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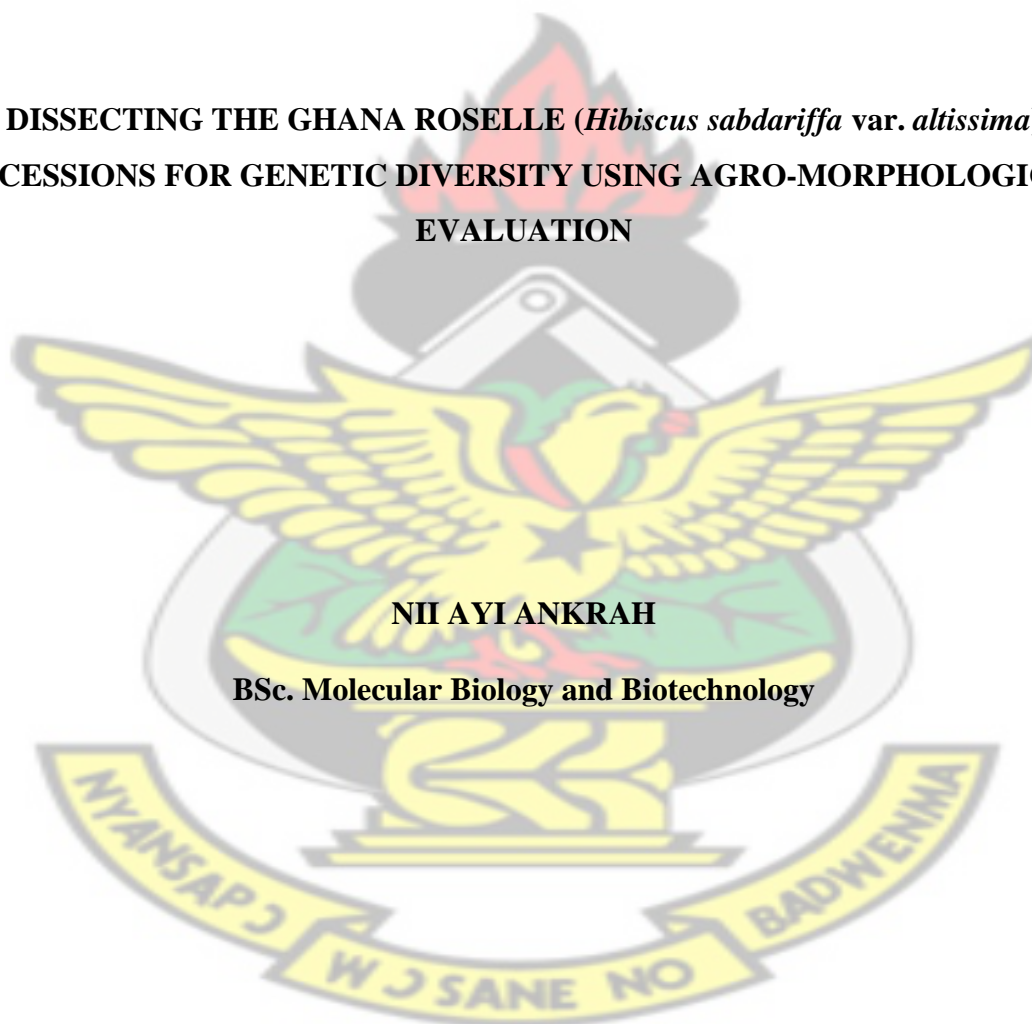
KUMASI, GHANA

DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

COLLEGE OF SCIENCE

KNUST

**DISSECTING THE GHANA ROSELLE (*Hibiscus sabdariffa* var. *altissima*)
ACCESSIONS FOR GENETIC DIVERSITY USING AGRO-MORPHOLOGICAL
EVALUATION**



NII AYI ANKRAH

BSc. Molecular Biology and Biotechnology

JUNE 2019

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EVALUATION**

KNUST

**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 SCIENCE IN BIOTECHNOLOGY**

NII AYI ANKRAH

(BSc. Molecular Biology and Biotechnology)

JUNE 2019

DECLARATION

I, do hereby certify for this thesis that:

- a. Except where due acknowledgement has been made, the work is the outcome of my own research;
- b. This thesis has not been submitted previously, in whole or in part to qualify for any degree or any other academic award;
- c. The content of the thesis is the result of work which has been carried out since the official commencement date of the approved research, and
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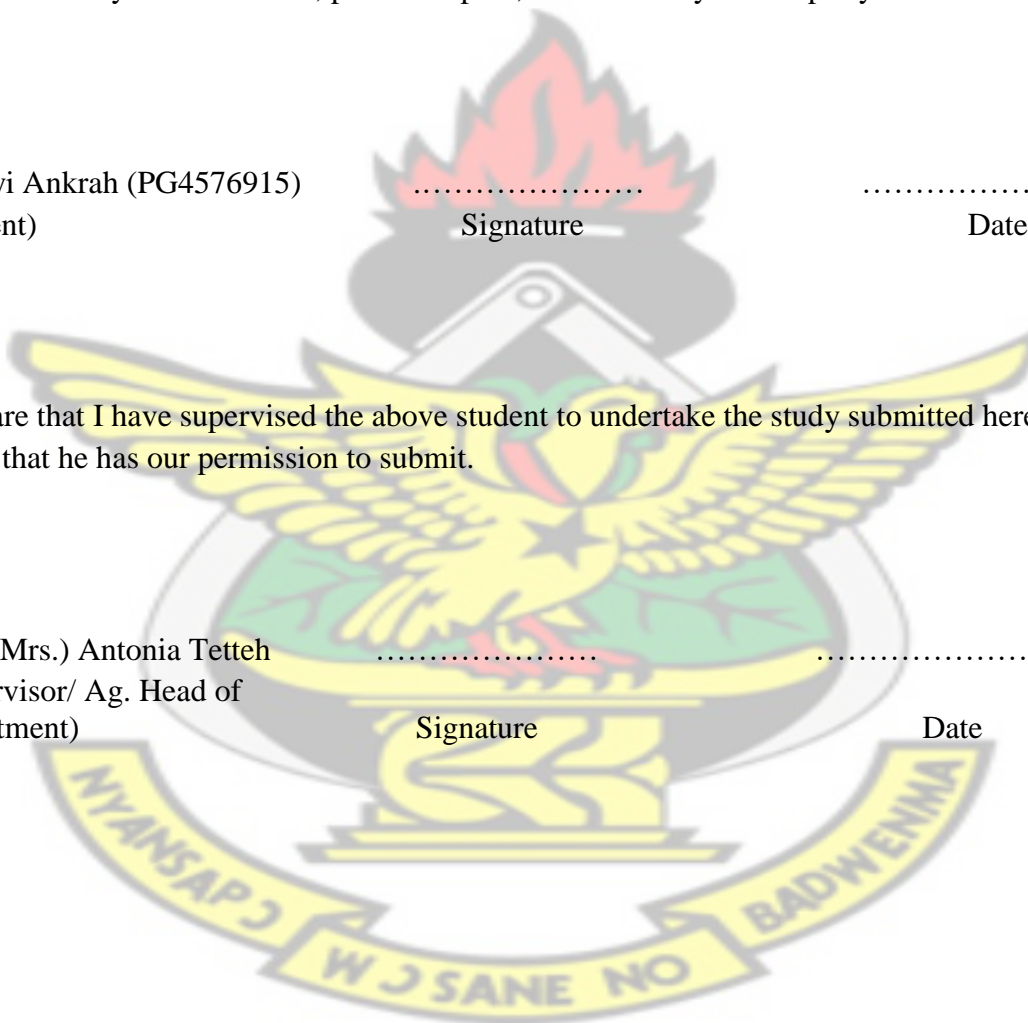
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I declare that I have supervised the above student to undertake the study submitted herein and verify that he has our permission to submit.

Prof. (Mrs.) Antonia Tetteh
(Supervisor/ Ag. Head of
Department)

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DEDICATION

I would like to dedicate this thesis to God Almighty for His protection, guidance and the knowledge he gave me when carrying out this research.

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Special thanks go to the KNUST Research and Conference Grants for the financial contribution made available through my supervisor. It is worth mentioning that without the emotional and financial support of my dear uncle and guardian, Mr. Michael Sowu, this work would not have seen completion.

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ABSTRACT

Roselle *altissima* is a bast fiber crop of global economic importance. Although West Africa is considered the centre of diversity, research and utilization in roselle is widely ignored. The awakening of industrialization in Ghana presents roselle as candidate crop for exploration, however, information on genotypes of economic importance is lacking. Characterization and genetic diversity estimation identifies genotypes having important traits for incorporation into breeding programmes, and reveal within and between population variability, divergence between genotypes and relationships among them. Our objective was to map roselle geographical origin in northern Ghana, determine regions of substantial genetic diversity, examine genetic variability, estimate the distance within the collection, and identify relationships in the genotypes. Sixty-one accessions collected from 21 districts were evaluated in a two-season field trial using a 6×6 lattice square design for 36 accessions and 5×5 lattice square design for 25 accessions in three replications each from July to November 2016 and 2017, respectively. The genotypes were evaluated using twelve qualitative and five quantitative morphological traits covering plant type, leaf, stem and flower characteristics, and growth habit. Data were analysed by Shannon-Weiner Diversity Index (SDI), analysis of variance, genetic distance, cluster, principal component, and stepwise discriminant analysis. Except for conserved traits in growth habit, calyx pubescence, and capsule shape, large variability was observed in plant type, branching habit, stem pubescence, leaf form, and calyx pigmentation. Large between population differences were identified in plant height, branch number, basal diameter, and days to flowering. A large within population variation of 0.74 to 0.85 for branch number, plant height, basal diameter, and days to flowering resided in the accessions. Height after first branching was somewhat identical in season 1. Mean plant height ranged from 184 cm to 355 cm, basal diameter 13 mm to 24 mm, height at first branching 6 cm to 157 cm, branch number 2 to 12, and days to 50 % flowering from 97 to 101 days after planting.

Twenty-one accessions were selected based on tall plant height with few branches at high branch points, and large basal diameter were HA-07, HA-11, HA-12, HA-21, HA-33, HA-37, HA-38, HA-39, HA-42, HA-43, HA-44, HA-45, HA-46, HA-47, HA-49, HA-50, HA-52, HA-54, HA-55, HA-57, and HA-58. Seven districts exhibited largest diversity by SDI estimates Kassena-Nankana (0.87), Bolgatanga Municipal (0.83), West Mamprusi (0.82), Savelugu-Nanton (0.73), Tamale Metropolitan (0.68), Gowri-Yorogo and Talensi (0.67). Mean district SDI values ranged from 0.53 to 0.87. Savelugu-Nanton district accessions had the highest mean plant height of 308.27 ± 48.91 cm, highest branching point at 107.19 ± 64.66 cm, and less than five branches. Low correlation coefficients of $r = 0.11$ in plant height and basal diameter to $r = 0.13$ in plant height and branch number was identified. Mean genetic distance based on Euclidean estimate was 3.03 ± 0.90 and ranged from 0.41 to 5.17 representing substantial genetic diversity in the region. Clustering based on Ward's minimum variance produced three clusters independent of their geographical origin suggesting seed flow in the region. The first three principal components explained 100 % of the total variance with the highest loadings from branch number, height at first branching and basal diameter, then plant height and days to 50 % flowering. Stepwise discriminant analysis identified branch number, plant height and days to 50 % flowering to be most important traits in the structuring of roselle *altissima*. The findings would be beneficial for planning a systematic collection, for conservation, and for trait improvement in breeding roselle.

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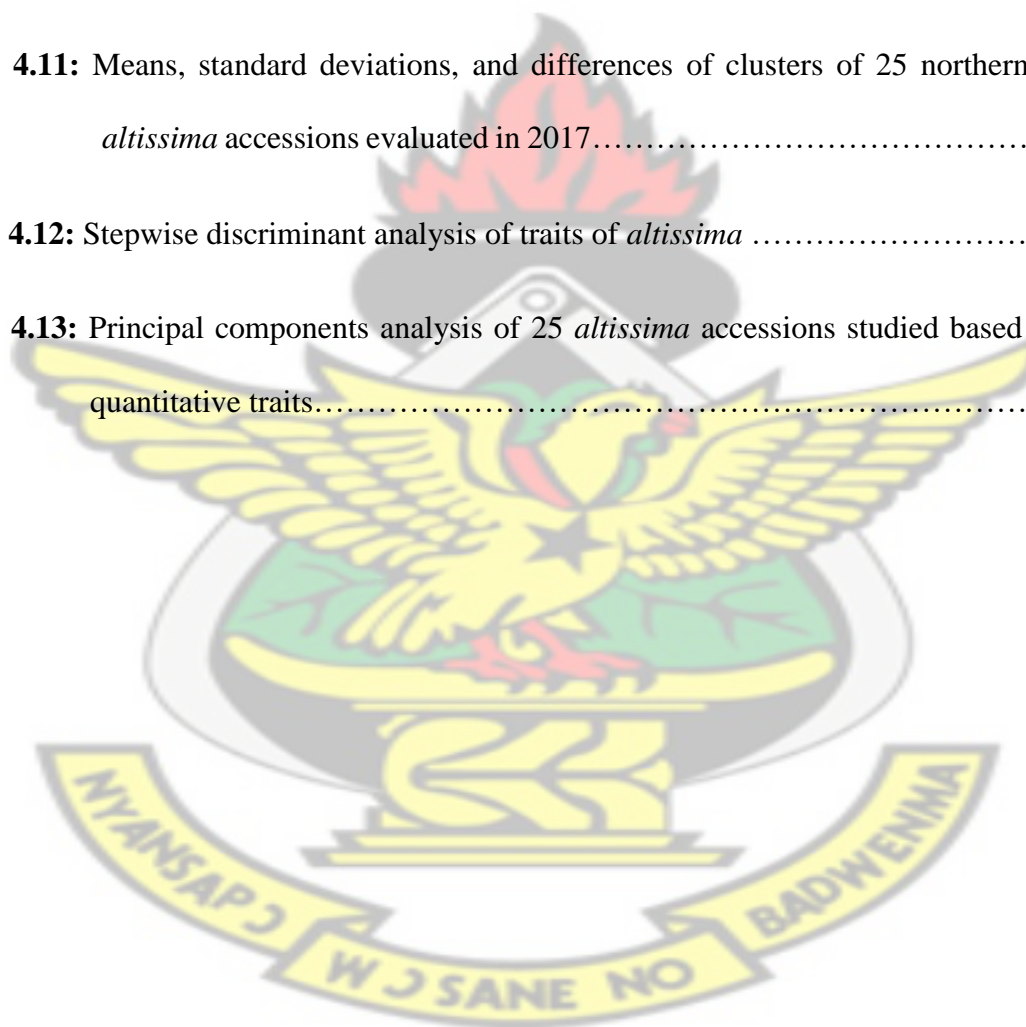
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CHAPTER ONE

1.0 Introduction

Roselle (*Hibiscus sabdariffa* var. *altissima*) commonly called roselle fiber type, hereinafter referred to as roselle *altissima*, is an important member of the bast fiber crops in the tropics. It is an annual herbaceous plant of the family Malvaceae which includes important crops such as cotton (*Gossypium hirsutum*), okra (*Abelmoschus esculentus*), jute (*Corchorus capsularis* and *Corchorus olitorius*), and kenaf (*Hibiscus cannabinus*). Roselle *altissima* is an erect, sparsely branched crop that grows to a height of about five meters under abundant sunlight and moderate rainfall (Crane, 1949; Morton, 1987). It is a self-pollinating allo-tetraploid species of $2n=4x=72$ (Akpan, 2000).

Roselle *altissima* is believed to be the product of a hybridization event between *H. asper* and *H. meeusei* (Singh, n.d., para. 15; Murdock, 1959). It is believed to originate from the East, Central, and West Africa (Morton, 1987; Rhodin and Panchoo, 1990). Records, however, indicate that the crop was discovered and studied after its seeds were found among a consignment shipped to the Philippines from the then Gold Coast (Ghana) in 1911 (Wester, 1912, 1914; Andrews, 1952). In 1912 an unusual variety of *Hibiscus* specimen collected from Gold Coast was identified in the Kew Gardens as a new variety of *H. sabdariffa* species (Imperial Institute, Great Britain, 1912).

A closely related genotype of roselle *altissima* is *H. sabdariffa* var. *sabdariffa*, commonly referred to as roselle vegetable type, with succulent and edible calyxes, whereas roselle *altissima* has fibrous inedible calyxes. Roselle vegetable is a relatively short, highly branched bushy type, principally cultivated for its edible calyx and leaves. Roselle *altissima* is similar to kenaf in height

and fiber characteristics, consequently, the two crops are collectively referred to as “mesta” among the Asian people (Singh, n.d.).

Roselle is known globally by many different names such as *roselle* or *sorrel* in Jamaica, Australia, France and Germany (Tindall, 1983; Schippers, 2000), as *karkadé* in Sudan and Egypt; *bissap* in Senegal and Mali; *sour sour* and *da* in Guinea, Burkina Faso, and Cote D’Ivoire. In Nigeria and Ghana it is known as *zooboo* and *sobooro*, respectively (Abu-Tarboush *et al.*, 1997; Akpan, 2000; Frimpong *et al.*, 2014). In northern Ghana, where roselle is more common, the local communities name roselle differently as ‘*saa bra*’ among the Dagbanli tribe, ‘*biuur*’ in Dagaare, and ‘*bito*’ among the Frafras (Coffie, 2016).

Roselle is essentially one of the traditional third world bast fiber crops targeted as a suitable annual renewable alternative to synthetic fibers (Fuqua *et al.*, 2012; Sathishkumar *et al.*, 2013). It is second in quality to kenaf as a jute fiber substitute. According to Mwasiagi *et al.* (2014), the physical and chemical properties of roselle are comparable to kenaf.

Roselle *altissima* has long been used for making salt-resistant cordage, burlaps and fishnets (Wester, 1907; Crane, 1949) and for making diverse paper products (Das Gupta, 1959). In meeting modern demands and diversification, the bast fiber of roselle has found application as automobile parts (Alves *et al.*, 2010), and for upholstery, fabric and shoes in the textile industry (Managooli, 2009). It finds other uses as bio-composites for building materials, such as fiber board (Junkasem *et al.*, 2006). In environmental bioremediation, roselle fiber is used as composite for sewage sludge and toxic waste clean-up (Manoj and Vasudevan, 2012). The economic potential of roselle as

source of bast fiber for making sacs for transport of produce has been ignored, while Ghana, imports sacs from Asia.

While roselle *altissima* is cultivated commercially in Asian countries for its bast fiber (Mohammed *et al.*, 2012) in Sub-Saharan Africa (SSA), it is under-exploited where its leaves and seeds are consumed as food, the stems for wood fuel, and its fiber is used for making ropes.

Ghana engaged in commercial cultivation of bast fiber crops including roselle to feed the newly built Kumasi Fiber and Bag Factory established in 1960, until 1991 when the factory was closed down (Asante and Amankwatia, 1992). After its closure, market for bast fiber and its products dropped substantially. Since then, cultivation of roselle has been restricted to subsistent scale farming in few growing areas in northern Ghana as vegetable food source. In southern Ghana, roselle *altissima* is unknown (personal communication).

Global restriction on non-biodegradable and synthetic fibers has led to interest in natural fibers of which roselle *altissima* is a good candidate (Aji *et al.*, 2009; Alves *et al.*, 2010; Ticoalu *et al.*, 2010; Begum and Islam, 2013), as the mechanical properties of its fiber is comparable to that of synthetic fibers (Junkasem *et al.*, 2006; Singha and Thakur, 2009; Nadlene *et al.*, 2016). Demand for bast fiber crops is expected to rise in SSA and genotypes of economic importance must be available for commercial cultivation. Currently, there is dearth of information on roselle *altissima* sources in northern Ghana and improved varieties are not available. Additionally, traits of economic importance, method of cultivation, seed availability, agronomic practices and processing for fiber extraction are not known. Indigenous knowledge from the aged folk indicates that northern Ghana has wide distribution of roselle. There is no single study that has identified

geographic locations of roselle diversity in northern Ghana besides the work of Coffie (2016) which examined genetic diversity in 35 roselle accessions (var. *sabdariffa*) from Senegal to Nigeria. This study revealed the area between Mali, Ouagadougou and northern Ghana as a centre of diversity for roselle *sabdariffa*.

Kwofie (2017) examined the hybrid potential of diallel crosses among 20 genotypes of *H. sabdariffa* and *H. altissima*. Successful crosses within *altissima* and within *sabdariffa* were achieved but crosses between *sabdariffa* and *altissima* were not successful due to compatibility barrier. Their findings indicate that to achieve genetic improvement in each subspecies, a genetic variability is required.

Despite the cleistogamous nature of roselle (Wilson and Menzel, 1964) and reports of narrow genetic variability among roselle genotypes of Asian origin (Heliyanto, 1992; Hanboonsong *et al.*, 2000), the findings of Coffie (2016) revealed substantial genetic diversity among the West African roselle *sabdariffa* landraces. It may therefore be possible to identify some variability within the roselle *altissima* accessions of Ghana, hence, a collection, characterization, and evaluation of genetic diversity in roselle *altissima* accessions in Ghana is needed.

Genetic diversity studies on *altissima* is essential for characterizing and identifying desirable genotypes with useful alleles that can be incorporated into crop improvement programmes. Additionally, genetic diversity estimates are employed for efficient management of germplasm conservation. It also provides information about the evolutionary history of a crop and the value that can be harnessed from it.

Genetic diversity within and among crop populations may be estimated by agro-morphological evaluation, biochemical and DNA-based molecular techniques (Corinne, 2002). According to

Karp *et al.* (1997), each of the methods used in estimating genetic diversity provides different information outcome based on the research objectives, availability of resources, and time. Of these, biochemical and DNA molecular markers are rapid, more accurate, insensitive to environmental influences and also estimate polymorphisms in enzymes or proteins, and DNA, respectively (Melchinger, 1993; Tanksley and Orton, 1983; Govindaraj *et al.*, 2015). Biochemical and DNA markers are however, expensive and require sophisticated equipment. Morphological evaluation is labour intensive, requires large population size, exhibit low rate of polymorphism, and is constrained by environmental sensitivity (Botha and Venter, 2000). However, because morphological evaluations are less costly, require readily assessable measurement tools and offer advantage of direct observation of traits of interest, it is often employed in developing countries where labor is cheap.

Very little research report is available on *altissima*. Siepe *et al.* (1997) examined genetic variability in 103 *H. cannabinus*, five *H. sabdariffa* var. *altissima*, 11 *H. sabdariffa*, var. *sabdariffa*, two *H. moschentos*, two *H. vulgaris*, one *H. mutabilis*, and one *H. gossypinus* and one *H. rosens* genotypes using morphological assessment. Morphological evaluation requires robust statistical analyses to reveal distinctness among accessions, and classify accessions into groups based on some distance measure. Cluster analysis and principal components analysis are often applied to morphological data to reveal between population differences. More often, positive significant correlation between morphological and molecular evaluations have been reported in comparative studies, such as in lentils ($r = 0.28$; $P < 0.0001$) (Fikiru *et al.*, 2010) and egg plant ($r = 0.68$) (Prohens *et al.*, 2005). To the best of my knowledge, this is the first report on examination of genetic variability in a large *altissima* collection, that is, 61 genotypes, in Ghana.

The overall objective of the present research was to determine genetic diversity and variability in 61 roselle *altissima* germplasm collected in Ghana, and to assess the geographical locations of substantial diversity.

Specific objectives include:

- (i) To collect roselle from northern Ghana and determine the regions of accumulation of genetic diversity
- (ii) To evaluate roselle *altissima* accessions for within-population and between-population variability
- (iii) To determine genetic diversity estimates within the collection
- (iv) To determine relationships and heterotic groups in the roselle populations

The findings of this research will be useful to the scientific community for further research on the crop, particularly to explore improvement in yield, quality, conservation, and development of other uses of the crop. In addition, promotion of roselle *altissima* bast fiber economic ventures in Ghana would provide employment and improve export potential.

1.1. Research Hypothesis

Genetic variability in populations are driven by evolutionary forces including mutation, gene flow, recombination and genetic drift. With no formal breeding practiced on roselle, many genotypes in Ghana are expected to possess a combination of genes arising from these forces, in addition to modulation by the environment. It should therefore be possible to identify variability in the Ghana roselle *altissima* populations.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Origin and History of Roselle

Hibiscus sabdariffa L. var. *altissima* Wester commonly referred to as roselle *altissima* is an economically important herbaceous shrub principally cultivated for its bast fiber. Roselle *altissima* variety is a wild type of its closely related genotype, *H. sabdariffa* var. *sabdariffa*, a usually short, highly branched bushy type, principally cultivated for its edible calyx and leaves. Roselle belongs to the family *Malvaceae* and is closely related to other horticultural crops such as cotton (*Gossypium hirsutum* L.), jute (*Corchorus capsularis* L. and *Corchorus olitorius* L.), kenaf (*Hibiscus cannabinus* L.) and okra (*Hibiscus esculentum* L.) (Boulanger *et al.*, 1984; Kalder, 1991). It originates from West and Central Africa (Morton, 1987; Rhodin and Panchoo, 1990).

Roselle altissima is believed to be the product of a hybridization event between *H. asper* and *H. meeusei* (Singh, n.d., para. 15; Murdock, 1959). Africans introduced seeds of roselle plant to India, West Indies, and parts of Central America in the latter part of the 16th century (Robyns, 1965; Andrews, 1952; Standley, 1923; Harris, 1913). A molecular-based assessment on a roselle collection from Senegal to Nigeria in West Africa by random amplified polymorphic DNA (RAPDs) confirmed the regions of Mali (Mopti), Burkina Faso (Ouagadougou) and northern Ghana (Bawku) as the plausible centre of diversity of roselle (Coffie, 2016). In this region, many roselle morphotypes including the green, red, pigmented, branched, sparsely branched, tall, short types, and a wide morphology of calyxes occur.

The fiber potential of roselle was first described by Herman (1687) and later by Royle (1855). The roselle *altissima* (var. *altissima*) was named by Khan (1930) but was originally described by Wester (1914) who accidentally identified roselle seeds from a seed consignment imported into the Philippines from then Gold Coast (Ghana) in 1911 (Wester, 1914, 1912,). The roselle *altissima* was later cultivated commercially in Cuba for its bast fiber in 1919 (Calvino, 1920), and thereafter gained global recognition as a potential substitute for jute fiber. Roselle has since become a commercial source of fiber in Asia. Roselle is now widely cultivated in many parts of the world including the Caribbean, Australia, Russia, Thailand and China for its stem fibers, leaves and seeds and has a myriad of common names.

Roselle is known in many parts of the world by many different names, “*roselle*” or “*sorrel*” in Jamaica, Australia, France and Germany, “*mesta*” as a common name for bast fiber crops including kenaf and roselle (Singh, n.d. ; Rao, 1996) among Asian people. In Africa, the plant is known as “*karkadé*” in Sudan and Egypt; “*wonjo*” in Gambia; “*bissap*” in Senegal and Mali; “*sour sour*” and “*da*” in Guinea, Burkina Faso, Cote D’Ivoire; “*zooboo*” in Nigeria and “*sooboro*” in Ghana (Arthney and Dennis, 1991; Abu-Tarboush *et al.*, 1997; Akpan, 2000; Frimpong *et al.*, 2014).

2.2. Indigenous Knowledge on Roselle Plant in Ghana

Roselle species is very common in northern Ghana, where it grows naturally in the wild and under homestead cultivation. After harvesting its leaves for food, the stem is processed to make tether for livestock. Despite the many cultivated varieties of roselle by farmers, intensive research has not been done in Ghana to collect, characterize and develop known varieties. In fact, little attention

is given to research and development of indigenous plant crops in Africa (Norman, 2003; Adeboye, 2011)

2.3. Taxonomical Classification and Morphology of *Hibiscus sabdariffa* L. var. *altissima* Wester

2.3.1. Classification of *Hibiscus sabdariffa* L. var. *altissima* Wester

In taxonomic classification, Roselle belongs to the section *Fucaria* with nearly 50 species characterized by prominent succulent calyces with ten prominent veins and stems and floral parts usually non-glabrous (Wilson and Menzel, 1964; Douglas-Wilson, 1993). Mclean (1973) and Wilson and Menzel (1964) both reported that roselle is a tetraploid with chromosome number of $2n=4x=72$, making their segregating population requiring longer periods for purification.

2.3.2. Morphology of *altissima*

Roselle *altissima* seeds sprout usually 2-3 days after planting in warm soil and grows into a non-bushy top-branching plant of about five meters high within five to seven months (Dempsey, 1975; Morton, 1987) when exposed to long hours of sunlight (Wester, 1914). Figure 2.1 shows a diagram of roselle plant.

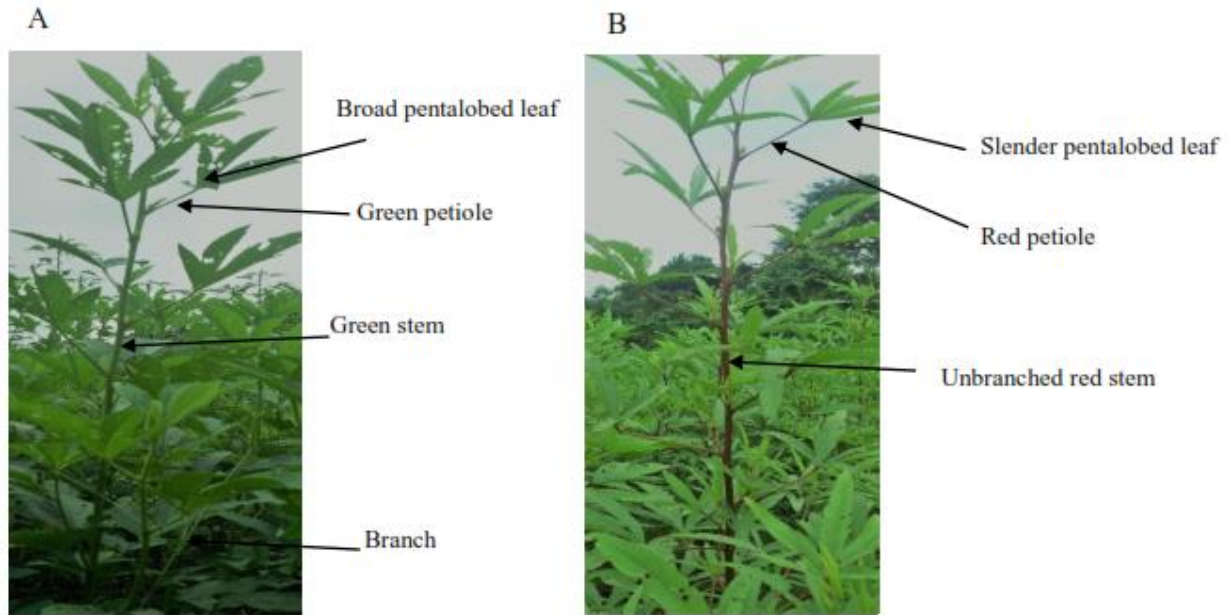


Plate 2.1. Image of roselle plant types at 60 days old. (A) Green stem genotype with branches standing at a height of 195 cm. (B) Unbranched red stem genotype standing at a height of 210 cm.

Roselle *altissima* is a self-pollinating day-length sensitive plant. Roselle is ambiphotoperiodic whereby it flowers in short days and in long days under warm temperature conditions (Mansour, 1975). Vegetative growth and flowering are critical factors in the growth cycle of roselle. A bright sunny day with day length no less than 12 h for four to six months is required to induce prolonged vegetative growth and delay flowering (Duke, 1983). In contrast, minimal day length of 8 to 10 h halts vegetative growth prematurely and induces early flowering, resulting in fruiting in less than three months (Hacket and Carolene, 1982; McClelland, 1924). The flowers of roselle which possess a glanded sepal wither and die off after successful pollination and the calyx enlarges into an inedible non-fleshy fibrous, hairy and irritating structure surrounding a round or semi-ovoid capsule (Wester, 1914). The capsules comprising five segments each, contains about 25 seeds (Dempsey, 1975) and mature within three months after pollination (Chakravarty and Basu, 1972). Coffie (2016) provided a comprehensive description of the five types of *altissima* available.

2.4. Roselle and Bast Fiber Market

The phloem or bast fiber obtained from the long non-branching stem of roselle is second in economic importance to kenaf (*H. cannabinus*) (Eltayeib and Elaziz, 2014). Roselle makes up about 20 % of the bast fiber market globally. Global roselle bast fiber production is concentrated in Asia, particularly, Thailand, where it became an important export commodity in the late 1950s after a seed consignment from Ghana was shipped to the Philippines in 1914 (Wester, 1914). Between 2012 and 2016, the annual production of the top six leading bast fiber producing countries were India (101,799.40 metric tons (mt)), Russia (50,547.80 mt), China (26,301.40 mt), Cuba (11,412.20 mt), Chile (9,396.40 mt), Malaysia (8,729.80 mt), and Democratic Republic of Congo (7,483.80 mt) (data.world, 2018).

Commercial production of bast fibers, in Africa dates back to 1961 where nine countries, Angola, Central African Republic, Democratic Republic of Congo, Ethiopia, Madagascar, Mali, Mozambique, Nigeria, and South Africa take part (data.world, 2018). Within the period 1961 to 2016, total bast fiber produced from the Americas increased from 12,000 to 15,000 hectares, equivalent to 25 % increase. In Africa, bast fiber production increased from 15,000 to 25,000 hectares, equivalent to 67 % increase. In contrast, production in Asia and Europe decreased substantially from 896,000 ha to 90,000 ha, equivalent to 90 % decrease, and from 27,000 to 13,000 ha corresponding to a 52 % reduction respectively (data.world, 2018). World leading producers and exporters of roselle are India (1,567,000 mt of jute and mesta per year), Bangladesh (800,000 mt in 2003), China (300 million mt in 2004) and Thailand (McClintock and El-Tahir, 2004, FAOSTAT, 2013). In recent years, environmental regulations and restrictions on non-biodegradable synthetic fibers (Padmaja *et al.*, 2014) has created a renewed interest in natural fiber crops.

2.5. Properties of Bast Fibers of Roselle

Roselle leaves, calyces, and stem bark are used as source of food, pigment, folk medicine, and antimicrobial agent. However, it is commercially known for its bast fiber in the stem. Other bast fiber crops include jute (*Corchorus olitorus* and *Corchorus capsularis*), kenaf (*Hibiscus cannabinus*), Congo jute (*Urena lobata*), and flax (*Linum usitatissimum*) (Chalbilendra, 2009).

Bast fiber, also known as phloem fiber, is obtained from the bast or outer layers around the stems of fibrous dicotyledonous plants. Roselle fibers are strong lignocellulose bundles of fibers, which form about a third of the weight of the plant. Figure 2.2 shows the fibers of roselle extracted from the stem.



Plate 2.2. Images of *altissima* fiber extracted from retted stem. (A) Retted fiber being removed from stem (B) Dried retted fibers of *altissima*

A single strand of roselle fiber measures averagely about 2.2 mm long and 21 microns in diameter (Olotuah, 2006). In the stem, the bundles of fiber are layered with pectin and calcium, hemicelluloses, and celluloses 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 (Thiruchitrambalam *et al.*, 2010; Mwasiagi *et al.*, 2014) to promote growth of

microorganisms with pectinolytic activity, after which the fibers are peeled off from the stem, washed, and dried in the shade to produce a soft, lustrous and cream or silvery-white product.

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 (Nadlene *et al.*, 2015).

Although African countries including Nigeria, Kenya, Cote D'Ivoire, Sudan, Uganda and Ghana grow roselle for a variety of reasons (Falusi *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).

Fiber quality parameters include fiber color and luster, gum content, fiber reed length, tensile strength and Young's modulus (Ramaswamy *et al.*, 1994), most of which are influenced by the day length during cultivation, age of the plant, and the retting procedure employed (Das *et al.*, 2014; Tahir *et al.*, 2011; Mansour, 1975).

2.6. Economic Importance of Roselle

Roselle is a multi-purpose plant with immense benefits to man. It has been used as food and source of pigment, fiber for handcraft purposes, and as preventive and therapeutic agent in folk medicine. Roselle fiber has long been commercially exploited in Asian countries like China, Thailand, Philippines, and India (Mohammed *et al.*, 2012). In Africa, the sale of roselle for vegetable offers an important source of income for women and girls in countries such as Ghana, Nigeria, Cote D'Ivoire, Burkina Faso, Senegal, and Mali (Obodai, 2007; Diouf *et al.*, 2007; McClintock, 2004).

Roselle fibers make salt-resistant cordage and fish net (Cook, 1960), shoes, bags, yarn, burlap, upholstery, and sacs. Some automobile companies such as BMW, Toyota and Ford have commenced usage of natural fibers in designing interiors of their new vehicle models (Akil and Zamri, 2014). A growing technology for value addition of bast fibers is to prepare mixtures of the fibers with materials, such as gypsum, thermoplastics, and rubber to make composite fibers (Babatunde et al., 2015; Domke and Mude, 2015; Pott, 2004) which have varying properties for wide applications. Notable among the composite fiber products is plasterboard and fiberboard as building materials (Zhang, 2003), electronic equipment (Bert, 2002), suitable fiberglass substitute in molded plastics and as a natural coagulant in wastewater treatment (Yong and Ismail, 2016). Roselle charcoal demonstrates good nitrate-nitrogen adsorbent properties and is listed as a wastewater treatment product (Yimrattanabovern and Chuersuwan, 2006).

National regulatory authorities advocate for total replacement of synthetic fibers or at least a natural-synthetic fiber composite of which roselle fiber is being explored as a reinforcement material for fiber composites (Favaro *et al.*, 2010; Nirmal *et al.*, 2011; Reem *et al.*, 2012; Nadlene *et al.*, 2015). 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 availability, light weight, ease of separation, high toughness, low density, and good thermal properties (Singha and Thakur, 2008; Mwasiagi *et al.*, 2014). The core wood of roselle stem is also used for making paper pulp.

The global shift in preference for “green” products has escalated the market for natural fibers for packaging agricultural produce and fiber composites, particularly, for automotive companies. Market for natural fiber composites valued at USD 4.46 billion in 2016 is projected to reach USD 5.10 billion in 2024, equivalent to 12.5 % increase in price value (Grand View Research, 2018). Scientists must therefore prepare to provide the genetic resources for natural fibers in order to meet the growing demand. The genetic resources include germplasm identification, germplasm improvement, seed availability, and development of processing technologies for extraction of fibers from the identified plants.

2.7. History of Fiber Research and Development in Ghana

Ghana’s drive to venture into the cultivation of fiber crops for commercial purpose was motivated by the first president, Dr. Kwame Nkrumah, in 1962 when the prices of jute and other allied fiber products shot up after World War II (Amanquah, 1968). Exploration and exploitation of fiber plants in Ghana started with collection, characterization and conservation with the hope to identify genotypes of commercial importance to feed the newly established Kumasi Jute Factory. Fiber crops that were cultivated for research at the time included white jute (*Corchorus capsularis*), tossa jute (*C. olitorius*), kenaf (*H. cannabinus*), Congo jute (*Urena lobata*) and roselle (*H. sabdariffa*) in Ashanti, Brong Ahafo and Northern regions of Ghana (Amanquah, 1968; Amankwatia, 1987).

Fiber crop varieties that were available for cultivation in the Guinea savannah zones of Northern Ghana after the research programme included Congo jute (Ex. Mokwa), kenaf (A63-440, A63-442, Cubano, Cuba 108, Cuba 2032, New Tafo Red, Fung Red, GT.3, GT.4, GT.7 and G.45 and

roselle. The roselle variety happened to be similar to the vegetable type, 'Thai Red' which Dr. Crandall, a plant breeder, and his team from Guatemala worked on. In the forest savannah transitional zones of Ghana, some kenaf varieties and Congo jute also did very well (Asante and Amankwatia, 1992). Both exotic roselle seeds and the local roselle variety were cultivated in Northern Ghana for comparative studies (Amankwatia, 1987).

The outcome of the experimental research at the time revealed that for good fiber yield and quality, ideal planting time was the long day length period which starts in May with the beginning of raining season in order to grow roselle plants with taller and larger stems which mature by 150 day after planting. Planting in August, the late planting season, resulted in relatively premature flowering and shorter plants with smaller stems. In their report, they concluded that taller roselle plants with bigger stems were ideal for good fiber production. For higher fiber yield and strong fiber, Jarman (1985) reported the ideal harvesting time for roselle is between 140 to 160 days after planting.

2.8. Current Status of Roselle Research and Development in Ghana

The bast fiber crops present a fortune for many developing countries as an industrial resource for creating a wide array of jobs. Being natural, they constitute the only replacement for synthetic fiber, which in recent times have received stringent environmental restrictions owing to their non-biodegradable nature. Regrettably, a roselle collection, conservation, and evaluation has not been carried out. Information on the centers of roselle diversity in northern Ghana is scanty (Coffie, 2016; Ankrah *et al.*, 2018). This has necessitated further collection and evaluation of roselle accessions in Ghana to reveal the diversity distributed among the populations, identify accessions

with commercial potential and to make this information available to breeders for genetic improvement.

Furthermore, climate anomalies in the tropics and genetic erosion have led to interest in genetic diversity of crops, particularly for widening the genetic base of populations and checking extinction.

In 2011, a bast fiber crops research team in the Kwame Nkrumah University of Science and Technology, Kumasi, embarked on a collection of 35 roselle accessions from Senegal to Nigeria. The accessions were evaluated using agro-morphological characterization and Random Amplified Polymorphic DNA (RAPD) marker profiling (Coffie, 2016). The work revealed that large variability existed in the West African genotypes to merit breeding for genetic improvement, contrary to reports of lack of variability in exotic phenotypes of roselle (Hanboonsong *et al.*, 2000; Yusof and Saud, 2009). The work further confirmed the region between Northern Ghana, Burkina Faso, and Mali as the most probable center of diversity (Coffie, 2016). Among the accessions of roselle studied, the only three fiber types identified among them were collected from Ghana. The findings of this work warranted further exploration into a larger collection of the roselle *altissima*. Kwofie *et al.* (2017) examined crossability among twenty accessions, namely, *H. sabdariffa* var. *altissima* and *H. sabdariffa* var. *sabdariffa* collected from Northern, Western, and Volta regions of Ghana and reported success in intra-varietal crosses but a hybridization barrier in the inter-varietal crosses. Their results indicate that an interspecific cross between the two genepools with the aim of creating variability will be challenging and for that matter, an aggressive search for variability within the *altissima* species and within the *sabdariffa* species is warranted.

A further collection of 61 *altissima* accessions has been carried out in northern Ghana and is awaiting characterization by both morphological and molecular approaches. A personal visit to Crop Research Institute(CRI) of the Council for Scientific and Industrial Research (-CSIR), Fumesua, and enquiries made at the Plant Genetic Resources Research Institute, PGRRI, Bunso, Ghana, revealed that these institutes do not have a roselle collection in their repository, hence, the need to conduct an expedition for roselle collection.

2.9. Economic Significance of Roselle Crop to Ghana

Ghana being an agricultural nation relies heavily on its agricultural commodities for her economic gains. Roselle crop is abundant and readily available in northern Ghana and the plant sprouts spontaneously after the first rains. Cultivation and processing of roselle in Ghana was an important economic activity from 1960 to 1991 when the Kumasi Fiber and Bag Factory of Ghana processed a composite of roselle, kenaf and jute fiber into cocoa sacs at about 5,000 mt/year and later expanded to 13,000 mt/year (Amanquah, 1968). A folding-up of the factory in 1991 led to a substantial drop in the bast fiber market and roselle use was limited to culinary aspects for its leaves, edible calyxes and seeds. Since then, the fiber-rich stem, is discarded or burnt as wood fuel. Only a minority of the indigenous folk in northern Ghana cultivate roselle for fiber for making tethers for their livestock.

Ghana's annual cocoa production has progressively increased from 400,000 mt in 1995 to 860,000 mt in 2016, equivalent to 112 % increment (Bangmarigu and Artan, 2018). During the period, an estimated 22 million fiber sacs was required annually to bag the cocoa beans alone not considering

other agricultural commodities. In 2015, the defunct Kumasi Fiber and Bag Factory was revamped under the name Jute Mills Ghana Limited to meet the country's demand for fiber products. With Ghana's target of achieving cocoa production of one million mt/year, the revamped factory would require about 44,000 cocoa sacs per day to store and transport 2,740 mt of cocoa produced each day of the year, which is equivalent to 1.0 million mt (16 million sacs) per year. A huge fortune in roselle cultivation would create jobs and livelihood, lead to industrialization as roselle fiber can give rise to other products such as fabric, carpets, upholstery, paper, fishing nets and building materials.

2.10. Genetic Diversity in *H. sabdariffa* var. *altissima*

Genetic diversity estimate is an essential resource utilized by breeders to identify economically important genotypes for trait improvement, cultivar development, and to widen the genetic base of populations. Genetic diversity estimate also guides germplasm conservation management, aids identification of heterotic groups, and reveals the evolutionary history of crop species (Dwivedi *et al.*, 2008; Mohammadi and Prasanna, 2003; Edmonds, 1991). Genetic diversity study in the Ghana roselle populations is important as many reports affirm the lack of variability in exotic roselle ecotypes from Asia and parts of Europe (Hanboonsong *et al.*, 2000; Yusof and Saud, 2009).

There is paucity of information on genetic variability within *altissima*, although some reports compare variability between kenaf and roselle (Sie *et al.* 2009; Cheng *et al.*, 2004; Siepe *et al.*, 1997). In contrast, genetic diversity and divergence studies abound for the var. *sabdariffa* (Satyanarayana *et al.*, 2015, 2016; Medagam *et al.*, 2015; El-Tahir and El-Gabri, 2013).

2.11. Genetic Diversity Estimation Methods

Methods for estimation of genetic diversity include morphological, biochemical, molecular and pedigree assessment. Of these, biochemical, which exploit isozymes and seed proteins, and the molecular approach present a more accurate and rapid estimation as they utilize proteins and DNA, respectively, and are not influenced by the environment (Govindaraj *et al.*, 2015). Besides, the codominant protein markers and the codominant DNA markers permit identification of polymorphisms for accurate estimation of genetic diversity (Melchinger, 1993; Tanksley and Orton, 1983; Farooq and Azam, 2002). Pedigree analysis relies on parentage of genotypes to assess variations in germplasms but cannot evaluate the effects of selection and genetic drifts (Messmer *et al.*, 1993). But for their expensive technique and requirement of sophisticated equipment in the cases of biochemical and molecular marker assessment, morphological markers are often desired.

Morphological evaluation constitute the first approach a breeder would use to characterize populations and identify important germplasm bearing the desired traits (Camussi *et al.*, 1985). Though morphological approach requires large sample size, is laborious, is influenced by environment and has low heritability of traits (Hartings *et al.*, 2008; Beyene *et al.*, 2005), in developing countries where labour is cheap, it is the most commonly used method. Agromorphological evaluation has been used successfully to investigate variation and diversity in roselle and other species. Studies on assessment and characterization of genetic diversity by agromorphological means include evaluation of six genotypes of roselle in Sudan (Ahmed *et al.*, 2009), assessment of yield character variability among nine roselle ecotypes in Niger (Attah *et al.*, 2011), evaluation of six accessions of roselle in Nigeria for yield attribute (Falusi *et al.*, 2014). Other important works include evaluation of 28 landraces of vegetable roselle from India (Medagam *et*

al., 2015), assessment of three mutant roselle cultivars in Malaysia (Osman *et al.*, 2011), and the evaluation of 51 kenaf accessions in Nigeria (Balogun *et al.*, 2008).

2.12. Measures of Genetic Diversity

Ranges of genetic diversity measures are available and application of each for quantifying variability within and between populations is contingent on factors, such as, type of population, including inbred lines, hybrids, or landraces; the status of the population, particularly, whether they are in Hardy-Weinberg equilibrium (Mohammadi and Prasanna, 2003), and the kind of data collected. The data may be qualitative or quantitative measures from agro-morphological evaluation, biochemical or molecular markers (Mohammadi and Prasanna, 2003). The diversity measures may be classified under four broad estimates, viz., genetic distance, within and between population variability, and detection of relationships in cluster analysis principal components analysis (Joliffe, 2002; Johnson and Wichern, 1992; Everitt, 1980; Anderberg, 1973) and discriminant analysis (Cacoullos, 1973) to reveal the power of each trait in distinguishing genotypes on the basis of minimum Wilk's lambda.

Within-population variation often employs some diversity index, such as Simpson diversity index (Simpson, 1949), Shannon-Weiner diversity index (SDI) (Shannon and Weaver, 1949), McIntosh index (McIntosh, 1967) and Berger-Parker index (Berger and Parker, 1970). Simpson's index most frequently estimates evenness and relies on the few most abundant species to estimate diversity without considering the rare species (Risser and Rice, 1971; Whittaker, 1972). Berger-Parker Index is used extensively to establish the more abundant species within a community and estimates species richness (May, 1975). Shannon's diversity combines both richness and evenness but is

influenced by the number of species (Pielou, 1969). DeJong (1975) reported that the McIntosh diversity index overcomes the sample size effect of Shannon's and estimates diversity as a percentage of the maximum possible diversity attainable by that sample size. The standardized Shannon diversity index, which ranges from 0 to 1.0 is more useful as it allows for comparison of accessions on the same scale.

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Qualitative data encompasses ordinal measures such as stem texture (smooth, hairy, or prickly), and binary measure (presence or absence). Genetic distance based on qualitative data include frequencies, principal components of qualitative data (Jolliffe, 1986), Dice coefficient (Dice, 1945), Jaccard's coefficient (Jaccard, 1908), simple matching (Sokal and Michener, 1958), to name a few.

Quantitative or interval data include measures such as plant height, seed yield, bast mass, and allele frequencies. Genetic distance measures of interval data may be variances, correlation coefficients, Nei's distance, Euclidean distance, etc. Genetic distance is the quantitative measure of genetic differences (similarity or dissimilarity) between individuals, populations, or species at the allelic level arising from a common ancestor (Beaumont *et al.*, 1998). In contrast, differences in populations arise from phenomenon such as founder effect, bottlenecks, gene flow or mutation (Nei, 1987; Kosman and Leonard, 2005). Populations with small genetic distance have close genetic relationships while those with large genetic distance are more or less diverged (Kosman and Leonard, 2005).

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). Common statistical analysis of distance measures are analysis of variance, neighbor joining, and bootstrapping of cluster analysis (Efron and Tibshirani, 1986).

2.13. Multivariate Techniques for Detection of Relationships

Various multivariate techniques and statistical tools are employed in genetic distance estimates to devise relationships among accessions and traits (Mohammadi and Prasanna, 2003). These statistical techniques offer a better graphical visualization of genetic distances among accessions regardless of the population size. The very common multivariate techniques employed includes cluster analysis, principal component analysis and multidimensional scaling (Melchinger, 1993; Johns *et al.*, 1997; Thompson *et al.*, 1998; Brown-Guedira *et al.*, 2000).

2.13.1. Cluster Analysis

Cluster analysis, developed by (Hair *et al.*, 1995) provides a graphical presentation of the distance matrix generated for pairwise comparisons of the genotypes. Genotypes that are similar are grouped together whereas dissimilar genotypes that have diverged from a common ancestor are widely spaced on a dendrogram. The Unweighted Pair Group with Arithmetic Means, (UPGMA) (Sokal and Michener, 1958) based on unweighted average distances between accessions pairs, and the Ward's minimum variance method (Ward, 1963) based on analysis of variance (ANOVA) are the two most popular clustering algorithms used in cluster analysis (Mohammadi and Prasanna, 2003).

A model-based Bayesian method, which relies on maximum likelihood estimation, is also commonly used (Pritchard *et al.*, 2000). Both UPGMA and Ward's minimum variance are agglomerative in that, they successively group individuals and merge them based on their similarities. In addition, both algorithms rely on Lance and William's recurrence formula to operate directly on any distance matrix or measurement (Lance and Williams, 1967).

Ward's minimum variance method of clustering was used to group 28 roselle *sabdariffa* landraces into two major clusters (Medagam *et al.*, 2015); and 51 roselle *sabdariffa* genotypes were grouped into eight major clusters (Pulli Bai, 2005). The UPGMA method of clustering has also been used in grouping roselle genotypes. Coffie (2016) used UPGMA to group 35 roselle *sabdariffa* ecotypes that included three *altissima* genotypes from West Africa into three major clusters. Other notable works that used UPGMA analysis include El-Tahir and El-Gabri (2013) who grouped 126 roselle *sabdariffa* genotypes into two main clusters, Alarcón Cruz *et al.* (2013) performed clustering of 47 Mexican roselle *sabdariffa* accessions into two major groups.

2.13.2. Principal Components Analysis (PCA)

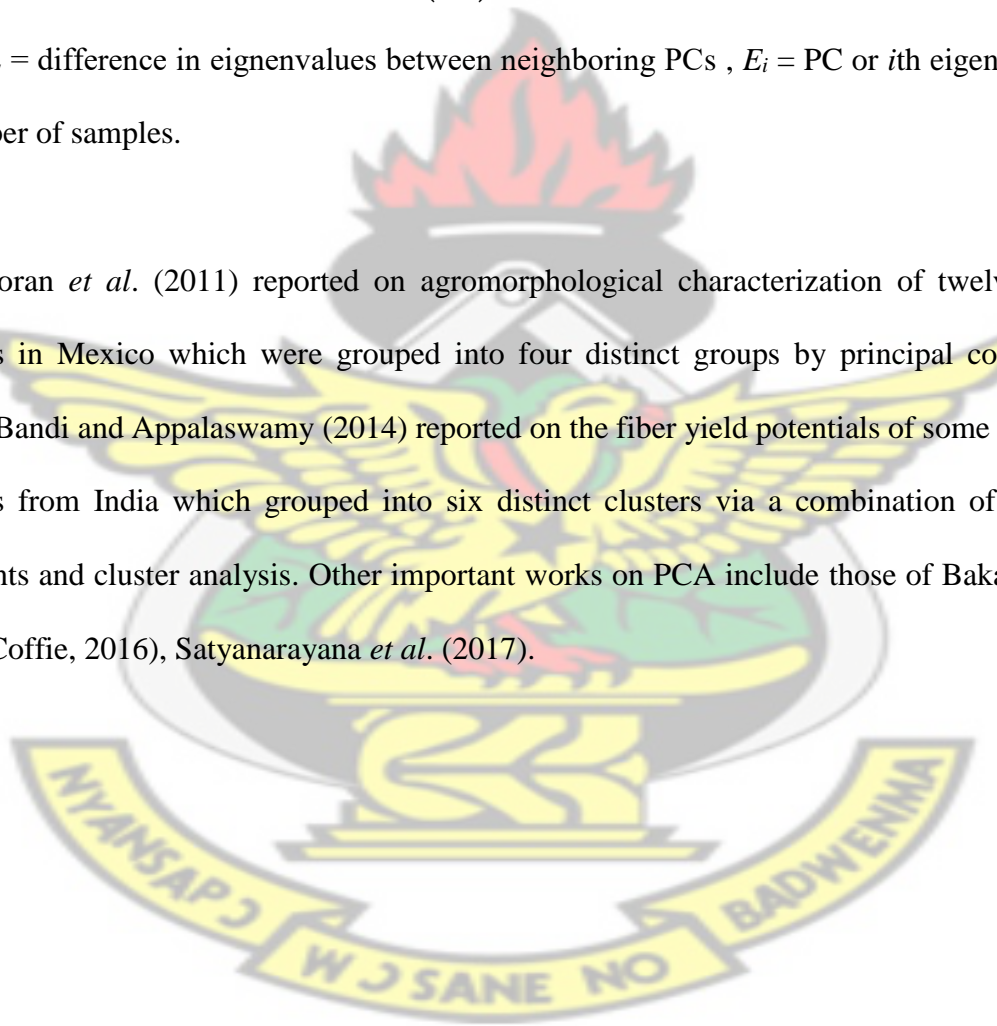
The concept of principal components analysis, PCA formulated by Pearson (1901) was later explicated by Hotelling (1933). The PCA computes properties of data matrices such as eigenvalues and eigenvectors, which reveal components that contribute substantial variance in the data, the rest being insignificant. Components that are grouped are correlated and those widely apart are uncorrelated. Essentially, PCA reduces dimensionality of multivariate data. The number of PCs is equivalent to the number of factors considered in the research, however, all PCs may or may not contribute substantially to the variance, as such a scree plot provides a guide.

North *et al.* (1982) provided information on the significance of a PC based on a sampling error associated with eigenvalues. Distance between two adjacent eigenvalues, if greater than the sampling error, interprets as a significance PC at 5 % error rate. Sampling error of principal components is calculated as:

$$\text{Sampling error} = \Delta E > E_i \left(\sqrt{\frac{2}{N}} \right) \dots \dots \dots (1)$$

where ΔE = difference in eigenvalues between neighboring PCs , E_i = PC or *i*th eigenvalue and N = number of samples.

Torres-Moran *et al.* (2011) reported on agromorphological characterization of twelve roselle genotypes in Mexico which were grouped into four distinct groups by principal components analysis. Bandi and Appalaswamy (2014) reported on the fiber yield potentials of some 60 roselle genotypes from India which grouped into six distinct clusters via a combination of principal components and cluster analysis. Other important works on PCA include those of Bakasso *et al.* (2013), (Coffie, 2016), Satyanarayana *et al.* (2017).



CHAPTER THREE

Characterization of roselle (*Hibiscus sabdariffa* L. var. *altissima* Wester) accessions by agro-morphological traits in Northern Ghana

3.0. Abstract

Roselle (*Hibiscus sabdariffa* L. var. *altissima* Wester) is a bast fiber crop of global economic importance. Although West Africa is considered the centre of diversity, roselle research and utilization is widely ignored. The awakening of industrialization in Ghana presents roselle as a candidate crop for exploration, however, information on genotypes of economic importance is lacking. Our objective was to map roselle population hotspots in northern Ghana and examine genetic variability therein. Thirty-six roselle accessions collected from five regions in Ghana were planted in field trials using a 6×6 lattice square design in three replications and evaluated for seven qualitative and four quantitative morphological traits covering plant type, leaf and stem characteristics, and growth habit. Data were analysed by Shannon-Weiner Diversity Index (SDI) and analysis of variance. A large variability was identified between the accessions. The mean SDI values in the 18 districts ranged from 0.53 to 0.73 with Savelugu-Nanton district having the largest diversity and having accessions with the highest mean plant height of 308.27 ± 48.91 cm, highest branching point at 107.19 ± 64.66 cm, and few branches not exceeding 5.0 in number. Majority of the accessions exhibited low branching points. The most variable trait was branch number with SDI of 0.83 ± 0.12 . Accessions HA-07, HA-11, HA-12, HA-21, and HA-33 ranked highest with respect to plant height with few branches at high branching points, and large basal diameter. The ample diversity in roselle and identification of genotypes of economic importance await their exploitation for genetic improvement, particularly for fiber yield.

Keywords: roselle, variability, genetic diversity, morphology

3.1. Introduction

Roselle (*Hibiscus sabdariffa* L. var. *altissima* Wester), the inedible calyx type of *H. sabdariffa* species is of the Malvaceae family which includes other fiber crops such as cotton (*Gossypium hirsutum* L.), jute (*Corchorus capsularis* L. and *Corchorus olitorius* L.), and kenaf (*Hibiscus cannabinus* L.). Roselle is believed to be native to Africa (Boulanger *et al.*, 1984; Gomez-Leyva *et al.*, 2008). A recent study of roselle collection from West Africa, specifically, Senegal to Nigeria, revealed that the region between northern Ghana to Mali through Burkina Faso is a center of rich diversity of roselle (Coffie, 2016). In this region, roselle grows in the wild and sprouts after the first rains. Here, roselle is known severally owing to the array of morphotypes available. While roselle is generally called *sobooro* or *suure*, indigenous information lists over 25 names for roselle among the tribal folks indicating a historical evidence of its domestication in northern Ghana. Some common names are “*biito*” among the Nankana and Frafra, “*vio*” in Grushi and Kasem, and “*tingyanbam*” in Konkomba. Together with its closely related variety, *H. sabdariffa* var. *sabdariffa*, which is grown principally for its edible calyx, both crops are under homestead cultivation by the traditional small-scale farmers in northern Ghana where their leaves, fibers and seeds are used for nutritional and medicinal purposes (Perry, 1980; Morton, 1987; Rhodin & Panchoo, 1990) and for tethering livestock.

Records indicate that roselle was accidentally carried from Gold Coast to the Philippines in 1911 where it was cultivated and studied (Wester, 1912, 1914; Andrews, 1952). Roselle variety specimens were also carried from Northern territories of Gold Coast (Ghana) to Kew Gardens for identification in 1912 (Imperial Institute, Great Britain, 1912).

The current stringent environmental regulations and restrictions on synthetic fibers have led to increase in demand for roselle as source of bast fiber for upholstery and sac making, for paper pulp, as a salt-resistant cordage, and as composite for electronic and building materials. In Ghana, roselle use is limited to culinary aspects for its leaves and seeds.

Current interest in roselle fiber for commercial cultivation in Ghana necessitates collection, evaluation, genetic improvement and conservation, however, scientific information on characterization of roselle types in Ghana is limited. An agro-morphological characterization and molecular profiling by Random Amplified Polymorphic DNA (RAPD) markers of roselle collection in Ghana contained only three *altissima* genotypes, the rest being *sabdariffa* types (Coffie, 2016). This study revealed that contrary to reports of lack of variability in exotic genotypes of roselle to merit success with genetic improvement (Hanboonsong *et al.*, 2000; Yusof and Saud, 2009; Omalsaad *et al.*, 2014), a large variability existed in the West African *sabdariffa* genotypes (Coffie, 2016).

Efficient utilization of a crop is largely dependent on the nature and magnitude of its diversity for genetic improvement in yield, disease resistance, and quality characteristics (Dudley and Moll, 1969; Chand *et al.*, 2008). Agro-morphological characterization is a common system for estimation of genetic diversity as it uncovers phenotypic differences, relationships among genotypes, and evolutionary history of the crop. Genetic diversity estimation by phenotypic characterization encompasses estimation of both within and between population variations. Within-population diversity estimates the number of unique classes within a population as richness, and the relative abundance of the genotypes within the classes as evenness. A population

with large number of diverse genotypes that are equally distributed will have high richness diversity index, whereas that with few diverse genotypic groups will have low diversity index (Brower and Zar, 1977). Four most common estimates that measure diversity as a composite of the two assessments include Berger–Parker index (Berger and Parker, 1970), McIntosh (1967), Simpson (1949), and Shannon-Weiner diversity index (SDI) (Shannon and Weaver, 1949). Simpson’s index, which predominantly estimates evenness, relies on the few most abundant species to estimate diversity without considering the rare species, (Risser and Rice, 1971; Loya, 1972; Whittaker, 1972). Berger-Parker Index has been used extensively to establish the more abundant species within a community and estimates species richness (May, 1975). Shannon’s diversity combines both richness and evenness but is influenced by the number of species (Pielou, 1969). DeJong (1975) reported that the McIntosh diversity index overcomes the sample size effect of Shannon’s and estimates diversity as a percentage of the maximum possible diversity attainable by that sample size. The standardized Shannon diversity index which ranges from 0 to 1.0 is more useful as it allows for comparison of accessions on the same scale. Medagam *et al.* (2015) reported SDI of Indian vegetable roselle (*H. sabdariffa* var. *sabdariffa*) for plant characteristics, leaf yield, and total biomass as 0.358 to 2.00. To the best of our knowledge, the diversity and genetic variability within the roselle *altissima* in Ghana has not been assessed. Similarly, the distribution of variation in roselle populations in the northern sector of Ghana where roselle originates is not known. We report the genetic variation in roselle *altissima* in a northern Ghana collection assessed by morphological traits.

3.2. Materials and Methods

3.2.1. Plant Material

Seeds of thirty-six accessions of roselle, designated HA-01 to HA-36 were collected from mature naturally growing wild plants on farmers' field in 18 districts covering five regions of Ghana at longitude 0.24°W in Bawku to 2.51°W in Wa and latitude 6.66°N in Kumasi to 11.05°N in Bawku. The elevations covered 132 to 408 m.a.s.l. (Figure 3.1). Seed collection was guided by farmers' indigenous knowledge. Seeds were planted from July to December 2016 on the research fields of the Department of Horticulture, Kwame Nkrumah University of Science and Technology, Kumasi, located at longitude 1.41°W to 1.60°W and latitude 6.71°N to 6.75°N. The experimental design was a 6 × 6 lattice square with three replications. Plot size was 0.5 m × 2.0 m with plant spacing of 25 cm × 50 cm separated by an alley of 0.5 m to give a plant density of 150,000 plants/ha. Irrigation was carried out as and when needed. Pre-emergence weeds were controlled with Round-Up Ready (glyphosate, 360 g/L) at a rate of 3.5 L/ha. Post-emergence weeds were cleared from the field by hand weeding using a hoe. The predominant insect pests, cotton stainer (*Dysdercus* sp.), cabbage fly (*Delia radium*), and thrips (*Thysanoptera*) were controlled with Sumitex (dimethoate, 400 g/L) applied at a rate of 1 L/ha.

3.2.2. Data Collection

At 120 days after planting, data were collected on 7-10 competitive plants per plot. Six qualitative traits on leaf, stem, and growth habit, and four quantitative traits, namely, plant height, height at first branching, branch number, and basal diameter were recorded. Leaf colour was evaluated as the predominant colour of the leaf blade (uniformly green=1, pigmented=2); and leaf size was recorded as appearance of width of leaf (slender=1, broad=3). Roselle leaves differentiate

progressively with maturation such that more than one leaf form can occur on one plant, hence leaf form was rated as the predominant shape of leaf in the mid-portion of the plant (entire=1, tri-lobed=3, penta-lobed=5). Plant type was scored as the predominant colour of the stem (uniformly green=1, pigmented=3, uniformly red=5); branching habit was recorded as the form of branching (few=1, intermediate=2, extensive=3); stem pubescence was determined as evenness of surface of stem (smooth=1, others=3), and branching height was measured as position of the branches with respect to the height of the plant (low and close to base=1, branching around the middle or intermediate=3, branching around the apex or high branching=5). For the quantitative traits, plant height was measured as height from ground level to the growing tip in cm, height at first branching was measured from ground level to first branch in cm using a meter rule. The basal diameter was measured at 5 cm above the ground using a micrometer screw gauge. The number of branches on a plant was scored as branch number.

3.2.3. Data Analysis

Frequencies were calculated for the qualitative data. For the quantitative data, means, standard deviations, minimum and maximum values, and coefficient of variation were computed, Analyses of variance were computed to estimate variation between population. The within population variation was assessed by the standardized Shannon-Wiener Diversity Index,

$$H' = \frac{-\sum (P_i \cdot \log P_i)}{\log n} \quad (1)$$

where, P_i is the fraction of the entire species population, n is the number of classes with observations, and $\log n =$ logarithm of n . For each trait, the population was subdivided into classes on the basis of the mean (Y) and standard deviation (σ) into six classes in the range of $(Y-2\sigma) > Y > (Y+2\sigma)$ where Y is response for each accession trait. The mean diversity index of

accessions in each district was calculated. All computations were carried out in SAS 9.3 software (SAS Institute, 2011).

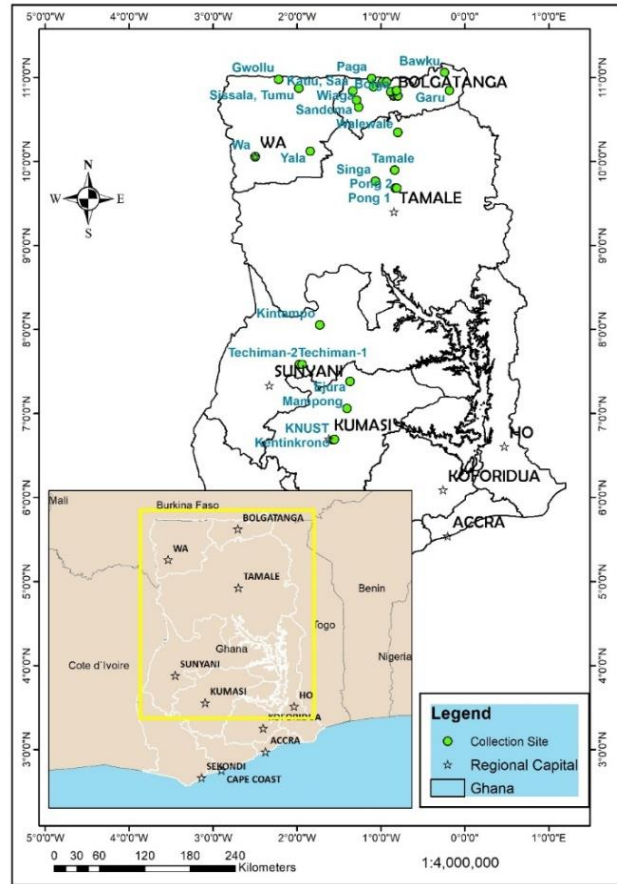


Figure 3.1. A schematic map of Ghana showing roselle collection sites from Kumasi (Ashanti) to Bolgatanga (Upper East) and Wa (Upper West).

3.3. Results

3.3.1. Variation in Plant Characteristics

The within and between population variation in 36 accessions of roselle *altissima* collected from 18 districts in Ghana were investigated by morphological trait assessment in a field study in Kumasi, Ghana. Bolgatanga district was represented by seven accessions (19.44 %), Kassena-

Nankana by five accessions (13.89 %), Kumasi and Savelugu-Nanton districts had three (8.33 %) accessions each, Ejura-Sekyedumase, Sissala, Tamale, and Techiman districts were represented by two (5.56 %) accessions each, while Bawku, Ejisu-Juaben, Garu-Tempene, Gowri-Yorogo, Kintampo, Mampong Sagnarigu-Garu, Talensi, Wa and West Mamprusi had one (2.78 %) accession each.

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In all, 703 plants were evaluated. Accessions exhibited ample variability in, leaf shape, plant type, and branching habit as they had three distinct categories of plant types. In contrast, leaf colour, leaf size, and stem pubescence were uniform and no variations (Table 3.1). The distribution of plant characteristics were 423 (63 %) uniformly green, 180 (27 %) pigmented, and 71 (10 %) uniformly red, 396 (59 %) plants with few branches, 238 (36 %) with intermediate branching, and 33 (5 %) plants with extensive branching. Branching height was less variable with 547 (82 %) having low branching points, 71 (11 %) with intermediate branching, and 48 plants (7 %) with high branching points. Stem pubescence was less variable. Majority of the plants (78 %) were hairy, rough, or prickly, and 22 % had smooth stems and leaves. Variation in leaf colour and leaf size was low. A large number, 646 plants (96 %) had green leaves, and 28 (4 %) were pigmented. Regarding leaf size, majority of the plants 639 (95 %) had slender leaves and 35 (5 %) had broad leaves. Ample variability was present in leaf shape with 456 (68%) being tri-lobed, 139 (21 %) were penta-lobed, and 79 plants (12 %) had entire leaves.

Table 3.1. Distribution of qualitative morphological traits in *H. sabdariffa* L. var. *altissima* collected from Northern Ghana and evaluated in 2016

Trait	Description	Score	Distribution	Percentage (%)
Plant type	Uniformly green	1	423	62.67
	Pigmented	3	180	26.71
	Uniformly red	5	71	10.53
Branching habit	Few	1	396	59.37
	Intermediate	2	238	35.68
	Extensive	3	33	4.95
Branching Height	Low	1	547	82.13
	Intermediate	3	71	10.66
	High	5	48	7.21
Stem pubescence	Smooth	1	146	21.66
	Other	3	528	78.34
Leaf colour	Uniformly Green	1	646	95.85
	Pigmented	2	28	4.15
Leaf size	Slender	1	639	94.81
	Broad	3	35	5.19
Leaf shape	Entire	1	79	11.72
	3-lobed	3	456	67.66
	5-lobed	5	139	20.62

3.3.2. Variation in Quantitative Traits

Mean squares of genotypes were highly significant ($P < 0.01$) for three traits, namely, plant height, height at first branching, and branch number. Basal diameter was also significantly different ($P < 0.05$) among the genotypes. Based on the coefficient of variation, height at first branching (16.39 %) and branch number (25.47 %) had more widely dispersed values than plant height (4.48 %) and basal diameter (10.81%) (Table 3.2). Plant height and height at first branching of the accessions were significantly different ($P < 0.05$) from one district to another, whereas branch number and basal diameter were not different (Table 3.3).

3.3.3. Variability in Districts Accessions

Variability in the accessions from different districts was remarkable. Analysis of variance for the district data produced highly significant ($P < 0.001$) mean squares for plant height, height at first branching, branch number and basal diameter (Table 3.3).

Overall mean plant height for the populations was 240.69 ± 23.22 cm and ranged from 164 to 389 cm. On the basis of mean of traits at the districts, plant height varied from 214.86 ± 19.72 cm in Gowri-Yorogo (Upper East) to 308.27 ± 48.91 cm in Savelugu-Nanton (Northern). Majority of the districts exhibited low values of height at first branching with a mean of 25.20 ± 25.97 cm and range of 1.2 to 215 cm. Again, Kintampo Municipal (Brong Ahafo) accessions exhibited the least height at first branching of 10.80 ± 4.56 cm and Savelugu-Nanton (Northern) had the highest value of 107.39 ± 64.66 cm. Overall mean of branch number of 5.64 ± 1.16 varied from 1.0 to 17. Based on district means, values ranged from 3.76 ± 1.73 in Ejisu-Juaben (Ashanti) to 8.27 ± 2.10 in Sagnarigu-Gariba (Upper East). The basal diameter had an overall mean of 18.52 ± 2.05 mm and ranged from 10.12 mm to 31.34 mm. The district means of these traits ranged from a minimum value of 13.51 ± 3.10 mm in the Mampong Municipal (Ashanti) to a maximum of 21.28 ± 4.60 mm in the Kassena-Nankana (Upper East) (Table 3.3). Accessions from four districts consistently had tall plants exceeding 250.0 cm. These sites were Bolgatanga Municipal (251.46 ± 41.72 cm), Kassena-Nankana (266.30 ± 58.94 cm), Savelugu-Nanton (308.27 ± 48.91 cm), and Techiman Municipal (287.26 ± 58.26 cm). Similarly, accessions from these districts showed intermediate branching points of 33.63 ± 47.25 cm, 45.00 ± 54.78 cm, 107.19 ± 64.66 cm and 88.84 ± 75.97 cm respectively. Number of branches per plant ranged from three to ten. Districts having least number of branches of three to four were Ejisu-Juaben (3.76 ± 1.73), Ejura-Sekyedumase ($4.42 \pm$

1.86), Mampong Municipal (4.25 ± 2.43), Savelugu-Nanton (4.69 ± 2.03), Sissala (4.81 ± 3.79), Talensi (4.88 ± 3.33), Techiman Municipal (4.61 ± 2.65), and Wa Municipal (4.42 ± 1.74) accessions. Mean basal diameter at the various districts ranged from 13.51 ± 3.1 mm in Mampong Municipal to 21.28 ± 4.60 mm in Kassena-Nankana accessions. Values exceeding 20 mm were found in accessions from Bolgatanga Municipal 20.61 ± 2.37 mm, Kassena-Nankana 21.28 ± 4.60 mm, and Savelugu-Nanton 20.06 ± 2.30 mm (Table 3.3).

Table 3.2. Mean squares of quantitative traits in roselle evaluated in field trial in Ghana in 2016

Source of Variation	df	Mean squares			
		Plant height	Height at first branching	Branch number	Basal diameter
Block(Replication)	15	141.09	21.84	1.76	3.72
Genotype	35	3778.02**	5021.88**	6.19**	8.18*
Error	48	124.80	28.36	2.21	4.12
CV (%)		4.48	16.39	25.47	10.81

* $P < 0.05$; ** $P < 0.01$

The most desirable trait in roselle is fiber yield, which is influenced by large values of plant height and basal diameter, high branching points, and low values of branch number (Maiti and Chakravarty, 1977). (Table 3.4) shows the means of the 36 accessions and their rank in terms of potential fiber yield where HA-11, HA-07, HA-21, HA-33, and HA-12 came up as the top five accessions. Accession HA-11 exhibited the highest plant height (355.88 ± 25.66 cm) a large value of height at first branching (130.42 ± 29.61 cm), fewest branch number (2.63 ± 1.10) and the largest basal diameter (23.32 ± 4.11 cm). Accession HA-07 ranked second with plant height 347.17 ± 14.94 cm, high branching point at 143.52 ± 27.18 cm above ground, few branches 3.78 ± 1.59 and large basal diameter of 20.52 ± 2.20 cm. Accession HA-21 was tall 339.92 ± 21.84 cm, highest

branching point at 157.08 ± 32.97 cm above ground, few branch number of 4.21 ± 1.53 , and a large basal diameter of 20.01 ± 2.98 cm. Accessions HA-33, and HA-12 with plant height, height at first branching, branch number, and basal diameter of 334.78 ± 29.40 cm, 137.13 ± 25.53 cm, 4.48 ± 1.62 , 19.62 ± 1.56 cm and 324.85 ± 29.93 cm, 144.78 ± 35.11 cm, 5.22 ± 1.97 and 21.05 ± 1.83 cm, respectively, ranked fourth and fifth in fiber yield potential. The remaining accessions exhibited relatively low mean values of plant height (214 to 249 cm), high mean branch number (5 to 8) at low mean branching points (8.59 to 20.90 cm), and smaller mean basal diameters of less than 20 cm.

3.3.4. Shannon-Wiener Diversity Index in *H. sabdariffa* var. *altissima* Accessions

Shannon-Weiner Diversity Index (SDI) assessed variation within the roselle populations on the quantitative traits. Mean SDI of the 36 populations was 0.63 ± 0.34 with a range of 0.00 to 1.00. The SDI values were very different among the traits. Largest variation resided in branch number 0.83 ± 0.12 , basal diameter 0.80 ± 0.11 , and plant height 0.75 ± 0.16 , whereas height at first branching was low at 0.13 ± 0.31 . Mean SDI values of individual accessions were high and ranged from 0.42 to 0.86. All accessions had mean SDI values above 0.50 except HA-10, which had a value of 0.42 (Table 3.5). Majority of the SDI values occurred within the ranges of 0.50 - 0.59 (13) and 0.60-0.69 (17) (Figure 3.2). Accessions with high SDI values were HA-07 (0.79), HA-11(0.79), HA-12 (0.86), HA-21 (0.76), and HA-33 (0.79).

Table 3.3. Mean, standard deviation, minimum, and maximum traits evaluated in 36 *H. sabdariffa* L. var. *altissima* accessions originating from 18 districts in Ghana in 2016

No.	District	Plant height (cm)	Height at first branching (cm)	Branch number	Basal diameter (mm)
1	Bawku Municipal	220.63±31.98 (188-285)	14.28±7.36 (4.3-29.4)	5.5±3.12 (1-9)	15.17±4.21 (10.13-21.2)
2	Bolgatanga Municipal	251.46±41.72 (191-380)	33.63±47.25 (1.2-200)	6.03±3.23 (1-17)	20.61±2.37 (17-24.18)
3	Ejisu-Juaben	231.67±12.72 (206-248)	10.82±6.88 (3.7-34.4)	3.76±1.73 (1-8)	19.44±3.97 (15.16-26.24)
4	Ejura-Sekyedumase	229.54±23.96 (199-289)	13.73±6.64 (2.5-32.6)	4.42±1.86 (2-9)	18.26±3.26 (12.38-24.3)
5	Garu-Tempne	242.40±23.25 (207-291)	11.10±4.56 (2.6-20)	6.65±2.96 (1-13)	18.73±3.77 (10.12-25.24)
6	Gowri-Yorogo	214.86±19.72 (187-255)	11.41±3.99 (2.4-17.1)	6.57±1.83 (5-10)	19.42±1.83 (16.14-22.31)
7	Kassena Nankana	266.30±58.94 (164-389)	45.00±54.78 (1.5-200)	6.46±3.43 (1-14)	21.28±4.60 (11.27-30.15)
8	Kintampo Municipal	228.83±22.26 (193-263)	10.8±4.56 (3.7-19.5)	6.33±2.46 (3-11)	21.57±2.79 (15-26.3)
9	Kumasi	234.36±14.02 (203-268)	12.84±6.38 (1.7-26.4)	6.1±3.23 (1-15)	18.22±2.9 (12.42-26.05)
10	Mampong Municipal	219.50±11.60 (202-234)	17.39±6.45 (9.9-28.2)	4.25±2.43 (1-8)	13.51±3.10 (10.47-19.47)
11	Sagnarigu-Gariba	248.64±16.97 (212-280)	15.06±5.93 (6.3-23)	8.27±2.10 (5-11)	18.22±2.77 (12.13-25.44)
12	Savelugu-Nanton	308.27±48.91 (229-387)	107.19±64.66 (5.9-215)	4.69±2.03 (1-10)	20.06±2.30 (14.14-24.4)
13	Sissala	221.37±15.06 (175-250)	14.30±6.18 (2.9-32)	4.81±3.79 (1-20)	17.41±3.92 (11.13-31.34)
14	Talensi	225.46±16.05 (202-258)	13.58±5.69 (1.9-27)	4.88±3.33 (1-14)	16.47±2.94 (11.26-22.32)
15	Tamale Metropolitan	233.11±16.90 (217-290)	14.92±6.68 (5.9-27.2)	5.44±1.79 (3-8)	17.53±3.12 (11.43-22.31)
16	Techiman Municipal	287.26±58.26 (198-378)	88.84±75.97 (3.6-211)	4.61±2.65 (1-14)	19.38±2.62 (15.3-26.07)
17	Wa Municipal	241.95±12.01 (222-269)	15.24±4.5 (9.3-23.4)	4.42±1.74 (2-8)	17.56±1.89 (15.16-21.47)
18	West Mamprusi	240.12±18.50 (212-286)	13.54±4.78 (8.1-25)	5.80±1.80 (2-10)	17.91±2.23 (13.26-21.34)
	Mean square	2,344.49*	3,096.44*	4.41	7.03

* P<0.05

Collectively, the accessions in the districts exhibited intermediate to high diversities. Savelugu-Nanton recorded the highest SDI value 0.73 ± 0.2 arising from the large diversity in plant height (0.75 to 0.93), branch number (0.84 to 0.94), and basal diameter (0.61 to 0.83) only. Diversity in height at first branching was minimal except in HA-07 and HA-12, which gave substantial values 0.77 and 0.93, respectively. Other districts with high SDI values between 0.60 and 0.70 were Bolgatanga Municipal (0.63 ± 0.34), Ejisu-Juaben (0.62 ± 0.42), Ejura-Sekyedumase (0.64 ± 0.42), Garu-Tempne (0.63 ± 0.42), Gowri-Yorogo (0.67 ± 0.45), Kassena-Nankana (0.63 ± 0.27), Kintampo Municipal (0.62 ± 0.43), Mampong Municipal (0.62 ± 0.43), Talensi (0.67 ± 0.45), Tamale Metropolitan (0.68 ± 0.46), and Wa Municipal (0.66 ± 0.45).

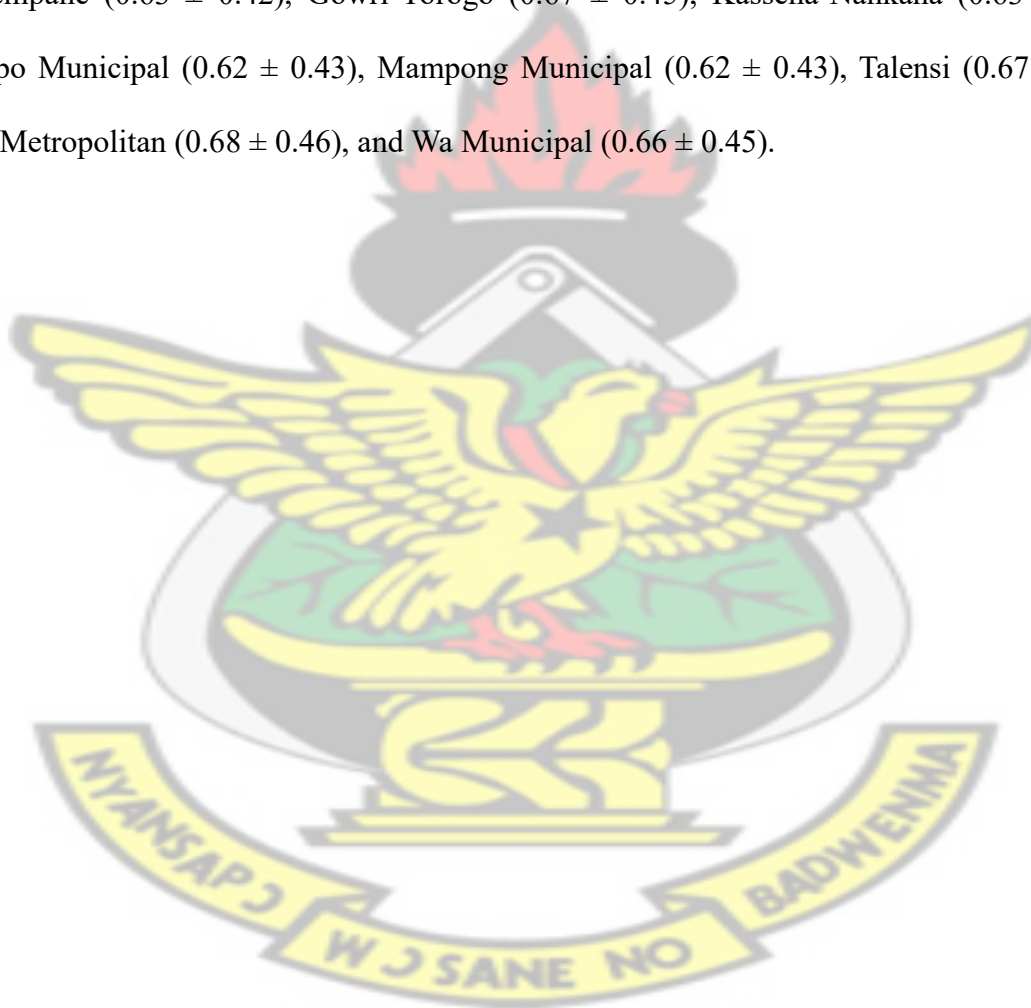


Table 3.4. Mean, standard deviation, minimum, maximum values of traits of 36 *H. sabdariffa* L. var. *altissima* accessions evaluated in Ghana in 2016 and their rank based on potential fiber yield

ACC	Plant height (cm)	Height at first branch (cm)	Branch number	Basal diameter (mm)	Rank
HA-01	241.95±12.01 (222-269)	15.24±4.50 (9.3-23.4)	4.42±1.74 (2-8)	17.56±1.89 (15.16-21.47)	12
HA-02	228.83±22.26 (193-263)	10.80±4.56 (3.7-19.5)	6.33±2.46 (3-11)	21.57±2.79 (15-26.3)	24
HA-03	248.64±16.97 (212-280)	15.06±5.93 (6.3-23)	8.27±2.10 (5-11)	20.61±2.37 (17-24.18)	8
HA-04	228.29±9.43 (203-243)	13.27±5.09 (3.5-22.4)	4.57±1.72 (2-8)	18.75±1.87 (15.29-22.41)	25
HA-05	248.05±20.73 (210-279)	20.90±21.38 (6-11)	7.00±3.01 (2-14)	17.56±2.39 (11.30-22.03)	9
HA-06	220.63±31.98 (188-285)	14.28±7.36 (4.3-29.4)	5.50±3.12 (1-9)	15.17±4.21 (10.13-21.2)	31
HA-07	347.17±14.94 (331-382)	143.52±27.18 (104-205)	3.78±1.59 (2-7)	20.52±2.20 (14.14-24.23)	2
HA-08	230.89±13.53 (213-259)	18.32±5.21 (8.7-27.3)	5.94±2.21 (3-10)	18.78±2.53 (14.08-22.31)	21
HA-09	238.80±20.16 (217-290)	11.37±6.61 (5.9-27.2)	5.40±1.71 (3-8)	16.13±2.92 (11.43-20.26)	19
HA-10	229.82±11.16 (198-248)	14.39±6.96 (3.6-25.3)	5.05±3.48 (1-14)	18.70±2.01 (15.3-24.12)	23
HA-11	355.88±25.66 (248-389)	130.42±29.61 (93-200)	2.63±1.10 (1-5)	23.32±4.11 (17.09-30.15)	1
HA-12	324.85±29.93 (264-387)	144.78±35.11 (93-215)	5.22±1.97 (2-10)	21.05±1.83 (18.35-24.4)	5
HA-13	239.05±14.04 (213-260)	16.52±5.70 (6.10-25.4)	6.48±3.92 (2-13)	18.70±3.50 (14.03-25.44)	18
HA-14	224.17±14.47 (193-252)	14.02±13.95 (5.4-77)	6.50±4.05 (1-17)	17.50±2.55 (12.13-22.7)	28
HA-15	249.19±12.87 (212-271)	15.10±7.96 (4.6-40)	4.38±2.46 (1-10)	18.78±2.86 (13.28-23.41)	7
HA-16	243.84±16.60 (210-267)	14.11±7.10 (4.8-30.2)	7.00±2.81 (3-12)	18.20±2.77 (12.26-23.11)	10
HA-17	206.89±8.65 (199-223)	11.62±5.95 (2.5-22.7)	6.00±2.00 (3-9)	19.62±1.49 (17.46-22.31)	35
HA-18	239.83±14.86 (218-268)	14.10±5.94 (3.7-26.4)	6.35±3.46 (2-15)	17.09±3.03 (12.42-26.05)	17
HA-19	231.67±12.72 (206-248)	10.82±6.88 (3.7-34.4)	3.76±1.73 (1-8)	19.44±3.97 (15.16-26.24)	20

Table 3.4 cont'd

HA-20	241.53±20.45 (204-289)	14.85±6.88 (5.7-32.6)	3.59±1.12 (2-6)	17.53±3.72 (12.38-24.3)	13
HA-21	339.92±21.84 (309-378)	157.08±32.97 (104-211)	4.21±1.53 (1-8)	20.01±2.98 (16.17-26.07)	3
HA-22	240.12±18.50 (212-286)	13.54±4.78 (8.10-25)	5.80±1.80 (2-10)	17.91±2.23 (13.26-21.34)	16
HA-23	225.46±16.05 (202-258)	13.58±5.69 (1.90-27)	4.88±3.33 (1-14)	16.47±2.94 (11.26-22.32)	27
HA-24	206.50±16.87 (164-236)	8.67±5.19 (1.7-17.3)	8.89±2.19 (6-13)	17.19±3.75 (11.27-24.25)	36
HA-25	240.67±17.91 (198-265)	12.82±7.37 (1.2-28.2)	5.67±3.62 (2-13)	16.47±2.18 (13.43-20.22)	15
HA-26	230.82±14.20 (214-250)	12.75±1.56 (10.7-15.4)	7.73±1.56 (5-11)	16.83±1.09 (15.22-19.14)	22
HA-27	249.76±12.04 (228-277)	13.04±5.11 (1.5-22.8)	7.76±3.83 (1-13)	19.15±3.66 (11.33-25.4)	6
HA-28	219.50±11.60 (202-234)	17.39±6.45 (9.9-28.2)	4.25±2.43 (1-8)	13.51±3.10 (9.47-19.47)	32
HA-29	217.24±16.81 (175-250)	12.53±4.98 (6-19.7)	7.06±5.03 (1-20)	20.21±4.62 (11.13-31.34)	33
HA-30	224.08±13.45 (204-248)	15.35±6.67 (2.9-32)	3.35±1.52 (1-7)	15.58±1.82 (12.05-20.48)	29
HA-31	242.40±23.25 (207-291)	11.10±4.56 (2.6-20)	6.65±2.96 (1-13)	18.73±3.77 (10.12-25.24)	11
HA-32	222.05±18.65 (191-280)	9.98±5.18 (3-18.1)	6.09±2.96 (1-11)	17.80±2.84 (12.28-24.23)	30
HA-33	334.78±29.40 (248-380)	137.13±25.53 (103-200)	4.48±1.62 (2-8)	19.62±1.56 (16.38-23.18)	4
HA-34	214.86±19.72 (187-255)	11.41±3.99 (2.4-17.1)	6.57±1.83 (5-10)	19.42±1.83 (16.14-22.31)	34
HA-35	241.15±15.38 (229-280)	14.66±4.82 (5.9-23.8)	5.00±2.27 (1-10)	18.20±1.95 (15.31-22.24)	14
HA-36	226.50±7.89 (210-239)	8.59±5.67 (1.7-23.4)	5.75±2.93 (1-11)	19.11±2.31 (13.31-23.41)	26

Table 3.5. Shannon Weiner Diversity Index of morphological evaluation of 36 *H. sabdariffa* L. var. *altissima* accessions from 18 districts conducted in Ghana in 2016

Acc	District	Plant height (cm)	Height at first branching (cm)	Branch number	Basal diameter (mm)	Accession mean	District mean	Rank
HA-06	Bawku Municipal	0.63	0.00	0.89	0.63	0.53±0.38	0.53±0.38	12
HA-13	Bolgatanga Municipal	0.96	0.00	0.97	0.78	0.68±0.46	0.63±0.34	6
HA-14		0.54	0.00	0.75	0.83	0.53±0.37		
HA-15		0.65	0.00	0.99	0.83	0.62±0.43		
HA-16		0.96	0.00	0.83	0.59	0.59±0.43		
HA-25		0.76	0.00	0.91	0.99	0.66±0.45		
HA-32		0.42	0.00	0.94	0.69	0.51±0.40		
HA-33		0.54	0.94	0.85	0.85	0.79±0.17		
HA-19	Ejisu-Juaben	0.92	0.00	0.85	0.71	0.62±0.42	0.62±0.42	7
HA-17	Ejura-Sekyedumase	0.81	0.00	0.89	0.81	0.63±0.42	0.64±0.42	5
HA-20		0.82	0.00	0.86	0.87	0.64±0.43		
HA-31	Garu-Tempane	0.85	0.00	0.83	0.83	0.63±0.42	0.63±0.42	6
HA-34	Gowri-Yorogo	0.84	0.00	0.99	0.85	0.67±0.45	0.67±0.45	3
HA-05	Kassena-Nankana	0.75	0.27	0.62	0.62	0.56±0.21	0.63±0.27	6
HA-11		0.61	0.86	0.74	0.93	0.79±0.14		
HA-24		0.81	0.00	0.78	0.64	0.56±0.38		
HA-26		0.95	0.00	0.72	0.99	0.66±0.46		
HA-27		0.67	0.00	0.85	0.85	0.59±0.40		
HA-02	Kintampo Municipal	0.92	0.00	0.66	0.89	0.62±0.43	0.62±0.43	7
HA-04	Kumasi	0.59	0.00	0.94	0.72	0.56±0.40	0.56±0.37	11
HA-18		0.99	0.00	0.55	0.78	0.58±0.43		
HA-36		0.67	0.00	0.65	0.83	0.54±0.37		
HA-28	Mampong Municipal	0.67	0.00	0.91	0.91	0.62±0.43	0.62±0.43	7
HA-03	Sagnarigu-Gariba	0.49	0.00	0.91	0.83	0.56±0.41	0.56±0.41	11
HA-07	Savelugu-Nanton	0.93	0.77	0.84	0.61	0.79±0.14	0.73±0.21	1
HA-08		0.85	0.00	0.93	0.79	0.64±0.43		
HA-12		0.84	0.93	0.87	0.82	0.86±0.05		
HA-35		0.75	0.00	0.94	0.83	0.63±0.43		
HA-29	Sissala	0.55	0.00	0.84	0.85	0.56±0.40	0.58±0.42	9
HA-30		0.50	0.00	0.99	0.91	0.60±0.45		
HA-23	Talensi	0.92	0.00	0.94	0.83	0.67±0.45	0.67±0.45	3
HA-09	Tamale Metropolitan	0.82	0.00	0.94	0.96	0.68±0.46	0.68±0.46	2
HA-10	Techiman Municipal	0.59	0.00	0.48	0.59	0.42±0.28	0.59±0.09	8
HA-21		0.54	0.93	0.78	0.78	0.76±0.16		
HA-01	Wa Municipal	1.00	0.00	0.76	0.87	0.66±0.45	0.66±0.45	4
HA-22	West Mamprusi	0.79	0.00	0.78	0.69	0.57±0.38	0.57±0.38	10
Mean		0.75±0.16	0.13±0.31	0.83±0.12	0.80±0.11			

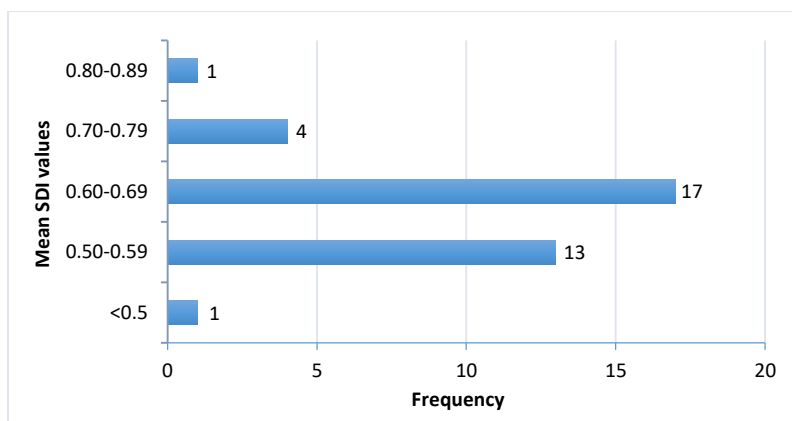


Figure 3.2. Frequency of Shannon Weiner Diversity Index among 36 roselle fiber accessions evaluated in Ghana in 2016

3.4. Discussion

Roselle *altissima* is an important commodity that is marketed around the globe for its multipurpose bast fiber. A recent interest in roselle fiber in Ghana for economic exploitation necessitates its evaluation to identify important economic traits, useful accessions of commercial value, and to estimate the genetic diversity therein for genetic improvement and conservation. The unequal distribution of the accessions in the study area indicates that roselle *altissima* is not very well known among the localities compared to the more common vegetable type, except for the indigenous folks who make tethers from roselle for their livestock. Rapid rate of urbanization in the northern sector of Ghana may have contributed to lack of knowledge of this germplasm. Additionally, seed morphology for the fiber and vegetable roselle are similar, and information on morphological differences in the seeds is not available, so that roselle *altissima* seed collection is fraught with challenges. An informed and well-planned seed collection by a team of taxonomists and the indigenous people should provide a more accurate roselle *altissima* germplasm collection and this is urgently needed. The large variability in plant type, branching habit, and leaf shape were also reported by Alam *et al.* (2006) among four Bangladesh *Hibiscus* species, the *H. sabdariffa*,

H. cannabinus, *H. acetosella* and *H. radiatus*. Plants having uniformly green stems, few branching and high branching points are more desirable for fiber production than pigmented, extensively branched and low branching types. Youngberg (1929) confirmed a 5% yield advantage in green over pigmented roselle. The indigenous folks testify that they extract fiber from green roselle but not from the pigmented genotypes for claim of good fiber yield and quality. This claim needs validation through a scientific research programme. The less variable height-at-first-branching, stem pubescence, leaf colour, leaf size, and stem pubescence appeared to be traits that are conserved in the bastfiber plants of *Hibiscus* species, including *H. sabdariffa* variety *sabdariffa* (Coffie, 2016; Siepe *et al.*, 1997), *H. cannabinus* (Faruq *et al.*, 2013), and *H. vulgaris*, *H. rosens*, *H. mutabilis* (Siepe *et al.*, 1997).

Large variability in plant height, branch number, and basal diameter were also identified in *H. sabdariffa* var. *sabdariffa* from West Africa (Coffie, 2016), Asia and Europe genotypes (Siepe *et al.*, 1997), *H. cannabinus* accessions from Kenya, China, U.S.A. and Australia (Faruq *et al.*, 2013). This large variability means that genetic improvement in these traits via selection is possible. The differences in the quantitative traits may be attributed to varying genotypic differences. A plant height of 164 to 389 cm falls within the range of plant height of *H. sabdariffa* var. *altissima*, which can grow from 250 to 500 cm and is usually branchless (Eltayeib and Elaziz, 2014). Plant height and branching in roselle is governed by day length, temperature, and its effect on flowering (Dempsey, 1975; Tindall, 1983). Roselle is ambiphotoperiodic whereby it flowers in both short days and in extremely long days (Mansour, 1975). Once roselle plants flower, vegetative growth slows down. Because roselle is used for fiber and paper pulp, the length and thickness of the stem determine fiber yield. Short stems arising from early flowering or poor nutrition and irrigation

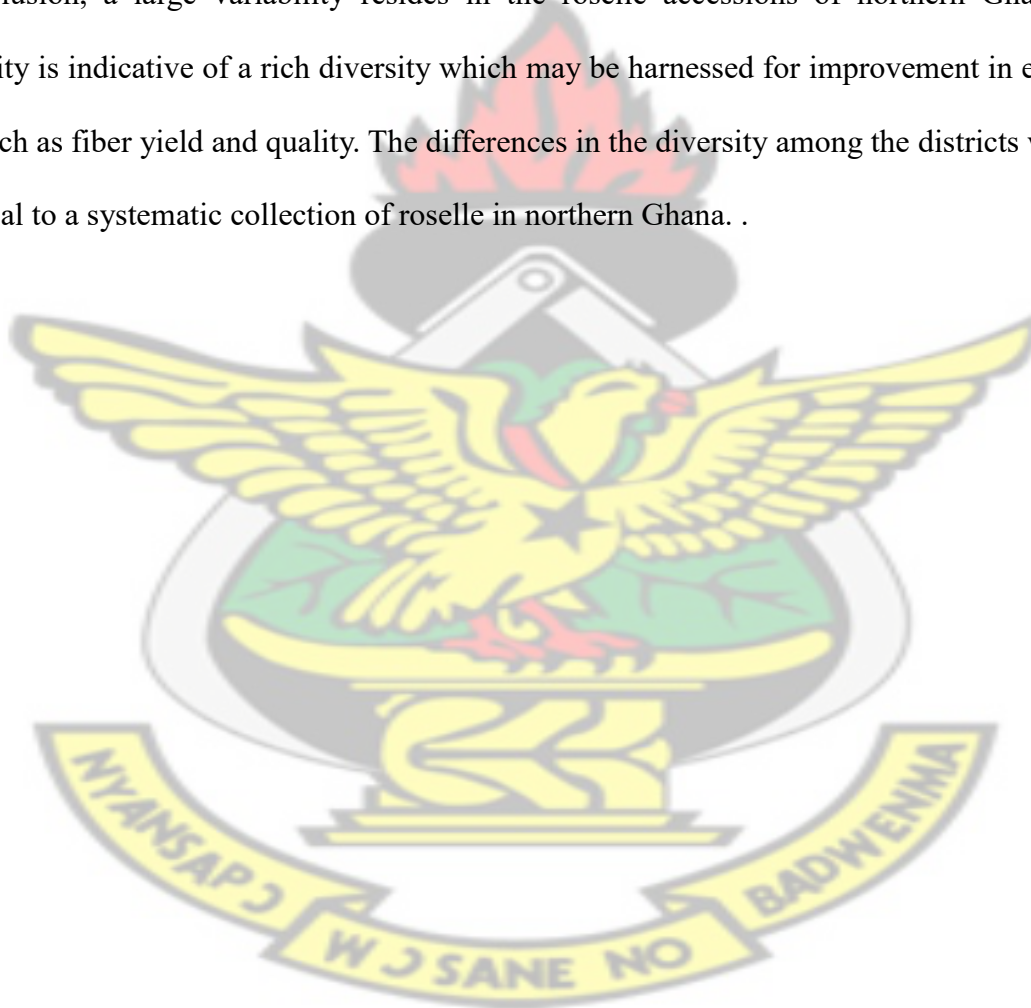
limit yield. A short day length of 8 h promotes more flowering, many branches, short plant height, and shallow tri-lobed leaves with upper slender leaves, whereas long days of 16-h light induces vegetative growth with few branches, tall plants, and deeply lobed leaves. The climate in the study site consisted of 12 h 30 min in July 2016, which reduced to 11 h 44 min in December 2016. Temperature ranged from 22 °C to 31 °C, a typical intermediate day length (World Weather and Climate Information, 2016). Although 106 plants were tall, only two accessions (HA-07 and HA-11) had few branches, while 188 plants had intermediate height with few branches. On the basis of tall plant height and large basal diameter, high but few branching the most important traits for selection of genotypes for bast fiber production and quality include HA-07, HA-11, HA-12, HA-21, and HA-33 from Techiman and Bolgatanga Municipal, Kassena-Nankana, and Savelugu-Nanton districts in the Brong Ahafo, Northern and Upper East regions of Ghana. These accessions had plant height in excess of 250 cm, basal diameter greater than 20 mm, maximum of four branches at branching heights exceeding 130 cm. Low branching points and extensive branching interrupt the fiber strands and reduces fineness. Maiti and Chakravarty (1977) indicated that the strong environmental influence on plant height and basal diameter makes prediction and selection for fiber yield a complicated task in roselle, however, an additional parameter, which is the length of the vegetative growth period highlights the plant's economic value for fiber. On the basis of diversity index and fiber yield characteristics, roselle hotspots in northern Ghana include Savelugu-Nanton, Tamale Metropolitan, Gowri-Yorogo and Talensi districts. Other districts of large diversity include Wa Municipal and Ejura-Sekyedumase. A more robust and well-planned collection of accessions from all the hot spot regions in northern Ghana is needed. In addition, evaluation of roselle accessions in their collection sites would be required to ascertain a genotype by environment interaction component that contributes to the variability. The fairly high variability

observed among the *H. sabdariffa* var. *altissima* accessions in this study was unexpected as roselle plant is self-pollinating. The environment and most probably mutation that had accrued over several generations may have contributed to the observed variability. To the best of our knowledge, this is the first report on *H. sabdariffa* var. *altissima* diversity in Ghana.

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3.5. Conclusion

In conclusion, a large variability resides in the roselle accessions of northern Ghana. This variability is indicative of a rich diversity which may be harnessed for improvement in economic traits such as fiber yield and quality. The differences in the diversity among the districts would be beneficial to a systematic collection of roselle in northern Ghana. .



CHAPTER FOUR

Variability and Genetic Diversity in 25 Roselle (*Hibiscus sabdariffa* var. *altissima*) Collection of Northern Ghana Using Agro-morphological Evaluation

4.0 Abstract

Roselle is a bast fiber crop that is well adapted to the hot climates in Sub-Saharan Africa and grows abundantly in northern parts of Ghana. Roselle *altissima* research and utilization is widely ignored, with scanty information on its genetic diversity. Little genetic variability is reported in exotic roselle genotypes for trait improvement. The awakening of industrialization in Ghana presents roselle as a candidate crop for exploration, however, information on genotypes of economic importance is lacking. Our objective was to investigate the distribution and diversity in roselle *altissima* in northern Ghana. Twenty-five roselle accessions seeds collected from two regions in northern Ghana were planted in field trials using a 5×5 lattice square design in three replications and evaluated for twelve qualitative and five quantitative morphological traits covering plant type, leaf and stem characteristics, flower characteristics, and growth habit. Data were analysed by Shannon-Weiner Diversity Index (SDI), analysis of variance, genetic distance, cluster, principal component, and stepwise discriminant analysis. A large variability was identified between and within the accessions. The mean SDI values in the seven districts ranged from 0.72 to 0.87 with Kassena-Nankana East having the largest diversity, however, West Mamprusi district had accessions with the highest mean plant height of 262.03 ± 17.04 cm, largest stem diameter at 22.63 ± 3.17 cm, and substantial branches not exceeding 7.0 in number. Majority of the accessions exhibited low branching points. The most variable traits were plant height and branch number with SDI of 0.83. Accessions HA-42, HA-52, HA-38, HA-43, and HA-47 ranked highest with respect to plant height with few branches at high branching points, large basal diameter, and late flowering

time. The ample diversity in roselle and identification of genotypes of economic importance await their exploitation for genetic improvement, particularly for fiber yield.

4.1. Introduction

Hibiscus sabdariffa var. *altissima* Wester is the cultivated fiber type of roselle having inedible calyx, tall stem with few branches, and distinct from the wild, vegetable type, *Hibiscus sabdariffa* var. *sabdariffa*. Although the plant is not widely known and utilized in Sub-Saharan Africa (SSA), it is an alternative source of bast fiber (Singh, n.d; Ergle *et al.*, 1945), the other sources being kenaf (*H. cannabinus*) and jute (*Corchorus capsularis*). Roselle accounts for about 20 % of bast fiber crops. The leading bast fiber producing countries annual production since 2012 are India (101,799.40 metric tons), China (26,301.40 metric tons), and Malaysia (8,729.80 tons) (data.world, 2018). From 1961 to 2016, nine countries in SSA engaged in commercial bast fiber crop production. These were Angola, Central African Republic, Democratic Republic of Congo, Ethiopia, Madagascar, Mali, Mozambique, Nigeria, and South Africa. Within this period, total bast fiber production increased from 15,000 to 25,000 hectares, equivalent to 67 % increase, whereas bast fiber yield dropped from 1.15 t/ha to 0.67 t/ha corresponding to 42 % reduction. Current bast fiber production in SSA amounts to 60,000 metric tons. Top producers in SSA since 2012, include the Democratic Republic of Congo with average annual cultivation of 9,289 ha and yield of 0.81 t/ha and Mozambique with 6,948 ha and yield of 0.54 t/ha (data.world, 2018). Records of roselle production in Ghana are not available, despite a wide diversity of this crop in the northern sector of the country (Ankrah *et al.*, 2018).

Roselle vegetable type is largely exploited as a vegetable crop in many countries, including Ghana, however, the fiber type roselle that provides a more economic advantage and a potential for industrial exploitation is not widely exploited. Roselle *altissima* provides a rich source of bast fiber and has for decades been the ideal fiber for making cordage due to its unique salt-resistant trait (Crane, 1949; Cook, 1960).

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In recent years of climate change phenomenon, where SSA is projected to be most seriously affected with loss of biodiversity (IPCC, 2014), the need for collection and conservation of underutilized genetic resources has become more important than ever. An efficient conservation and utilization of biodiversity rely on genetic diversity information. In northern Ghana, the indigenous folk testify that roselle is threatened, as previous morphotypes are no longer common (personal communication).

In a recent study on 36 accessions of roselle *altissimas*, the regions of large occurrence, as well as widest genetic variability residing within and between populations in northern Ghana were reported (Ankrah *et al.*, 2018). The region of largest roselle *altissima* diversity was identified in Upper East, particularly, Savelugu-Nanton, Kasena-Nankana districts and Bolgatanga Municipal, and Techiman district in the Brong-Ahafo region. The Shannon-Weaver Diversity index for these regions were 0.73, 0.63, 0.63, and 0.59, respectively. Additionally, the large and significant mean squares reported indicated a large between-population variability in the collection. The widely reported lack of variability in roselle germplasm which is hampering efforts for genetic improvement is a legitimate concern (Omalsaad *et al.*, 2014; Yusof and Saud, 2009; Hanboosong *et al.*, 2000).

Genetic diversity is a dynamic property of germplasm. It is driven by evolutionary forces of gene flow, genetic drift, mutation, and recombination and modulated by the environment (Dwivedi *et al.*, 2008; Baudoin *et al.*, 2002). Genetic diversity studies, based on morphological evaluation provide information on crop characteristics and reveal estimates of genetic variability and alleles of interest, particularly, those for yield improvement, biotic and abiotic stress tolerance, and improvement in horticultural and agronomic performance (Camussi *et al.*, 1985). Genetic diversity estimates may be based on morphological evaluation, biochemical, or molecular assessment (Bhandari *et al.*, 2017). Biochemical and molecular markers estimate polymorphisms in protein or enzymes, and DNA, respectively. They are desired because they are rapid, robust and insensitive to environmental influences (Govindaraj *et al.*, 2015) however, they are expensive. Morphological characterization, is labour intensive, require large plant population size, exhibit low rate of polymorphism and are constrained by environmental sensitivity and higher risks of biased estimation of diversity (Botha and Venter, 2000). However, their less cost and readily assessable measurement tools make them attractive to breeders for a genetic structuring programme.

Statistical analyses of morphological data assess within- and between-population variability, compute genetic distance, group the accessions, and estimate discriminatory power of the trait markers by means of multivariate techniques (Mahalanobis, 1936). Principal components analysis (PCA) and cluster analysis are commonly employed to dissect genetic diversity in populations. Torres-Moran *et al.* (2011) reported on agromorphological characterization of 12 roselle genotypes in Mexico which were grouped into four distinct groups. Bandi and Appalaswamy (2014) reported on the fiber yield potentials of 60 roselle genotypes from India which grouped into six distinct clusters via a combination of principal components and cluster analysis. Discriminant analysis is

also used in population genetic diversity study to assess relationships between the different clusters. Discriminant analysis was carried out on ten agro-phenological traits in 124 roselle ecotypes in Niger, and further clustered into two distinct groups using a Bayesian method (Bakasso *et al.*, 2013).

Genetic diversity studies on roselle *altissima* is rather scanty and limited to work reported by Ankrah *et al.* (2018) who assessed thirty-six wild growing roselle fiber accessions in Ghana. Another study done on roselle fibre type was a bast fiber characterization study in Kenyan roselle (Mwasiagi *et al.*, 2014). A further collection of 25 roselle accessions in northern Ghana has been carried out by the current Bast Fiber Research project team. Genetic diversity information on the new collection is required. The objective of this research was to estimate genetic diversity among 25 accessions of roselle in northern Ghana based on agro-morphology evaluation.

4.2. Materials and Methods

4.2.1. Plant Material

Twenty-five accessions of roselle seeds were supplied by farmers located in seven districts in northern Ghana covering a geographical area of latitude 9° 39' and 10° 59' N to longitude 0° 47' and 1° 23' W with an elevation within the range 119 to 238 masl (Figure 4.1). The accessions and their designations are presented in Table 4.1. Field trials were carried out from June 26, 2017 to November 30, 2017 on the research fields of the Department of Horticulture, Kwame Nkrumah University of Science and Technology, Kumasi. This site is located at latitude 6° 40'39 N and longitude 1° 33'58 W with an elevation of 258 masl in the semi-deciduous forest zone of Ghana.

Average monthly rainfall within this period was 1.8 mm to 11.0 mm. The soil type was sandy loam Auroso Orchrosols with a pH of 4.90.

Seeds were planted in a 5 × 5 lattice square design in three replications on 0.5 m × 2.0 m plot spaced at 20 cm × 50 cm with an alley of 1.0 m to give a plant density of 20 plants/plot. Irrigation was carried out as and when required. Weeds, nut grasses (*Cyperus rotundus*) and *Panicum maximum* were controlled by WeedKill (glyphosate, 400 g/L) and was applied at a rate of 3.0 L/ha. Hand weeding with a hoe was carried out to weed out post-emergence weeds. The predominant insect pests, cotton stainer (*Dysdercus* sp.) and thrips (*Thysanoptera*), were controlled with Sumitex (dimethoate 400 g/L) at a rate of 1 L/ha.

Table 4.1. Accessions of roselle *altissima* evaluated in Ghana in 2017

Accession	Collection site	District	Accession	Collection site	District
HA-37	Sumbrungu	Bolgatanga Municipality	HA-50	Yorogo	Bolgatanga Municipality
HA-38	Sirigu	Kassena-Nankana West	HA-51	Nawasa	Gonja North
HA-39	Chuchuliga	Builsa North	HA-52	Korania	Kassena-Nankana East
HA-40	Bolgatanga	Bolgatanga Municipality	HA-53	Nawasa	Gonja North
HA-41	Yua	Kassena-Nankana West	HA-54	Wiasa	West Mamprusi
HA-42	Pungu	Kassena-Nankana East	HA-55	Yua	Kassena-Nankana West
HA-43	Sirigu	Kassena-Nankana West	HA-56	Navrongo	Kassena-Nankana East
HA-44	Manyoro	Kassena-Nankana East	HA-57	Dua	Bongo
HA-45	Korania	Kassena-Nankana East	HA-58	Korania	Kassena-Nankana East
HA-46	Chuchuliga	Builsa North	HA-59	Navrongo	Kassena-Nankana East
HA-47	Manyoro	Kassena-Nankana East	HA-60	Gowrie	Bongo
HA-48	Saboro	Kassena-Nankana East	HA-61	Zaare	Bolgatanga Municipality

HA-49	Bolgatanga	Bolgatanga Municipality	
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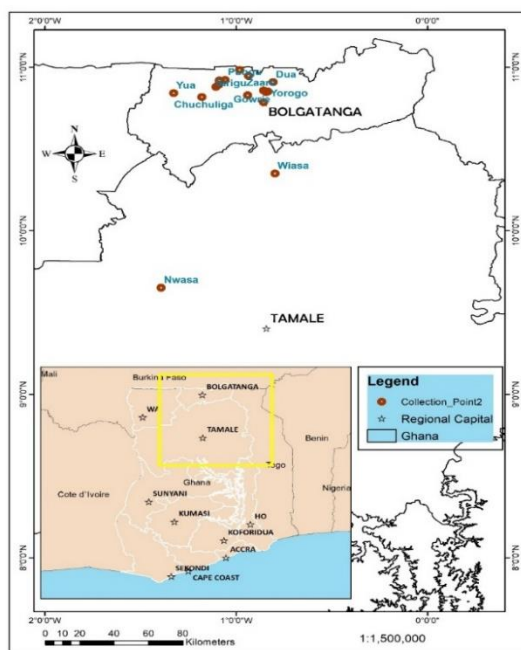


Figure 4.1. A schematic map of Ghana showing roselle *altissima* seed collection sites in the Northern and Upper East regions

4.2.2. Data Collection

Days to 50% flowering were recorded from day 90. At 150 days after planting, 12 qualitative and five quantitative traits on 10 competitive plants per plot were collected. The descriptors of roselle *altissima* were adapted from El-Naim *et al.* (2012) and Coffie (2016). The qualitative traits included plant type, (PT, as the predominant colour of the plant: green (1), pigmented (3), red (5)); branching habit, (BH, as extent of branching: few (1), intermediate (2), extensive (3)); growth habit, (GH as the form of growth: non-bushy (1), bushy (2)); stem pubescence, (SPB as the feel of the stem: smooth (1), hairy (2), rough (3), spiny (4)).

Other traits were leaf form, (LF, as the shape of leaf: entire (1), trilobed (3), pentalobed (5)); size of leaf, (LS, as slender (1), broad (2)); leaf pubescence, (LPB, as presence or absence of hair: smooth (1), hairy (2)). Calyx pigmentation, (CPG, as the predominant colour of the calyx: green (1), pigmented (2), red (3), calyx pubescence, (CPB, as presence or absence of hair: smooth (1), hairy (2)); capsule shape, (CSH, as the predominant shape of capsule: ovoid (1), round (2)) were also evaluated. The remaining traits were petal colour, (PC, as the predominant colour of the petals: yellow (1), purple (2)), and throat colour, (TC, as colour of the flower throat: yellow (1), crimson (3)).

The quantitative traits were days to 50 % flowering (D50), measured as number of days from planting to 50 % of plants in a plot having at least one open flower. Plant height (PH, cm) was estimated as height from ground level to growing tip, height at first branching (HFB, cm) as distance from ground level to first primary branch, and basal diameter (BD, mm) as diameter of the stem at 5 cm above ground. Finally, branching number (BN) was determined by counting the number of primary reproductive branches along the stem. Micrometre screw gauge and meter rule were used to measure diameter and heights, respectively.

4.2.3. Statistical Analysis

The 12 qualitative traits were analysed by calculating frequencies and percentages to reveal morphological variabilities. Principal Components Analysis (PCA) was also performed on the qualitative data to reveal the discriminatory power of the traits and reveal groups among the accessions. The quantitative traits were analyzed by computing means, standard deviation, minimum and maximum values and coefficient of variation (CV). Entry means (X_i) and standard

deviation (σ) were used to divide accession scores into five phenotypic classes (x_i) of equal width of 1.0σ , for the entire data spanning $(x_i - 2\sigma) \geq X_i \geq (x_i + 2\sigma)$. The frequency of genotypes in the i th class (P_i) was used to deduce the standardized Shannon-Wiener Diversity Index (SDI) for within-population variation, (Shannon, 1948), where:

$$H' = -\sum \frac{(P_i * \ln P_i)}{\ln n} \dots \dots \dots (1)$$

The SDI values ranges between 0 and 1. Values close to 1 were considered highly diverse compared to values close to 0. P_i was computed as n_i/N , where n_i is the number of individuals of the i th class, and N is the total number of individuals; n is the number of classes. The between-population variation was assessed by analysis of variance of the lattice square design based on the random effects model presented as

$$Y_{ijk} = \mu + R_j + B(R) + G_i + \epsilon_{ijk} \dots \dots \dots (2)$$

In this model, Y_{ijk} is response from genotype, G_i , in replication R_j , in block B_k and ϵ_{ijk} is the error associated with the genotype $i = 1 \dots, t$, replication $j = 1 \dots, r$; and block nested within replication $k = 1 \dots, s$. The expected mean squares (EMS) were derived from the analysis of variance (Table 4.2).

Table 4.2 Analysis of variance for obtaining variance components

Source	df	MS	Expected Mean Square
Rep	$r-1$	M_R	$\sigma^2_E + r\sigma^2_{B(R)} + st\sigma^2_R$
Block(replication)	$s(r-1)$	$M_{B(R)}$	$\sigma^2_E + t\sigma^2_{B(R)}$
Genotype	$t-1$	M_G	$\sigma^2_E + r\sigma^2_G$
Error	$rs(t-1)$	M_E	σ^2_E

Pairwise genetic similarity based on Euclidean distance for the accessions were computed as:

$$d(x, y) = \sqrt{\sum_i^n (Px_i - Py_i)^2} \dots\dots\dots (5)$$

where d = the Euclidean distance; i = trait; n =total number of traits; x = value for trait x ; and y = value for trait y . Mean genetic similarity for each accession was calculated as the average of between-population Euclidean distances across accessions.

Cluster analysis was performed on the distance matrix using Ward's method of minimum variance (Ward, 1963). Stepwise discriminant analysis was carried out to determine traits that contributed most to the variance by minimizing Wilk's lambda (Wilk, 2006). Principal components analysis (PCA) was performed on the distance matrix in order to depict non-hierarchical relationships among the genotypes and determine the traits that are most effective in discriminating between accessions. The eigenvalues and eigenvectors, and cumulative proportions of the total variance expressed by each trait were calculated followed by a scatterplot of the first and second principal components to reveal relationships between traits and between accessions. The SAS 9.3 programme (SAS Institute Inc, 2011) was employed for all statistical computations.

4.3. Results

4.3.1. Variability in Qualitative Traits

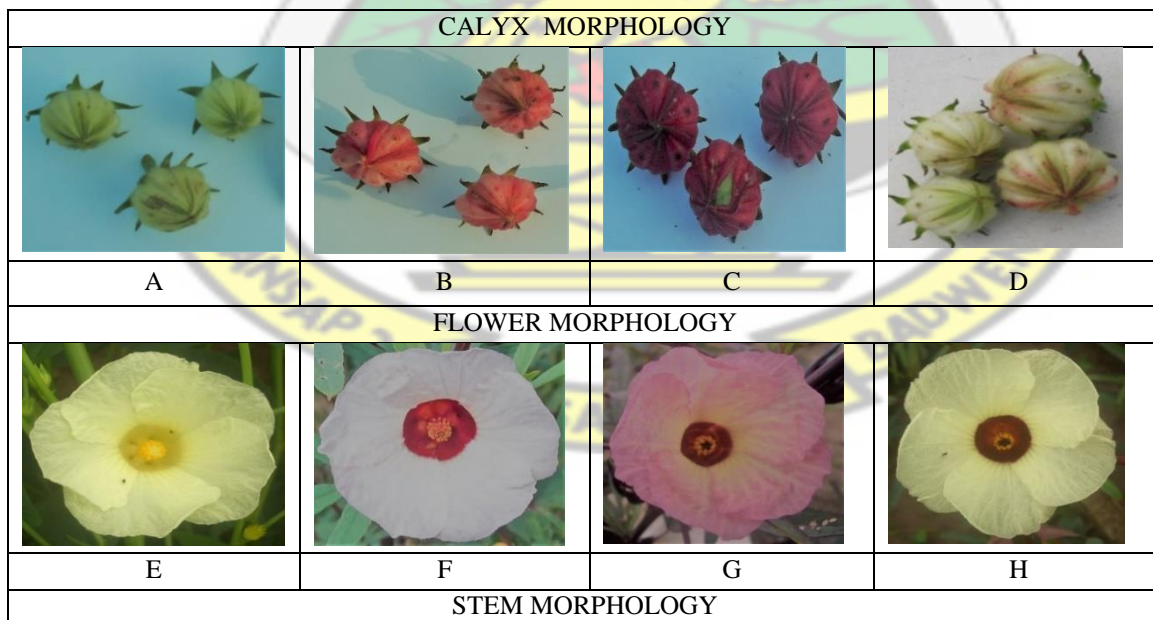
The roselle *altissima* collection was represented by nine accessions (36 %) from Kassena-Nankana East, five accessions (20 %) from Bolgatanga Municipal, and four (16 %) from Kassena-Nankana West. The others were two accessions (8 %) each from Bongo, Builsa-North, and Gonja-North districts and one (4 %) accession from West Mamprusi. A total of 750 roselle *altissima* plants were evaluated by pheno-morphological traits.

The roselle *altissima* collection exhibited large variability in all qualitative traits except growth habit, calyx pubescence and capsule shape in which all plants exhibited non-bushy growth, with hairy calyx and round capsules, respectively. Leaf size and petal colour were somewhat variable with 80 % slender leaves, 20 % having broad leaves, and 72 % yellow and 28 % purple petals, respectively.

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The highly variable traits were plant type (40 % uniform green, 32 % pigmented, 28 % red), branching habit (25 % few, 60 % intermediate, 15 % extensive), stem pubescence (45 % smooth, 26 % hairy, 28 % rough, 1.5 % spiny). The others were leaf form (6 % entire, 30 % tri-lobed, 64 % penta-lobed), leaf pubescence (62 % smooth, 38 % hairy), calyx pigmentation (40 % uniform green, 32 % pigmented, 28 % red), and throat colour (41 % yellow, 59 % crimson) (Table 4.3).

Plate 4.1 shows images of calyx, flower, stem and leaf morphology in the collection and the variations therein.



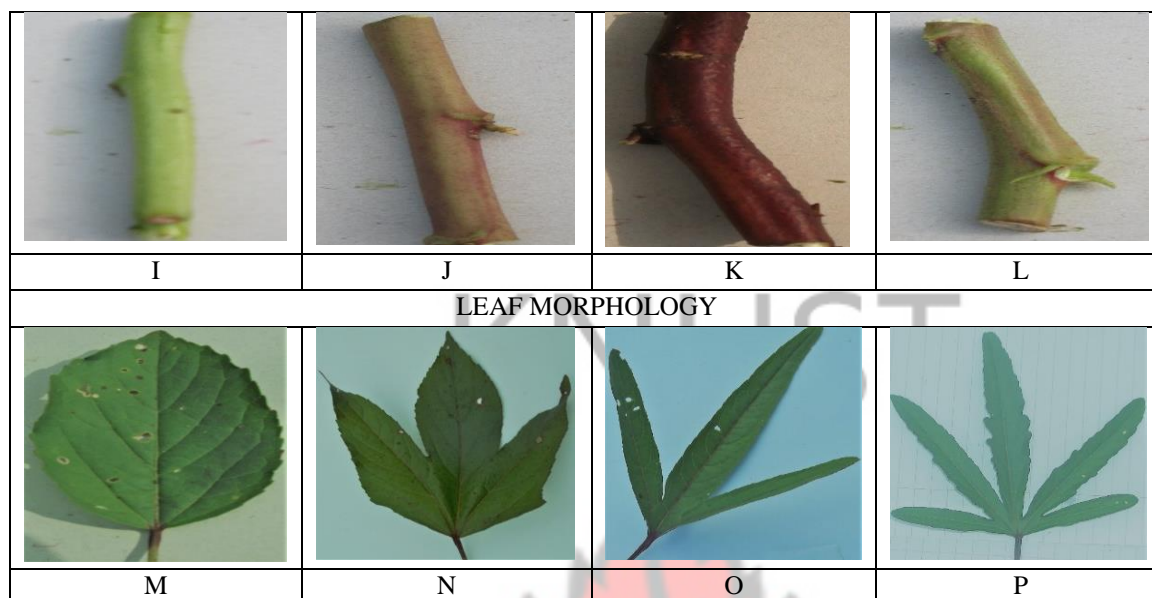


Plate 4.1. Image of plant parts of a matured *altissima* plant. (A) Round fruit of a full green *altissima* plant enclosed in inedible fibrous green calyx. (B) Round fruit of a pigmented (light brown) *altissima* plant enclosed in inedible fibrous pink calyx. (C) Round fruit of a full red *altissima* plant enclosed in inedible fibrous red calyx. (D) Round fruit of a green-pigmented *altissima* plant enclosed in inedible fibrous green-pigmented calyx. (E) Bright yellow flower with deep-yellow throat on a full green *altissima* plant. (F) Pale purple flower with crimson throat on a pigmented (light brown) *altissima* plant. (G) Purple flower with crimson throat on a red *altissima* plant. (H) Pale yellow flower with deep-crimson throat on a green-pigmented *altissima* plant. (I) Full green stem of an *altissima* plant. (J) Pigmented (light brown) stem of an *altissima* plant. (K) Full red stem of an *altissima* plant. (L) Green-pigmented stem of an *altissima* plant. (M) Broad entire leaf of an *altissima* plant. (N) Broad trilobed leaf of an *altissima* plant. (O) Slender trilobed leaf of an *altissima* plant. (P) Slender pentalobed leaf of an *altissima* plant.

4.3.2. Principal Component Analysis of Qualitative Data

A scree plot revealed that the first three principal components (PC) with eigenvalues greater than 1.0 had large contribution to the variance (Figure 4.2A). The first two PCs accounted for 65 % of the total variance, where PC1 accounted for 43.64 % and PC2 21.71 %. Based on length of the vectors, plant type and calyx pigmentation exerted greatest contribution to the variance, followed by petal colour and throat colour, and branching habit contributed least (Figure 4.2B).

Four major groups of roselle *altissima* were identified, namely, group I, consisting of accessions HA-42, HA-44, HA-49 which were predominantly penta-lobed, with rough and spiny stems, and hairy leaves with few branching; group II with genotypes HA-38, HA-51, HA-54, HA-57, HA 59 distinguished by extensive branching; group III accessions HA-39, HA-41, HA-60 were clustered entirely on their broad leaf trait, group IV genotypes, HA-48, HA-53, HA-55, HA-56, HA-58 had green plant type, few branching, smooth stem, entire, slender and smooth leaves, green calyx, yellow petals and yellow throat colour. Accessions HA-46 and HA-47 were clustered according to pigmented plant type with pigmented calyx. Accessions HA-37, HA-43, HA-45, HA-52, and HA-61 were isolated from the other groups. Growth habit, calyx pubescence, and capsule shape were not discriminatory.

Table 4.3. Distribution of qualitative morphological traits in *H. sabdariffa* var. *altissima* collected from Northern Ghana and evaluated in 2017

Trait	Description	Score	No. of plants	Percentage (%)
Plant type (PT)	Uniformly green	1	305	40.7
	Pigmented	3	238	31.7
	Uniformly red	5	207	27.6
Branching habit (BH)	Few	1	188	25.1
	Intermediate	2	441	60.1
	Extensive	3	111	14.8
Growth habit (GH)	Non-bushy	1	750	100
	Bushy	2	0	0
Stem pubescence (SPB)	Smooth	1	334	44.5
	Hairy	2	196	26.1
	Rough	3	209	27.9
	Spiny	4	11	1.5
Leaf form (LF)	Entire	1	45	6.0
	3-lobed	3	227	30.3
	5-lobed	5	478	63.7
Leaf size (LS)	Slender	1	601	80.1
	Broad	2	149	19.9
	Smooth	1	466	62.1

Leaf pubescence (LPB)	Hairy	2	284	37.9
Calyx pigmentation (CPG)	Green	1	305	40.7
	Pigmented	2	238	31.7
	Red	3	207	27.6
Calyx pubescence (CPB)	Smooth	1	0	0
	Hairy	2	750	100
Capsule shape (CSH)	Ovoid	1	0	0
	Round	2	750	100
Petal colour (PC)	Yellow	1	543	72.4
	Purple	2	207	27.6
Throat colour (TC)	Yellow	1	305	40.7
	Crimson	3	445	59.3

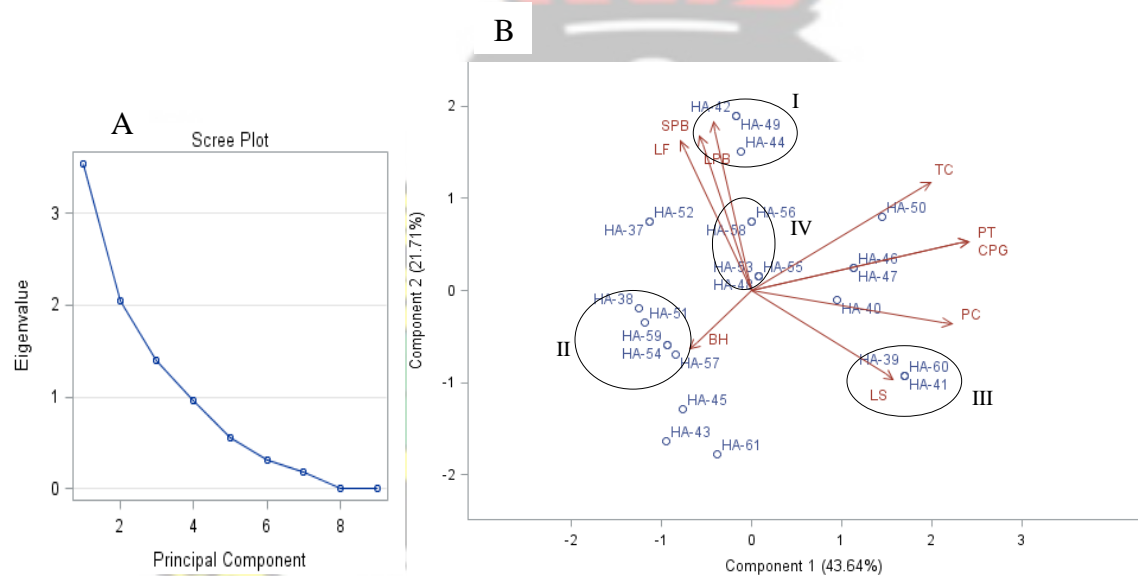


Figure 4.2. Principal components analysis of 25 northern Ghana roselle *altissima* accessions evaluated in 2017 on 12 qualitative traits. (A) Scree plot; (B) Biplot of PC1 and PC2

4.3.3. Within-population Variation in Quantitative Traits

The Shannon-Weiner Diversity Index (SDI) assessed diversity within the roselle fiber populations. Values ranged from 0.00 to 1.00 with a mean of 0.82 ± 0.19 . All traits exhibited high mean SDI values of 0.74 to 0.85 with an error rate of 10 to 50 % (Table 4.4). Plants of all accessions exhibited wide variation in number of days to flowering with SDI values as high as 0.92 to 1.00 except HA-43, HA-51, HA-53, HA-55, and HA-60 whose individual plants consistently flowered on the same day, hence their SDI values were 0.00. Based on accession means across traits, HA-38, HA-42, HA-47, HA-57 and HA-58 were the most variable with SDI values of 0.90 ± 0.08 to 0.93 ± 0.05 . The most variable accessions in the individual traits were for plant height HA-45 , HA-51 and HA-59 (SDI: 0.97 to 1.00); height after first branching HA-43 and HA-58 (SDI: 0.97); branch number HA-37, HA-50, HA-47, HA-55, HA-57, HA-58, HA-60 (SDI: 0.97 to 1.00), and for basal diameter HA-44 and HA-57 (SDI: 0.95 to 0.96). Districts of highest roselle fiber diversity by rank were Kassena-Nankana East (0.87 ± 0.09), Bolgatanga Municipal (0.83 ± 0.10) and West-Mamprusi (0.82 ± 0.09) (Table 4.4).

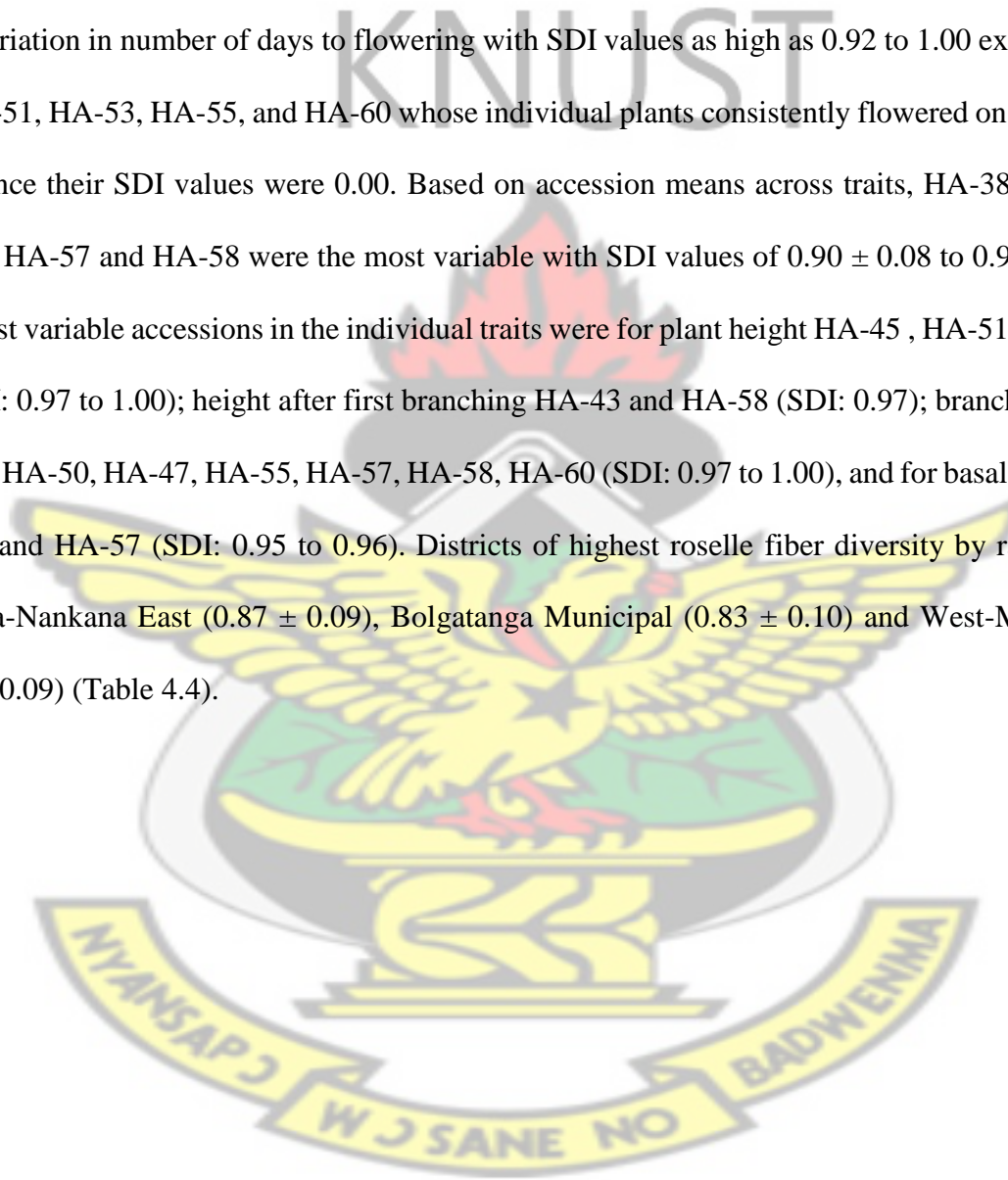


Table 4.4. Shannon Weiner Diversity Index of morphological evaluation of 25 roselle *altissima* accessions in Ghana in 2017

Acc	District	Plant height (cm)	Height at first branch (cm)	Branch number	Basal diameter (mm)	Days to 50% flowering	Accession mean	District mean	Rank
HA-37	Bolgatanga Municipal	0.74	0.87	0.99	0.75	0.92	0.85±0.11	0.83±0.10	2
HA-40		0.69	0.88	0.76	0.84	0.92	0.82±0.09		
HA-49		0.89	0.84	0.81	0.88	0.94	0.87±0.05		
HA-50		0.86	0.81	0.97	0.77	0.92	0.87±0.08		
HA-61		0.57	0.71	0.68	0.82	0.92	0.74±0.14		
HA-57	Bongo	0.89	0.88	1.00	0.95	0.92	0.93±0.05	0.80±0.29	4
HA-60		0.75	0.91	0.97	0.73	0.00	0.67±0.39		
HA-39	Builsa-North	0.84	0.8	0.88	0.61	0.92	0.81±0.12	0.77±0.29	6
HA-46		0.92	0.95	0.92	0.89	0.00	0.74±0.41		
HA-51	Gonja-North	0.98	0.69	0.89	0.80	0.00	0.67±0.39	0.72±0.27	7
HA-53		0.67	0.74	0.74	0.78	0.92	0.77±0.09		
HA-42	Kassena-Nankana East	0.95	0.91	0.75	0.96	0.92	0.90±0.08	0.87±0.09	1
HA-44		0.90	0.82	0.68	0.71	1.00	0.82±0.13		
HA-45		0.97	0.86	0.88	0.82	0.92	0.89±0.06		
HA-47		0.83	0.94	1.00	0.86	0.94	0.91±0.07		
HA-48		0.92	0.78	0.76	0.92	0.92	0.86±0.08		
HA-52		0.87	0.86	0.75	0.72	0.92	0.82±0.08		
HA-56		0.89	0.68	0.96	0.62	0.92	0.81±0.15		
HA-58		0.86	0.97	0.97	0.87	0.92	0.92±0.05		
HA-59		1.00	0.79	0.76	0.88	0.92	0.87±0.10		
HA-38	Kassena-Nankana West	0.94	0.99	0.83	0.88	0.92	0.91±0.06	0.79±0.28	5
HA-41		0.86	0.69	0.81	0.93	0.92	0.84±0.10		
HA-43		0.84	0.97	0.84	0.91	0.00	0.71±0.40		
HA-55		0.83	0.78	0.99	0.91	0.00	0.70±0.40		
HA-54	West Mamprusi	0.72	0.80	0.74	0.90	0.92	0.82±0.09	0.82±0.09	3
Mean		0.85±0.10	0.84±0.09	0.85±0.11	0.83±0.10	0.74±0.38			

4.3.4. Between-population Variation in Roselle *altissima* Accessions

Analysis of variance revealed a strong replication effect ($P \leq 0.05$) for all traits except height at first branching. Genotype effect was important ($P \leq 0.05$) except for basal diameter. Block nested within replication was not important (Table 4.5).

Table 4.5. Mean squares of traits of *altissima* accessions evaluated in a lattice square design in 2017 in Ghana

Source	df	PH	HFB	BN	BD	D50
Replication	2	2844.30**	1.00	14.41**	64.35**	4.51*
Block (Replication)	8	191.39	0.70	2.05	4.41	0.81
Genotype	20	2606.79**	1.11	14.48**	6.41	3.39**
Error	40	449.40	1.80	1.76	6.74	1.19

PH= Plant height; HFB= Height at first branching; BN= Branch number; BD= Basal diameter; D50= Days to 50 % flowering

Mean plant height of the collection was 244.85 ± 37.49 cm and ranged from 154 cm to 342 cm. Mean height at first branching was at 8.05 ± 2.39 above ground level beginning at a minimum height of 2.40 cm to 19.50 cm. Branch number ranged from 4 to 21 with mean of 8.71 ± 3.22 . Basal diameter varied within wide limits in a range of 12.19 to 32.59 mm with a mean of 21.04 ± 3.50 mm. Roselle fiber plants in current study flowered somewhat at the same time beginning from 96 to 104 days after planting. On average, flowering occurred at 99.40 ± 1.34 days. On the basis of coefficient of variation, the trait with highest variability was branch number (37.01 %) and the least variable was days to 50 % flowering (1.35 %) (Table 4.6).

Table 4.6 Means, standard deviations, range, and coefficient of variation of traits evaluated in 25 *altissima* accessions collected from northern Ghana in 2017

Trait	Mean	SD	Min - Max	CV (%)
Plant height (cm)	244.85	37.49	154.00 - 342.00	15.31
Height at first branching (cm)	8.05	2.39	2.40 - 19.50	29.67
Branch number	8.71	3.22	4.00 - 21.00	37.01
Basal diameter (mm)	21.04	3.50	12.19 - 32.59	16.65
Days to 50 % flowering	99.40	1.34	96.00 - 104.00	1.35

Based on accession means, plant height varied from 184.83 ± 14.81 cm to 283.50 ± 27.00 cm. Typically short genotypes were HA-46 (184.43 ± 14.81 cm), HA-59 (187.30 ± 15.82 cm), HA-61 (202.87 ± 12.37 cm), HA-41 (209.27 ± 21.88 cm), and HA-51 (216.97 ± 28.43 cm). The tall genotypes were HA-44 (275.37 ± 30.87 cm), HA-47 (277.40 ± 26.30 cm), HA-43 (277.57 ± 21.88), HA- 38 (279.00 ± 22.92), HA- 52 (280.77 ± 22.54), and HA-42 (283.50 ± 27.00 cm). A lower standard deviation of 22.54 cm for HA-52 than 27.00 cm for HA-42 shows that majority of HA-52 plants were taller. All accessions exhibited branching, but to varying extents and somewhat at the same height above ground. On accession mean basis, height at first branching ranged from 6.86 ± 1.62 cm in HA-60 to 9.60 ± 3.69 cm in HA-57 with branches numbering between 6.40 ± 1.45 in HA-49 to 12.97 ± 3.59 in HA-51 (Table 4.7). On individual plant basis, as few as four and as many as 21 branches were present (Table 4.7). Accessions with few branches were HA-49 (6.40 ± 1.45), HA-45 (6.53 ± 1.61), HA-58 (6.73 ± 1.66), HA-46 (6.73 ± 1.91), and HA-39 (6.77 ± 1.36). Accessions with extensive branching were HA-56 (10.40 ± 3.57), HA-48 (10.50 ± 2.87), HA-61

(10.70 ± 2.60), HA-43 (11.63 ± 3.80), HA-42 (11.83 ± 3.31), HA-38 (12.87 ± 3.40), and HA-51 (12.97 ± 3.59).

Mean basal diameter ranged from 18.96 ± 1.89 mm in HA-56 to 24.55 ± 2.99 mm in HA-61. Other accessions with large mean basal diameter were HA-57 (22.11 ± 2.86 mm), HA-58 (22.31 ± 3.34 mm), HA-42 (22.38 ± 2.94 mm), HA-54 (22.63 ± 3.17 mm), HA-55 (23.45 ± 3.45 mm) (Table 4.7). Flowering in the roselle fiber plants occurred about the same time with accession means ranging from 97.67 ± 0.96 to 101.33 ± 1.92 days after planting. Three accessions HA-47, HA-54, and HA-59 flowered at 97.67 ± 0.96 days while only HA-37 flowered at 101.33 ± 1.92 days (Table 4.7). Based on fiber yield, accession ranking by tallest plants, highest branching points, fewer branch numbers, and largest basal diameter identified HA-42, HA-52, HA-38, HA-43 and HA-47 to be the top five with economic value (Table 4.7).

Based on district means, maximum plant height of 262.03 ± 17.04 cm was recorded in West Mamprusi and the least plant height of 214.82 ± 40.57 cm was recorded in Builsa-North. District mean for height at first branching varied from 7.74 ± 0.03 cm in Builsa-North to 8.32 ± 0.31 cm in Bolgatanga Municipal. On district mean basis, branch number recorded least value of 6.75 ± 0.03 in Builsa-North to highest value of 10.73 ± 2.59 in Gonja-North. For basal diameter, the district mean ranged from 22.63 ± 3.17 mm in West Mamprusi to 19.25 ± 0.00 in Builsa-North. Genotypes from the districts showed intermediate flowering period and varied from (99.84 ± 0.23) in Bongo to (97.67 ± 0.96) in West Mamprusi (Table 4.8). On the basis of fiber yield, district ranking by tallest plants, highest branching points, fewer branch numbers, and largest basal diameter identified West Mamprusi, Kassena-Nankana West and Bongo as the top three localities with roselle fiber potentials (Table 4.8).

Table 4.7. Means, standard deviations, and range of phenotypic traits of 25 roselle *altissima* accessions evaluated in Ghana in 2017

Accession	Plant height (cm)	Height at first branch (cm)	Branch number	Basal diameter (mm)	Days to 50% flower	Rank
HA-37	250.40±19.40 (219-297)	8.05±2.85 (4.40-17.50)	7.07±1.74 (4.00-11.00)	21.83±3.02 (15.67-28.71)	101.33±1.92 (100-104)	12
HA-38	279.00±22.92 (231-328)	7.42±2.10 (3.40-10.50)	12.87±3.40 (7.00-20.00)	20.59±2.25 (17.42-25.20)	99.67±0.96 (99-101)	3
HA-39	245.20±34.91 (200-314)	7.72±3.19 (3.50-17.20)	6.77±1.36 (5.00-10.00)	19.25±2.76 (15.32-29.18)	98.00±1.44 (96-99)	14
HA-40	233.23±26.18 (179-289)	8.19±1.97 (4.60-12.70)	8.43±2.80 (4.00-16.00)	20.62±3.15 (15.67-27.38)	99.67±0.48 (99-100)	17
HA-41	209.27±21.88 (160-248)	7.52±1.48 (4.50-11.50)	9.60±3.04 (5.00-18.00)	20.61±5.85 (12.57-31.23)	99.33±0.48 (99-100)	22
HA-42	283.50±27.00 (239-341)	7.60±1.75 (4.20-10.30)	11.83±3.31 (8.00-21.00)	22.38±2.94 (17.89-28.18)	99.33±0.48 (99-100)	1
HA-43	277.57±21.88 (240-316)	7.75±2.00 (4.20-12.00)	11.63±3.80 (7.00-20.00)	20.87±4.47 (14.80-31.41)	100.00±0.00 (100-100)	4
HA-44	275.37±30.87 (229-332)	8.15±2.37 (4.30-15.20)	8.73±2.03 (5.00-14.00)	19.58±1.56 (15.24-22.13)	100.33±1.27 (99-102)	6
HA-45	233.03±14.68 (206-261)	7.46±2.07 (4.70-12.50)	6.53±1.61 (4.00-10.00)	20.87±3.09 (15.63-27.80)	98.33±0.96 (97-99)	18
HA-46	184.43±14.81 (160-210)	7.76±2.00 (4.10-11.20)	6.73±1.91 (4.00-11.00)	19.25±3.86 (12.47-26.07)	100.00±0.00 (100-100)	25
HA-47	277.40±26.30 (212-323)	7.25±1.82 (4.20-11.30)	7.37±1.50 (5.00-10.00)	21.99±3.45 (15.81-29.30)	97.67±0.96 (97-99)	5
HA-48	225.37±12.12 (208-251)	8.60±3.2 (4.30-19.50)	10.50±2.87 (7.00-18.00)	19.62±1.96 (16.36-23.81)	100.67±0.96 (100-102)	20
HA-49	238.73±32.03 (202-300)	8.69±2.29 (4.50-14.00)	6.39±1.45 (4.00-9.00)	20.34±4.00 (14.32-28.80)	99.33±0.48 (99-100)	16
HA-50	250.43±25.84 (213-305)	8.04±3.11 (4.10-16.30)	7.03±1.50 (4.00-10.00)	21.07±3.42 (16.15-29.61)	99.33±0.48 (99.00-100)	11
HA-51	216.97±28.43 (163-261)	8.69±2.37 (5.20-15.40)	12.97±3.59 (8.00-21.00)	21.63±3.42 (16.61-29.66)	99.00±0.00 (99-99)	21
HA-52	280.77±22.54 (238-317)	8.26±2.90 (4.40-18.30)	8.77±3.02 (5.00-19.00)	21.94±2.58 (18.93-30.00)	98.33±0.96 (97-99)	2
HA-53	232.30±20.87 (190-277)	8.34±2.16 (4.10-12.80)	8.90±2.63 (5.00-15.00)	20.29±2.37 (16.55-27.28)	99.67±0.48 (99-100)	19
HA-54	262.03±17.04 (220-293)	7.82±2.23 (4.10-13.10)	7.47±1.94 (4.00-13.00)	22.63±3.17 (18.13-30.12)	97.67±0.96 (97-99)	9
HA-55	266.82±29.30 (219-331)	8.58±2.14 (4.50-14.30)	7.04±1.35 (5.00-10.00)	23.45±3.45 (18.40-32.59)	100.36±0.49 (100-101)	7
HA-56	248.87±30.80 (203-321)	8.16±1.33 (4.70-10.50)	10.40±3.57 (5.00-17.00)	18.96±1.89 (13.15-22.70)	99.67±0.48 (99-100)	13
HA-57	266.40±33.10 (208-331)	9.60±3.69 (2.40-17.00)	7.13±1.81 (4.00-10.00)	22.11±2.86 (16.36-27.65)	99.67±0.48 (99-100)	8
HA-58	251.40±32.83 (209-342)	8.25±2.34 (4.10-12.40)	6.73±1.66 (4.00-9.00)	22.31±3.34 (17.41-29.24)	100.67±2.40 (99-104)	10
HA-59	187.30±15.82 (154-210)	7.71±1.68 (4.10-11.30)	8.97±3.90 (4.00-20.00)	19.52±4.59 (12.19-30.41)	97.67±0.96 (97-99)	24

Table 4.7 cont'd

Accession	Plant height (cm)	Height at first branch (cm)	Branch number	Basal diameter (mm)	Days to 50% flower	Rank
HA-60	245.07±29.10 (198-319)	6.86±1.62 (4.30-9.70)	7.10±1.54 (4.00-10.00)	19.82±2.07 (15.20-23.77)	100.00±0.00 (100-100)	15
HA-61	202.87±12.37 (172-223)	8.70±2.20 (4.50-17.00)	10.70±2.60 (7.00-17.00)	24.55±2.99 (19.78-31.09)	99.30±0.47 (99-100)	23

Table 4.8. Mean, standard deviation, and range of phenotypic traits evaluated in 25 roselle *altissima* accessions from 7 districts in northern Ghana in 2017

District	Plant height (cm)	Height at first branch (cm)	Branch number	Basal diameter (mm)	Days to 50% flower	Rank
Bolgatanga Municipal	234.38±19.42 (172-305)	8.32±0.31 (4.10-17.50)	7.92±1.72 (4.00-17.00)	21.68±1.70 (14.32-31.09)	99.80±0.87 (99-104)	5
Bongo	255.74±15.08 (198-331)	8.23±1.94 (2.40-17.00)	7.12±0.02 (4.00-10.00)	20.97±1.62 (15.20-27.65)	99.84±0.23 (99-100)	3
Builsa-North	214.82±42.97 (160-314)	7.74±0.03 (3.50-17.20)	6.75±0.03 (4.00-11.00)	19.25±0.00 (12.47-29.18)	99.00±1.41 (96-100)	7
Gonja-North	224.91±10.45 (163-277)	8.32±0.03 (4.10-15.40)	10.73±2.59 (5.00-21.00)	20.88±0.83 (16.65-29.66)	99.34±0.47 (99-100)	6
Kassena-Nankana East	251.28±32.02 (154-342)	7.93±0.44 (4.10-19.50)	8.87±1.81 (4.00-21.00)	20.80±1.40 (12.19-30.41)	99.20±1.23 (97-104)	4
Kassena-Nankana West	258.17±33.05 (160-331)	7.82±0.53 (3.40-14.30)	10.29±2.55 (5.00-20.00)	21.38±1.39 (12.57-32.59)	99.84±0.44 (99-101)	2
West Mamprusi	262.03±17.04 (220-293)	7.82±2.23 (4.10-13.10)	7.47±1.94 (4.00-13.00)	22.63±3.17 (18.13-30.12)	97.67±0.96 (97-99)	1

4.3.5. Correlation of Quantitative Traits in *altissima* Accessions

The Pearson correlation coefficients, r , were low, -0.01 to 0.13. Basal diameter showed positive significant correlation with plant height ($r = 0.11$; $R^2 = 0.012$) and branch number ($r = 0.13$; $R^2 = 0.017$) but a negative significant correlation with height at first branching ($r = -0.08$; $R^2 = 0.006$). Variation in basal diameter is explained by 1.21 % of plant height, 1.70 % of branch number and 0.64 % of height at first branching. Height at first branching showed a positive significant

relationship with days to 50 % flowering ($r = 0.12$; $R^2 = 0.014$) accounting for 1.40 of the variation. The remaining traits showed non-significant correlations be it positive or negative.

Table 4.9. Pearson correlation of five quantitative traits of *altissima* accessions

	Plant height (cm)	Height at first branching (cm)	Branch number	Basal diameter (mm)
Height at first branching	-0.02			
Branch number	0.03	0.01		
Basal diameter	0.11**	-0.08*	0.13**	
Days to 50 % flowering	-0.03	0.12**	-0.02	-0.01

**($P < 0.01$); * ($P < 0.05$)

4.3.6. Genetic Distances Among the 25 roselle *altissima* Accessions

The overall mean genetic distance of the 25 roselle fiber accessions was 3.03 ± 0.90 covering a range of 0.41 to 5.17 (Table 4.10). Very low distances were recorded between accessions HA-40 and HA-53 (0.41), HA-27 and HA-58 (0.84), and HA-38 and HA-43 (0.91), whereas large distances were recorded between accessions HA-55 and HA-59 (5.02), HA-57 and HA-59 (5.11), and HA-60 and HA-61 (5.17). Based on means across pairwise distances, which lied between 2.22 and 3.94, three accessions were divergent, namely, HA-61 (3.94), HA-57 (3.66) and HA-59 (3.63). Accessions HA-40 (2.22), HA-50 (2.30) and HA-53 (2.30) represented the closest pairs (Table 4.10).

Table 4.10. Euclidean distances of the 25 northern Ghana *altissima* accessions evaluate by morphological characterization in 2017

Accession	Mean ± SD	Min - Max	Accession	Mean ± SD	Min - Max
HA-37	3.05 ± 0.86	0.84 – 4.73	HA-50	2.30 ± 0.67	1.05 – 3.64
HA-38	3.35 ± 0.93	0.91 – 4.77	HA-51	3.41 ± 0.63	2.41 – 4.63
HA-39	3.04 ± 0.88	1.35 – 4.94	HA-52	2.82 ± 0.69	1.44 – 4.40
HA-40	2.22 ± 0.71	0.41 – 3.31	HA-53	2.30 ± 0.74	0.41 – 3.64
HA-41	2.71 ± 0.66	1.56 – 4.38	HA-54	3.12 ± 0.82	1.21 – 4.39
HA-42	3.19 ± 0.81	1.31 – 4.83	HA-55	3.19 ± 0.89	1.17 – 5.02
HA-43	2.90 ± 0.86	0.91 – 4.24	HA-56	2.73 ± 0.80	1.35 – 4.39
HA-44	2.76 ± 0.80	1.46 – 4.65	HA-57	3.66 ± 0.87	1.95 – 5.11
HA-45	2.82 ± 0.83	1.35 – 4.17	HA-58	2.82 ± 0.86	0.84 – 4.48
HA-46	3.47 ± 0.91	2.06 – 4.83	HA-59	3.63 ± 0.87	2.05 – 5.11
HA-47	3.40 ± 0.92	1.21 – 4.79	HA-60	3.23 ± 0.82	2.16 – 5.17
HA-48	3.04 ± 0.85	1.45 – 4.79	HA-61	3.94 ± 0.64	2.41 – 5.17
HA-49	2.70 ± 0.76	1.33 – 4.06	Overall mean	3.03 ± 0.90	0.14 – 5.17

4.3.7. Cluster Analysis of roselle *altissima* Accessions

Accessions were clustered into three distinct groups (Figure 4.3). Cluster I comprised of 13 accessions; IA: HA-37, HA-40, HA-44, HA-48, HA-49, HA-50, HA-51, HA-53, HA-55, HA-56, HA-57, HA-58, HA-61 with a mean genetic distance of 2.58 ± 0.89 . Cluster I was grouped based on highest branching points and late flowering (Table 4.11). Accessions HA-57, HA-61, HA-49, HA-48, and HA-55 exhibited branching points at heights above 8.50 cm. Similarly, accessions HA-37, HA-48 and HA-58, flowered after 100 days. Mean branching point and flowering were 8.47 ± 2.54 cm and 99.92 ± 1.21 days, respectively.

Cluster II was made up of six accessions: HA-38, HA-42, HA-43, HA-47, HA-52, HA-54 with a mean genetic distance of 2.36 ± 0.92 . The six accessions of cluster II were separated based on highest plant height with mean of 276.71 ± 23.87 cm, high branching number (9.98 ± 3.65), and

highest basal diameter (21.73 ± 3.27 mm) (Table 4.11). The very tall accessions of interest were HA-42 (283.50 ± 27.00 cm), HA-52 (280.77 ± 22.54 cm), HA-38 (279.00 ± 22.92 cm), HA-43 (277.57 ± 21.88 cm), HA-47 (277.40 ± 26.30 cm), HA-54 (262.03 ± 17.04 cm). Accessions with large basal diameter in excess of 22.30 mm were HA-61, HA-55, HA-54, HA-42, and HA-58.

Accessions HA-39, HA-41, HA-45, HA-46, HA-59, and HA-60 of cluster III had an overall mean genetic distance of 2.40 ± 0.48 and were segregated on the basis of short plant height (217.38 ± 34.16 cm), least number of branches (7.62 ± 2.67), smallest basal diameter (19.89 ± 3.91 mm) and lowest branching point (7.51 ± 2.08 cm) (Table 4.11). Accessions of interest having the least number of branches were HA-49 (6.39 ± 1.45), HA-45 (6.53 ± 1.61), HA-58 (6.73 ± 1.66), HA-46 (6.73 ± 1.91), and HA-39 (6.77 ± 1.36).

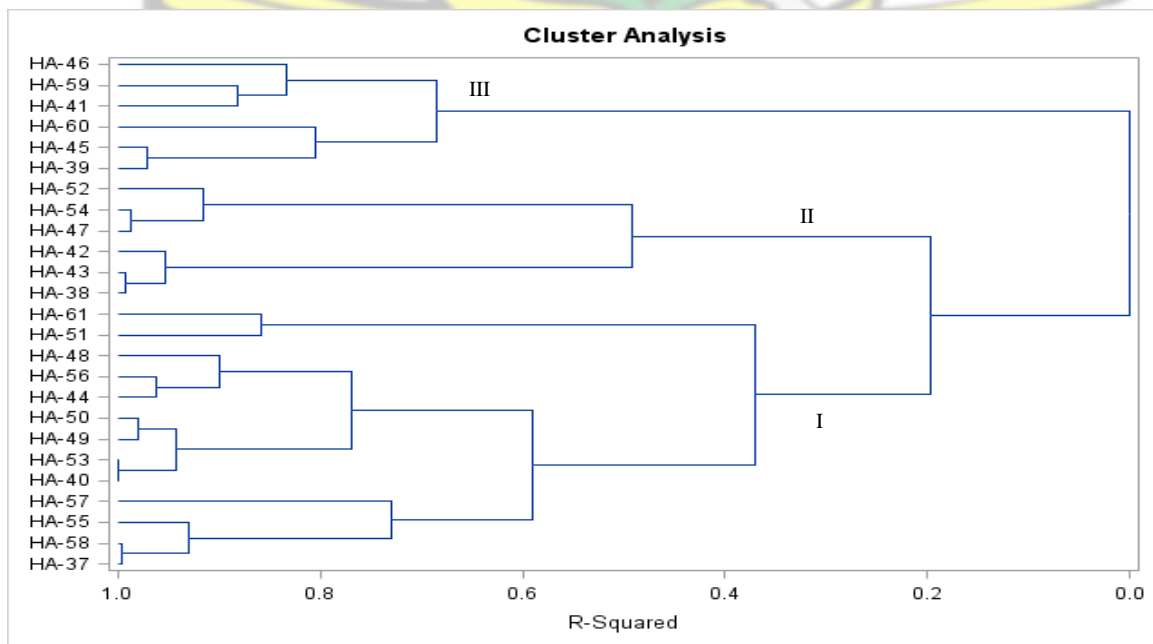


Figure 4.3. A dendrogram based on Ward's minimum variance of 25 northern Ghana roselle *altissima* accessions evaluated by morphological traits in field trials in 2017

Table 4.11. Means, standard deviations, and differences of clusters of 25 northern Ghana *altissima* accessions evaluated in 2017

Traits	Overall means	Cluster I	Diff	Cluster II	Diff	Cluster III	Diff
PH	244.85±37.49	242.83±32.92	-2.02	276.71±23.87	31.86	217.38±34.16	-27.47
HFB	8.05±2.39	8.47±2.54	0.42	7.68±2.16	-0.37	7.51±2.08	-0.54
BN	8.71±3.22	8.62±3.05	-0.09	9.98±3.65	1.27	7.62±2.67	-1.09
BD	21.04±3.50	21.25±3.29	0.21	21.73±3.27	0.69	19.89±3.91	-1.15
DTF	99.40±1.34	99.92±1.21	0.52	98.78±1.23	-0.62	98.89±1.25	-0.51

PH = Plant height; HFB = Height at first branching; BN = Branch number; BD = Basal diameter; DTF = Days to 50 % flowering; Diff = cluster means – overall means (standard deviations excluded)

4.3.8. Stepwise Discriminant Analysis

Three of the five morphological traits evaluated produced adequate discrimination of the accessions based on minimization of Wilk's lambda. Branch number (0.27), contributed the most variance to the data, followed by plant height (0.08) and then days to 50 % flowering (0.04), each having significant minimization (Table 4.12). Height at first branching and basal diameter were not discriminatory.

Table 4.12. Stepwise discriminant analysis of traits of *altissima*

Trait	Wilk's lambda	F-value
Branch number	0.27**	5.54
Plant height (cm)	0.08**	4.84
Days to 50 % flowering	0.04**	2.51

4.3.9. Principal Component Analysis

The first three principal components (PCs) with eigenvalues greater than 1.00 contributed 100 % to the variance in the data (Table 4.13). Contributions to the total variance were for PC1 (40.05 %) with major loading in height at first branching (0.74) and basal diameter (0.71), and for PC2 (31.77 %) with major loading in plant height (0.69) and days to 50 % flowering (0.56). Total contribution of PC1 and PC2 to the variance was 71.82 %. The PC3 accounted for 28 % of the total variation, with much contribution from branch number (0.86) (Table 4.13).

Table 4.13. Principal components analysis of 25 *altissima* accessions studied based on five quantitative traits

Trait	PC1	PC2	PC3
Plant height (cm)	0.38	0.69	0.17
Height at first branching (cm)	0.74	-0.42	-0.23
Branch number	0.18	0.12	0.86
Basal diameter (mm)	0.71	0.41	-0.30
Days to 50 % flowering	0.48	0.56	0.34
Eigenvalues	1.45	2.61	3.62
Cumulative eigenvalues	1.45	2.61	3.62
Percentages	40.05	31.77	28.18
Cumulated percentages	40.05	71.82	100.00

Biplot of PC1 and PC2 revealed three major uncorrelated groups of accessions. First group of accessions (HA-38, HA-42, HA-43 HA-52, HA-54) were based on large values of plant height and basal diameter, the second group of accessions (HA-39, HA-45, HA-60) were clustered based on least number of branches per plant. Roselle fiber accessions in the third group (HA-37, HA-40, HA-44, HA-49, HA-50, HA-51, HA-53, HA-56and HA-58) were separated according to least values of plant height, basal diameter, branch number and medium values of height at first branching and days to flowering. Accessions HA-46, HA-47, HA-57 and HA-59 were separated from the rest.

All traits contributed positively to the total variance as they grouped to the right. Positive correlation was observed between plant height and basal diameter as well as plant height and branch number. Negative correlation was observed for height at first branching and days to flowering (Figure 4.3B).

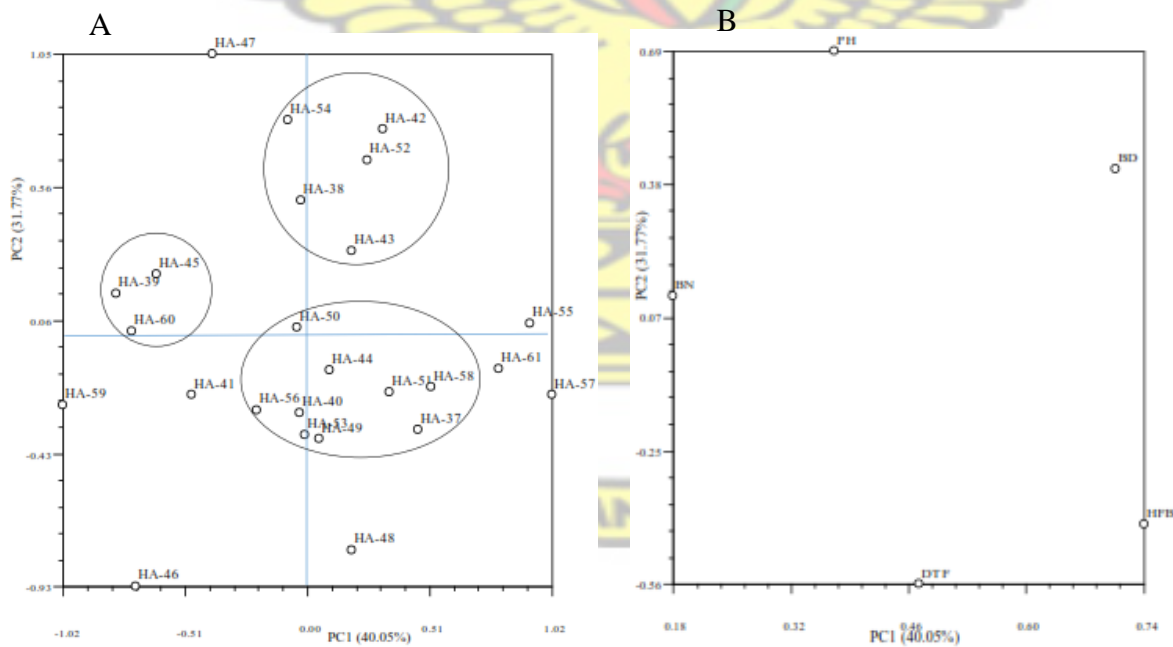


Figure 4.4 Principal components biplots of (A) 25 accessions and (B) five quantitative traits of *altissima*.

4.4. Discussion

Roselle is a crop that is well adapted to the hot climates of SSA and has thrived over several decades in limiting soil nutrients and marginal environments. In Ghana, indigenous communities that have fair knowledge of roselle *altissima* utilize them in homesteads for domestic fiber production for making ropes. In contrast, roselle is commercially cultivated in Asian countries where its bast fiber is exploited in industry. Since the study of Coffie (2016), who reported that the center of diversity of roselle vegetable type lies along a northern Ghana, Ouagadougou, and Mali belt, no study has been carried out to investigate the distribution and diversity in roselle *altissima* in northern Ghana. Additionally, there is dearth of knowledge on the economic potential of roselle *altissima* in Ghana. Hence, this work was carried out to investigate the distribution and diversity in roselle *altissima* in northern Ghana, to delineate traits of commercial importance, and to identify genotypes of commercial value based on superior traits.

The roselle *altissima* seed collection was obtained from seven districts in northern Ghana where Kassena-Nankana East was represented more than the other districts. This non-uniform collection could not be avoided owing to the widespread lack of knowledge on roselle *altissima*. In addition, similarity in seed morphology with the vegetable type posed challenges in obtaining adequate information on the fiber type. Despite these drawbacks, indigenous knowledge of the farmers provided ample guide to the locations of roselle *altissima* cultivation. Chivenge *et al.* (2015) stated that aged folk possessed sufficient indigenous knowledge about under-utilized crops in SSA, and that, this knowledge needs to be harnessed in a rapid manner for utilization, conservation, and cultivar development in the midst of devastating climate change effects of excessive heat and drought.

A large variability in qualitative traits was identified. The diverse forms of leaf form, plant type, calyx and flower pigmentation were consistent with the morphology of the vegetable roselle. Coffie (2016), in her study of 35 roselle vegetable genotypes from across West Africa reported on substantial variability among the accessions. On plant type, roselle *altissima* had more green genotypes (40 %) with substantially fewer branching (25 %) than the vegetable type (9% green; 1% with few branching). In current study, roselle *altissima* leaves were predominantly slender (80 %) and penta-lobed with few tri-lobed (64 % and 30 %, respectively) forms, contrasting with the fundamentally broad (68%), tri- (49%) and pentalobed (36 %) leaves of *sabdariffa*. Calyx pigmentation in *altissima* was similar to that in *sabdariffa*, but majority of the plants had yellow petals (72 %) and somewhat equal distribution of yellow (41 %) and crimson (59 %) throat. In *sabdariffa*, however, 39 % had yellow petals and 91% crimson throat.

The qualitative characteristics of roselle *altissima* concur with an earlier study on 36 roselle fiber genotypes from northern Ghana (Ankrah *et al.*, 2018), which exhibited ample variability in plant type, branching habit, stem pubescence and leaf form. The absence of variability in growth habit, calyx pubescence and capsule shape indicates that these traits are conserved in roselle *altissima*. On the contrary, there are reports of variability in growth habit and capsule shape of roselle vegetable type (El-Tahir and El-Gabri, 2013; Coffie, 2016). Because qualitative traits are not influenced by environment, the variations identified in roselle *altissima* are largely genetic. For the purpose of fiber production, roselle *altissima* with tall green stem and few branches at high branching points are most desirable. Indigenous knowledge purports that green stems produce high fiber yield of better quality. While selection methods based on the phenotypic expression would likely achieve the desired improvement in trait, further work is needed to verify this claim.

Principal components analysis on the qualitative data revealed that the first two PCs cumulatively explained 65 % of the total variance. Of the nine qualitative traits that were significant for the structuring of roselle genotypes the contribution of four traits, namely, plant type, calyx pigmentation, petal and throat colour were substantial (Figure 4.3). Further work on roselle *altissima* should concentrate on these four traits.

The high SDI values observed in the 25 roselle accessions and within the seven districts, is indicative of a large within-population variation. Notwithstanding, the high values could have been caused to some extent, by mixture of seeds. Further work is needed to clean up the seeds. To the best of my knowledge, this is the first report, which estimates SDI in roselle *altissima*. Medagam *et al.* (2015) estimated SDI values of agro-economic traits of vegetable roselle to be 0.32 to 2.00. On the basis of large values of plant height, high branching points, and large basal diameter, the most desired accessions with huge fiber yield potential included HA-38, HA-42, HA-47, HA-57 and HA-58. The districts of largest diversity in a decreasing order were Kassena-Nankana East, Bolgatanga and West Mamprusi. The import of this finding is that, future collection of roselle in Ghana should focus on these districts. Other notable districts with large diversity of roselle were Bongo and Kassena-Nankana West.

Except for height at first branching, a strong genotype main effect for plant height, branch number, basal diameter and days to 50 % flowering indicate a large between population variability. The large replication effect confirmed mixing up of seeds at the various collection points. It is therefore important to make provision for a seed-cleaning programme for roselle, in which case, the large

between-population variability could facilitate the exercise. Strong genotype effect in plant height and branch number (Ibrahim *et al.*, 2013; Abou El-Nasr *et al.*, 2014; Javadzadeh and Saljooghianpour, 2017) was identified in some Sudan, Egypt and Iran roselle *sabdariffa* collections, respectively. Similarly, a large genotype effect in number of days to flowering in roselle *sabdariffa* was reported by Ibrahim *et al.* (2013). The high variability was unexpected as roselle is cleistogamous, and selfing more often restricts variability. Indeed, roselle genotypes that were carried from Ghana to the Philippines in early 1900's and were later multiplied and distributed in Malaysia, Thailand, and other Asian countries have failed to accrue variation hence researchers in these regions have explored mutation breeding, howbeit with limited success (Osman *et al.*, 2011; Heliyanto, 1992). The large between-population variability expresses the differences in the phenotypes of the accessions. Phenotypic differences arise from genotypic and environmental components. Chief among the environmental factors is the day length effect (Warner and Erwin, 2003; Mansour, 1975). Accessions in current study flowered at 96 to 104 days after planting. At flowering, growth in height and stem diameter slow down and limit increase in plant height as occurs in kenaf (Dempsey, 1975). The wide differences in plant height in roselle *altissima* could be attributed to the significant genotype effect for days to flowering. In contrast, no accession differences were observed for basal diameter as all stems were of almost similar girth. This characteristic of roselle warrants further study into the performance of roselle at various geographical areas in Ghana for identification of best growing areas and identification of accessions with best economic value in terms of plant height and basal diameter. Webber *et al.* (2002) reported that tall kenaf plants with large stem diameter produced high fiber yield. The roselle collection in this study could be a germplasm resource in breeding programmes aimed at improving fiber yield and quality.

The large values of branch number at predominantly low branching points in roselle was unexpected. Although, there is no defined planting distance for roselle, the planting distance of 20 × 50 cm within and between rows, respectively, may have contributed to the extensive branching, together with other environmental influences. The import of this finding suggest a much narrower planting distance to increase plant height and decrease number of branches. Sermsri *et al.* (1987) suggested planting distance of 5 to 15 cm for within row spacing and 20 to 40 cm for between row spacing as ideal to maximizing fiber yield potential of roselle *altissima* as well as its plant density. In addition, few reports have confirmed that wider plant spacing of 50 to 80 cm in roselle (*sabdariffa*) increased branching while reducing plant height (Okosun *et al.*, 2006; Shalaby and Razin, 1989).

Basal diameter was found to have significant positive correlation with plant height and branch number, but negative correlation with height at first branching. This association appears to be beneficial since tall plants with large basal diameter would have branching, if any, at high points. For high yield and good quality fiber, plants with high branching points are desired as the long fiber strands would have few or no interruptions. The low correlation coefficients indicate that only 1.21 % and 1.70 % of the variation in plant height is explained by variation in basal diameter and branch number, respectively. Similarly, 0.64 % of the variation in basal diameter is explained by height at first branching (Table 4.9). Coffie (2016) reported low to moderate positive correlation coefficient of $r = 0.11$ to 0.41 in plant height with number of internodes, branch number, leaf area and height at first branching in 35 *sabdariffa* genotypes from West Africa. Very low to moderate correlation coefficients were reported in basal diameter and plant height of 0.56 ($P < 0.01$), basal

diameter and number of branches of 0.42 ($P < 0.05$), basal diameter and days to 50 % flowering of 0.047 ($P > 0.05$), in kenaf genotypes in Bangladesh (Mostofa *et al.*, 2002). Knowledge regarding association of agronomic traits and their yield potential provides guidelines for breeders to make improvement in crops based on correlated traits, especially for characters that are difficult to evaluate or take long time to express.

Roselle is ambiphotoperiodic as it can flower both in short days or long days (Mansour, 1975) depending on the day length. The duration of the growing season and length of day are critical factors that have significant influence on the fiber yield characteristics of roselle (Dempsey, 1957). Roselle *altissima* typically grows to a height exceeding 250 cm with very few branches at high branching points at optimal environmental and climate conditions, which includes adequate irrigation, good soil nutrients, warm temperature and minimum day length of about 12 h 30 min. With a typical tropical day length that consisted of 12 hr 30 min and an average temperature of 25 °C (World Weather and Climate Information, 2017) during the trial period, the roselle genotypes studied were expected to have tall plant height. Plant height ranged from 154 to 342 cm and majority exhibited extensive and low branching. The unusual poor growth of some roselle genotypes may be attributed to biotic rather than abiotic stresses. High soil moisture conditions prevailed during the growing season, and plants were infected with *Fusarium oxysporum* during flowering. Low branching points and extensive branching are hindrance to fiber quality as fineness of fiber strands are reduced. Of the 25 roselle genotypes, only five accessions HA-39, HA-45, HA-46, HA-49 and HA-58 exhibited few branches to merit selection for improvement. On the basis of tall plants exceeding 250 cm and large stem diameter greater than 20 mm, twelve accessions, HA-37, HA-38, HA-42, HA-43, HA-44, HA-47, HA-50, HA-52, HA-54, HA-55, HA-

57, HA-58 were selected for further studies on yield improvement. At the district level, West Mamprusi, Kassena-Nankana West, Kassena-Nankana East and Bongo districts had tall plants with large stem diameter while accessions from Builsa-North had least number of branches.

The mean genetic distance of 3.03 ± 0.90 based on Euclidean estimates represents a substantial genetic diversity in the region. Coffie (2016) reported a mean genetic similarity distance of 0.27 ± 0.26 based on correlation coefficient among 35 *sabdariffa* landraces from West Africa. The fairly large genetic distances suggest that the accessions were divergent. Because the genetic distance was based on morphological evaluation, the influence of environment on the Euclidean estimates cannot be ruled out. Despite being a self-pollinating plant, the unexpected wide genetic variability may have arisen from forces such as gene or seed flow, climate and environmental variability, or mutation. An outcrossing rate of less than 1 % in roselle (Young, 1995; Vaidya, 2000) over many generations could create large variability.

Clustering of the accessions was independent of geographical origin, suggesting movement of seeds across the region. Accessions in cluster I were associated with late flowering and fairly high branching points. Cluster II accessions had very tall plants exceeding 250 cm with very thick stems and cluster III were grouped based on accessions having less number of branches. Each of the three clusters comprised at least one desirable trait of economic value. Hence, for any genetic improvement for better yield and quality in terms of bast fiber potential, there should be selection of parents across the three clusters.

The findings of Bakasso *et al.* (2013) revealed two major clusters in 124 roselle genotypes collected from Niger on the basis of ten agro-morphological traits which included plant height,

branch number and basal diameter. Coffie (2016) reported clustering of 35 roselle *sabdariffa* accessions into three main groups on the basis of six agro-morphological descriptors. Satyanarayana *et al.* (2015) reported of clustering of 60 roselle *sabdariffa* genotypes from India into seven clusters based on eleven agro-phenological traits. In their work, they showed that fiber yield per plant and dry stick weight were the most important while plant height and basal diameter were the least contributors to the variance.

Three of the five quantitative traits, namely, branch number, plant height, and days to 50 % flowering achieved the largest minimization of Wilk's lambda and were the most efficient traits in discriminating among the accessions. Further studies on structuring and genetic diversity in roselle *altissima* should consider these three traits. Coffie (2016) in evaluation of traits in roselle (var. *sabdariffa*) collected from West Africa, plant height, number of internodes and basal diameter were most important for structuring the genotypes, while Bakasso *et al.* (2013) found flowering time and 100-seed weight to be the two main discriminatory traits in evaluation of 124 roselle *sabdariffa* genotypes.

The first three PCs that explained 100 % of the total variance revealed that all five traits were relevant in structuring of *altissima* accessions. However, from the PCs, height at first branching, number of branches and height of plants are very critical in providing a selection guide when considering fiber potential of roselle *altissima* plants. Based on that, it is beneficial to select tall plants that have many branches at high branching points rather than tall plants with few branches at low branching points.

Delineating accessions into different groups have proved relevant for selecting desirable parents to maximize genetic variance in breeding programmes (Chakravarty and Basu, 1972). Hybridization of genotypes from uncorrelated groups is therefore expected to result in beneficial improvement in agronomic character performances. In contrast, the tetraploid nature in *altissima* rather promotes breeding success for trait improvement with members in similar groups than in uncorrelated groups. The biplots of the PCA showed a good contribution of the accessions to the variance, however, accessions HA-46, HA-47, HA-57 and HA-59, contributed substantially to the variance based on their projections from the origin. Overall, the large biodiversity residing in roselle *altissima* genotypes was revealed, hence, trait improvement in these accessions is possible. Similarly, the traits revealed an array of angles among themselves, indicating the diverse relationship between plant height, branch number, basal diameter, height at first branching and days to 50 % flowering.

4.5 Conclusions

Roselle *altissima* is not widely known in Ghana as a potential bast fiber crop despite its widespread occurrence in the northern part of the country. This research was conducted to provide information on potential regions of roselle collection, carry out a representative roselle collection, and estimate the genetic diversity therein. Twenty-five roselle *altissima* accessions from northern Ghana were evaluated for genetic diversity based on qualitative and quantitative measures of agromorphological traits. The qualitative descriptors revealed wide differences in morphology with a good number being uniformly green, with intermediate branching, smooth slender pentalobed leaves, yellow petals and crimson throat. All plants exhibited nonbushy growth habit, had hairy calyxes, and round capsule. The genotypes split into four distinct groups having similar qualitative

traits, each group having three to five accessions, while nine accessions were scattered and isolated from the groups. Of the four, Group IV with uniformly green with few branches was the most important considering economic value. Green roselle are known to possess higher stalk yield than the red types and few branching is desirable for higher quality fiber. On this basis, accessions HA-48, HA-53, HA-55, HA-56, and HA-58 were selected for breeding programme. Branching habit being qualitative trait is not enough to distinguish accessions. Nevertheless, branching habit may be considered as a quantitative trait upon counting the number of branches and analyzing the data statistically. As such, individual plants within the three categories of few, intermediate and extensive can be distinguished. The power of the test and informativeness can then be improved. On quantitative trait, the extensive branching was unexpected and represented a departure from roselle *altissima* accessions previously described. Earlier description of roselle *altissima* was based on genotypes that had been introduced to regions outside the center of diversity, which were unbranched. The discovery of branched types in the current study indicates the existence of other morphotypes in the roselle gene pool which have not yet been characterized. Further collection in the centers of diversity, followed by a comprehensive characterization and documentation is needed.

The large within and between population variability, and the large genetic distance demonstrate that the accessions were divergent. Wide genetic variability is a prerequisite for making progress in trait improvement during breeding.

Roselle *altissima* plants that exhibited tall plant height and few or many branches at high branching points were selected for incorporation into a breeding programme. Sixteen accessions selected on the basis of tall plant height and few branches were HA-37, HA-49, HA-50, HA-55, HA-57, and HA-58 in cluster I, HA-38, HA-42, HA-43, HA-47, HA-52, and HA-54

in cluster II, and finally, HA-39, HA-44, HA-45, and HA-46 in cluster III. The correlated accessions that were grouped on the biplot was in agreement with clustering. This observation indicates the power of cluster analysis and PCA in identifying relationships among genotypes. The discriminant analysis revealed that branch number, plant height, and days to 50% flowering were the most important traits that contributed to the variance in the data. Future studies on roselle should incorporate these three traits. Because roselle is day length sensitive, evaluation in another geographical location would be necessary. To overcome the environmental sensitivity among roselle genotypes, further work on molecular genotyping is required.



CHAPTER FIVE

5.0. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

In summary, the goal of this study was to evaluate a representative collection of 61 roselle *altissima* accessions from northern Ghana and to estimate the genetic diversity and relationships that lies therein based on agro-morphological trait evaluation. Motivation for the study was that there had not been any study to evaluate and estimate genetic diversity in roselle *altissima* in Ghana. With no breeding programme in roselle *altissima* in Ghana, no genotype has been exploited to identify useful traits for bast fiber improvement nor inform conservation management of its germplasm. Moreover, renewed interest in and increasing global demand for natural bast fiber presents roselle *altissima* as a candidate crop for exploration and exploitation to the benefit of Ghana, where the crop grows abundantly. Unfortunately, commercialization of roselle *altissima* in Ghana is non-existent but rely on bast fiber imports from Asian countries for bagging cocoa beans and other agriculture produce.

Furthermore, there are reports of narrow genetic variability in roselle *sabdariffa* genotypes of Asian origin, while mutation breeding resorted to, in introducing variability in roselle is fraught with many limitations including physiological damage to plant and distortion of leaf shape and flowers, among other undesirable outcomes. The assertion that West Africa is the probable center of roselle diversity needs validation as, there is dearth of information on systematic collection and conservation in roselle *altissima*.

While extensive studies have been carried out in roselle *sabdariffa* in other countries, there is very little information available on roselle *altissima* and in Ghana, information is absent. It was imperative to carry out this research to investigate the nature and magnitude of genetic diversity that resides in roselle *altissima* collections from northern Ghana, to identify genotypes worth incorporating into breeding programmes, to determine groupings and relationships among the genotypes for effective breeding systems. The adopted approach involved a systematic collection of 61 roselle *altissima* accession seeds from various locations in northern Ghana and evaluating the genotypes using agro-morphological markers in field trials over two seasons (2016 and 2017) to estimate genetic variability within and between the roselle populations.

The study revealed substantial variation in both qualitative and quantitative traits. Qualitative traits exhibited varying discrete classes and revealed wide variations in phenology for plant, stem, leaf, calyx and flower characteristics in *altissima* while, growth habit, calyx pubescence and capsule shape were rather conserved. The rest of the traits exhibited many discrete classes including plant type, stem pubescence, branching habit, and leaf form. These wide differences in morphology based on qualitative descriptors may be controlled by gene(s) rather than environmental influence. Roselle plants having uniformly green stems, few branching and high branching points are more desirable for fiber production than pigmented, extensively branched, and low branching types.

On the basis of quantitative evaluation, the large within and between population variability, and the large genetic distance demonstrated that the *altissima* genotypes were divergent. The large variability revealed in plant height, basal diameter, branch number, height at first branching and days to 50 % flowering based on the moderate to large coefficient of variation, and highly significant mean squares are very important for breeders for incorporation into breeding

programmes. Wide genetic variability is a prerequisite for making progress in trait improvement during breeding.

Roselle *altissima* plants that exhibited tall plant height and few or many branches at high branching points and large stems were identified to be good sources of bast fiber and warrants further breeding programmes aimed at improving desirable traits for higher fiber yield and quality. Twenty-one accessions were selected on the basis of tall plant height with branches at high branching and large basal diameter were HA-07, HA-11, HA-12, HA-21, HA-33, HA-37, HA-38, HA-39, HA-42, HA-43, HA-44, HA-45, HA-46, HA-47, HA-49, HA-50, HA-52, HA-54, HA-55, HA-57, and HA-58.

Based on SDI, seven districts of roselle *altissima* diversity in decreasing order were Kassena-Nankana (0.87), Bolgatanga Municipal (0.83), West Mamprusi (0.82), Savelugu-Nanton (0.73), Tamale Metropolitan (0.68), Gowri-Yororgo (0.67) and Talensi (0.67).

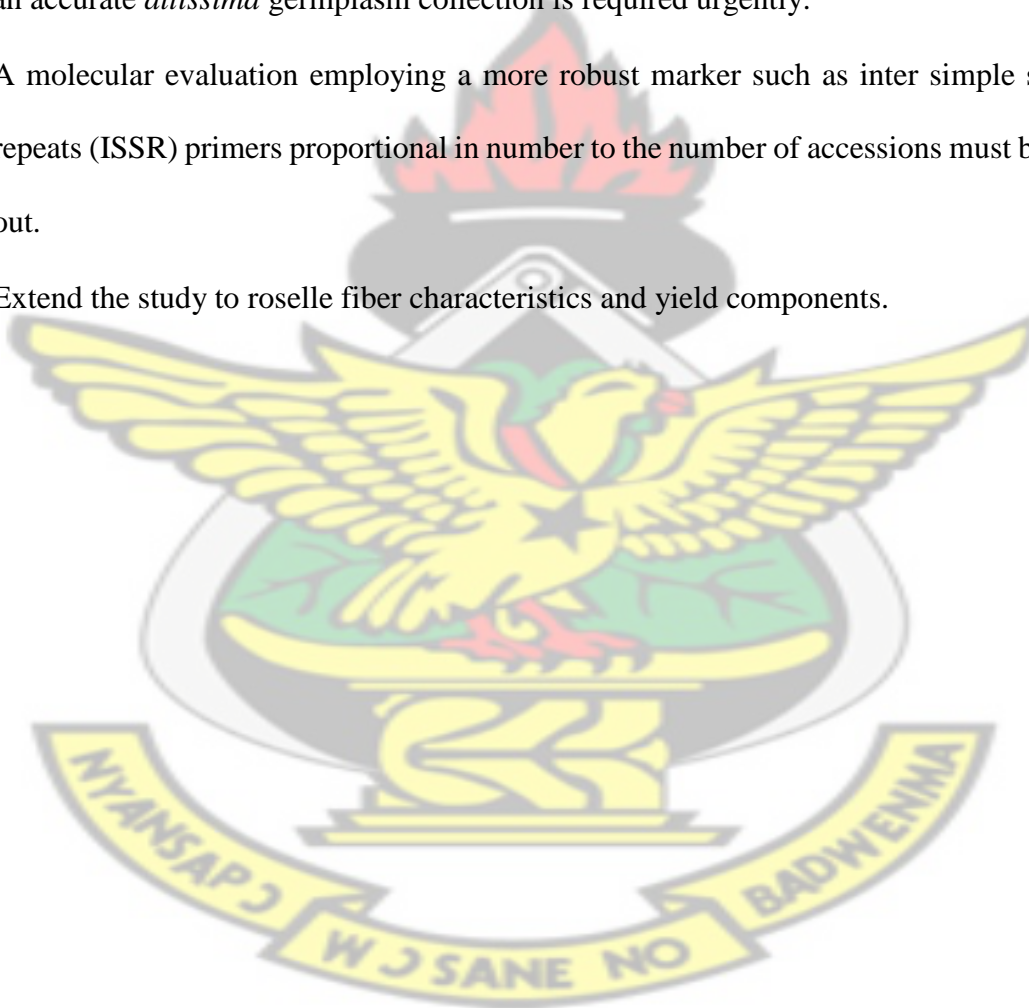
This result holds true with previous work that the roselle genotypes in West Africa are endowed with wide genetic variability in pheno-morphological traits, besides the identification of genotypes of economic importance await their exploitation for genetic improvement, particularly for fiber yield.

The major limitations encountered during the research were unequal distribution of accessions collected from the various towns in northern Ghana and not including a DNA-based molecular assessment in the genetic diversity studies of roselle *altissima* that may have revealed more subtle genetic information.

5.2. Recommendations

Based on the outcome and the limitations of the current study, the following recommendations are made:

- A wider collection of *altissima* genotypes representative of various geographical locations in northern Ghana is required.
- A well-planned seed collection by team of taxonomist and the indigenous folk to provide an accurate *altissima* germplasm collection is required urgently.
- A molecular evaluation employing a more robust marker such as inter simple sequence repeats (ISSR) primers proportional in number to the number of accessions must be carried out.
- Extend the study to roselle fiber characteristics and yield components.



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