	0.01	0.06	0.07	0.36	0.56	0.15	0.23	0.37	0.54	0.75	0.09	0.59	0.01	0.45	0.45	0.93	0.03	0.92	0.41	0.37			
HS30	0.00	0.04	0.16	0.87	0.39	0.56	0.01	0.26	0.75	0.46	0.15	0.76	0.03	0.00	0.00	0.07	0.52	0.52	0.03	0.74	0.00	0.00	0.05	0.49	0.08	0.00	0.68	0.57	0.07	0.11	0.16	0.29	0.20	0.07	MIN	0.00

APPENDIX C: Molecular distance matrix

		HS77	HS22	HS08	HS30	HS70	HS41	HS75	HS65	HS84	HS81	HS32	HS13	HS29	HS04	HS16	HS48	HS50	HS02	HS25	HS20	HS56	HS59	HS11	HS85	HS24	HS58	HS14	HS86	HS78	HS88	HS83	
HS77	Ouaga																																
HS22	Ouaga	0.57																															
HS08	Mali	0.3	0.35																														
HS30	Ouaga	0.19	0.27	0.35																													
HS70	Ouaga	0.35	0.56	0.55	0.39																												
HS41	Ouaga	0.44	0.44	0.53	0.29	0.61																											
HS75	Nigeria	0.47	0.3	0.3	0.35	0.43	0.5																										
HS65	Ouaga	0.37	0.53	0.36	0.39	0.57	0.58	0.6																									

HS84	Ouaga	0.37	0.3	0.3	0.29	0.43	0.3	0.6	0.52													
HS81	Senegal	0.09	0.04	0.17	0.26	0.14	0.16	0.19	0.19	0.24												
HS32	Ouaga	0.57	0.31	0.3	0.23	0.4	0.67	0.56	0.53	0.47	0.22											
HS13	Nigeria	0.41	0.4	0.32	0.32	0.39	0.5	0.43	0.5	0.43	0.2	0.79										
HS29	Ouaga	0.33	0.27	0.38	0.32	0.33	0.42	0.58	0.5	0.67	0.3	0.56	0.6									
HS04	Lome	0.24	0.3	0.38	0.35	0.45	0.35	0.35	0.41	0.35	0.3	0.17	0.25	0.36								
HS16	Mali	0.28	0.33	0.17	0.26	0.29	0.23	0.25	0.3	0.25	0.29	0.22	0.21	0.21	0.32							
HS48	Mali	0.17	0.23	0.29	0.28	0.31	0.35	0.27	0.38	0.38	0.33	0.4	0.35	0.35	0.23	0.35						
HS50	Ouaga	0.06	0.06	0.06	0.06	0.06	0	0	0	0	0.18	0.06	0.06	0	0.12	0.06	0.13					
HS02	Cote D'Ivoire	0.23	0.17	0.29	0.3	0.38	0.41	0.22	0.29	0.26	0.35	0.35	0.32	0.39	0.45	0.42	0.61	0.18				
HS25	Ouaga	0.29	0.38	0.35	0.37	0.58	0.56	0.33	0.53	0.32	0.25	0.33	0.3	0.3	0.53	0.42	0.43	0.12	0.61			
HS20	Ghana	0.37	0.32	0.15	0.35	0.32	0.38	0.28	0.39	0.28	0.27	0.35	0.38	0.26	0.35	0.36	0.38	0.13	0.47	0.55		
HS56	Ouaga	0.3	0.44	0.15	0.23	0.38	0.32	0.28	0.39	0.28	0.15	0.35	0.36	0.2	0.24	0.43	0.38	0.13	0.33	0.53	0.6	
HS59	Cote D'Ivoire	0.23	0.37	0.25	0.24	0.38	0.32	0.14	0.23	0.19	0.24	0.23	0.25	0.2	0.35	0.43	0.38	0.06	0.53	0.5	0.39	0.39
HS11	Ghana	0.15	0.27	0.16	0.36	0.28	0.29	0.25	0.3	0.3	0.25	0.25	0.28	0.28	0.25	0.45	0.41	0	0.41	0.39	0.45	0.43	0.43

HS85	Senegal	0.24	0.39	0.24	0.37	0.45	0.41	0.29	0.42	0.29	0.26	0.41	0.38	0.26	0.35	0.4	0.47	0.13	0.57	0.64	0.53	0.5	0.59	0.53													
HS24	Ouaga	0.31	0.5	0.19	0.14	0.32	0.33	0.17	0.29	0.23	0.21	0.26	0.32	0.26	0.29	0.33	0.4	0.13	0.35	0.41	0.44	0.42	0.69	0.32	0.47												
HS58	Lome	0.29	0.2	0.29	0.18	0.24	0.28	0.14	0.19	0.14	0.2	0.21	0.1	0.1	0.14	0.14	0.08	0.25	0.13	0.19	0.14	0.14	0.08	0.05	0.1	0.1											
HS14	Ouaga	0.13	0.14	0.12	0.27	0.19	0.14	0.07	0.19	0.1	0.24	0.12	0.12	0.07	0.12	0.12	0.1	0.29	0.17	0.22	0.19	0.11	0.19	0.04	0.13	0.13	0.39										
HS86	Mali	0.13	0.22	0.1	0.24	0.25	0.25	0.21	0.33	0.21	0.2	0.33	0.39	0.25	0.2	0.25	0.33	0.08	0.19	0.33	0.38	0.41	0.28	0.3	0.33	0.41	0.06	0.11									
HS78	Ghana	0.17	0.22	0.19	0.24	0.27	0.26	0.17	0.23	0.17	0.17	0.19	0.24	0.18	0.32	0.29	0.4	0.23	0.41	0.33	0.23	0.23	0.5	0.24	0.25	0.47	0.05	0.24	0.24								
HS88	Lome	0.31	0.29	0.17	0.14	0.22	0.27	0.18	0.29	0.17	0.15	0.27	0.12	0.12	0.1	0.17	0.22	0.31	0.16	0.2	0.21	0.22	0.16	0.12	0.13	0.29	0.1	0.4	0.2	0.31							
HS83	Cote D'Ivoire	0.29	0.25	0.19	0.18	0.35	0.33	0.29	0.39	0.29	0.17	0.29	0.2	0.2	0.37	0.23	0.42	0.2	0.42	0.41	0.24	0.24	0.3	0.14	0.28	0.35	0.1	0.17	0.19	0.6	0.46						
HS87	Ghana	0.29	0.17	0.13	0.17	0.24	0.22	0.24	0.33	0.24	0.19	0.24	0.21	0.21	0.32	0.25	0.3	0.2	0.39	0.44	0.24	0.19	0.25	0.15	0.16	0.24	0.11	0.25	0.29	0.64	0.36	0.56	MIN	0			

APPENDIX D: Binary scoring of RAPD amplification products of 32 West African roselle accessions.

	H577	H512	H508	H530	H570	H541	H575	H563	H584	H581	H532	H513	H529	H504	H516	H548	H550	H502	H525
OPA01a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OPA01b	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
OPA01c	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
OPA01d	1	1	0	0	1	0	1	1	1	0	0	0	0	1	1	0	0	0	1
OPA01e	0	0	1	0	0	1	0	0	0	0	1	1	1	0	0	1	0	1	0
OPA02a	1	9	1	1	1	1	1	1	1	1	1	9	9	1	1	1	9	1	1
OPA02b	0	9	0	0	0	0	0	0	1	0	0	9	9	0	0	0	9	0	0
OPA02c	0	9	0	0	0	0	1	0	0	0	0	9	9	0	0	0	9	0	0
OPA02d	1	9	0	0	0	1	0	1	0	0	1	9	9	0	0	0	9	1	1
OPA03a	0	9	0	1	0	0	0	0	0	0	0	9	9	0	0	1	9	1	1
OPA11a	0	0	0	0	0	0	1	1	1	0	9	0	1	1	0	0	9	9	0
OPA11b	0	0	0	0	0	0	0	0	0	1	9	0	0	0	0	0	9	9	0
OPA11c	0	0	0	0	0	0	0	0	0	1	9	0	1	0	0	0	9	9	0
OPA11d	0	1	1	0	1	1	0	1	0	0	9	0	0	0	1	1	9	9	1
OPA11e	0	1	0	1	1	1	0	1	0	0	9	1	0	0	0	1	9	9	1
OPA11f	1	0	0	0	0	0	0	0	0	0	9	1	0	0	0	0	9	9	0
OPA12a	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	1	0	0
OPA12b	0	0	1	1	1	9	0	0	0	0	0	0	0	1	0	0	1	1	1
OPA12c	0	0	0	0	0	9	0	0	0	1	0	1	1	1	1	0	0	1	1
OPA12d	1	1	1	1	1	9	0	0	0	0	0	0	0	1	1	0	0	1	1
OPA12e	1	1	1	1	1	9	1	1	1	0	1	1	1	0	0	1	0	0	0
OPA16a	0	0	0	9	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
OPA16b	0	0	0	9	0	0	0	0	0	1	0	0	0	1	1	1	1	1	1
OPA16c	1	0	1	9	1	1	1	0	1	0	1	1	1	0	0	0	0	0	0
OPA16d	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0
OPA16e	1	1	1	9	1	1	1	1	1	0	1	1	1	0	0	0	0	0	1
OPA16f	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OPA20a	0	0	0	0	0	0	0	0	0	9	9	0	0	0	9	0	9	0	1
OPA20b	0	0	0	0	1	0	0	0	0	9	9	0	0	0	9	0	9	1	0
OPA20c	0	0	0	0	0	0	0	0	1	9	9	0	0	0	9	1	9	0	0
OPA20d	1	1	1	1	0	1	1	1	0	9	9	1	1	1	9	0	9	0	0
OPG05a	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
OPG05b	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
OPG05c	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OPG05d	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1
OPG05e	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OP808a	9	1	1	0	1	1	0	0	0	0	0	0	0	1	0	0	9	9	9
OP808b	9	0	0	1	0	0	1	1	1	1	1	1	1	0	1	1	9	9	9
OP808c	9	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	9	9	9
OP808d	9	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	9	9	9
OP808e	9	1	0	1	1	1	1	1	0	0	1	1	0	0	1	0	9	9	9
OP808f	9	0	0	1	0	0	1	0	1	0	0	0	1	0	0	0	9	9	9
OP808g	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	9	9
OPH13a	9	9	9	9	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0
OPH13b	9	9	9	9	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
OPH13c	9	9	9	9	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
OPH13d	9	9	9	9	1	1	1	1	1	1	1	1	1	1	0	1	9	1	1
OPH13e	9	9	9	9	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0
OP802a	1	0	0	0	0	1	1	0	0	9	1	0	0	0	0	0	9	0	9
OP802b	0	1	1	1	1	0	0	1	1	9	0	1	1	1	0	0	9	0	9
OP802c	0	0	0	0	0	0	0	0	0	9	0	0	0	0	1	1	9	1	9
OP802d	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	9	0	9
OP802e	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	9	0	0
OPG03a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OPG03b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
OPG03c	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0
OPG03d	1	1	0	0	0	0	0	0	1	1	1	1	1	0	1	1	0	1	0
OPG03e	0	0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0	1	1
OPA08a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
OPA08b	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
OPA08c	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1
OPA08d	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
OPA08e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0