INTRA-SPECIFIC HYBRIDISATION AND PERFORMANCE EVALUATION OF HYBRID LINES OF ROSELLE (*Hibiscus sabdariffa* L.)

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By

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DECLARATION

This thesis is the outcome of research work undertaken by Joel Kwofie in the Department of Nuclear Agriculture and Radiation Processing of School of Nuclear And Allied Sciences, University Of Ghana, under the supervision of Dr. H. M. Amoatey and Dr. S. Amiteye.

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DEDICATION

This work is dedicated foremost to the Lord God Almighty for his protection and guidance and to my late father Mr. J. W. Kwofie, my loving and caring mother, Mrs. Veronica Kwofie and siblings Justice Kwofie and Juliana Kwofie for their support and prayers.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of Variance
BNARI	Biotechnology and Nuclear Agriculture Research Institute
BSc	Bachelor of Science
Ca	Calcium
Cm	Centimetre
FAO	Food and Agriculture Organisation
FAOSTAT	Food and Agriculture Organisation's Statistical Database
Fe	Iron
Fig.	Figure
GAEC	Ghana Atomic Energy Commission
ha	Hectare
IBPGR	International Board for Plant Genetic Resources
IPGRI	International Plant Genetic Resources Institute
K	Potassium
M	Meter
Mg	Milligramme
Mg	Magnesium
mg/g	Milligramme per gramme
MPhil	Master of Philosophy
Na	Sodium
PGRRI	Plant Genetic Resources Research Institute
pH	Hyrogen ion concentration
RCBD	Randomised Complete Block Design
SNAS	School of Nuclear and Allied Sciences
UG	University of Ghana
UNESCO	United Nations Educational, Scientific and Cultural Organisation
US	United States

ABSTRACT

Roselle (*Hibiscus sabdariffa* L.) is an important multi-purpose crop in Ghana and across West Africa, used as a source of food as well as raw material for medicinal and industrial applications. However, due to limited research efforts to gather relevant information on breeding behaviour and inheritance of desirable traits of existing cultivars to enhance yield and nutritional contents, economic potential of the crop has not been fully exploited. The present study was therefore initiated with a broad objective of assessing breeding behaviour and nature of inheritance of selected qualitative and quantitative traits and also variability with respect to agro-morphological characteristics, as well as nutritional composition of leaves and calyces, among 20 local accessions of roselle (*Hibiscus sabdariffa* L.); comprising 6 accessions of *H. sabdariffa* var *altissima* and 14 accessions of *H. sabdariffa* var *sabdariffa*. The accessions were collected from three major production regions of Ghana (Northern, Volta and Western). The investigations were carried out at the research fields and laboratories of Biotechnology and Nuclear Agriculture Research Institute (BNARI) from April 2013 to November 2014. All six accessions of var *altissima* were able to hybridize with one another in both direct and reciprocal cross combinations with high degree of crossability success (30 % to 80 %). On the other hand, cross compatibility among the 14 accessions of var *sabdariffa* was direction-dependent, and crossability success was low (0.00 % to 43.33 %), as some parents could only be used as males or females. Parental lines A3 and A11 emerged as most compatible male and female respectively. Crossability success was relatively high during early hours of the day but decreased continuously in subsequent hours. Fourteen parental accessions and 25 F1 progenies of var *sabdariffa* evaluated for 23 agro-morphological traits exhibited significant variations in all quantitative traits studied.

Clustering pattern based on both qualitative and quantitative traits largely reflected colour and size of calyx. Leaf and calyx traits showed strongest positive association among pairs of quantitative traits investigated. Accessions D2, D3, D4 and D6 exhibited highest general combining ability for major yield traits; days to first flowering, number of fruits per plant, plant height and stem basal diameter, while D2 x D5, D5 x D2, D2 x D4, D6 x D5, D3 x D6 and D6 x D4 emerged as most promising in specific cross combinations following a diallele experiment. Concentrations of the various nutrients were generally higher in calyces compared to the leaves. Except for total titrable acid and vitamin C contents, F1 offspring performed better on all other parameters than their parental accessions and control varieties. Calyces of A11 x A1 and A11 x A13 contained highest protein, ash and pH contents and relatively low moisture as well as highest concentration of K (3284.04±0.01 mg/100 g), Fe (19.45±0.02 mg/100 g) and Ca (1360.70±1.0 mg/100 g), while A4 x A1 recorded highest concentrations of Mg (4.30±0.88 mg/100 g). With regards to the leaf samples, A7 x A12 produced highest values for Ca (1440.20±1.00 mg/100 g) and Fe (15.59±0.01 mg/100 g), while A11 x A2 gave highest Mg (2.72±0.13 mg/100 g) and Na (32.93±1.15 mg/100 g) contents. Similarly leaves of A2 gave highest concentration of K (1641.08±0.01 mg/100 g). These results demonstrate possibility of producing superior hybrids of roselle through artificial cross-pollination. Key recommendations based on these findings include: i) ascertaining specific barriers to crossability between accessions of *H. sabdariffa* var *sabdariffa* and *H. sabdariffa* var *altissima* to aid transfer of useful traits between them. ii) use of molecular markers to confirm results of morphological characterisation and also to better understand inheritance of qualitative traits. iii) composite breeding to incorporate as many desirable traits as possible into one or two improved (standard) variety/varieties.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background

Roselle (*Hibiscus sabdarifa* L.) is an annual or biennial crop cultivated within tropical or sub-tropical regions (Adamson and 1981) which provides food (vegetables, source of oil, refreshing drink), medicine and raw materials for industries (Gautam, 2004). It belongs to the family Malvaceae which consists of many important species including a number of other food, fibre, and medicinal crops such as cotton, okro, and kenaf (Anderson and Pharis, 2003).

Morphologically, roselle (*Hibiscus sabdarifa* L.) is closely related and shares similar traits such as leaf and flower structures with kenaf (*Hibiscus cannabinus* L.), but can be distinguished by its flower colour and size of the calyx (Morton, 1987). Two sub-species *H. sabdariffa* var. *sabdariffa* and *H. sabdariffa* var. *altissima* are recognised, distinguishable based on their growth habits and end use. Var. *sabdariffa* comprises bushy and pigmented types cultivated for their edible calyces, whereas var. *altissima* consists of tall-growing, usually unbranched types which produce inedible calyces and are mainly cultivated for stem fibre (Gautam, 2004).

Roselle is also popularly known as „sorrel‟ in English speaking regions (Gautam, 2004), „karkade‟ in the Middle East and North Africa, and „bissap‟ in West Africa (Omemu *et al*., 2006). In Ghana, it is usually called „sobolo plant‟ in the southern part of the country, a name derived from a popular non-alcoholic beverage made from the calyces. However, in the northern part of Ghana where the crop is widely cultivated it is popularly known as „surre‟, apparently an altered form of „sorrel‟.

Roselle is a valuable food resource due to its store of nutrients; largely proteins, fats, vitamins and essential elements. The seed contains substantial amounts of fibre and valuable micro-nutrients as well as high amounts of calories and proteins which are higher than those of most common legumes cultivated in the tropics such as cowpea (*Vigna unguiculata* L.), pigeon pea (*Cajanus cajan* L.), soya bean (*Glycine max* L.) and groundnut (*Arachis hypogaea* L.) (FAO, 2001). Additionally, oil extracted from the seed is low in cholesterol (Ahmad *et al*., 1979).

The leaves, which are usually consumed as green vegetables in soups and stews contain considerable amounts of micronutrients such as iron, manganese, copper and zinc (Sena *et al*., 1998; Smith *et al*., 1996). The most widely utilised part of the plant, the calyx (cup-like structure which encloses the fruit capsule) is highly touted in culinary and medicinal applications for its outstanding nutritional and phytochemical properties. It contains high amounts of organic acids (Cissé *et al*., 2009) including protocatechuic acid (Dickel *et al*., 2007; Herrera-Arellano *et al*., 2004), and all essential amino acids (Glew *et al*., 1997).

In addition, it contains greater amounts of anthocyanins than most edible crops (Mazza and Miniati, 1993). Anthocyanins are large groups of water soluble pigments highly valued mainly for their antioxidant and anti-cancer properties (Philpott *et al*., 2004). Moreover, the calyx which contains nine (9) times more vitamin C than citrus (*Citrus sinensis*) provides readily available and inexpensive source of vitamin C (Amin *et al*., 2008).

Besides, it also contains hydroxycitric acid (HCA), an organic compound used as weight loss agent (Mohamed *et al*., 2007) and holds high amount of mucilage and pectin which are very essential in manufacturing jam, liquor, and jellies (Akindahunsi and Salawu, 2005).

Roselle has also received enormous attention in traditional medicine for its antiseptic, aphrodisiac, astringent, diuretic, emollient, purgative, and sedative properties (Mahadevan and Kamboj, 2009). Fibre obtained from the stem also provides raw material for the manufacture of a wide range of fibre and paper-based products (McClintock *et al*., 2004).

1.2 Statement of problem

Despite the high nutritional and phytochemical properties and hence potential as food, medicinal or industrial crop, roselle still remains highly underutilised and genetically unimproved in Ghana. Cultivated types are typically local landraces, which in spite of their adaptation to marginal conditions, exist in primitive state and are characterised by slow growth and low yields (Atta *et al*., 2011).

Besides, roselle germplasm are threatened by a number of biological and socio-economic factors; cleistogamous mode of reproduction (obligate self-fertilising) and substitution by other crops predispose the crop to narrow genetic diversity and high risk of germplasm erosion (El Tabir and El Gabri, 2013). Moreover, anthocyanins from roselle are known for their instability, a factor which limits their application as colourants in complex food formulations (Mazza and Miniati, 2000; Esselen and Sammy, 1975).

Currently, there is low adoption of roselle by local farmers in Ghana due to a combination of factors; including incidence of pests and diseases. Even with increasing exploitation of the crop and its products, particularly the delectable "sobolo" drink across the country, a chunk of the raw material (dry calyces) is imported from neighbouring countries such as Cote d'voire and Burkina Faso (Blench, 2007). Unfortunately, very little research attention by way of breeding has been directed to the study of genetic diversity and breeding behaviour of existing local landraces in order to facilitate improvement of the crop. Consequently, the full economic potential of roselle has not been realised in Ghana.

1.3 Justification

Intra-specific hybridisation offers a remedy for introducing genes for resistance and other agronomically desirable traits such as high yield into the locally adapted cultivars of roselle in order to create segregating populations from which new varieties with combined desirable characteristics may be selected. Successful hybridisation will help to raise the output of existing local cultivars, to expand adoption by farmers in Ghana and across the West African sub-region for commercial cultivation. In addition, it will help to expand the genetic base of the crop and bring greater diversity in cropping systems to enhance food security to especially subsistence farmers.

Development of superior varieties with high nutritional and phytochemical contents will also open up new options for diversified use of roselle in order to meet the varied preferences of end-users and provide potential market benefits to farmers.

Moreover, intra-specific hybridisation among existing cultivars could produce new varieties with greater stability of anthocyanin to permit market entry of roselle as a natural colourant, estimated at 940 million US dollars ($) per year, compared to the market for artificial colourants (400 million US dollars) (Tsai *et al*., 2002).

However, the outcome of hybridisation depends on the extent of genetic diversity and compatibility between genotypes used as parents, as well as adequate knowledge of the nature of inheritance of desirable traits. Concerted efforts are therefore needed to gather relevant information on genetic potential and breeding behaviour of roselle germplasm scattered across Ghanaian order to undertake hybridisation towards improvement of existing local cultivars. Characterisation and investigations of crossability and combining would provide good basis studies for selecting potential parents to be utilised in future breeding work.

1.4 Objective

The main aim of this study was to assess breeding behaviour of 20 local landraces of roselle (*H. sabdariffa* L.) and subsequently investigate the nature of inheritance of selected qualitative and quantitative agro-morphological traits through characterisation of the parental varieties and their intra-specific F1 offspring to identify superior hybrids for selection.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and Distribution

Roselle (*Hibiscus sabdariffa* L.) is widely accepted to have originated from West Africa where diverse wild types are found particularly in Ghana, Niger, Nigeria (Wilson, 2006), Senegal and Mali (McClintock and El Tahir, 2004). The species (*Hibiscus sabdariffa* L.), is believed to have originated from hybridisation between *Hibiscus cannabinus* (AA) and an unknown Y genome species (YY) followed by doubling of the chromosomes of the resulting diploid species (Satya *et al*., 2013).

The crop was first domesticated in western Sudan before it was disseminated throughout the tropical regions of the world such as Asia where it was developed as a fibre crop and Central and Northern America (Wilson, 2006). Roselle is also known by different names across the world (Table 2.1).

Table 2. 1. Names of roselle in diferent regions of the world.

Region	Vernacular name	Source
Caribbean	Sorrel	Halimatul *et al*., 2007
Latin America	Jamaica	
Florida	Cranberry	
Asia	Mesta, Meshta	
Sudan and Middle East	Karkade	Abu-Tarboush *et al*.,1997
Southern Africa	Omutete	Omemu *et al*., 2006.
West Africa	Bissap	
Ghana	Riaripari (Guan), Siiro, Soboro or Rarna in (Hausa), Digbeme or Injamgbam (Dagbani) Sakpa (Ga), Evema (Ewe) Bito (Moshii),	Irvine, 1961.

Adapted from: Mehdi *et al*., (2013).

The largest producers of roselle are China and Thailand with minor producers being Mexico, Egypt, Senegal, Tanzania, Mali and Jamaica whose productions are mostly used domestically (FAO, 2007).

2.2 Morphological Description

The roselle plant (*H. sabdariffa*) comprises two main cultivated types; var *sabdariffa* L. and var *altissima* W. Var *sabdariffa* includes cultivated types which produce large fleshy calyces, predominantly used as food, while var *altissima* on the other hand comprises generally tall growing, unbranched types which bear inedible calyces, and are cultivated for their stem fibre (Gautam, 2004) (Fig.2.1).

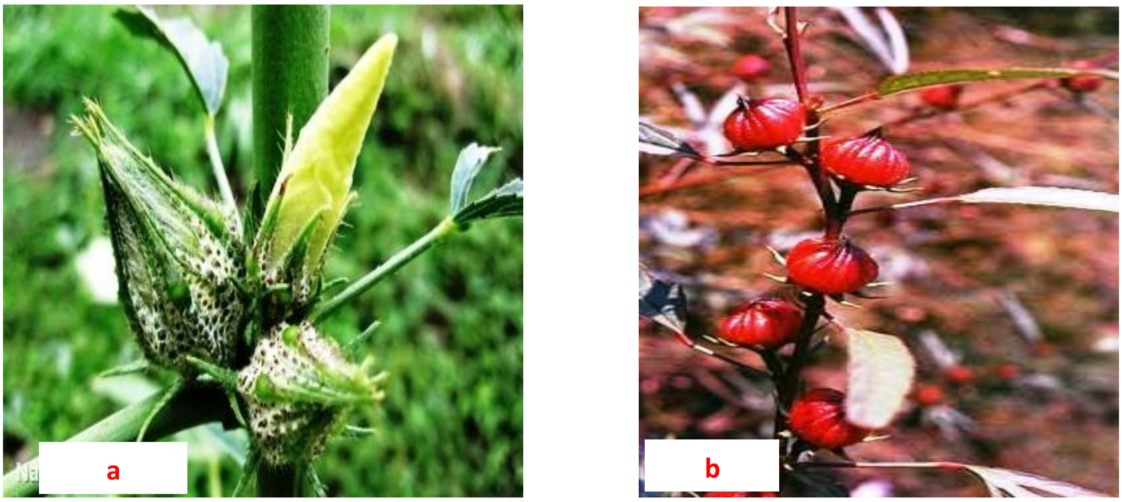

Fig. 2.1: Cultivated types of roselle; (a) var. *altissima* and (b) var. *sabdariffa*.

Source: Qi, *et al*., (2005).

In most West African countries including Ghana, the green and red cultivars of var *sabdariffa* are commonly cultivated as leafy vegetable and to supply raw material for making the very popularly beverage known as „„bissap‟‟ across the Sub-region respectively (McClintock and El Tahir, 2004).

Roselle plant generally grows to a height of 3.5 m tall and has smooth or slightly rough, cylindrical dark green to red stems. The leaves range from 7.5 cm to12.5 cm in length, and are usually green or may have reddish veins with long or short petioles. The leaves of young seedlings and young leaves of older plants are simple but mature leaves may be 3 to 5 or even 7 – lobed and with serrated margins (McClintock and El Tahir, 2004).

Very bright yellow or red hibiscus-like flowers measuring about 12.5 cm wide are borne singly in the leaf axils of roselle which change to pink as they wither. The calyces (cup-like structures which enclose the fruit capsules) consist of 5 large sepals with 8 to 12 thin, pointed bracts (epicalyx) around the base of each flower (Fig. 2.2) (McClintock and El Tahir, 2004).

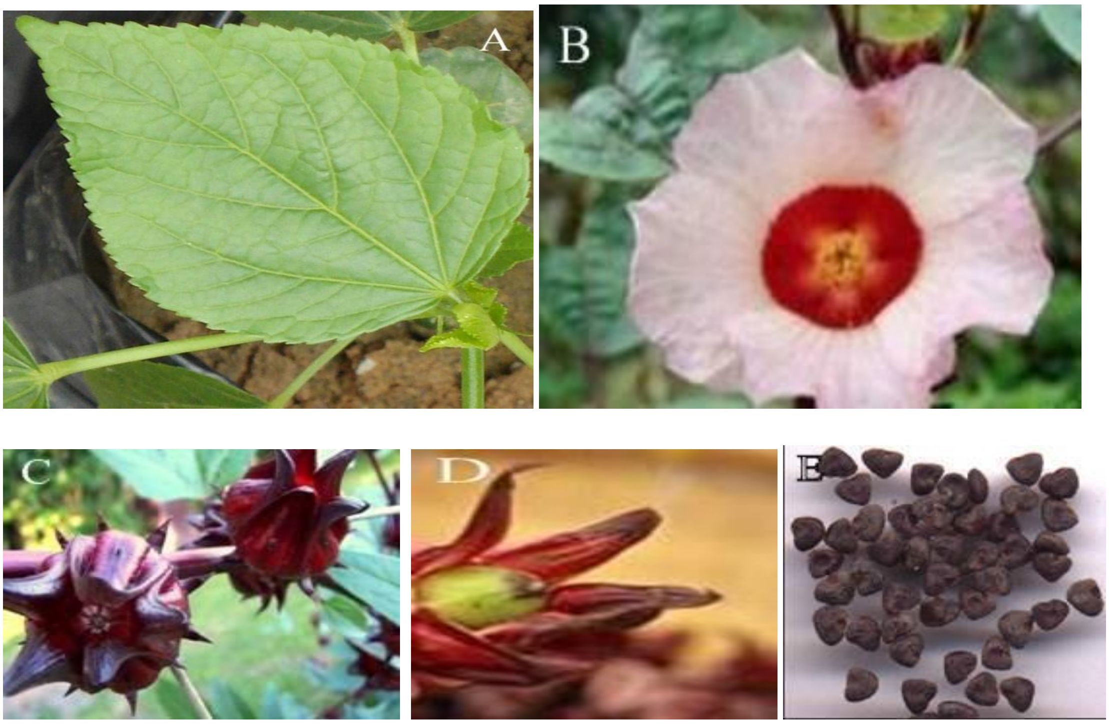

Fig. 2.2: Morphology of roselle (*H. sabdariffa* L.) plant; (A) Non-lobed leaf, (B) flower with red colouration of petal base, (C) red calyces, (D) fruit capsule and (E) dark brown kidney-shaped seeds.
Source: Mc Clintock and El Tahir (2004).

The fruit is a 5-valved velvet capsule measuring 1.25 cm – 2 cm long which is green when immature, but turns brown and splits open when mature and dry. Each valve contains 3 – 4 kidney-shaped dark-brown seeds which measure 3 mm – 5 mm long (McClintock and El Tahir, 2004).

2.3 Classification and Cytology

Taxonomically, roselle (*Hibiscus sabdariffa* L.) is classified into the family Malvaceae, genus *Hibiscus* and along with 40 – 50 other species into section Furcaria. Thirteen genomes have been identified among species of Furcaria (A, B, C, D, E, G, H, J, P, R, V, X, Y) of which nine (9) (A, B, C, D, E, G, H, X, Y) are distributed in Sub-Saharan Africa, being the centre of genome diversity (Wilson, 1999).

The A, X, and Y genomes occur in only diploid and tetraploid species in Africa which include *H. sabdariffa* (AAYY, 2n = 4x = 72), *H. cannabinus* (AA, 2n = 2x = 36), *H. acetosella* (AABB) and *H. radiatus* (AABB) (Satya *et al.*, 2012; Wilson, 2006). Roselle is an allotetraploid species (AAYY, 2n = 4x = 72) which is closely related to kenaf (*H. cannabinus* L.) (AA, 2n = 2x = 36) (Satya *et al*., 2013; Akpan, 2000).

2.4 Environmental adaptation

Roselle exhibits wide adaptation to different environmental and ecological conditions across many countries, hence can grow in almost every continent on the globe. However, it thrives best in warm and humid tropical climate, up to an altitude of 900 m above sea level. It tolerates a temperature range of 18 °C to 35 °C, but 25°C is required for optimum growth (McClintock, 2004).

Roselle is a short-day plant which is very sensitive to photoperiod; it requires daily light phase of 11 hours during the first four weeks of its growth in order to flower and flowering may delay up to five months if daily light illumination exceeds 13 hours (Adamson and O'Bryan (1981). Rainfall of 400 mm to 500 mm per annum (Augstburger, 2000) or minimum of 100 mm to 150 mm per month (McClintock and El Tahir, 2004) is required during its cultivation to achieve optimum yield. Though it tolerates a wide variety of soils, moderate to slightly acidic, well-drained, heavy loam soils ensure better growth of roselle (Das and Das, 2000).

2.5 Propagation and cultivation

The roselle plant is usually propagated from seed but can also be planted vegetatively from stem cuttings. The seeds are sown through broadcasting or drilling at a spacing of 40 cm – 60 cm between rows and 20 cm – 300 cm within rows (McClintock and El Tahir, 2004).

Traditionally, roselle is cultivated as a subsistence crop by small-scale farmers under rain-fed conditions along field boundaries or intercropped with staple crops. It requires little attention during cultivation with very little or no fertilizer application (Abdelatif, 2009). The vegetative period of roselle ranges from 130 – 180 days, but it takes about six months to mature (McClintock and El Tahir, 2004).

2.6 Harvesting and Post-Harvest processing

Harvesting and post-harvest processing can greatly affect the quality of products obtained from roselle. The fruits are harvested when matured but tender and processed through decoring or scouring techniques (Fig. 2.3) to separate the calyces from seed capsules (Duke, 2004). To obtain the fibre, the stems are soaked in water for about two weeks followed by stripping of the bark and beaten to detach the fibre (Fig. 2.3).

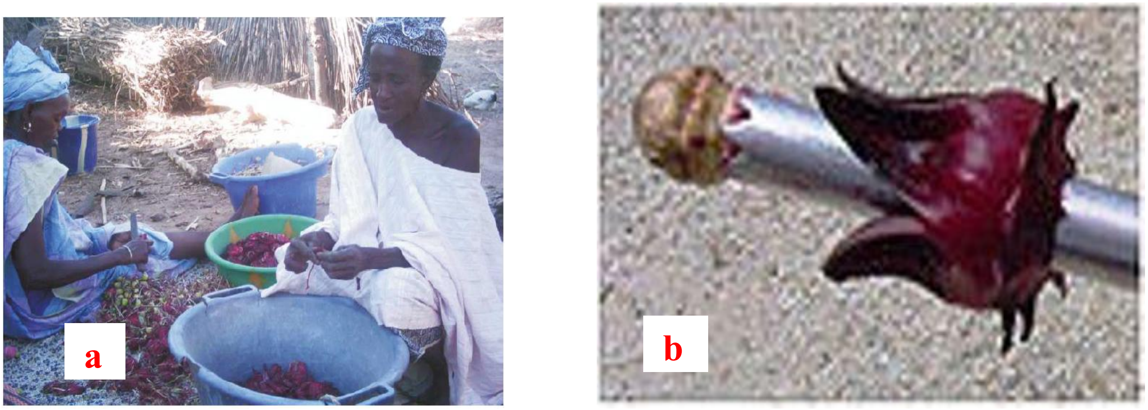

Fig 2.3a: Post harvest operation for separating calyx from fruit capsule of roselle; (a) Manual scouring and (b) decoring.

Source: Cisse *et al*., (2009).

Fig. 2.3b: Extracting bast fibre from roselle; (1) soaking of stems, (2) detached fibres and (3) drying of detached fibres.

Source: Mwasiagi *et al*., (2014).

2.8.1 Nutritional Composition

The roselle plant is bestowed with a wealth of nutritional qualities which are superior to those of several food crops cultivated in the tropics (FAO, 2001). Proximate examination reveals substantial amounts of protein, carbohydrates, fat, crude fiber, and ash in the flowers, calyces, and seeds on dry weight basis (Table 2.2). Predominant minerals found in the calyces are potassium, magnesium, calcium, phosphorus, sodium, iron and manganese (Table 2.3).

Table 2.2. Proximate composition (mg / 100 g) of different organs of roselle

Nutrient	Flower	Red Calyx	Green Calyx	Seed
Ash	9.75±0.59	12.24	6.83	6.89
Fat	0.59±0.06	2.01	2.17	21.60
Crude Fibre	33.9±3.59	4.69	6.75	4.12
Protein	9.87±0.28	4.71	6.45	31.02
Moisture	4.38±0.05	7.60	6.24	9.25
Carbohydrate	4.38 ±0.05	68.75	71.56	36.37

Source: Sayago-ayerdi *et al*., 2007; Hainida *et al*., 2007.

Table 2.3 Elemental Composition of calyxof roselle

Element	Composition(mg / 100 g)
Sodium	96. 66
Potassium	49. 35
Calcium	12.65
Magnesium	38.65
Iron	3.22
Zinc	12.22
Manganese	2.39
Phosphorus	36.30

Source: Adanlawo and Ajibade, 2006.

The calyces also contain major organic acids such as citric acid, malic acid and tartaric acid resulting in the low pH (2 – 2.5) (Ali *et al*., 2005) as well as vitamins and bioactive compounds (Cissé *et al*., 2009). .

2.8.2 Phytochemical content

Numerous chemical compounds which are of immense benefit to human health such as flavonoids, phenolic compounds, organic acids and alkaloids have been identified in various organs of the roselle plant (Ramirez-Rodrigues *et al*., 2011; Christian and Jackson 2009; Olaleye 2007; Tsai *et al*., 2002).

The calyces contain anthocyanins; delphinidin-3-sambubioside and cyanidin-3-sambubioside which are responsible for the red colour (Mazza and Miniati, 1993) and major contributors to antioxidant activity (Tsai *et al*., 2002). Also, the calyx contains gossypetin, saponins, protocatechuic acid, glycoside and hibiscin (Olaleye 2007). Moreover, extracts from the calyx contain up to 28 % of hyrocitric acid (HCA) a chemical compound which is used as weight loss agent (Mohamad, *et al*., 2007).

2.9. Uses of roselle

2.9.1. Uses of roselle as food

Despite being cultivated on marginal scale, roselle occupies a very prominent place in the diets of many households living especially in the tropics by supplying cheap and readily available source of nutritional supplements; almost every part of the plant, from the leaf to the root is consumed as food (Morton 1987). The leaves are predominantly used to prepare potherb or sauce which is consumed with tubers or cereals particularly among rural communities across West Africa (Amusa *et al*., 2005).

The seed is consumed as substitute for meat by rural communities across West Africa (Parkouda *et al*., 2008), after subjecting it to a kind of fermentation process to produce a condiment popularly known as „dawadawa-botso‟ (Niger), „bikalga‟ (Burkina Faso), „datou‟ (Mali), and „furundu‟ (Sudan). It is also consumed as substitute for groundnut in some parts of Africa (Augstburger *et al*., 2000). Oil obtained from the seed is also used for cooking in Tanzania, China and West Africa (Halimatul *et al*., 2007).

2.9.2 Use of roselle as beverage

Dried calyces of roselle are used to make a kind of non-alcoholic beverage which is consumed in different parts of the world. The beverage is prepared by boiling the dried calyces with mint or ginger and may be sweetened with sugar. „„Sobolobo‟‟‟or „„Bissap‟‟‟ as it is popularly known in Ghana and across West Africa, is usually bottled and sold in markets, along roadsides or in restaurants. It also forms a prominent part of refreshment packages served at social gatherings such as weddings, funerals and out-doorings (Morton 1987).

The dried calyces are also used to make wine, jelly, syrup, gelatin, pudding as well as flavour for liqueurs (Furia, and Bellanca, 1971). The seed is processed into „‟tea bag‟‟‟ which is used as substitute for coffee especially by people who are sensitive to stimulants (Augstburger *et al*., 2000).

2.9.3 Other uses of roselle

Roselle serves as an attractive garden plant; the flowers and red stalks are used for decoration. The calyx extract is used as a dye (Grubben and Denton, 2004), while bast fibre obtained from the stem is used as substitute for jute fibre in making twines, cordage, rope, netting and sacks, and as raw material for the paper industry in the United States and Asia (Adamson and O'Bryan, 1981).

Oil extracted from the seed is used for making soap (Morton, 1987), and the residue after extracting the oil as well as the calyces and seeds are valued as high-protein feed for cattle and chicken (Hiernaux and Herault, 2003).

2.10. Therapeutic properties

Medicinal qualities of roselle have long been recognised in folk medicine. The leaves and seeds are also used for treating scurvy, sores, and ulcers and as emollient for the treatment of coughs and after effects of drunkenness (Prasongwatana *et al*., 2008).

Roselle (*Hibiscus sabdariffa*) plant is rich in antioxidants such as anthocyanins, phenolic compounds (Obah and Rocha, 2008), and a mixture of organic acids such as citric, malic and tartaric acids (Ali *et al*., 2005), which are of immense value in treating diseases. Recent studies demonstrate the efficacy of extracts of roselle calyces for treating gastrointestinal, kidney, liver and cardio-vascular disorders such as coronary heart disease and high blood pressure (Khalid *et al*., 2012; Alarcón-Alonso *et al*., 2012; Odigie *et al*., 2003). Besides, quercetin of the calyx is used to control *Mycobacterium tuberculosis* (Alarcón-Alonso *et al*., 2012) and leukemia (Tseng *et al*., 2000).

2.11 Socio-economic importance of roselle plant

Information on production and marketing of roselle in Ghana is not readily available. Yet, estimates indicate that it provides substantial income to many farmers, wholesalers, street vendors and exporters, who engage in its cultivation and marketing.

Production of roselle in Senegal is estimated at 700 metric tonnes per year, which involves 30 000 to 40 000 producers and an average cultivated area of 65 million m^2 (6500 ha) (Cisse *et al*., 2009). Exported quantities from Sudan in 2011 and 2012 were 18531 metric tonnes and 15656 metric tonnes, with corresponding total income of 17.59 and 14.09 million US dollars respectively (Central Bank of Sudan, 2012).

Export of roselle comprises mainly calyces to the United States, France and Germany (McClintock and El Tahir, 2004). Quantities being exported are far below the demand for roselle products internationally (Cisse *et al*., 2009). Women benefit mostly from cultivation of roselle, particularly in Africa where access to land is often a major challenge for women since it is usually cultivated along field boundaries (McClintock and El Tahir, 2004).

The beverage also forms a key part in the organisation of social events such as weddings, funerals and out-dooring ceremonies across West Africa (Schippers, 2000).

2.12 Pests and Diseases of roselle

The roselle plant is host to several pests including root-knot nematode (*Heterodera rudicicola*) and beetles (*Nisotra breweri* and *Lagris cyanea*) which attack the roots and leaves.

Other pests such as scales, *Coccus hesperidum* and *Hemichionaspis aspidistrae*, yellow aphid (*Aphis gossypii*) cotton stainer (*Dysdercus suturellus*) also cause minor damages to leaves, flower buds and ripening calyces (McClintock and El Tahir, 2004).

Common diseases of roselle are root rot, stem rot, leaf spot (Eslaminejad *et al*., 2012), leaf curl, and yellow vein mosaic (Chatterjee *et al*., 2005). Crop losses from disease attack on roselle can be significant. Estimated crop losses for the period 1995 to 1998 in Grenada alone were 18.3 million US dollars and the cost of control was 1.1 million US dollars (Kairo *et al*., 2000).

2.13 Natural crossing of roselle

Roselle is naturally self-pollinating due to the cleistogamous nature of flowers. Results of various crossability studies in roselle so far indicate that natural cross-pollination rate is very low, ranging from 0.20 % to 0.68 % (Vaidya, 2000) which suggests obligate self-pollination of the crop.

2.14 Breeding of roselle

Roselle germplasm utilisation has mostly involved selection of wild types and introduction of existing cultivars with desirable characteristics such as disease and pest resistance or wide adaptability. Due to the cleistogamous mode of reproduction, cross-pollination among cultivars of roselle does not occur naturally leading to low genetic variability (Vaidya, 2000).

As a result, the primary objective of roselle breeding is to widen the genetic base of existing cultivars in order to create a large population from which variants with desirable traits such as high yield and nutritional or phytochemical contents could be selected.

Over the years, a number of techniques of crop improvement have been adopted by plant breeders. These include intra-specific hybridisation for new gene combination, wide hybridisation to introgress desirable genes from related species, induction of mutations for modifying existing alleles and exploitation of somaclonal variation (Scowcroft and Larkin, 1982).

Genetic potential of wild relatives of cultivated crop species has been demonstrated in plant breeding. Wild relatives have helped to fill the voids in traditional breeding programmes towards improvement of several crops (Stalker, 1980).

Successful introgressing of desirable genes of roselle such as root knot nematode resistance to kenaf has been achieved through inter-specific hybridisation (Satya *et al*., 2012; Wilson and Menzel, 1967). However, despite several efforts to introgress genes from other related species of roselle such as African rose mallow (*H. acetosella*) and kenaf (*H. cannabinus*), no success has been achieved (Wilson and Menzel, 1967).

So far, success in breeding of roselle has been limited to intra-specific hybridisation among accessions of var *altissima* (Louis *et al*., 2013; Veni, 2001). No major breakthrough has been achieved with regards to breeding of var *sabdariffa*.

2.14.1 Improvement of roselle through mutation breeding.

Mutation breeding offers an alternative means of creating and increasing genetic variability and overcoming problems of incompatibility of crop species (Anon, 1977). Over the past two decades, induction of variation in roselle using mutation breeding techniques has been attempted (Sherif *et al*, 2011; Mohamad *et al*., 2005; Young, 1995; Vaidya, 1994).

These initiatives have resulted in successful development of new mutant varieties with shorter maturity period and reduced plant height (Sherif *et al*, 2011; Harding and Mohamad, 2009). Despite these achievements no major success in terms of improved nutritional and phytochemical contents has been achieved so far, through mutagenic induction.

In view of the achievements and constraints of earlier breeding work of roselle, intra-specific hybridisation among existing genotypes offers great prospects for creating the desired variation for improving yield as well as nutritional and phytochemical contents of the crop.

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CHAPTER THREE

3.0 Crossability Studies among Twenty Local Landrace Varieties of Roselle (*Hibiscus sabdariffa* L.).

3.1 INTRODUCTION

Several limiting factors, including cleistagamous nature of pollination have impeded manual crossing of roselle (*H. sabdariffa* var *sabdariffa*). These physiological constraints, coupled with its tetraploid nature, have impaired proper understanding of breeding behaviour and successful development of new varieties of roselle (*H. sabdariffa* var *sabdariffa*) through hybridisation.

Artificial sexual hybridisation is a conventional breeding approach which involves crossing of different genotypes in order to introduce new genetic variability for generating new or novel varieties (Sharma, 1994). This is mostly aimed at incorporating genes for desirable traits such as disease resistance and high yield present in one genotype into the genetic background of the other genotype to produce superior hybrids. It is a very useful approach in quantitative genetic analysis such as studies of combining ability and expression of heterosis towards improvement of targeted traits.

In cross-pollinated species such as sweet potato, the phenomenon of self-incompatibility and high levels of cross-compatibility promotes high rates of cross-fertilisation after manual pollination (Jousselin *et al*., 2004; Ahn *et al*., 2002).

However, due to genetic and reproductive barriers such as homogamy in which synchronisation of anther dehiscence and stigma receptivity or close proximity of stigmas with anthers, self-pollinated crops generally exhibit very low cross-fertilisation rates after artificial pollination (Bhojwani and Razdan, 1983; Yeung and Thorpe, 1981).

Differences in genetic and floral morphology such as the effects of different style length among genotypes utilised as parents have been identified to contribute to the low success rates after artificial pollination (Valdiani *et al.*, 2012; Lattoo *et al*., 2006; De-Block and Igersheim, 2001). For instance in cassava, the genotype of the female plays prominent role in determining success of the crosses than the pollen source (Hershey, 1981).

Apart from floral and genetic factors, environmental conditions particularly low temperature and high humidity are also known to play very important roles in determining hybridisation success after manual pollination (Ahn *et al*., 2002; Ma *et al*., 1996). Consequently, assessment of crossability relationships among genotypes of self pollinating crops such as roselle is a first step for systematic and effective planning of crop improvement programmes through artificial hybridisation in order to efficiently utilise genetic diversity (Valdiani *et al.,* 2012).

Roselle (*Hibiscus sabdariffa* L.) is an underutilised multi-purpose crop cultivated throughout the tropical regions of the world with huge potential for a wide range of applications such as food, medicine as well as raw material for industry. Traditionally, it is cultivated along field boundaries as a subsistence crop in many rural communities across West Africa (Morton, 1987). Consumer preferences currently trending towards "natural food”, demand for the non-alcoholic beverage of roselle has increased considerably in Ghana and across West Africa in recent years (Schippers, 2000).

There is also a resurgence of interest in roselle as a natural colouring agenton the part of many food, beverage and pharmaceutical industries across the world, which anticipate stringent restrictions on synthetic colourants by Food and Drug Authorities (Morton, 1987).

Indeed, recent investigations reveal that the roselle plant (*H. sabdariffa*) is a suitable source for many culinary and medicinal applications due to its outstanding phytochemical and nutritional contents (Christian and Jackson 2009, Olaleye 2007).

Despite the numerous prospects offered by roselle, it has been overlooked by scientists and neglected from organised agriculture in Ghana. Consequently, the raw material for the popular beverage („sobolo bo"") is imported as net production is below demand. Though its robust nature and adaptation to marginal conditions have made it a subsistence crop, existing cultivars are still primitive, characterised by low yields, conspicuous pubescence on their leaves, stems and fruits and susceptible to diseases and pests particularly root-knot nematodes (Acquah, 2007). Hence, are unsuitable for commercial cultivation.

Therefore, there is the need to enhance the productivity of the local landrace varieties of roselle through breeding to boost farmers" interest in large-scale cultivation of the crop in Ghana. Intra-specific hybridisation among locally adapted cultivars may play a very important role in producing a broad-based segregating population from which recurrent selection could be carried out to obtain hybrids which combine high yield and disease resistance with other desirable traits such as high nutritional and anthocyanin contents.

As with most obligate self-pollinating species, roselle exhibits very low natural cross-pollination rate due to its cleistogamous nature of reproduction which facilitates self-pollination of flowers at the bud stage (Vaidya, 2000), but very little is known about crossability behaviour of various genotypes after artificial pollination. This is a challenge to breeders in choosing the appropriate breeding design towards improvement of the crop through hybridisation. Crossability studies would provide baseline information on fertility status of the various local cultivars of roselle for utilisation in future breeding programmes in Ghana.

3.1.1 Objectives of the study

The main aim of this study was to investigate crossability relationships among 20 local cultivars of roselle selected for key traits such as number of fruits per plant, plant height, fruit size and calyx colour in order to obtain relevant information on their breeding behaviour to be utilised in future breeding programmes towards improvement of the crop in Ghana.

The specific objectives were;

1. To assess the possibility of cross fertilisation among accessions of var *altissima* and var *sabdariffa*.
2. To investigate cross compatibility among six accessions of var *altissima* and 14 accessions of var *sabdariffa*.
3. To estimate hybridisation success for the various crosses.
4. To determine the best time of the day for crossing roselle in Ghana.
5. To investigate mode of inheritance of some key qualitative traits of roselle.

3.2 MATERIALS AND METHODS

3.2.1 Experimental site

The experiment was carried out at the research farms of Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC) at Kwabenya, near Accra. The experimental site is located at 05º 40' N and longitude 0º 13' W at an elevation of 76 m above sea level within the coastal savannah agro-ecological zone. The soil at site belongs to the Nyigbenya-Haatso series, which is typically well-drained savannah Ochrosol (Ferric Acrisol), derived from quartzite Schist (FAO/UNESCO, 1994).

3.2.2 Germplasm Assembly

Twenty (20) local accessions of roselle (*H. sabdariffa*) comprising 6 cultivars of var *altissima* and 14 cultivars of var *sabdariffa* collected from three geographic regions of Ghana (Northern, Volta and Western) were used as parents to produce F1 hybrids. This sampling was done in order to compare the extent of crossability between genotypes belonging to different sub-species as well as rates of crossability success among members of the same sub-species.

3.2.3 Planting of parent plants for the crossing

Planting was done in the field and in pots (Fig. 3.1).

Fig. 3.1: Roselle plants growing in (a) the field and (b) in pots.
Source: Field experiment 2013.

Prior to sowing of seeds, the field was cleared to remove the vegetation cover followed by ploughing to loosen the soil. Seeds of each accession were sown manually on the field in a row of ten stands separated at a distance of 60 cm between rows and 30 cm within rows. Seeds of each accession were also planted in pots filled with potting media comprising three parts of soil to one part of manure. Five seeds were initially sown but later thinned to three plants per pot. Weed control at two-week intervals and daily watering were also carried out.

3.2.4 Emasculation and crossing operation

At flowering, ten healthy plants of each accession comprising five plants from the field and five from the pots were selected to serve as parents for hybridisation. Emasculation was carried out prior to pollination by removing anthers from matured buds which would open the next day (Fig.3.2) to prevent self pollination.

Tools used for the emasculation process were, an improvised pair of forceps, 70 % alcohol, cotton wool, a stapler, and pieces of paper (Fig. 3.2).

Fig 3.2: (a) Flower bud (red arrow) prior to emasculation and (b) tools used for the emasculation.

Pollination was performed from 6:00 am to 10:00 am by dusting the stigma of the emasculated flower with pollen from selected male parents. For each cross thirty (30) flowers were pollinated and reciprocal crosses were also made. Immediately after emasculation and pollination, the flower buds were covered with paper bags and clipped to prevent contamination from undesirable pollen and properly tagged in order to identify the pollen donor and recipient as well as the time that each particular cross was made.

Between successive emasculation of flowers belonging to different accessions, the pair of forceps was sterilised with alcohol to prevent contamination with pollen from earlier sources. Three days after pollination, the paper bags were removed to allow the fruits to develop properly.

3.2.5. Evaluation of hybridisation success.

Compatibility was assessed through observation of each flower up to two weeks after pollination. Fertilised flowers developed fruit capsules with seeds between 7 to 14 days after pollination depending on the accessions crossed, but where fertilisation failed the flowers dropped off 2 to 3 days after pollination without developing any fruit capsules.

3.2.6 Estimation of hybridisation success

Hybridisation success of each cross was calculated according the formula of Nunekpeku *et al.*, (2012).

$$\text{Hybridisation success (\%)} = \frac{\text{Number of fruits formed}}{\text{Number of flowers pollinated}} \times 100$$

3.27. Mode of inheritance of five qualitative traits

Mode of inheritance of five qualitative traits namely, leaf structure, leaf colour, stem colour, flower colour and colouration of petal base were studied by following their transmission from parents to offspring in direct and reciprocal crosses.

Genotypes belonging to both sub-species with contrasting phenotypic expression of the characters of interest and their F1 hybrids were used for the inheritance studies.

3.3 RESULTS

3.3.1 Cross compatibility among accessions of var *altissima* and var *sabdariffa*.

Table 3.1 displays crossability success among accessions of var *altissima* and var *sabdariffa*. Successful hybridisation between the two crossing groups was not achieved, since all attempted crosses failed to develop fruit capsules and dropped off two or three days after pollination.

Table 3.1: Crossability success among accessions of var *altissima* and var *sabdariffa*.

Cross	Number of crosses			Compatibility	Comment
Female/Male	Attempted	Successful	Unsuccessful		
A1 X D2	30	0	30	No	No fruits formed
D2 X A1	30	0	30	No	No fruits formed
A3 X D6	30	0	30	No	No fruits formed
D6 X A3	30	0	30	No	No fruits formed
A9 X D1	30	0	30	No	No fruits formed
D1 X A9	30	0	30	No	No fruits formed
A2 X D5	30	0	30	No	No fruits formed
D5 X A2	30	0	30	No	No fruits formed
A11 X D3	30	0	30	No	No fruits formed
D3 X A11	30	0	30	No	No fruits formed

However, hybridisation attempts among accessions belonging to each mating group, was successful though to varying levels in different cross combinations.

Cross-compatibility success among accessions of var *sabdariffa* was patchy (Table 3.2), with only a total of 30 successful hybrids recorded. Accessions A11and A3 emerged as most compatible female and male parents, yielding 11 and 6 successful hybrids respectively. On the contrary, accessions A2, A13, and A14 failed to produce any hybrids when crossed as female parents. Similarly, accession A4 produced no successful progeny in the reciprocal direction as a male parent.

However, due to poor synchronization of flowering, and inability of some of the accessions to produce enough flowers complete pairwise crossing was not achieved among all the accessions of var *sabdariffa*.

Table 3.2: Cross compatibility success among 14 local accessions of *var sabdariffa*.

Female parent (♀)	Male parent (♂)													
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**A10**	**A11**	**A12**	**A13**	**A14**
A1	X	N	N	N	N	*	N	N	N	N	√	N	*	*
A2	*	X	*	N	N	N	*	N	N	N	N	N	N	N
A3	N	N	X	*	N	*	√	N	*	N	N	N	N	√
A4	√	√	√	X	N	N	N	N	N	*	√	√	N	*
A5	N	*	√	*	X	N	*	N	N	N	N	N	N	N
A6	N	N	N	N	*	X	N	N	N	*	√	N	*	N
A7	N	N	N	N	N	N	X	*	N	N	N	√	N	*
A8	N	N	*	N	√	N	N	X	N	N	*	N	N	N
A9	√	N	√	N	√	N	√	*	X	N	*	N	N	N
A10	N	N	√	N	N	N	√	*	*	X	√	N	*	N
A11	√	√	√	*	√	√	√	√	√	√	X	*	√	√
A12	N	N	√	N	N	*	N	N	*	*	N	X	N	N
A13	N	*	N	N	*	N	N	N	N	N	N	*	X	N
A14	*	N	N	N	N	N	*	N	N	N	N	N	*	X

√ = Successful cross X = Self N = Not successful * = Cross not carried out

Contrariwise, direct and reciprocal crosses (whether used as male or female) among all six accessions of var *altissima* were successful (Table 3.3).

Table 3.3: Compatibility relationship among six local accessions of *var altissima* in pairwise crosses.

Female parent (♀)	Male parent (♂) D1	D2	D3	D4	D5	D6
D1	X	√	√	√	√	√
D2	√	X	√	√	√	√
D3	√	√	X	√	√	√
D4	√	√	√	X	√	√
D5	√	√	√	√	X	√
D6	√	√	√	√	√	X

√ = successful cross   X = self

3.3.2.2 Hybridisation success among accessions of var *sabdariffa* in pairwise crosses.

Table 3.4 shows estimates of hybridisation success among 14 accessions of var *sabdariffa* crossed in pairwise manner. Generally, hybridisation success was low ranging from 0.00 % for most crosses to a highest of 43.33 % for paired cross between accessions A12 (female) and A3 (male).

On average, crosses in which accession A11 was used as a female parent yielded the highest hybridisation success (23.00 %). In contrast crosses where A2, A13 and A14 were utilised as maternal parents produced no success. Accession A3 gave highest average crossability success (13.00 %) in reciprocal crosses as a male parent to the other accessions, while A4 recorded no success in all crosses as a male parent.

Table 3.4: Hybridisation success (%) among 14 accessions of var *sabdariffa* in pairwise crosses.

Male parent (♀)	Female parent (♂)														
Accession	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**A10**	**A11**	**A12**	**A13**	**A14**	**Mean**
A1		0.00	0.00	0.00	0.00	*	0.00	0.00	0.00	0.00	3.33	0.00	*	*	0.00
A2	*		*	0.00	0.00	0.00	*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A3	0.00	0.00		*	0.00	*	32.33	0.00	*	0.00	0.00	0.00	0.00	16.67	2.08
A4	16.67	3.33	10.00		0.00	0.00	0.00	0.00	0.00	*	16.67	19.35	0.00	*	5.50
A5	0.00	*	23.33	*		0.00	*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.30
A6	0.00	0.00	0.00	0.00	*		0.00	0.00	0.00	*	23.33	0.00	*	0.00	2.30
A7	0.00	0.00	0.00	0.00	0.00	0.00		*	0.00	0.00	0.00	16.67	0.00	*	1.50
A8	0.00	0.00	*	0.00	23.33	0.00	0.00		0.00	0.00	*	0.00	0.00	0.00	2.60
A9	36.67	0.00	30.00	0.00	40.00	0.00	30.00	*		0.00	*	0.00	0.00	0.00	12.42
A10	0.00	0.00	20.00	0.00	0.00	0.00	13.33	*	*		26.67	0.00	*	0.00	6.00
A11	16.67	13.33	16.67	*	23.33	36.67	26.67	20.00	30.00	16.67		*	36.67	16.67	**23.00**
A12	0.00	0.00	**43.33**	0.00	0.00	*	0.00	0.00	*	*	0.00		0.00	0.00	4.30
A13	0.00	*	0.00	0.00	*	0.00	0.00	0.00	0.00	0.00	0.00	*		0.00	0.00
A14	*	0.00	0.00	0.00	0.00	0.00	*	0.00	0.00	0.00	0.00	0.00	*		0.00
Mean	5.38	1.5	**13.03**	0.00	7.22	5.58	5.38	2.22	2.31	2.08	5.13	3.00	5.24	2.08	

Bolded values represent highest and least hybridisation success. * = Cross not carried out

3.3.2.1 Hybridisation success (%) among accessions of var *altissima* in pairwise crosses.

Table 3.5 displays hybridisation success among accessions of var *altissima* in pairwise crosses. Generally success rates were high among accessions belonging to this crossing group than were obtained for var *sabdariffa*. On average, success rate ranged from 46.67 % (D4) to 55.33 % (D2) when the accessions were crossed as only female parents in succession (direct crosses) and 42.67 % (D1) to 55.33 % (D2) in reciprocal crosses in which each accession was utilised as male only.

However, on individual basis, crosses involving D3 (female) and D4 (male) scored the highest success (80 %). On the other hand D1, D3 and D6 recorded least success rates (30%) as female parents when crossed to D5, D1, and D1 respectively.

Table 3.5: Hybridisation success (%) among six accessions of var *altissima* in pairwise crosses.

Female parent (♀)	Male parent (♂)						
Accession	D1	D2	D3	D4	D5	D6	Mean
D1		63.30	50.00	40.00	30.00	56.67	48.00
D2	73.33		46.67	36.67	63.33	56.67	**55.33**
D3	30.00	46.67		**80.00**	41.94	40.00	48.00
D4	36.67	50.00	60.00		33.33	53.33	46.67
D5	43.33	40.00	53.33	50.00		50.00	47.33
D6	30.00	76.67	50.00	50.00	43.33		50.00
Mean	42.67	**55.33**	54.67	53.33	42.67	51.33	

Bolded and underlined values represent highest and least hybridisation success respectively.

3.3.3 Crossability success with respect to time of the day.

Table 3.6 shows crossability success in relation to the time of the day during which crosses were made among 20 accessions of roselle. Hybridisation success was relatively high during the early hours of the day but decreased steadily in subsequent hours. Crosses made at 6 am – 7 am yielded the highest success (15.18 %), followed by 7 am – 8 am (10.00 %) and8 am – 9 am (8.94 %), while the least success 7.62 % was recorded at 9 am – 10 am.

Table 3.6: Crossability success with respect to time of the day.

Time	Number of crosses			Crossability Success (%)
	Attempted	Successful	Unsuccessful	
6 am – 7 am	939	143	796	**15.18**
7 am – 8 am	779	78	701	10.00
8 am – 9 am	498	45	453	8.94
9 am – 10 am	729	56	673	7.62
Mean	736.25	80.5	655.75	10.44

3.3.4. Inheritance of qualitative traits of roselle (*H. sabdariffa* L.).

Table 3.7 shows mode of inheritance of five qualitative traits of roselle (*H. sabdariffa*) based on phenotypic features of parental accessions and F1 offspring produced from reciprocal crosses among the accessions. It emerged that green with reddish vein (GR) leaf colour is dominant to green (G), while deeply incised narrowly-lobed (DN) is dominant to narrowly incised broad-lobed (DB) leaf structure as evident from outcome of crosses between accessions A1 and A11 (Fig. 3.3). Similarly, red (RS) and brownish green (BS) stem colours are dominant to green (GS) stem colour.

In like manner, red flower colour (RF) and red colouration of petal base (RP) are dominant to whitish yellow flower colour (YF) and yellow colouration of petal base respectively. This is demonstrated by the outcome of crosses between accessions A1 and A4 (Fig. 3.3).

Table 3.7: Mode of inheritance of qualitative traits of roselle (*H. sabdariffa* var *sabdariffa* L.).

Parent/ F1 Offspring	Leaf colour (1)	Leaf structure (2)	Stem colour (3)	Flower colour (4)	Colouration of petal base(5)	Mode of inheritance
A1	GR	A	RS	RF	RP	1. GR > G
A6	G	A	GS	YF	YP	2. A > B
A11	G	B	BS	YF	RP	3. RS > GS;
						BS > GS
A11 x A6	G	A	BS	YF	RP	
A6 x A11	G	A	BS	YF	RP	4. RF > YF
A1 x A11	GR	A	RS	RF	RP	5. RP > YP
A11 x A1	GR	A	RS	RF	RP	

A = deeply incised narrowly-lobed; GR = green with reddish veins; G = green
B = shallowly incised broad-lobed; BS = brownish green; RS = red; GS = green
RF = red; YF = whitish yellow RP = red; YP = Yellow

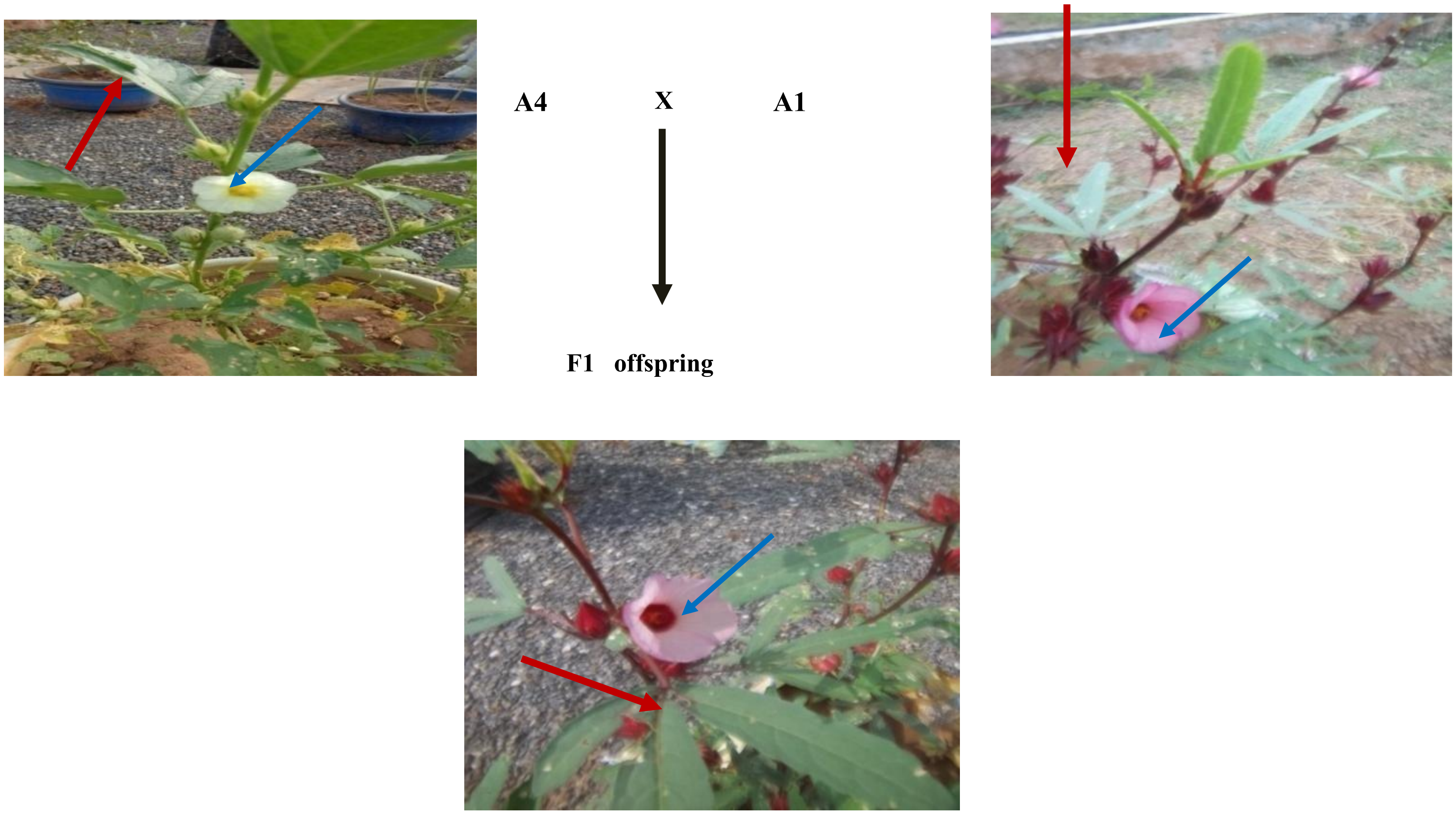

Fig. 3.3 a: Inheritance of qualitative traits of roselle in F1; red arrow: deeply incised broad-lobed leaf structure of A4 recessive to deeply incised narrowly lobed of A1. Blue arrow: yellowish white with no colouration of petal base flower colour of A4 recessive to red flower colour with red colouration of petal base of A1.

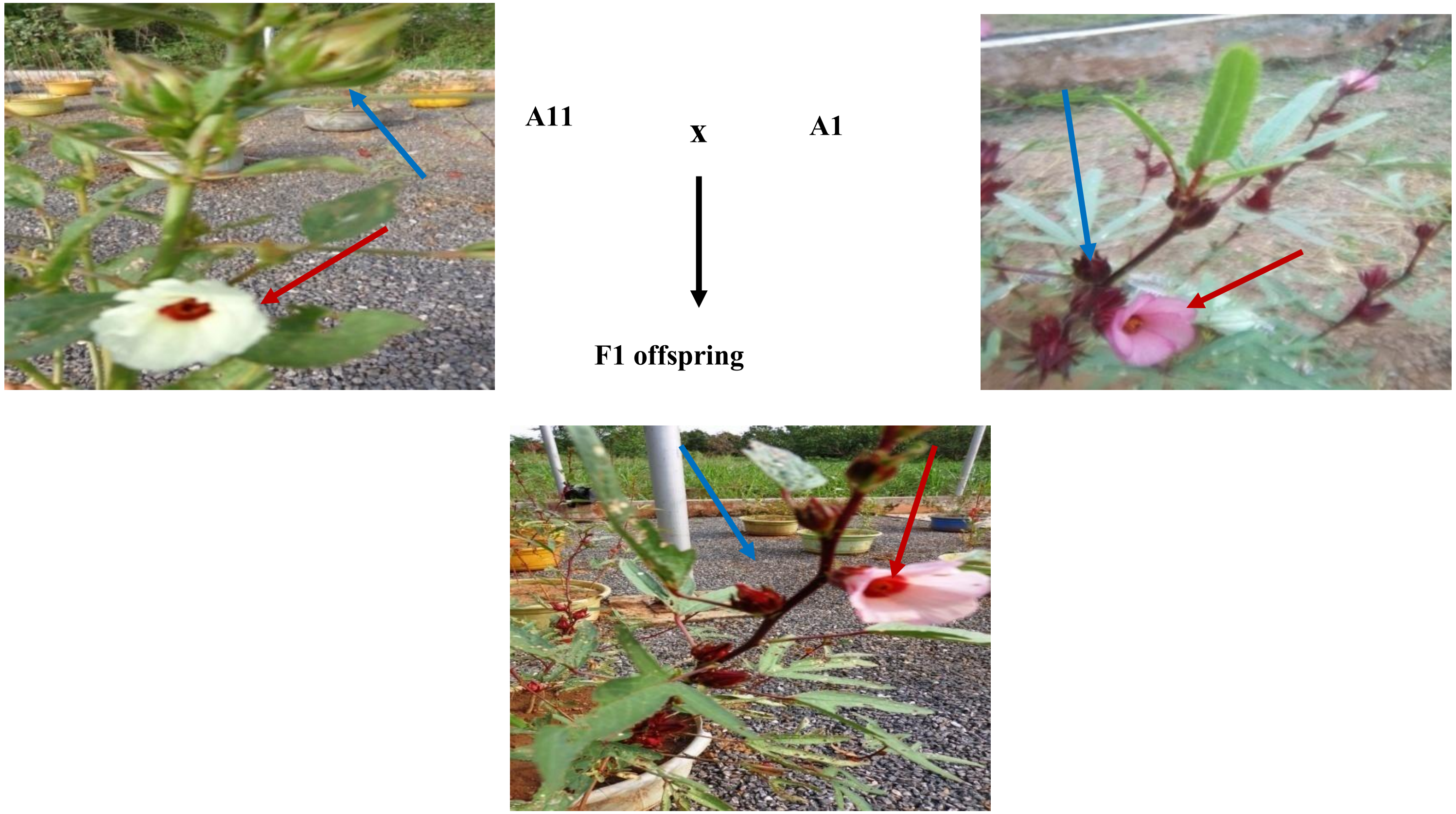

Fig. 3.3 b: Inheritance of qualitative traits of roselle in F1; blue arrow: green stem and calyx colour of A11 recessive to red stem and calyx colour of (A1).red arrow: yellowish white with red colouration of petal base of A11 recessive to red flower colour with red colouration of petal base of A1.

3.4 Discussions

3.4.1 Cross compatibility success among accessions of var *altissima* and var *sabdariffa*.

The results of the investigation reveal that the two sub-species of roselle are not cross compatible with each other since all hybridisation attempts between accessions belonging to these groups were unsuccessful. It also emerged that only few accessions of var *sabdariffa* are compatible with one another. This may suggest a possible cross fertilisation barrier among genotypes belonging to this sub-species.

In contrast, it was revealed that all six accessions of var *altissima* utilised in this study are compatible with one another in both direct and reciprocal crosses, a strong indication of no major crossability barriers among genotypes of this sub-species of roselle. Indeed, results of earlier workers (Louis *et al*., 2013; Veni, 2001) affirm that accessions of var *altissima* are cross-compatible with one another in all possible cross combinations.

Outcomes of crossability studies help breeders to determine appropriate strategies or breeding designs to adopt to transfer genes for desirable traits present in one genotype into the genetic background of other genotypes to produce novel varieties (Ahn *et al*., 2002). Where genotypes are compatible with one another in both direct and reciprocal crosses as was achieved for accessions of var *altissima* in this study, a full diallele analysis can be carried out. However, where complete pairwise crosses are not achieved as it came out for accessions of var *sabdariffa*, partial diallele or other breeding designs whereby genotypes can be used as either male or female parents only such as North Carolina design would be suitable (Todd, 2013; Griffing, 1956).

Accessions A4 and A11 stood out as most compatible male and female parent respectively. This shows their inherent potential as pollen donor and recipient respectively, since all accessions were crossed under the same conditions. Hence, A4 and A11 would be suitable maternal and paternal parents respectively to cross with the other accessions in future breeding of roselle through intra-specific hybridisation.

The results also show that three accessions A2, A13 and A14 produced no success in all crosses in which they were utilised as female parents, which indicates that they should only be used as pollen donors in future breeding programmes. A4 also failed to register any success as a male parent, thus could be utilised only as maternal parent in breeding programmes. Similar results were reported by Nunekpeku *et al.,* (2012) and Sheffield *et al.,* (2005) who studied crossability in cassava and apple respectively.

3.4.2 Hybridisation success among accessions of var *altissima* and var *sabdariffa* in pairwise crosses.

Results of the investigation reveal that crossability estimates of all accessions of var *altissima* utilised in the study are high which indicates high fertility rates of the genotypes of this sub-species of roselle. On the other hand, hybridisation success of crosses among accessions of var *sabdariffa* was generally low, which agrees with their very low natural crossing estimates (Vaidya, 2000) and may thus suggest low fertility rate of genotypes belonging to this group.

In line with observations of Valdiani *et al.,* (2012) as well as De-Block and Igersheim, (2001), positive relationship between similar floral morphology and high crossability success appeared to have accounted for the results recorded in this study since most crosses which yielded high hybridisation success involved accessions with similar style length. Floral structures of accessions of var *altissima* are very identical, which is reflected in high hybridisation success rates among them.

Similarly, crosses among genotypes of var *sabdariffa* which gave high crossability success such as A12 x A3 involved accessions with similar floral structures. Conversely, those which yielded low success such as A4 x A2 differed in style length. Several researchers (Jousselin *et al.*, 2004; Tupac and Ackerman, 2002; Bolat and Pirlak, 1999) have also observed that differences in floral structures such as style length may affect stigma receptivity and pollen germination as well as tube migration through the style which can completely prevent fertilisation.

However, similar floral structures did not always result in high crossability success in the reciprocal direction with respect to accessions of var *sabdariffa*. Albeit having similar floral structures, crossability success among accessions A12 and A3 differed according to direction. For example the cross A12 x A3 emerged as the most successful (43.33 %) in one direction but failed to record any success in the opposite direction.

Genetic relationships among genotypes are known to play an important role in determining hybridisation success after artificial pollination (Koelling *et al.*, 2011; Lattoo *et al.*, 2006; Ogburia and Okele, 2001). Consequently, genetic difference among the accessions of var *sabdariffa* utilised in this study could have also contributed to the observed variations in reciprocal crosses.

3.4.3 Crossability success with respect to time of the day

Results of the study reveal that the best time for crossing roselle is 6 am – 7 am under the local conditions, which is comparable to observations of Samba (2013) who investigated crossability of sweet potato in Ghana. However, it contrasts those of Li (2000) who observed 9 am – 11 am as the best time for pollinating kenaf, a species closely related to roselle which also belongs to the same genus Hibiscus.

Since roselle exhibits cleistogamy, it was expected that maturation of pollen and stigma receptivity would occur simultaneously before opening of floral bud. Also, during the study it was observed that stigma desiccation and abscission were more pronounced in later hours of the day resulting in low success after pollination. Thus, pollinations made in early hours of the day (6am – 7am), immediately after opening of flowers were likely to fall on receptive stigmas than those made during the subsequent hours of the day.

Several researchers including Ahn *et al*., (2002) and Ma *et al*., (1996) observed that favourable environmental conditions such as low temperature, high humidity and water availability can also affect success rates of hybridisation after artificial pollination. These favourable environmental conditions were observed in the early hours of the day during the study, which could have also accounted for the higher crossability success during this period of the day.

4.4.5 Inheritance of qualitative characters of roselle (*H. sabdariffa* var *sabdariffa*).

Most qualitative characters, such as flower colour have little influence on productivity or performance. Nevertheless knowledge of mode of inheritance of such characters is of immense importance to breeders.

Due to tetraploid nature of roselle, segregation ratios are quite complex, hence large numbers of progenies are required in order to accurately fix fitting ratios in the F2 generation to adequately explain transmission of traits from parents to offspring. This could not be achieved in this study. Therefore, in this study mode of inheritance of qualitative traits was determined on the basis of phenotypic features of parental accessions and F1 offspring produced from direct and reciprocal crosses among the accessions.

Results obtained in this study corroborate those of Adamson (1983) who also reported that, shallowly incised broad-lobed leaf structure found in most edible roselle is recessive to both the deeply incised narrowly-lobed and to obscurely-lobed (non-lobed). This is similar to the inheritance of narrowly-lobed leaf structure in cassava which is dominant to broad-lobed leaf structure (Fukuda *et al,* 2002).

It also emerged that, red pigmentation of leaf, stem and flower of roselle is dominant to green pigmentation as earlier suggested by Falusi (2008).

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CHAPTER FOUR

4.0 Agro-morphological characterisation of 14 local accessions of roselle (*Hibiscus sabdariffa* var *sabdariffa* L.) and their intra-specific F1 offspring.

4.1 INTRODUCTION

One essential aspect of crop improvement is assessment of genetic diversity of desirable characteristics such as increased yield, wide adaptability, pests and diseases resistance among other traits which exist within populations of crop species (Beeching *et al*., 1993). Characterisation and identification of genetic variability within germplasm collections are a preliminary requirement for the exploitation of useful traits in plant breeding (Oka, 1991).

Characterisation of genetic resources refers to the process by which accessions are identified, differentiated or distinguished according to their characteristics (Oseikita and Akinyele, 2008). Among other purposes, characterisation plays a key role in investigations of genetic diversity patterns and identification of duplicates within crop germplasm collections. It also facilitates studies of correlation among characteristics of agronomic importance (CIAT, 2007). The efficacy of the method deployed for the characterisation process largely determines the potential genetic value of a particular germplasm (De vicente *et al*, 2005).

Generally, characterisation and genetic assessment is centered on use of genetic markers; any measurable character (phenotypic or genotypic whose inheritance can be traced through different generations) which is capable of detecting a variation in either a protein or DNA sequence to identify the characteristics of a genetic material (De vicente *et al.*, 2005).

A number of morphological, biochemical and molecular (DNA) markers have been developed and widely deployed to investigate diversity in plant genetic resources. However, resolution of diversity using biochemical analyses has received little attention due to their reliance on proteins/enzymes which are usually limited for most traits in plant germplasm (De vicente *et al.*, 2005).

Morphological markers are traditionally proven to indicate differences among accessions in plant genetic diversity studies. Despite the challenge of their ambiguity due to contribution of multiple genes and modifications or interactions with the environment, they still remain very useful primary methods for germplasm characterisation (Staub, *et al*., 1996). Moreover, morphological characterisation techniques require little skills and are relatively inexpensive to carry out (Hoogendijk and William, 2001).

Morphological characterisation of crops is facilitated by the use of standard descriptors, which provide an international format for producing a universally understood language for plants genetic resources (De vicente *et al*., 2005). Sometimes, published descriptors for conserved species may be modified to account for distinct characteristics of other crops (Hanson and Nairot, 1991).

On the contrary, molecular techniques which comprise a large variety of DNA-based markers are very efficient for analysis of variation in germplasm collections due to their ability to detect or amplify anonymous loci (expressed or non-expressed sequences). However, due to requirement of high expertise and sophisticated facilities, molecular techniques are very expensive to carry out (Soni *et al*., 2010).

Recent discovery of roselle (*H. sabdariffa* var *sabdariffa* L.) as a source of food and raw material for pharmaceutical and industrial applications due to high phytochemical and nutritional content (Mohamad, *et al*., 2007; Adanlawo and Ajibade, 2006), has attracted the attention of researchers to identify superior genotypes in order to maximize its exploitation. Consequently, a number of local landraces and exotic cultivars of roselle have been collected and screened in many countries (Mohamed and Gabri, 2013; Torres-Morán *et al*., 2011).

These notwithstanding, it is extremely important to consolidate the existing knowledge on genetic diversity of the crop. Therefore, further characterisation and investigation into genetic diversity and relatedness of roselle germplasm resources in other regions which have received limited attention, remain a matter of interest.

Ghana abounds in a number of different landraces of roselle which are predominantly cultivated in the Northern, Volta and Western Regions as leafy vegetables and to provide raw material for the popular non-alcoholic beverage („„sobolobo"") consumed across the country. Unfortunately, there are few attempts to characterise these local germplasm of roselle to elucidate their genetic potential.

This presents a challenge to breeding efforts through hybridisation towards improvement of these locally adapted cultivars to enhance their productivity. It is therefore crucial to assemble and characterise the available local germplasm of roselle distributed across the country in order to gain meaningful insight into their genetic potential with respect to yield and yield-determining parameters. Genetic diversity studies will provide baseline information to curators interested in conservation of the crop and breeders desirous of carrying out improvement of useful characters. It will also provide the opportunity to identify and remove duplicates within the collection in order to achieve efficient management of roselle germplasm in Ghana.

4.1.1 Objective of the study

The main objective of this study was to morphologically characterise fourteen local landraces of roselle (*H. sabdariffa* var *sabdariffa* L.) and 25 F1 off spring obtained from intra-specific hybridisation among the accessions in order to identify promising genotypes for selection. The specific objectives were;

1. To assess the level of genetic variability among the accessions for fourteen qualitative and nine quantitative traits in order to identify and recommend lines with superior agro-morphological characteristics for selection.
2. To determine genetic relatedness among parental accessions and the F1 offspring in order detect possible duplicates.
3. To study the degree of association between the quantitative traits to determine appropriate strategies to adopt in future hybridisation programmes towards improvement of useful traits.
4. To identify agro-morphological parameters which contribute most to total variability among the accessions to be utilised in future breeding work.

4.2 MATERIALS AND METHODS

4.2.1. Experimental site

The research was carried out at the research farm of Biotechnology and Nuclear Agriculture Research Institute (BNARI) of Ghana Atomic Energy Commission (GAEC), Kwabenya, near Accra. The experimental site is located at 05º 40' N and longitude 0º13'W at an elevation of 76 m above sea level within the coastal savannah agro-ecological zone. The soil at the Nyigbenya-Haatso series, which is typically well-drained savannah Ochrosol (Ferric Acrisol), derived from quartzite Schist (FAO/UNESCO, 1994).

4.2.2 Planting material and planting

Planting material for the study comprised fourteen local landraces of roselle (*H. sabdariffa* var *sabdariffa* L.) collected from three geographical regions of Ghana and 25 F1 hybrids derived from intraspecific hybridisation among the accessions. The seeds were sown in plastic bags (measuring 6 cm x 12 cm) filled with potting medium (Fig. 4.1 a) comprising three parts of soil and one part manure.

Fig. 4.1: (a) Seedlings of roselle prior to transplanting and (b) one week after transplanting.
Source: Field experiment, 2013.

Exactly one week after germination, six seedlings were transplanted into each pot and later thinned to three plants per pot (fig. 4.1 b). The pots were arranged in rows at a spacing distance of 30 cm within rows and 1m between rows. Optimal management practices such as monthly weed control and daily watering were carried out. However, no pesticides or fertilizers were applied during the study period since it requires little very little or no fertilizer application for its cultivation (Das and Das, 2000). Experimental design used was randomized complete block design (RCBD) with three replicates. Each replicate comprised ten pots of 30 plants per accession.

4.2.3 Data collection

Data were collected on 14 qualitative and 9 quantitative traits using 10 randomly selected plants per accession. Qualitative data were taken on leaf (structure, colour, pubescence and number of leaf lobes.), stem (colour, pubescence), petiole (colour, pubescence), flower (colour and colouration of petal base) and calyx (colour and pubescence) characteristics.

The data were collected using the International Plant Genetic Resource Institute (IPGR, 1991) Descriptor list for okra, a related crop belonging to the same family Malvaceae, with modifications where necessary. Quantitative data comprised number of days to first flowering, fresh calyx weight, fresh fruit weight, branches per plant, leaf length, leaf width, number of fruits per plant, plant height and petiole length.

4.2.4 Data analysis

Qualitative data were evaluated based on the morphological descriptors to identify the extent of variation within the roselle population for the selected qualitative traits. The quantitative data were subjected to Analysis of variance (ANOVA) to determine the level significance of variability for the various parameters. A p-value of 0.05 or less was considered as statistically significant. Duncan"s multiple range test was deployed to determine differences among means.

Cluster analysis based on similarity matrices was performed to generate a dendrogram in order to determine genetic relationships among the genotypes. Correlation analysis was also carried out to determine degree of association between the quantitative agro-morphological traits.

Contribution of each trait to total genetic diversity within the roselle population studied was determined via principal component analysis based on correlation matrix of agro-morphological variables.

Genstats Statistical Software Package (12th edition), Statsgraphics Centurion software (version 16.1) and Microsoft Excel Software (2010 edition) were used for the Data analyses.

4.3 Results

4.3.1 Variation in qualitative traits among 14 accessions of roselle (*H. sabdariffa* var *sabdariffa* L.) and their F1 offspring.

Table 4.1 shows variability of qualitative traits among 14 local accessions of roselle (*H. sabdariffa* var. *sabdariffa* L.) and 25 F1 offspring. The accessions exhibited widest variability with respect to calyx colour. In general, five groups of colour namely, red, green, whitish green, dark red, whitish green with red patches were recorded across both parents and F1 offspring while two F1 offspring A7 x A10 and A7 x A10 (2) expressed whitish green with red stripes bringing to a total of six colours.

With respect to stem and petiole colour, four types emerged; Green, Brownish Green, Red and Dark red but majority (64.10 %) expressed brownish green while only two accessions showed Dark red. Expression of pubescence on leaf, stem and calyx varied from glabrous, slight to conspicuous pubescence. However, most of the accessions (57.14 %) produced conspicuous pubescence with the exception of accessions A1, A2, A7, A11, and A13 and F1 offspring (A11 x A1, A11 x A2, and A11 x A13), which expressed glabrous or downy pubescence.

Flower and fruit sizes were categorised into large, medium and small. Majority (76 %) of the F1 offspring produced large or medium fruit size with only six recording small. Relatively, fewer (42.86 %) of the parental lines produced large fruits while the rest gave small fruit size. Flower colour, colouration of petal base and mature leaf colour gave the least variation of two categories each; red or whitish yellow, red or yellow and green or green with red veins respectively. Four types of leaf structure were observed among the parental accessions, but almost all (80 %) F1 offspring had deeply incised narrowly lobed.

Table 4.1: Variation in qualitative agro-morphological traits among 14 local accessions of roselle (*H. sabdariffa* var *sabdariffa*) and their F1 offspring.

Parent/ F1 Offspring	SC[1]	SP[2]	FC[3]	CPB[4]	FS[5]	MLC[6]	LS[7]	NLL[8]	LP[9]	SF[10]	CC[11]	FP[12]	PC[13]	PP[14]
A1	R	G	R	R	M	GWRV	A	5	G	L	R	D	R	G
A2	BG	G	YW	R	M	G	B	3	G	L	G	D	BG	G
A3	BG	C	YW	R	S	G	A	5	G	S	WG	SR	BG	S
A4	G	C	YW	Y	S	G	D	3	SR	S	WG	SR	G	C
A5	G	C	YW	Y	S	G	A	5	G	S	WG	SR	G	S
A6	G	C	YW	Y	S	G	A	5	C	S	WG	P	G	C
A7	BG	G	YW	R	L	G	A	5	G	L	WGRP	D	BG	G
A8	BG	C	YW	R	S	G	A	5	C	S	WGRP	P	BG	C
A9	BG	C	YW	R	S	G	B	3	C	S	WGRP	SR	BG	C
A10	G	C	YW	Y	S	G	B	3	G	S	WG	SR	G	S
A11	BG	G	YW	R	L	G	C	1	G	L	G	D	BG	G
A12	R	C	YW	R	S	GWRV	A	5	S	S	R	P	R	S
A13	DR	G	R	R	S	GWRV	B	3	G	L	DR	D	DR	G
A14	BG	S	YW	R	M	G	A	5	G	L	G	D	BG	G
A3 X A7	BG	S	YW	R	S	G	A	5	S	M	WGRP	P	BG	S

1.Stem colour (G = Green, BG = Brownish Green, R = Red, DR = Dark red)
2. Stem pubescence (G = Glabrous, S = Slight, C = Conspicuous)
3. Flower colour (WY = Whitish yellow, R = Red)
4. Colouration of petal base (R = Red, Y = Yellow)
5. Flower size (S = Small, M = Medium, L= Large)
6. Mature leaf colour (G = green; GWRV = Green + red veins)
7. Leaf structure (A = deeply incised narrowly lobed; B = narrowly incised broad lobed; C = non-lobed; D= deeply incised broad lobe)
8. Number of leaf lobes at flowering (1, 3, 5)
9. Leaf pubescence (G = Glabrous, SR= Slight rough, P = Prickly)
10. Fruit size (S = Small, M = Medium, L= Large)
11. Colour of calyx (G = Green; W = White, R = Red, WGRP = Whitish green with red patches, WGRS = Whitish green with red stripes , DR = Dark red).
12. Fruit pubescence (G = Glabrous, SR= Slight rough, P = Prickly)
13. Petiole colour (G = Green, BG = Brownish Green, R = Red, DR = Dark red)
14. Petiole pubescence (G = Glabrous, S = Slight, C = Conspicuous)

Table 4.1(continued): Variation in 14 qualitative agro-morphological traits among 14 local accessions of roselle (*H. sabdariffa* var *sabdariffa*) and their F1 offspring.

F1 Offspring	SC[1]	SP[2]	FC[3]	CPB[4]	FS[5]	MLC[6]	LS[7]	NLL[8]	LP [9]	SF[10]	CC[11]	FP[12]	PC[13]	PP[14]
A4 x A1	R	S	R	R	M	GWRV	A	5	G	M	R	SR	R	S
A4 x A3	BG	C	YW	R	S	G	A	5	S	S	WGRP	P	BG	C
A4 x A11	BG	C	YW	R	S	GWRV	D	3	S	M	WGRP	P	BG	C
A5 x A3	BG	S	YW	R	S	G	A	5	S	S	WGRP	SR	BG	S
A6 x A11	G	C	YW	R	S	GWRV	A	5	C	S	WGRP	P	G	C
A7 x A12	R	S	R	R	S	G	A	5	G	M	R	SR	R	S
A9 x A1	R	S	R	R	M	G	A	5	G	M	R	SR	R	S
A9 x A3	BG	S	YW	R	S	G	A	5	S	M	WGRP	P	BG	S
A9 x A5	BG	S	YW	R	S	G	A	5	G	S	WGRP	SR	BG	S
A9 x A7	BG	C	YW	R	M	G	A	5	S	M	WGRP	P	BG	C
A10 x A3	BG	S	YW	R	S	G	A	5	G	S	WGRP	SR	BG	S
A10 x A7	BG	S	YW	R	M	G	A	5	S	L	WGRP	P	BG	S

1.Stem colour (G = Green, BG = Brownish Green, R = Red, DR = Dark red)
2. Stem pubescence (G = Glabrous, S = Slight, C = Conspicuous)
3. Flower colour (WY = Whitish yellow, R = Red)
4. Colouration of petal base (R = Red, Y = Yellow)
5. Flower size (S = Small, M = Medium, L= Large)
6. Mature leaf colour (G = green; GWRV = Green + red veins)
7. Leaf structure (A = deeply incised narrowly lobed; B = narrowly incised broad lobed; C = non-lobed; D= deeply incised broad lobe)
8. Number of l leaf nodes at flowering (1, 3, 5)
9. Leaf pubescence (G = Glabrous, SR= Slight rough, P = Prickly)
10. Fruit size (S = Small, M = Medium, L= Large)
11. Colour of calyx (G = Green; W = White, R = Red, WGRP = Whitish green with red patches, WGRS = Whitish green with red stripes , DR = Dark red).
12. Fruit pubescence (G = Glabrous, SR= Slight rough, P = Prickly)
13. Petiole colour (G = Green, BG = Brownish Green, R = Red, DR = Dark red)
14. Petiole pubescence (G = Glabrous, S = Slight, C = Conspicuous)

Table 4.1(continued): Variation in 14 qualitative agro-morphological traits among 14 local accessions of roselle (*H. sabdariffa* var *sabdariffa*) and their F1 offspring.

F1 Offspring	SC[1]	SP[2]	FC[3]	CPB[4]	FS[5]	MLC[6]	LS[7]	NLL[8]	LP[9]	SF[10]	CC[11]	FP[12]	PC[13]	PP[14]
A10 x A7 (2)	BG	S	YW	R	M	G	A	5	S	L	WGRS	P	BG	S
A10 x A11	BG	S	YW	R	M	G	B	3	S	L	WGRS	SR	BG	S
A10 x A11 (2)	BG	S	YW	R	M	G	B	3	S	L	WGRP	SR	BG	S
A11 x A1	R	G	R	R	M	GWRV	A	5	G	L	R	G	R	G
A11 x A2	BG	G	YW	R	M	G	B	3	G	L	WGRP	G	BG	G
A11 x A5	BG	C	YW	R	M	G	A	3	S	M	WGRP	P	BG	C
A11 x A6	BG	C	YW	R	S	G	A	5	C	M	G	P	GB	C
A11 x A7	BG	G	YW	R	M	G	A	5	G	L	WGRP	G	BG	G
A11 x A9	BG	C	YW	R	S	G	D	3	S	L	WGRP	P	BG	C
A11 x A10	BG	C	YW	R	M	GWRV	B	3	S	L	WGRP	P	BG	C
A11 x A13	DR	G	R	R	M	G	A	5	G	L	DR	G	DR	G
A12 x A3	R	C	R	R	S	G	A	5	S	S	R	P	R	C

1.Stem colour (G = Green, BG = Brownish Green, R = Red, DR = Dark red)
2. Stem pubescence (G = Glabrous, S = Slight, C = Conspicuous)
3. Flower colour (WY = Whitish yellow, R = Red)
4. Colouration of petal base (R = Red, Y = Yellow)
5. Flower size (S = Small, M = Medium, L= Large)
6. Mature leaf colour (G = green; GWRV = Green + red veins)
7. Leaf structure (A = deeply incised narrowly lobed; B = narrowly incised broad lobed; C = non-lobed; D= deeply incised broad lobe)
8. Number of leaf nodes at flowering (1, 3, 5)
9. Leaf pubescence (G = Glabrous, SR= Slight rough, P = Prickly)
10. Fruit size (S = Small, M = Medium, L= Large)
11. Colour of calyx (G = Green; W = White, R = Red, WGRP = Whitish green with red patches, WGRS = Whitish green with red stripes , DR = Dark red).
12. Fruit pubescence (G = Glabrous, SR= Slight rough, P = Prickly)
13. Petiole colour (G = Green, BG = Brownish Green, R = Red, DR = Dark red)
14. Petiole pubescence (G = Glabrous, S = Slight, C = Conspicuous)

4.3.2 Variations in 9 quantitative agro-morphological traits of 14 accessions roselle (*H. sabdariffa* var *sabdariffa*) and their F1 offspring.

Table 4.2 displays 9 quantitative agro-morphological traits of 14 accessions of roselle and 25 F1 offspring obtained from intra-specific hybridisation between the accessions. Highly significant variation ($p \leq 0.01$) was observed among the accessions for all 9 quantitative traits (Appendix 9.1.). Generally, values for days to first flowering (DF), number of branches per plant (NBPP), plant height (PH) and petiole length (PL) recorded for parental accessions were higher than those recorded for the F1 offspring.

However, for fresh calyx weight (FCW), fresh fruit weight (FFW), leaf length (LL), leaf width (LW) and number of fruits per plant (NFPP) values obtained for the F1 offspring were comparatively higher than those of parental accessions.

Overall, A13, A11 x A13, A11 x A1, A3, A5, A7 recorded highest values for DF, FCW, FFW, NBPP, PH and PL respectively, while A7 x A12 gave the best DF and LL and LW respectively. On the other hand, A4 x A3, A10, A8, A4, A11, and A2 recorded lowest values for DF, FCW, FFW, LL, LW and PH respectively.

Similarly, parental line A5 registered lowest values for NBPP, NFPP and PL.

Table 4.2: Variations in quantitative agro-morphological traits of 14 accessions of roselle (*H. sabdariffa* var *sabdariffa*) and their F1 offspring

Accession	DF(Days)	FCW (g)	FFW (g)	LL (cm)	LW (cm)	NBPP	NFPP	PH (cm)	PL (cm)
A1	43.00±1.00 ^{h}	39.32±3.62 ^{a}	66.68±1.55 ^{b}	11.40± 0.61 ^{d}	10.10±0.30 ^{d}	6.67±0.58 ^{cd}	45.33±1.53 ^{de}	70.00±0.00 ^{i}	7.10±2.19 ^{a}
A2	55.00±1.00 ^{f}	**43.95±4.50 ^{a}**	**68.28±2.61 ^{a}**	12.27± 0.25 ^{c}	7.40±0.26 ^{fg}	5.67±0.58 ^{e}	33.33±1.53 ^{h}	50.33±2.08 ^{j}	7.13±0.99 ^{a}
A3	40.00±1.00 ^{i}	22.67±2.52 ^{e}	44.44±4.01 ^{d}	14.21±0.02 ^{a}	14.45±0.33 ^{a}	**8.33±0.58 ^{a}**	**79.33±2.52 ^{a}**	81.00±1.00 ^{g}	4.80±0.265 ^{bcd}
A4	43.00±1.00 ^{h}	21.33±1.53 ^{ef}	34.93±2.14 ^{e}	6.53± 0.35 ^{g}	7.00±0.10 ^{g}	6.00±0.00 ^{de}	66.00±1.00 ^{b}	85.67±1.15 ^{f}	6.00±0.50 ^{abc}
A5	44.33±1.58 ^{h}	20.00±2.00 ^{efg}	32.12±1.76 ^{e}	11.13±0.13 ^{d}	10.97±0.12 ^{c}	3.67±0.58 ^{g}	32.33±1.53 ^{i}	**148.00±1.00 ^{a}**	3.70±0.00 ^{d}
A6	45.33±0.58 ^{gh}	16.33±1.53 ^{fg}	32.17±1.27 ^{e}	8.77±0.25 ^{f}	7.31±0.27 ^{fg}	7.33±0.58 ^{bc}	62.33±2.53 ^{c}	71.33±1.15 ^{hi}	3.70±0.30 ^{d}
A7	57.67±2.51 ^{e}	38.67±5.51 ^{ab}	67.79±2.08 ^{ab}	**14.03±1.34 ^{a}**	**14.50±0.36 ^{a}**	4.67±0.58 ^{f}	43.00±1.00 ^{ef}	73.33±3.06 ^{h}	**7.40±2.23 ^{a}**
A8	63.67±3.21 ^{c}	16.33±0.58 ^{fg}	26.89±2.33 ^{f}	6.69±0.21 ^{g}	7.67±0.21 ^{f}	7.67±1.58 ^{ab}	38.67±1.53 ^{gh}	94.00±1.73 ^{d}	4.07±0.21 ^{cd}
A9	47.33±0.58 ^{g}	17.00±2.00 ^{efg}	35.65±1.69 ^{e}	8.63±0.25 ^{f}	8.73±0.25 ^{e}	4.67±0.58 ^{f}	45.00±1.00 ^{e}	97.33±2.08 ^{c}	4.10±0.89 ^{cd}
A10	44.67±0.58 ^{h}	15.33±1.15 ^{g}	32.71±2.83 ^{e}	8.42±0.39 ^{f}	8.99±0.40 ^{e}	7.33±0.58 ^{bc}	65.33±1.15 ^{b}	88.67±1.15 ^{e}	3.83±0.76 ^{d}
A11	56.67±0.58 ^{ef}	37.00±1.00 ^{bc}	62.94±1.02 ^{bc}	8.43±1.02 ^{f}	5.1±0.17 ^{i}	4.67±0.58 ^{f}	48.00±1.00 ^{d}	86.33±1.15 ^{ef}	4.23±0.58 ^{cd}
A12	60.00±1.00 ^{d}	29.48±1.31 ^{d}	47.69±4.19 ^{d}	13.22±0.48 ^{b}	13.23 ±0.25 ^{b}	4.00±0.00 ^{fg}	67.67±1.53 ^{b}	124.33±2.52 ^{b}	6.20±1.06 ^{ab}
A13	**75.33±1.52 ^{a}**	37.00± 4.58 ^{bc}	64.84±5.68 ^{b}	11.33±0.49 ^{d}	6.25±0.22 ^{h}	4.00±0.00 ^{fg}	36.67±1.53 ^{hi}	84.00±1.00 ^{f}	4.80±0.52 ^{bcd}
A14	66.33±0.58 ^{b}	32.67±5.51 ^{cd}	60.00±1.0 ^{c}	10.23±0.21 ^{e}	6.2±0.35 ^{h}	5.67±0.58 ^{e}	40.67±2.08 ^{fg}	85.67±1.15 ^{f}	4.00±1.00 ^{d}
Mean	53.21±7.88	27.97±10.80	48.62±16.10	10.26±2.87	8.933.57	3.41±1.67	47.57±15.20	83.76±23.99	5.08±1.61
LoS	***	***	***	***	***	***	***	***	***
CV (%)	20.40 %	38.59 %	33.12 %	24.37 %	33.13 %	26.96 %	28.69%	26.23 %	31.70 %

FCW = Fresh Calyx weight   NBPP = Number of branches per plant   LL = Leaf length   PH = Plant height   DF = Days to first flowering
FFW = Fresh Fruit weight   NFPP = Number of fruits per plant   LW = Leaf width   PL = Petiole length.

± sd = Standard deviation. **Bolded** and underlined values represent maximum and minimum values respectively for each trait.
Means in same column with same alphabets are not significantly different according to Duncan multiple range test.
LoS, ***, CV represent level of significant, significant at $P \leq 0.001$ and total coefficient of variation respectively.

Table 4.2 (continued): Variations in quantitative agro-morphological traits of 14 accessions of roselle (*H. sabdariffa* var *sabdariffa*) and their F1 offspring.

F1 Offspring	DF(Days)	FCW (g)	FFW (g)	LL (cm)	LW (cm)	NBPP	NFPP	PH (cm)	PL (cm)
A3 X A7	37.33±0.58^{k}	25.82±1.02^{g}	48.67±7.23^{bc}	9.60±0.95^{bcdefghi}	11.93±2.06^{bc}	4.67±0.58^{bcdef}	36.67±3.51^{hi}	81.33±4.16^{de}	3.90±0.36^{fghijkl}
A4 x A1	39.00±1.00^{k}	30.44±1.41^{ef}	53.08±2.12^{b}	11.03±1.70^{bc}	9.50±1.25^{defg}	4.00±1.00^{defg}	51.67±3.79^{abcde}	88.67±9.61^{cd}	5.27±1.04^{bcdefgh}
A4 x A3	37.00±1.00^{k}	18.67±1.40^{h}	31.73±2.20^{i}	8.33±0.91^{ghi}	7.60±1.06^{ghij}	4.67±0.58^{bcdef}	49.00±3.61^{bcdefg}	82.33±3.21^{de}	4.20±0.36^{efghijkl}
A4 x A11	45.00±1.00^{gh}	26.95±1.54^{fg}	37.14±2.84^{ghi}	8.60±1.23^{efghi}	6.43±0.51^{jk}	5.67±0.58^{abc}	43.33±5.51^{defghi}	100.0±11.14^{bc}	4.30±0.61^{efghijk}
A5 x A3	44.00±1.00^{hi}	19.67±1.21^{h}	35.21±3.66^{hi}	9.63±0.60^{bcdefghi}	13.00±1.15^{b}	3.33±0.58^{fg}	50.00±5.20^{abcdef}	124.67±1.53^{a}	3.77±0.25^{hijkl}
A6 x A11	45.00±1.00^{gh}	21.14±2.07^{h}	37.18±2.72^{ghi}	5.77±1.55^{j}	7.77±0.25^{fghij}	5.67±0.58^{abc}	52.67±2.31^{abcd}	86.67±9.50^{cde}	2.87±0.32^{kl}
A7 x A12	49.33±1.53^{de}	30.34±2.76^{ef}	53.14±2.58^{b}	**14.33± 0.29^{a}**	**15.20±0.96^{a}**	4.00±1.00^{defg}	48.00±5.20^{bcdefgh}	110.67±18.15^{b}	5.47±0.50^{abcdefg}
A9 x A1	44.33±1.15^{gh}	34.17±7.39^{cde}	53.98±1.50^{b}	10.60±0.53^{bcde}	9.90±0.85^{cdefgh}	3.33±0.58^{fg}	38.67±2.08^{fghi}	85.67±8.14^{cde}	3.87±0.57^{ghijkl}
A9 x A3	49.00±1.00^{e}	22.17±2.01^{h}	38.33±4.93^{gh}	8.40±1.15^{fghi}	9.30±2.26^{defg}	4.00±1.00^{efg}	38.33±4.93^{ghi}	86.67±1.15^{cde}	3.47±0.50^{ijkl}
A9 x A5	46.33±1.53^{fg}	19.60±1.32^{h}	35.82±2.75^{hi}	10.37±1.21^{bcdefg}	11.53±1.79^{bcd}	3.67±0.58^{efg}	46.33±3.06^{cdefghi}	89.00±9.17^{cd}	4.10±1.15^{efghijkl}
A9 x A7	45.00±1.00^{gh}	31.57±0.79^{e}	40.78±2.48^{fgh}	9.73±1.12^{bcdefghi}	9.97±1.15^{cdefg}	3.33±0.58^{fg}	38.00±4.36^{ghi}	88.67±9.02^{cd}	3.87±0.98^{ghijkl}
A10 x A3	41.67±2.08j	19.93±2.30^{h}	35.24±3.01^{hi}	8.07±0.90^{hi}	10.20±0.98^{cde}	5.67±0.58^{abc}	41.00±8.89^{efghi}	84.67±12.42^{de}	2.60±0.66^{l}
Mean	44.83±6.72	28.14±2.05	46.26±10.77	9.53±1.91^{ghi}	9.14±2.48	4.53±1.15	46.58±8.44	88.41±14.34	4.74±1.40
CV (%)	15.98 %	24.41 %	23.29 %	20.08 %	27.10 %	25.47 %	18.14%	16.21 %	29.57 %

FCW = Fresh Calyx weight NBPP = Number of branches per plant LL = Leaf length PH = Plant height DF = Days to first flowering
FFW = Fresh Fruit weight NFPP = Number of fruits per plant LW = Leaf width PL = Petiole length.

± sd = Standard deviation. **Bolded** and underlined values represent maximum and minimum values respectively for each trait.
Means in same column with same alphabets are not significantly different according to Duncan multiple range test.
LoS, ***, CV represent level of significant, significant at P ≤ 0.001 and total coefficient of variation respectively.

Table 4.2 (continued): Variations in quantitative agro-morphological traits of 14 accessions of roselle (*H. sabdariffa* var *sabdariffa*) and their F1 offspring.

F1 Offspring	DF(Days)	FCW (g)	FFW (g)	LL (cm)	LW (cm)	NBPP	NFPP	PH (cm)	PL (cm)
A10 x A7	42.00±1.00^{ij}	32.71±1.54^{de}	47.68±2.76^{bcde}	8.27±2.03^{ghi}	8.93±1.07^{efghi}	3.67±0.58^{efg}	43.33±5.51^{defghi}	83.67±11.72^{de}	5.27±0.87^{bcdefgh}
A10 x A7 (2)	44.00±1.00^{hi}	30.34±1.86^{ef}	47.90±2.82^{bcde}	9.17±0.31^{cdefghi}	9.83±0.58^{cdefgh}	3.67±1.15^{efg}	56.00±9.64^{abc}	87.67±2.31^{cde}	5.63±0.67^{abcde}
A10 x A11	48.00±1.00^{ef}	31.25±3.16^{e}	48.22±2.70^{bcd}	10.77±1.01^{bcd}	7.27±1.27^{ijk}	4.33±0.58^{cdefg}	56.33±10.07^{abc}	80.33±0.58^{de}	6.97±0.38^{a}
A10 x A11(2)	39.00±1.00^{k}	30.68±1.43^{ef}	45.98±4.99^{cdef}	13.37±1.46^{a}	9.93±2.00^{cdefgh}	5.00±1.00^{abcde}	59.00±7.00^{ab}	86.00±5.29^{de}	**7.03±0.81^{a}**
A11 x A1	51.33±1.53^{d}	36.26±1.26^{bcd}	**68.52±0.81^{a}**	11.27±0.84^{b}	10.13±1.51^{cdef}	6.00±1.00^{ab}	46.33±6.11^{cdefghi}	86.67±9.94^{cde}	5.53±0.67^{abcdef}
A11 x A2	60.67±2.08^{b}	42.55±7.08^{ab}	67.39±0.94^{a}	9.17±0.64^{cdefghi}	5.27±0.86^{k}	5.33±0.58^{abcd}	47.00±1.00^{cdefghi}	74.33±1.53^{de}	4.63±0.47^{defghij}
A11 x A5	57.67±1.53^{c}	18.55±4.13^{h}	38.25±2.12^{ghi}	8.03±0.85^{i}	8.93±1.07^{efghi}	3.00±0.50^{g}	41.00±6.25^{efghi}	**126.00±7.94^{a}**	5.97±2.39^{abcd}
A11 x A6	46.33±1.53^{fg}	18.39±0.40^{h}	41.63±1.14^{efgh}	10.47±0.55^{bcdef}	11.43±0.93^{bcd}	5.67±1.15^{abc}	46.00±7.00^{cdefghi}	80.33±0.58^{de}	4.93±1.01^{cdefghi}
A11 x A7	48.00±1.00^{ef}	38.77±0.84^{b}	64.88±5.46^{a}	9.10±0.75^{cdefghi}	8.27±0.68^{efghij}	5.00±1.00^{abcde}	45.00±4.00^{cdefghi}	82.33±0.58^{de}	6.70±0.37^{ab}
A11 x A9	48.00±2.00^{ef}	30.67±3.44^{ef}	43.52±3.50^{cdefg}	8.33±0.58^{ghi}	6.07±0.91^{jk}	5.00±0.00^{abcde}	46.00±5.57^{cdefghi}	81.00±4.00^{de}	3.73±0.68^{hijkl}
A11 x A10	43.00±2.00^{hij}	32.39±2.76^{de}	40.31±6.95^{fgh}	10.17±1.04^{bcdefgh}	7.57±1.33^{ghij}	3.33±0.58^{fg}	36.33±7.51^{i}	83.00±6.25^{de}	5.27±0.87^{bcdefgh}
A11 x A13	**70.33± 1.53^{a}**	**44.04±3.59^{a}**	64.48±2.59^{a}	8.70±1.05^{defghi}	7.53±1.82^{hij}	5.00±1.00^{abcde}	39.00±7.21^{fghi}	74.33±1.53^{de}	6.40±0.52^{abc}
A12 x A3	41.33±1.53^{j}	30.01±0.35^{ef}	41.80±1.73^{defgh}	8.27±2.03^{ghi}	8.83±0.47^{efghi}	**6.33±0.58^{a}**	**60.33±4.51^{a}**	83.67±11.72^{de}	4.57±0.38^{defghij}
Mean	44.83±6.72	28.14±2.05	46.26±10.77	9.53±1.91^{ghi}	9.14±2.48	4.53±1.15	46.58±8.44	88.41±14.34	4.74±1.40
CV (%)	15.98 %	24.41 %	23.29 %	20.08 %	27.10 %	25.47 %	18.14%	16.21 %	29.57 %

FCW = Fresh Calyx weight NBPP = Number of branches per plant LL = Leaf length PH = Plant height DF = Days to first flowering
FFW = Fresh Fruit weight NFPP = Number of fruits per plant LW = Leaf width PL = Petiole length.

± sd = Standard deviation. **Bolded** and underlined values represent maximum and minimum values respectively for each trait.
Means in same column with same alphabets are not significantly different according to Duncan multiple range test.
LoS, ***, CV represent level of significant, significant at P ≤ 0.001 and total coefficient of variation respectively.

4.3.3 Clustering pattern of 14 local accessions of roselle (*H. sabdariffa* var *sabdariffa*) and their intra-specific F1 offspring.

Fig 4.2 displays a furthest neighbour dendrogram showing genetic relatedness of 14 local accessions of roselle and 25 F1 offspring obtained from intra-specific hybridisation among the accessions.

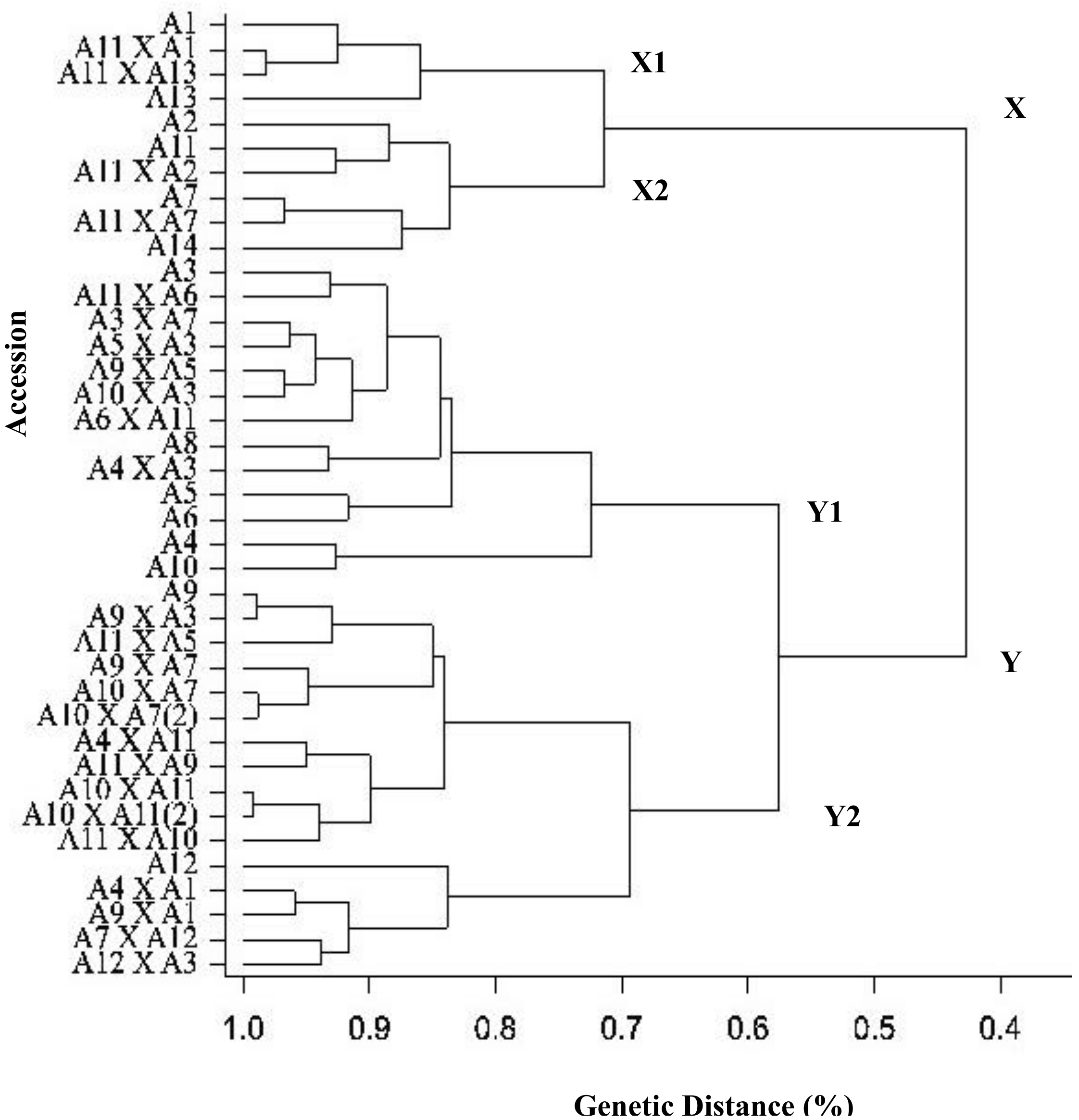

Fig. 4.2: Dendrogram showing relatedness of 14 accessions of roselle and 25 F1 offsprings based on 9 quantitative and 14 qualitative traits.

All entries were initially grouped into two major clusters, X and Y at genetic similarity distance of 45.0 %. Each cluster was further divided into two sub-clusters at 58 % (Y1 and Y2) and 70 % (X1 and X2) respectively. Accessions A1, A13 and F1 offspring from crosses among A1, A11 and A13 (A11 x A1 and A11 x A13) clustered into sub-cluster X1, while X2 comprised A2, A7, A11, A14, and one hybrid progenies (A11 x A2 and A11 X A7).

Sub-cluster Y1 was further separated into two sub-groups at a genetic distance of 72 %; the first sub-group consisted of six parental accessions and seven hybrid progeny; A3, A5, A6, A7, A8, A11 x A6, A3 x A7, A5 x A3, A9 x A5, A10 x A3, A6 x A11 and A4 x A3, while the second sub-group comprised only A4 and A10.

Similarly, the entries of sub-cluster Y2 also separated into two at a genetic distance of 69 %; the first group comprised one parental accession, A9 and eight F1 offspring, A9 x A3, A4 x A5, A9 x A7, A10 x A7, A4 x A11, A11 x A9, A10 x A11 and A11 x A10, while the second group also contained one parental accession A12, along with four F1 offspring, A4 x A1, A9 x A1, A7 x A12 and A12 x A3.

Additionally, each sub-cluster had at least one pair of entry which shared genetic similarity index above 95 %; X1 (A11 x A1 and A11 x A3), X2 (A7 and A11 x A7) Y1 (A3 x A7 and A5 x A3, A9 x A5 and A10 x A3) and Y2 (A9 and A9 x A3, A10 x A7 and A10 x A7 (2), A10 x A11 and A10 x A11 (2) and A4 x A1 and A9 x A1)

4.3.4 Principal Components Analysis (PCA).

Table 4.3 displays factor scores of nine quantitative traits for four principal components accounting for variability among 14 local accessions of roselle (*H. sabdariffa* var *sabdariffa*) and 25 F1 offspring obtained from intra-specific hybridisation among the accessions as well as contributions and eigen values of each principal component.

Table 4.3: Association of nine quantitative traits with four principal components accounting for total variability among 14 accessions of roselle and their F1 offspring.

Trait	Principal Component (PC)			
	1	**2**	**3**	**4**
Days to flowering	0.2459	-0.1352	-0.3635	**0.6786**
Fresh calyx weight	**0.5452**	-0.0266	-0.0995	-0.0734
Fresh fruit weight	**0.5322**	0.0160	-0.0092	-0.1778
Leaf length	**0.3061**	**0.5193**	0.0788	-0.1136
Leaf width	-0.0586	**0.6623**	0.0250	-0.1350
Number of branches per plant	-0.0141	-0.3024	**0.5982**	0.0883
Number of fruits per plant	-0.1170	**0.2330**	**0.5726**	**0.3384**
Plant height	-0.3042	**0.3374**	-0.2813	**0.4208**
Petiole length	**0.3942**	0.1133	**0.2937**	**0.4148**
Eigen value	3.0053*	1.7791	1.4242	1.04236
% variance	33.3920*	19.7681	15.8240	11.5820
Cumulative % variance	33.3920	53.1610	68.9851	**80.5670***

Bolded values represent variables which made significant contribution to total variance in respective axes. Maximum eigen value and percent variance are asterisked (*); minimum eigen value and percent variance are underlined. Maximum cumulative percent variance of the 4 principal components is bolded and asterisked.

Contributions of the four principal components were 33.39 %, 19.77 %, 15.82 % and 11.58 % for the first (PC1), second (PC2), third (PC3) and fourth (PC4) components respectively, with corresponding eigen values of 3.0053, 1.7791, 1.4242 and 1.04236 respectively, cumulating into maximum of 80.57 % of total variance.

Factor scores of the variables indicate that fresh calyx weight, fresh fruit weight, leaf length and petiole length shared significant positive association with PC1. For PC2, significant variables were leaf length, leaf width, plant height and number of fruits per plant. Number of branches per plant, number of fruits per plant and petiole length made significant positive contribution to the genetic variance in PC3, while days to flowering, number of fruits per plant, plant height and petiole length contributed significantly to PC4. Fig. 4.3 displays component weight of the nine quantitative agro-morphological characters.

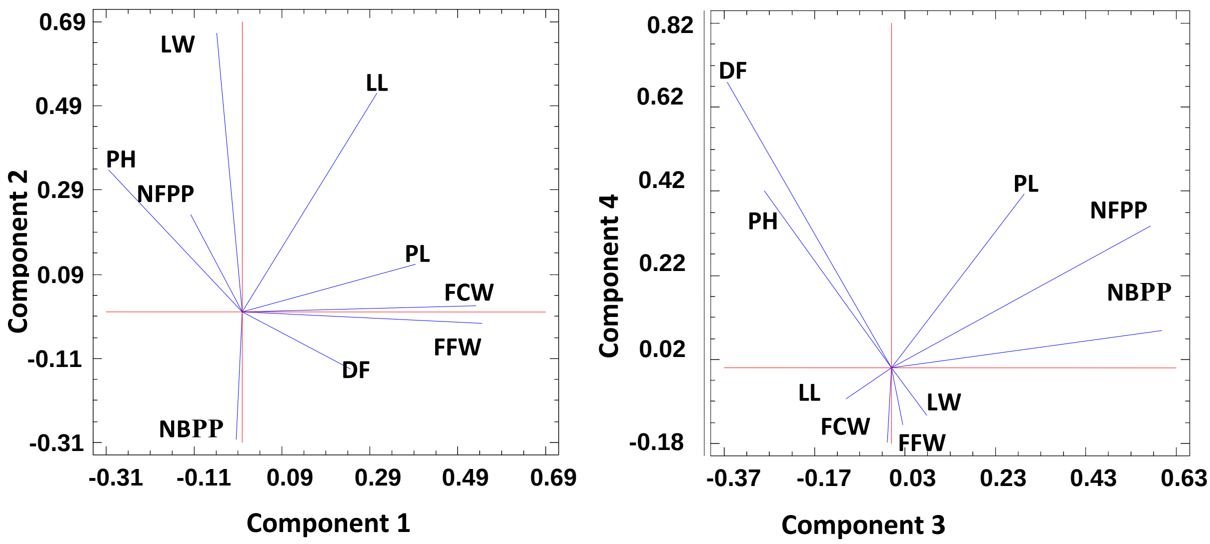

Fig. 4.3: Plot of component weight of 9 quantitative agro-morphological traits of roselle (*H.sabdariffa* var *sabdariffa*).

FCW = Fresh Calyx weight; NFPP = Number of fruits per plant; LL = Leaf length;PH = Plant height
FFW = Fresh Fruit weight; NBPP = Number of branches per plant; LW= Leaf width;PL = Petiole length
DF = Days to flowering.

4.3.5 Correlation studies among nine quantitative traits of roselle.

Table 4.4 displays degree of association among 9 quantitative traits of 14 local accessions of roselle and their intra-specific F1 hybrids. Days to first flowering (DF) and number of branches per plant (NBPP) did not show significant ($p \leq 0.05$) association with any of the other traits. Additionally, DF was negatively correlated to leaf width (LW), number of fruits per plant (NFPP) and plant height (PH), while NBPP also exhibited negative association with all the other traits except DF, number of fruits per plant (NFPP) and petiole length (PL).

A coefficient of correlation of r = 0.9052 reveals highly significant ($P \leq 0.01$) positive association between fresh fruit weight (FFW) and fresh calyx weight (FCW). Also, both FFW and FCW displayed highly significant positive correlation ($p \leq 0.01$) with leaf length (LL) (r = 0.4114; 0.4641) and petiole length (PL) (r = 0.5328; 0.5110) but exhibited negative association with NBPP (r = -0.1134; -0.0036), leaf width (LW) (r = -0.1117; -0.0300), number of fruits per plant (NFPP) (r = -0.2520; -0.2374) and plant height (PH) (r = -0.4705; -0.4580).

Leaf traits, LL and LW shared significant ($p \leq 0.05$) positive association (r = 0.4679) with each other and were also positively correlated to NFPP (r = 0.0988; 0.1824) and petiole length (PL) (r = 0.3880; 0.0186). However, LW displayed significant ($p \leq 0.05$) positive association with PH (r = 0.3664), while LL showed negative association (r = -0.0621). Finally, NFPP recorded non-significant positive relationship ($p \leq 0.05$) with PH and PL, while PH and PL shared negative association (r = -0.1964).

Table 4.3: Correlation matrix for nine quantitative traits of roselle (*H. sabdariffa* var *sabdariffa*).

	DF	FCW	FFW	NBPP	LL	LW	NFPP	PH	PL
DF									
FCW	**0.1178**^{ns}								
	0.4751								
FFW	**0.1087**^{ns}	**0.9052***							
	0.5100	0.0000							
NBPP	**0.0352**^{ns}	**-0.1134**^{ns}	**-0.0036**^{ns}						
	0.8315	0.4920	0.9828						
LL	**0.1341**^{ns}	**0.4114**	**0.4641**	**-0.1851**^{ns}					
	0.4156	0.0093	0.0029	0.2592					
LW	**-0.1807**^{ns}	**-0.1117**^{ns}	**-0.0300**^{ns}	**-0.2263**^{ns}	**0.4679**				
	0.2710	0.4985	0.8560	0.1659	0.0027				
NFPP	**-0.2604**^{ns}	**-0.2520**^{ns}	**-0.2374**^{ns}	**0.1717**^{ns}	**0.0988**^{ns}	**0.1824**^{ns}			
	0.1094	0.1217	0.1457	0.2958	0.5494	0.2665			
PH	**-0.0471**^{ns}	**-0.4705**	**-0.4580**	**-0.2136**^{ns}	**-0.0621**^{ns}	**0.3664**	**0.0461**^{ns}		
	0.7759	0.0025	0.0034	0.1916	0.7073	0.0218	0.7806		
PL	**0.0676**^{ns}	**0.5328***	**0.5110***	**0.1761**^{ns}	**0.3880**	**0.0186**^{ns}	**0.1733**^{ns}	**-0.1964**^{ns}	
	0.6827	0.0005	0.0009	0.2837	0.0146	0.9106	0.2915	0.2309	

FCW = Fresh Calyx weight NBPP = Number of branches per plant LL = Leaf length PH = Plant height DF = Days to first flowering
FFW = Fresh Fruit weight NFPP = Number of fruits per plant LW = Leaf width PL = Petiole length.

Below each correlation coefficient (**bolded)** is P-value (underlined). *, **, *** = significant at P ≤ 0.05, 0.01, 0.001 respectively
Ns = not significant at P ≤ 0.05.

4.4 Discussions

The next logical step for exploitation of genetic diversity after determination of crossability relationships among genotypes is the assessment of quantum of variability generated and then a study of inheritance patterns of targeted or key characteristics to facilitate efficient utilization of segregating generations. Identification and understanding of relationships between genotypes also ensures effective conservation of germplasm (De vicente *et al*., 2005).

4.4.1Variations in qualitative and quantitative agro-morphological traits of 14 accessions of roselle (*H. sabdariffa*) and their intra-specific F1 hybrids.

Minimal variation in flower colour, colouration of petal base and mature leaf colour was observed among the accessions investigated in this study which corresponds to results of Omalsaad *et al*., (2014), implying that these traits are not useful for studying genetic diversity of roselle germplasm.

On the other hand, six calyx colours namely, red, green, whitish green, dark red, whitish green with red patches and whitish green with red stripes were recorded in this study being the most variable among the qualitative traits. These observations disagree with those of Omalsaad *et al*., (2014) and El Tahir and El Gabri, (2013) who observed only red, dark red and whitish green. However, it is not surprising since the F1 hybrids apparently contributed to the high variation obtained in this study. This is supported by the fact that, F1 hybrids expressed a new colour combination (whitish green with red stripes) which was not observed in any of the existing cultivars evaluated in this study or in the earlier works. This underscores the contribution of hybridisation in expanding genetic variability in a base population.

The analyses also reveal that majority of the F1 offspring produced large or medium-size fruits compared to the parental accessions. Similarly, conspicuous pubescence was also observed on leaves, stems and calyces of most of the local cultivars used as parents in this study, pointing to their incomplete domestication status since expression of high degree of pubescence is an evolutionary adaptation to prevent insect attack in the wild. However, four parental lines namely, A1, A2, A7, A11 and A13 expressed glabrous or downy pubescence which could possibly be as a result of being purposefully selected over the years by farmers.

Calyx colour, fruit size and expression of pubescence are important characteristics for consumer acceptability and are used as selection criteria for grading cultivars of roselle. Cultivars with large fruit size normally attract premium prices due to their greater capacity to store photosynthates (Barrera *et al*., 2008), while those with red calyces and smooth surfaces are preferred by consumers and farmers as raw material for the popular beverage and non-irritating nature during cultivation and harvesting (Das and Das, 2000). Hybrid lines A11 x A1, A11 x A2, and A11 x A13 combined all of these attributes indicating their greater genetic and economic potential over the existing local cultivars used as parents hence could possibly be suitable substitutes as standard varieties.

Moreover, both red and green varieties have greater overall antioxidant and cyclo-oxygenase inhibitory activity than white varieties (Christian *et al.,* 2006). Thus F1 hybrids produced from crosses between red and green parents such as A11 x A13, A11 x A1 and A11 x A7 may possibly offer greater prospects for superior anthocyanin content.

The outcome of the assessment reveals significant variation among the accessions with respect to all quantitative traits evaluated in this study. This indicates a broad range of genetic variability offering an opportunity for selection among hybrid lines for a wide array of applications or hybridisation using parents with desirable attributes. In line with findings of Mohamad *et al*., (2011), fresh calyx weight, fresh fruit weight, leaf width and petiole length, accounted for the greatest variability among the accessions. These results also agree with the output of the principal component analysis thus, emphasising the relevance of these traits in future breeding work of roselle.

Generally, the F1 offspring registered significantly higher values for all the yield parameters; fresh calyx weight, fresh fruit weight, and number of fruit per plant. Overall A7 x A12 recorded the highest leaf length and leaf width which shows that it has the largest leaf surface area. Therefore, it could possibly intercept more light from the sun to produce more photosynthates than all the other accessions.

Apart from their other outstanding qualities hybrid lines A11 x A13 and A11 x A1 also produced significantly higher fresh calyx weight and fresh fruit than were obtained by the parental lines investigated in this study as well as those recorded by Mohamad *et al*., (2011). This provides some basis for their selection for further evaluation. Despite expressing conspicuous pubescence, A4 x A1 and A11 x A5 may serve as source of breeding material for introgression of desirable genes for earliness, high fruit yield and plant height, since they performed significantly better in these traits.

The analysis also reveals that A4 x A3 displayed the greatest capability to flower early, while A11 x A2 and A11 x A13 recorded shortest plant height.

These could impact positively on their yield since early flowering is an escape mechanism especially in the event of adverse environmental conditions such disease and pest incidence (Demirsoy and Demirsoy, 2004), while short plants are usually less prone to lodging in the events of heavy down pour and wind storms (Akinyele and Oseikita, 2006).

4.4.2 Genetic relationship among 14 local accessions of roselle and their intra-specific F1 hybrids based on cluster analysis.

Variations among genotypes do not only reflect their genetic constitution but also their interactions with the environmental. Hence combining qualitative and quantitative traits gives more desirable results in cluster analysis (Dixon and Nukenine, 2000).

The pattern of clustering from the cluster analysis based on both quantitative and qualitative traits, generally reflected variability in terms of calyx characteristics, as all entries of cluster X possessed relatively large calyces compared to those of cluster Y. Separation of the entries into sub-clusters also reflected similarity based on red pigmentation of the calyx, since members of sub-cluster X1 and Y2 exhibited red pigmentation of calyx, but X2 and Y1 produced no pigmentation.

Any pair of genotypes which share genetic similarity of above 95 % may be considered identical (Anderson *et al*., (2007). By applying this criterion to the results of the correlation analysis, eight pairs of entries are possible duplicates. These include offspring produced from crosses among same parent; A10 x A7 and A10 x A7 (2) or A10 x A11 and A10 x A11 (2). Since roselle is a tetraploid species, both parents transfer two copies (alleles) of each gene to their offspring and thus, offspring with different phenotypes but similar genetic constitution could be produced from crosses between the same parents.

4.4.3 Contribution of 9 quantitative traits to total variability via principal components analysis (PCA).

The main aim for undertaking principal component analysis in genetic diversity studies is to identify variables which contribute most to genetic variability to be selected for characterising genotypes (Johnson and Wichern, 1992).

Results of the principal component analysis show that 80.57 % of the total variability among the roselle accessions evaluated in this study was accounted for by the first four principal components thus, greater percentage of the total variance was explained by these components. This compares with 75.77 % and 82.97 % reported by earlier researchers (Torres-Morán *et al*., 2011; Ahiakpa *et al*., 2013) who also evaluated 12 local cultivars of roselle cultivated in Mexico and 30 accessions of Okra (*Abelmoschus* spp L.) in Ghana respectively. They however, differ from findings of Doku, (2011) who observed 91.87% contribution of first four principal axes to total variance among 17 accessions of African rice (*Oryza glaberrima* Steud) in Ghana.

Calyx and leaf parameters namely fresh calyx weight, fresh fruit weight, leaf length and petiole length shared significant positive association with the first principal axes (PC1) which contributed most (33.39 %) to the total genetic variance. This is in consonance with results of the quantitative analysis and also similarly to results recorded elsewhere (Torres-Morán, 2011). This implies that genes controlling the inheritance of these traits accounted for most of the genetic divergence as pointed out by Adeniji and Aremu, (2007). Therefore, it provides good basis for their selection for future investigations pertaining to genetic diversity of roselle germplasm.

4.4.4 Correlation analysis of 9 quantitative agro-morphological traits of roselle (*H. sabdariffa* var *sabdariffa*).

Calyx and leaf traits are perhaps the most important traits in roselle and their improvement is of particular interest in roselle breeding programmes. Results of the correlation analysis reveal strong positive association between fresh fruit weight and fresh calyx weight which agrees with the report by Ibrahim *et al*., (2013). This implies that component breeding would lead to significant increase in both fresh fruit weight and fresh calyx weight as pointed out by Hazra and Basu, (2000).

Improvement of fresh fruit weight and fresh calyx weight could also be accomplished indirectly through selection for leaf length since both calyx traits shared strong positive association with leaf length. However, breeding for plant height and number of branches per plant would have negative effect on both fresh fruit weight and fresh calyx weight since they exhibited negative association with these traits. Days to first flowering did not show strong correlation with the other traits. Hence, breeding for earliness would not have any significant effect on other traits of importance.

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CHAPTER FIVE

5.0 COMBINING ABILITY AND ESTIMATES OF HETEROSIS IN 6 X 6 DIALLELE CROSS OF ROSELLE (*Hibiscus sabdariffa* var. *altissima* W.).

5.1 Introduction

Roselle has in the last decade gained significant attention as a substitute to traditional fibre crops such as jute (*Corchorus olitorius*) and kenaf (*Hibiscus cannabinus*). Due to its wide ecological adaptability, roselle is more tolerant to drought and grows fairly well in areas with less rainfall than kenaf (FAO, 2003; Liu, 2003), but produces bast fibre of comparable quality to jute fibre (Baker, 1970).

Indeed, roselle plant possesses enormous potential for economic benefit as a fibre crop. This has been demonstrated in regions where it has received considerable attention through breeding. However, in West Africa and Ghana in particular, cultivation and utilisation of roselle is very low as a result of gross neglect by breeders to enhance the genetic and yield potential of existing cultivars.

Even though it has the potential to grow well across Ghana, roselle is predominantly cultivated in the northern part of the country, where the bast fibre (obtained from the stem) is used for a number of applications such as packaging materials for transporting farm produce (such as yam and millet). Also, yarns obtained from the fibre are used as roofing material and by livestock farmers for tying animals (Blench, 2007).

Despite the enormous potential for fibre production, a combination of factors including inadequate supply of raw material, has contributed to the collapse of the Kumasi Jute Factory which was established to supply jute bags for exporting cocoa. Consequently, Ghana currently imports jute sacs from other countries (GNA, 2014).

However, the recent „„home-grown"" policy of the Government has redirected focus towards the development of the local fibre industry in order to locally produce jute bags for exporting cocoa and other agricultural commodities and also to create jobs (GNA, 2014). One of the strategies to achieve this goal is promotion of research into locally available but under-exploited fibre resources such as roselle (*Hibiscus sabdariffa* var. *altissima* W.) and provision of other support services for their large-scale cultivation in Ghana.

Despite huge prospects of economic benefits as a fibre crop, large-scale cultivation of roselle (*Hibiscus sabdariffa* var. *altissima*) in Ghana is constrained by unavailability of improved varieties which are adapted to the growing conditions of Ghana. Existing cultivars are low yielding and susceptible to diseases and pests, hence are unsuitable for large-scale cultivation. As a result, roselle is typically cultivated along field boundaries as vegetable crop (Abbey *et al*., 2006).

Research indicate that, considerable genetic potential exist among available landrace varieties of roselle (Asante and Amankwa-Tia, 1992). This could be incorporated in the breeding programme to develop superior varieties with improved yield and yield-related parameters such as disease and pest resistance in order to renew farmers" interests in commercial cultivation to tap into the numerous opportunities offered by the crop.

Development of improved varieties has been very critical in boosting production of many important crops (Low *et al*., 2009), which is evident in Ghana for cocoa, oil palm and pineapple (Conley *et al*., 2001).

Artificial sexual hybridisation among locally adapted cultivars offers a good option for generating a wide range of segregating populations from which recurrent selection can be carried out to obtain superior varieties. However, the outcome of any hybridisation programme depends on combining ability (ability to transfer desirable traits to offspring in hybrid combinations) of genotypes selected as parents (Mohammad, 2003).

Apart from facilitating identification of promising genotypes for future breeding programmes, knowledge of combining ability also helps to better understand the nature of gene action underlying inheritance of various traits, thereby ensuring efficiency in breeding efforts (Basbag, 2007). Consequently, investigation of combining ability is a prerequisite in plant breeding programmes aimed at improving yield and other quantitative traits through artificial hybridisation (Mohammad, 2003).

Crossing in diallel fashion is an effective method which has been exploited by a host of researchers to study combining ability (Mohammed and Rasheed, 2011; Mostofa *et al*., 2011; Viana, and Matta, 2003). By allowing partitioning of variance components for a given trait into additive and dominance effects coupled with simplicity in computation, diallel analysis allows crossing and selection procedures to be better optimized for traits of interest (Pickett, 1993). By this, early testing of genotypes is achieved to identify genotypes which can be used to produce superior hybrids and thus bring efficiency into the breeding process (Pickett, 1993).

Studies of heterosis provide relevant information to complement studies of combining ability to offer solid basis for selecting parent for future breeding programmes. Heterosis or hybrid vigour refers to superiority of a hybrid line over the mean of its parents (mid-parent heterosis) or over the mean of the better parent (better-parent heterosis) (Bernado, 2002). Studies of expression of heterosis are fundamental to hybrid breeding programmes aimed at improvement of desirable agronomic traits. Good heterotic responses are obtainable when crosses are made between parents originating from genetically different populations (Hallauer and Miranda, 1988). For better exploitation of heterosis in hybrid breeding, heterotic groups and patterns need to be established (Melchinger and Gumber, 1998).

Expression of heterosis in roselle has been reported elsewhere (Louis *et al*., 2013; Mostofa *et al*., 2011; Gasim, 1994). This notwithstanding, there is still the need to establish heterotic groups and patterns among the local germplasm resource of roselle. Study of combining ability and expression of heterosis among the local cultivars of roselle would provide relevant information to fully explore the possibility of hybrid breeding in Ghana.

5.1.1 Objectives of the study

The main objective of this study was to investigate genetic variability and combining abilities of six local accessions of roselle (*H. sabdariffa var altssima*) and to estimate expression of heterosis by F1 offspring obtained from intra-specific hybridisation among the accessions for seven key quantitative agro-morphological traits.

The specific objectives were to;

1. Assess the extent of variability for seven quantitative agro-morphological traits among six local accessions of roselle.
2. Investigate general and specific combining abilities of the six accessions.
3. Assess the nature of gene action underlying the inheritance of each of the selected traits.
4. Estimate expression of heterosis by 30 F1 offspring for the seven quantitative agro-morphological traits.

5.2 Materials and Methods

5.2.1 Experimental site

The experiment was conducted at the Research Farms of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC), Kwabenya, in the Greater Accra Region of Ghana. The BNARI Research Farm is located at latitude 05° 40′ N and longitude 0° 13′ W, and elevated at 76 m above sea level within the coastal savannah agro-ecological zone. Kwabenya has an annual average temperature of 28 °C and receives an annual rainfall less than 1000 mm (Ghana Meteorological Authority, 2005). The soil at site is the Nyigbenya-Haatso series, which is a typically well-drained savannah ochrosol (Ferric Acrisol) derived from quartzite schist (FAO/UNESCO, 1994).

5.2.2 Planting material

Six local accessions of roselle (*H. sabdariffa* var *altissima*) and 30 F1 hybrids obtained from crosses among the accessions were used for the study. Total entries were 36 including six parents (inbred lines), 15 crosses and 15 reciprocals according to Method 1 of the diallele mating design by Griffing (1956).

5. 2.3 Experimental design and planting

The seeds were manually sown in plastic bags (measuring 12 cm x 6 cm) filled with potting medium comprising three parts of top soil and one part manure. Seedlings were transplanted into the pots exactly one week after germination. Optimal management practices such weed control at two-week intervals and daily watering for up to 14 days were carried out. However, no pesticides or fertilizers were applied throughout the duration of the experiment.

The experiment was set up in randomized complete block design (RCBD) with three replicates. Each replicate comprised ten pots of 30 plants per accession separated at a distance of 30 cm within rows and 1 m between rows.

5.2.4 Data collection

Using ten randomly selected plants per entry, data were collected on seven quantitative agro-morphological traits namely;

(i) Number of fruits per plant (NFPP)

(ii) Days to initial flowering (DF)

(iii) Stem basal diameter (SBD)

(iv) Petiole length (PL)

(v) Leaf length (LL)

(vi) Leaf width and (LW)

(vii) Plant height (PH)

5.2.5 Data analysis

Means were subjected to Analysis of variance (ANOVA) to determine the level significance of variability among accessions for the each of the seven parameters. Analysis of the combining ability was carried out following Griffing‟s Method 1, where parents, F1s and reciprocals are included. Genstats Statistical Software Package (12th edition), Statsgraphics Centurion software (version 16.1) and Microsoft Excel Software (2010) edition were used for the Data analyses.

5.2.6 Estimation of gene action

The ratio of general combining ability to specific combining ability (GCA: SCA) was estimated to measure the relative importance of additive and non-additive gene action for inheritance of each of the seven traits.

5.2.7 Determination of general and specific combining ability

General combining ability (G.C.A) and specific combining ability (SCA) were estimated using the formulae of Singh and Chaudhary, (2004). General combining ability was computed as:,

$$GCA = \overline{y} - Y$$

Where $\overline{y}$ = mean of parent,

Y = grand mean

Similarly, specific combining ability was given as:

$$SCA = \overline{yij} - \hat{g}j - \hat{g}i + Y$$

Where, $\overline{yij}$ = mean value of F1 from *ith* and *jth* parents

$\hat{g}j$ = general combining ability of *jth* parent,

$\hat{g}i$ = general combining ability of *ith* parent.

Y = grand mean

5.2.8 Estimation of heterosis.

Percent mid parent heterosis (MPH %) and better parent heterosis (BPH %) were calculated based on the formulae of Falconer and Mackay, (1996) ;

$$(1)\ MPH\ \% = \frac{(F1 - [Pi + Pj] / 2)}{[Pi + Pj] / 2} \times 100$$

$$(2)\ BPH\ \% = \frac{(F1 - BP)}{B} \times 100$$

Where, MPH % = mid parent heterosis of F1 hybrid,

F1 = mean value of F1 hybrid,

Pi = mean value of first parent,

Pj = mean value of second parent,

BPH % = heterobeltiosis of F1 Hybrid and

BP = mean value of better parent.

5.3 RESULTS

5.3.1 Variation in quantitative agro-morphological traits among six accessions of var *altissima*.

Table 5.2 shows variation in seven quantitative agro-morphological traits among six inbred lines of roselle. Significant variation ($p \leq 0.01$) was observed among the accessions for all parameters investigated (Appendix 9.2).

Accession D1 recorded highest number of fruits per plant (NFPP), while D6 scored highest leaf length (LL), leaf width (LW) and stem basal diameter (SBD), though values obtained by D3, D4 and D5 are not statistically different from those of D6 with respect to SBD. Similarly, D4 registered the highest days to first flowering (DF) although not significantly different from those of D2, D3 and D6. Correspondingly, D3 gave highest plant height (PH), but did not differ from values obtained by D5 and D6. Likewise, D2 scored highest petiole length (PL), but similar to the value of accession D4.

Conversely, D2 and D5 recorded lowest NFPP and PL respectively, while D1 registered lowest values for PH, LL and LW and SBD.

Table 5.1: Variation in seven quantitative traits among six local accessions of roselle (*H. sabdariffa var altissima*).

Parent	NFPP	DF (days)	PH (cm)	LL (cm)	LW (cm)	PL (cm)	SBD (cm)
D1	**70.33±4.51 [a]**	40.00±1.00 [b]	195.00±3.00 [c]	7.33±0.81 [c]	5.30±0.30 [b]	7.20±1.18 [c]	1.67±0.51 [b]
D2	54.00±5.00 [b]	44.00±1.00[a]	203.00±3.60 [bc]	10.27±0.81 [ab]	5.47±0.67 [ab]	**12.57±1.50 [a]**	2.31±0.50 [ab]
D3	66.00±16.52 [ab]	44.33±1.53 [a]	**228.00±3.60 [a]**	8.67±1.53 [bc]	5.57±0.99 [ab]	11.33±0.58 [ab]	2.60±0.50 [a]
D4	64.33±5.13 [ab]	**44.67±1.53 [a]**	204.67±1.15 [b]	10.80±2.71 [ab]	7.10±1.28 [ab]	12.33±3.51 [a]	2.59±0.21 [a]
D5	64.67±1.15 [ab]	38.00±1.00 [b]	224.00±9.17 [a]	8.63±0.93 [bc]	5.50±0.36 [ab]	6.90±1.06 [c]	2.43±0.15 [a]
D6	66.33±2.56 [ab]	42.67±0.57 [a]	226.33±5.13 [a]	**11.40±0.40 [a]**	**8.67±4.16 [a]**	8.67±0.76 [bc]	**2.80±0.36 [a]**
CV %	12.82	6.43	6.57	19.74	32.03	28.62	20.89

DF = Days to first flowering PH = Plant height LL = Leaf length SBD = stem basal diameter

NFPP = Number of fruits per plant PL = Petiole length LW = Leaf width

Bolded and underlined values represent highest and least values respectively for each trait. Means in same column with same alphabets are not significantly different according to Duncan multiple range test. CV = total coefficient of variation.

5.3.4 General combining ability.

Table 5.2 shows general combining ability of six local accessions of roselle (*H. sabdariffa* var *altissima*) used as parents for the study. For days to first flowering (DF), accessions D1, D2 and D3 recorded positive values while D4, D5 and D6 gave negative, with D2 and D5 emerging with highest (4.11**)** and least (-6.29) respectively.

With 9.41 and -6.11, D4 and D5 scored highest and least values respectively for plant height, with only D3 and D4 recorded positive values. Accession D6 gave highest values for the two leaf traits, leaf length and leaf width, while D4 and D1 scored least values respectively. Similarly, values of D2 (1.68 and 0.41**)** and D4 **(**1.34) emerged highest for number of fruits per plant (NFPP), petiole length (PL) and stem basal diameter (SBD) respectively. On the other hand, D6 (-0.88) and D1 (-0.50 and -1.14) emerged with least values for PL, SBD and NFPP respectively.

Table 5.2: General combining ability (GCA) of six accessions of roselle.

Parent	DF	PH	LL	LW	NFPP	PL	SBD
D1	2.31	-5.47	-0.14	-0.88*	-0.29	-0.50*	-1.14*
D2	**4.11**	-3.99	0.99	-0.37	**1.68**	**0.41**	-0.68
D3	3.45	8.75	0.81	-0.2	0.07	0.17	0.47
D4	-1.49	**9.41**	-4.85*	0.07	0.2	-0.2	**1.34**
D5	-6.29*	-6.11*	1.03	-0.29	-0.37	-0.12	-0.25
D6	-2.09	-2.59	**2.16**	**1.68**	-0.88*	0.23	-1.10

DF = Days to first flowering PH = Plant height ; LL = Leaf length; LW = Leaf width
NFPP = Number of fruits per plant; PL = Petiole length and SBD = Stem basal diameter.

Bolded and asterisked (*) values represent best and worst general combiners respectively for each trait.

5.3.5 Specific combining ability (SCA) effects

Table 5.3 displays specific combining ability of six inbred lines of roselle in pairwise crosses with each other.

Table 5.3: Specific combining ability (SCA) of six local accessions of roselle.

F1 offspring	DF	PH	LL	LW	NFPP	PL	SBD
D1 x D2	-0.91	13.77	-0.33	0.42	41.78*	-0.72	-1.10*
D1 x D3	2.75	-9.27	-1.21	1.70	54.02	0.95	0.35
D1 x D4	4.36	-26.63*	2.295	-3.05*	66.72	-1.45	-0.36
D1 x D5	-2.18	-0.11	0.745	1.63	61.64	-0.05	0.47
D1 x D6	-1.71	16.77	-1.639	-1.59	58.62	0.29	0.65
D2 x D1	5.09	15.47	-0.359	0.39	64.11	-0.82	0.55
D2 x D3	-7.38	3.95	-2.61	-1.43	58.45	-2.08	-0.12
D2 x D4	**6.02**	-25.41	2.95	-1.96	59.82	-2.68*	0.07
D2 x D5	2.69	7.11	0.54	1.86	67.07	**4.27**	0.12
D2 x D6	-1.51	-5.11	0.28	0.76	62.39	1.72	-0.11
D3 x D1	2.42	3.73	-1.01	-0.26	47.35	0.25	2.25
D3 x D2	0.28	4.95	-2.75	-1.57	60.78	-1.28	1.99
D3 x D4	-2.45	-14.45	3.31	-1.61	44.74	-0.80	1.74
D3 x D5	5.22	1.67	-1.11	0.22	48.32	-2.44	0.08
D3 x D6	-2.84	12.85	2.54	**3.40**	43.30	4.43	-0.90
D4 x D1	-4.31	9.07	5.40	0.05	62.72	-0.41	0.59
D4 x D2	4.89	9.59	2.52	-2.39	42.49	-2.71	-0.42
D4 x D3	-0.45	4.85	5.66	3.02	53.04	1.77	-0.36
D4 x D5	-2.71	-1.99	5.06	0.28	67.68	0.45	-0.01
D4 x D6	1.09	-12.11	4.33	-1.28	58.00	0.70	0.46
D5 x D1	-4.51	13.89	-2.52	-1.63	55.64	-1.26	-1.13
D5 x D2	-9.31*	0.81	1.67	2.99	**69.74**	2.04	0.10
D5 x D3	1.35	-5.93	1.50	2.82	59.66	-1.98	1.09
D5 x D4	-7.59	-7.59	2.43	-2.35	45.02	1.25	-0.28
D5 x D6	6.22	-7.29	-6.08*	-2.13	50.25	-0.18	-0.30
D6 x D1	-0.71	8.77	0.39	0.44	57.96	0.79	2.35
D6 x D2	-3.18	1.89	0.95	1.43	58.72	-0.61	1.35
D6 x D3	-2.51	-12.45	0.10	0.58	55.96	1.30	-0.45
D6 x D4	2.09	-21.51	**5.73**	0.12	60.67	0.67	**3.45**
D6 x D5	2.22	**20.71**	-5.51	-0.88	58.25	-1.91	1.05

Bolded and asterisked (*) values represent best and worst specific combiners respectively

DF = Days to first flowering PH = Plant height; LL = Leaf length; LW = Leaf width; NFPP = Number of fruits per plant; PL= Petiole length and SBD = Stem basal diameter.

Values of D2 x D5 (4.27) and D5 x D2 (69.74) emerged highest for petiole length (PL) and number of fruits per plant (NFPP) respectively. Similarly, D2 x D4, D6 x D5, D3 x D6, gave highest values for days to first flowering (DF) (6.02), plant height (PH) (20.71), and leaf width (LW) (3.40) respectively, while values of D6 x D4 scored highest leaf length (LL) (5.73) and stem basal diameter (SBD) (3.45).

On the other hand, D5 x D2, D5 x D6, and D2 x D4 produced least values for DF (-9.31), LL (-6.08), and PL (-2.68) respectively. In like manner, D1 x D2 and gave least values for NFPP (41.78) and SBD (-1.10) while D1 x D4 also produced least values with respect to PH (-26.63) and LW (-3.05).

5.3.6 Estimates of gene action

Table 5.4 shows the ratios of general combining ability to specific combining ability (GCA: SCA) for six quantitative agro-morphological traits investigated in this study. Apart from leaf length (LL) and stem basal diameter (SBD), GCA: SCA of all the traits was less than one (1).

Table 5.4: Estimates of gene action for six agro-morphological traits.

Trait	DF	PH	LL	LW	NFPP	PL	SBD
GCA:SCA	0.20	0.57	1.63	0.43	0.47	0.49	1.08

DF = Days to first flowering; PH = Plant height; LL = Leaf length; LW = Leaf width; NFPP = Number of fruits per plant, PL = Petiole length and SBD = Stem basal diameter.

5.3.7 Estimates of heterosis.

Table 5.5 shows estimates of mid parent heterosis (MPH) and better parent heterosis (BPH) expressed by 30 F1 hybrids obtained from intra-specific hybridisation among six local landrace varieties of roselle for seven agro-morphological quantitative traits. Generally extensive variation was recorded with respect to both MPH and BPH ranging from -51.35 to 78.85 and -52.63 to 59.58 respectively.

Most crosses produced positive MPH and BPH for days to first flowering (DF), length width (LW) and stem basal diameter (SBD), but relatively few of the F1 offspring gave positive values for plant height (PH), leaf length (LL), number of fruits per plant (NFPP) and petiole length (PL).

D2 x D4 produced highest MPH (33.33) and BPH (27.27) for DF, while D5 x D2 (-19.51 and -25.00) recorded least values for MPH and BPH with respectively. Values recorded by D6 x D5 (6.64 and 4.12) and D1 x D4 (-11.90 and -13.69) emerged highest and least for MPH and BPH respectively for PH. Similarly, D6 x D4 recorded highest MPH (45.41) and BPH (34.08) for LL, while D5 x D6 (- 51.35 and - 52.63) gave least values.

Correspondingly, D3 x D6 registered highest MPH (78.85) and BPH (59.58) for LW, while D1 x D4 gave the least values (- 26.87 and - 40.37). With respect to NFPP, values of D5 x D2 (31.42 and 20.56) and D1 x D2 (- 24.18 and - 26.50) gave highest and least MPH and BPH respectively. Also D5 x D2 (69.44 and 65.08) and D5 x D4 (-35.26 and - 46.78) scored highest and least values for PL, while D6 x D4 (30.40 and 27.20) and D6 x D1 (-9.40 and -11.70) emerged highest and least for stem SBD.

Table 5.7: Estimates of percent (%) Mid Parent Heterosis (MPH) and Better Parent Heterosis (BPH) expressed by 30 F1 offspring of roselle for seven quantitative traits.

F1	DF		PH		LL		LW		NFPP		PL		SBD	
Offspring	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH
D1 x D2	19.05	13.64	3.00	1.10	-9.98	-26.05	26.83	24.86	<u>-24.18</u>	<u>-26.50</u>	1.13	-0.42	1.10	-13.20
D1 x D3	25.70	19.56	-5.99	-11.18	-22.97	-32.19	12.32	11.33	-6.77	-6.98	-6.06	-24.09	10.30	6.70
D1 x D4	17.33	11.19	-<u>11.90</u>	<u>-13.69</u>	-44.97	-49.37	<u>-26.87</u>	<u>-40.37</u>	13.32	11.61	-39.62	-52.72	-6.40	-8.20
D1 x D5	-1.72	-.4.17	-5.56	-8.01	-2.84	-14.65	11.21	10.91	5.34	4.07	7.07	2.77	10.80	3.20
D1 x D6	4.03	0.77	2.08	-2.53	-21.83	-25.70	-23.15	-31.97	-2.88	-5.65	2.24	-7.73	7.80	-1.00
D2 x D1	18.03	17.15	3.83	1.91	-10.30	-26.31	55.00	53.69	19.70	8.59	-0.28	-1.80	6.30	-3.10
D2 x D3	1.14	0.77	-0.89	-4.68	-9.12	-16.38	10.95	8.26	11.67	1.51	-30.17	-42.89	-5.90	-6.91
D2 x D4	**33.33**	**27.27**	-11.01	-11.50	-22.78	-35.18	41.98	28.85	14.96	5.75	6.70	4.60	17.10	2.90
D2 x D5	9.76	2.27	-3.16	-3.91	31.20	21.32	5.55	3.64	26.92	16.43	**69.44**	**65.08**	8.30	-0.80
D2 x D6	3.84	2.27	-9.23	-11.75	28.75	10.32	55.97	36.20	14.24	0.99	30.16	19.12	21.10	12.00
D3 x D1	24.91	18.73	0.09	-5.43	-20.98	-30.44	-6.34	-7.18	-16.85	-17.03	-13.71	-30.27	27.3	17.80
D3 x D2	18.49	17.98	-0.44	-4.24	-10.87	-17.76	3.03	0.54	15.55	5.04	-21.53	-35.83	-7.80	-10.10
D3 x D4	-1.12	-1.50	-3.70	-6.89	-22.65	-30.28	-15.73	-30.79	-19.15	-20.20	-39.73	-42.17	17.10	2.90
D3 x D5	15.80	7.38	-3.65	-6,63	4.05	3.81	38.57	37.70	-13.80	-14.65	-41.12	-50.84	7.70	4.60
D3 x D6	-1.15	-3.00	0.98	-0.13	5.65	3.05	**78.85**	**59.58**	-17.42	-25.08	28.00	12.97	10.80	3.20

Bolded and underlined values represent highest and lowest heterosis combiners respectively for each trait.

DF = Days to first flowering; PH = Plant height; LL = Leaf length; SBD = Stem basal diameter;
NFPP = Number of fruits per plant; PL = Petiole length and LW = Leaf width.

Table 5.7 (continued): Estimates of percent (%) Mid Parents Heterosis (MPH) and Better Parent Heterosis (BPH) expressed by 30 F1 offspring of roselle for seven quantitative traits.

F1	DF		PH		LL		LW		NFPP		PL		SBD	
Offspring	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH
D4 x D1	-3.15	-8.21	1.01	-1.04	-23.42	-25.44	1.84	-16.95	7.20	5.58	-28.84	-44.28	-8.20	-8.90
D4 x D2	17.29	16.41	5.64	5.06	-27.52	-39.17	-25.12	-39.68	-14.34	-21.20	-43.98	-55.64	1.50	-6.80
D4 x D3	4.05	3.81	7.70	4.60	13.32	11.61	-1.72	-.4.17	7.07	2.77	-6.06	-24.09	-1.72	-.4.17
D4 x D5	-17.74	-23.89	-1.65	-1.88	3.96	-13.61	-13.48	-29.30	16.79	16.43	-18.67	-34.31	7.40	6.70
D4 x D6	-3.82	-5.98	-6.75	-8.86	-7.64	-9.91	11.50	0.35	-1.93	-6.12	-17.14	-29.44	-5.90	-3.00
D5 x D1	-7.69	-10.00	1.21	-1.41	-35.40	-43.24	-2.83	-3.09	-3.82	-4.98	-16.27	-19.63	-8.80	-5.40
D5 x D2	-19.51	-25.00	-6.15	-6.88	45.36	34.41	7.41	5.45	**31.42**	**20.56**	39.28	35.70	23.8	13.80
D5 x D3	4.46	-3.00	-7.11	-9.99	34.10	33.79	56.64	55.65	3.55	2.53	-36.26	-46.78	13.50	5.80
D5 x D4	-11.28	-17.91	-4.30	-4.52	-31.03	-37.96	-7.83	-8.03	-18.34	-18.59	-43.68	-55.39	2.10	-7.70
D5 x D6	4.95	-0.80	-10.66	-12.78	-51.35	-52.63	-5.87	-16.48	-13.58	-17.03	-2.83	-8.88	-8.10	-10.50
D6 x D1	6.45	3.12	-1.70	-6.14	-3.09	-7.89	57.52	39.44	-3.85	-6.59	8.63	-1.96	-9.40	-11.70
D6 x D2	-0.01	-1.52	-5.97	-8.56	36.36	16.84	29.03	12.68	8.33	-4.22	0.82	-7.73	7.90	-1.40
D6 x D3	-0.39	-2.25	-10.32	-11.31	19.64	10.32	52.64	36.20	-5.60	-8.49	-3.3	-14.65	22.90	18.80
D6 x D4	-1.53	-3.74	-11.10	-13.10	**45.41**	**34.08**	-2.73	-11.53	2.03	-2.32	-17.43	-23.43	**30.40**	**27.20**
D6 x D5	-0.10	-4.97	**6.64**	**4.12**	-38.94	-43.82	-15.40	-24.93	-1.73	-5.65	-24.11	-29.12	20.20	13.50

Bolded and underlined values represent highest and lowest heterosis combiners respectively for each trait.

DF = Days to first flowering; PH = Plant height; LL = Leaf length; SBD = Stem basal diameter;
NFPP = Number of fruits per plant; PL = Petiole length and LW = Leaf width.

5.4.0 Discussion

5.4.1 Variation in quantitative agro-morphological traits among six local accessions of roselle (*H. sabdariffa var altissima* W.).

Results of the analyses reveal significant variation among the accessions for all the quantitative agro-morphological traits investigated. This outcome is in agreement with the observations by previous researchers (Asante and Amankwa-Tia, 1992), who also investigated genetic diversity of some selected local accessions of roselle in the guinea savanna ecological zone of Ghana. This broad genetic variability among the local landrace varieties of roselle gives a wider scope for manipulation through breeding to improve yield and related parameters of the crop.

Extensive variability in useful traits such as leaf length, leaf width, plant height and stem basal diameter, among the accessions evaluated in this study indicates that, selection could be made for further hybridisation towards improvement of fibre attributes of roselle. Leaf area, plant height and stem basal diameter are positively correlated to biomass production, which is a major determinants of yield and quality of fibre obtained from roselle (Danalatos and Archontoulis, 2010). Large leaf area increases the capacity to undertake photosynthesis and hence greater amount of assimilates necessary for growth and biomass production (Li *et al*., 2009). Similarly, tall plant height and thick stems possess better ability to store photosynthates which also leads to greater accumulation of biomass.

The outcome of the analyses reveal that maximum leaf area, stem basal diameter and plant height were obtained by parental line D6 and D3 respectively, and thus could be expected to accumulate greater levels of biomass. Therefore, high priority should be given to these varieties during selection of genotypes to be used in breeding programmes towards improvement in fibre related attributes of roselle.

On the other hand, least number of days to flowering and highest number of fruits per plant recorded by accessions D5 and D2 respectively could be detrimental to their ability to accumulate biomass since early flowering and fruit production limit accumulation of cellulose and hemicelluloses (bast fibre reserves) in the stem (Danalatos and Archontoulis, 2010).

5.4.2 General and specific combining abilities of six local accessions of roselle.

General combining ability (GCA) is the relative performance of a parental line among similar groups of genotypes when crossed to a number of heterogeneous testers (Cruz and Regazzi, 1994). It is a critical factor used in selecting varieties for inclusion in elite germplasm pools for future use in breeding work. Small or negative combining ability indicates poor ability of a particular parent to transfer its genetic superiority to offspring in hybrids combination. A high or positive GCA value correlates to good ability of a parental line to produces superior offspring (Cruz and Regazzi, 1994).

Accessions D2, D6 and D4 came out as best general combiners for days to first flowering, leaf length and leaf width and plant height respectively. These traits are critical factors for evaluating the potential of a genotype to produce offspring with superior yield and fibre attributes (Ogunbodede and Ajibade, 2001). This implies that segregating population of these cultivars will perform better than should be expected for the others and thus gives credence for their selection and inclusion in future breeding work.

Specific combining ability (SCA) refers to progeny performance resulting from a particular cross as related to the performance of other crosses of similar nature. SCA indicate which particular hybrid combination may be doing relatively better or worse than would be expected from the average of the two parents. Specific combining ability is attributed to non-additive genetic variance.

As evident from the analyses, cross between D2 x D5, D2 x D4, D6 x D5, D3 x D6 and D6 x D4 produced highest SCA for major yield and fibre related attributes (days to first flowering, plant height, leaf length, and leaf width and stem basal diameter), hence paired crosses between these genotypes could give better results towards improvement of yield of roselle.

In contrast to the results recorded by Louis *et al*., (2013) in which desirable crosses for every trait involved at least one highest general combiner, in this study high specific combining ability did not always involve parents with high general combiners. Epistatic interactions between genes of two worse combiners could result into superior hybrids as suggested by Baker, (1978).

5.4.3 Estimates of gene action

The relative magnitude of general combining ability to specific combining ability (GCA: SCA) indicates whether inheritance of a character is mainly determined by additive or non-additive gene action (Singh and Chaudhary, 1985). If the ratio of GCA variance to SCA variance is greater than one, inheritance is determined by additive genetic effects otherwise, non-additive or dominant genetic variances is prevalent (Baker, 1978).

Results obtained in this study indicates that greater portion of heritable variation was of additive nature for plant height and stem basal diameter, but inheritance of other traits are large controlled by dominant gene effect.

These results correlates with findings of (Louis *et* al., 2013; Mostofa *et al*., 2011; Gasim, 1994), implying that both the additive and non-additive gene effects control inheritance of useful traits in the genetic of roselle. Consequently, a breeding approach which could mop up the fixable (additive) genes (additive genes) and also maintain considerable heterozygosity for exploiting the dominant gene action such as recurrent selection would be suitable for simultaneous improvement of the useful traits of roselle as suggested by Ahmad (2002).

5.4.4 Expression of Heterosis by F1 offspring

Heterosis (hybrid vigour) is the superior performance of a hybrid compared with the performance of its parents. Expression of heterosis depends on the differences in the gene frequency of the parental materials that are used for hybridisation.

Mid-parent heterosis (MPH) refers to the superiority of a hybrid over the mean of its parents, while better heterosis refers superior performance of a hybrid line over the mean performance of the better parent for a given trait (Bernado, 2002). These results are in agreement with observations of previous workers (Louis *et al*., 2013 Mostofa *et al*., 2011), who also recorded significantly high heterosis over mid and better parent(s) for all studied traits. Wide genetic variability among the parents utilised for the hybridisation could have accounted for greater hybrid vigour of the F1 offspring (Hallauer and Miranda, 1988).

In recent years, development of new varieties with greater capacity for stalk biomass accumulation has assume central focus in hybrid breeding programmes due to the positive relationship between biomass and yield of bast fibre (Webber and Bledsoe, (2002). Consequently, superior performance of a hybrid line over the better parent for traits which contribute significantly to accumulation of biomass such as plant height, leaf area and stem diameter is desirable for developing new varieties of roselle with improved fibre yield.

Hybrid lines D2 x D4, D5 x D2 and D3 x D6 came out as highest BPH for days to first flowering, plant height and leaf width respectively, while D6 x D4 recorded highest for leaf length and stem basal diameter, hence segregation populations of these hybrids offers an opportunity for developing new varieties with superior yield and fibre output over the existing cultivars of roselle.

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CHAPTER SIX

6.0 NUTRITIONAL COMPOSITION OF LOCAL CULTIVARS OF ROSELLE (*Hibiscus sabdariffa* var *sabdariffa* L.) AND THEIR INTRA-SPECIFIC F1 HYBRIDS.

6.1 INTRODUCTION

Growing awareness among consumers of health issues has engendered rising interest in the quality of food products. As a result enhancement in value-added traits such as high nutritional and phytochemical contents has become an essential component of crop improvement programmes. Neglected and underexploited traditional crops constitute a key source of these nutrients in the diets of local communities, providing both macro- and micro-nutrients (Kwenin *et al.*, 2011; Smith and Eyzaguirre, 2007; Moore and Raymond, 2006; National Academies Press, 2006).

Although they constitute a small fraction of whole diets, macro- and micro-nutrients play an important role in various metabolic processes essential for maintaining good health and prevention of diseases and their deficiency is usually detrimental to normal biochemical function of the human body (Akhter *et al*., 2004). Elements considered essential as macro-elements sodium, magnesium, phosphorus, potassium and calcium are needed in large amounts while micro- and trace-elements (manganese, iron, copper and zinc) are only required in small quantities (Roe *et al*., 2013; Paul and Southgate, 1988).

The World Health Organisation (WHO) recommends daily intake of 400 g of fresh fruits and vegetables, due to their high mineral and vitamin contents which play very critical role in reducing risk of diseases such as diabetes and cancer (Matthews, 2006; WHO/FAO, 2005). In tropical Africa where daily diets are dominated by starchy staples, indigenous leafy vegetables such as roselle (*H. sabdariffa* var *sabdariffa*) serve as cheap and readily available sources of nutrients (vitamins and essential elements) to ensure a well-balanced nutrition (Martin and Meitner, 1998).

Indeed, roselle plant contains precious nutrients such as vitamin C and protein (Rao, 1996; Mahadevan *et al*., 2009. The dry calyces contain all essential amino acids (Glew *et al*., 1997) and major organic acids such as citric acid, malic acid and tartaric acid (Ali *et al*., 2005). The plant is also a store house of precious dietary minerals, particularly calcium, magnesium, potassium, sodium, iron and phosphorus (Abou-Arab *et al*., 2001; Babalola *et al*., 2001).

Although generally a rich source of vital nutrients, composition of various nutrients in roselle varies significantly depending on location, variety and plant organ analysed. Research indicates that, red calyces contain significantly higher protein and ash contents than the green types, while green calyces contain higher amounts of crude fibre and vitamin C (Ascorbic acid) than the red types (Abdallah *et al*., 2011; Adanlawo and Ajibade 2006). This offers opportunity for breeders to undertake hybridisation between varieties with red and green calyces to produce hybrids with superior nutritional contents.

Despite being cultivated on subsistence basis, the roselle plant is a source of ingredients for the preparation of many local cuisines across West Africa. In Ghana, the leaves have considerable importance among rural communities in the northern parts of the country because they provide nutritional supplements to their diets which are largely composed of cereals such as millet and sorghum (Ataogye, 2012).

The calyx also serves as a source of raw material for the preparation of the popular „sobolobo"drink, widely consumed across the country (Blench, 2007).

Owing to increasing demand for the beverage in recent years, there is huge prospect for large-scale cultivation of roselle in Ghana, yet current level of production and consumption is much lower than it is for exotic vegetables such as cabbage (Blench, 2007). However, improvement of productivity and nutritional status of existing cultivars through hybridisation would most likely increase the competitiveness to boost interest in widespread utilisation and cultivation of the crop.

Generation of new varieties with reduced acidity would also meet the specific preference of a large section of consumers who are sensitive to the high acidity of the roselle beverage and thereby expand the market of „„sobolo"". New varieties with improved nutritional output would also help to overcome malnutrition, boost food security and increase personal incomes particularly of rural women, who are the major producers of the crop.

6.1.1 Objectives of the study

The main objective of this study was to investigate proximate and elemental composition of the leaves and calyces of 8 local landrace varieties of roselle (*H. sabdariffa* var *sabdariffa*) and their F1 hybrids in order to identify superior lines for selection.

The specific objectives were;

1. To determine moisture, ash, protein, pH, total titratable acidity (TTA) and vitamin C contents in leaf and calyx samples of the parental varieties of roselle and their F1 hybrids.

2. To assess concentration of five essential elements in leaf and calyx samples of the parental varieties of roselle and their F1 hybrids.

3. To determine the degree of association among the various elements detected in leaf and calyx samples to facilitate future breeding programmes to improve upon the elemental composition of the crop.

4. To identify lines with superior nutritional composition for selection and inclusion in future breeding programmes.

6.2 Materials and Methods

6.2.1 Experimental site

The experiment was carried out at research farms and laboratory of Biotechnology and Nuclear Agriculture Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC), near Kwabenya. The experimental site is located within the Coastal Savanna agro-ecological zone on latitude 05° 40° N and longitude 0° 30° W. It is situated 76 m above sea level 20 km north of Accra.Annual rainfall distribution pattern ranges from 700 mm to 1000 mm (Morris *et al*., 1999). The predominant soil type found in the area is well-drained Savanna Ochrosol, derived from quartzite schist (Ferric Acrisol, locally called Haatso series) (FAO/UNESCO, 1994).

6.2.2 Experimental material

The experimental material comprised 19 leaf samples (6 parental varieties and 13 F1 hybrids) and 16 calyx samples (6 parental varieties, 8 F1 hybrids, and one each of a local and imported varieties sold on the open market which served as control). Leaf samples of these were however, not available on the market. Table 6.1 shows identities of the accessions and the plant organ (s) used for the experiment.

Table 7.1 Identities of accessions and the plant organ (s) used for the experiment

Accession	Plant organ analysed	
	Calyx	Leaf
A1	√	
A2		√
A4		√
A5		√
A7	√	√
A9	√	√
A10	√	√
A11	√	√
A13	√	
A3 x A7		√
A4 x A12		√
A4 x A1	√	√
A4 x A11		√
A7 x A12		√
A9 x A7	√	
A10 x A7	√	√
A10 x A11		√
A11 x A1	√	
A11 x A2	√	√
A11 x A5		√
A11 x A7	√	√
A11 x A9	√	√
A11 x A13	√	
A12 x A3		√
Control 1	√	
Control 2	√	
Total	16	19

√ = plant organ used for the analyses.

6.2.3 Sample preparation

Mature fruits of commercial value of each accession were randomly selected and harvested from the field. After harvesting, the fruit were washed thoroughly with distilled water to remove any dirt followed by manual scouring to separate the seed capsules from the calyces. The calyces were dried in the sun to a constant weight.

Freshly harvested leaves of each accession were obtained from the field and transported to laboratory and washed in distilled water to remove all foreign particles. Leaf samples for the elemental analyses were frozen at -20 ℃ in a freeze dryer (DELTA- 24 LSC) for 72 hours. Both leaf and calyx samples were grounded separately into smooth powder in order to obtain a homogenous mixture for the analyses.

6.2.4 Determination of proximate composition.

6.2.4.1 Determination of moisture, pH, crude protein and ash contents.

Proximate composition of the samples were determined following the AOAC approved modified methods. Moisture, pH, crude protein and ash contents were analysed by following the 925.10 (1990) 15th Edition, 984.13 (1990) 15th Edition and 923.03 (2000) 17th Edition of AOAC approved modified methods respectively.

6.2.4.2 Determination of total titratable acidity

Ten (10) g of each sample was weighed into a clean dry erlemeyer flask and 100 ml of boiled water was added and shaken until particles were evenly suspended. The mixture was digested for 30 minutes and allowed to stand for ten minutes.

The supernatant was decanted into the 250 ml beaker followed immediately by titration with 0.1M NaOH, using 0.3 ml phenolphthalein. The titre value was noted and used for the calculations (expressed as percentage Malic acid) as follows;

$$\text{Malic acid (\%)} = \frac{A \times 0.009}{V} \times 100$$

Where: A = volume of NaOH used for the titration, V = volume of sample.

6.2.4.3 Determination of Vitamin C

Vitamin C contents of the samples were determined by titration using the method described by Pongracz *et al.*, (1971). Ten 10 g of each sample was soaked in 40 ml metaphosphoric acid-acetic acid (2 %, w/v) for 10 minutes. The mixture was then centrifuged at 3000 rpm for 20 minutes and the supernatant obtained was diluted with distilled water to 50 ml. Ten (10 ml) of the mixture was titrated with dichlorophenol-indophenol (DCPIP) 0.5 g / L.

6.2.5 Determination of elemental composition of samples using Atomic Absorption Spectrometry (AAS).

Five (5) g of of each sample was weighed into a Teflon beaker and dissolved in 20 ml of 10 % HNO_3 and 1 ml of 30 % H_2O_2 was added. The mixture was digested in an ETHOS 900 microwave. After the digestion, the inside walls of the Teflon beakers were washed with distilled water and made to a final volume of 20 ml.

Each sample was transferred into a 250 ml test tube and assayed for each element at the specific wavelength using a calibrated spectrometer (S Series 711239v1.27, USA). Reference standards for the elements of interest, blanks and repeats of each sample were used as internal positive control.

The final concentration for each of the samples was calculated using a standard curve as follows;

$$\text{Final concentration (mg/100 g)} = \frac{\text{Concentration x dilution factor x nominal volume}}{\text{Weight of sample in grams}}$$

Concentration = reading from spectrometer

Nominal volume = final volume after reagent and water were added after digestion.

6.2.6 Data analyses

Means were subjected to Analysis of variance (ANOVA) to determine the level of significance of variability among the accessions for the various parameters. A p-value of 0.05 or less was considered as statistically significant. Duncan‟s multiple range test was deployed to determine differences among means. Data were analysed using Statsgraphics Centurion software (version 16.1) and Microsoft Excel Software (2010 edition).

6.3 Results

6.3.1Proximate composition of leaves of roselle (*H. sabdariffa* var *sabdariffa* L.).

Table 6.2 shows proximate composition of 6 parental varieties and 13 F1 hybrids of roselle. Apart from moisture, total titratable acidity (TTA) and vitamin C contents, F1 hybrids generally scored higher values than were recorded by the parental lines. There were significant variations ($p \leq 0.05$) found among the samples for all parameters investigated (Appendix 9.3.1).

Moisture contents were generally high ranging from 79.83±0.49 % to 83.88±00.12 %. With respect to ash, most of the F1 hybrids obtained higher values than was recorded by their respective parents. Similarly, pH values of hybrid lines were higher than those of parental varieties, ranging from 2.60±0.00 to 2.99±0.00 and 2.54±0.01 to 2.88±0.00 respectively. Values for protein content ranged from 4.38±0.00 % to 13.13±0.00 %, but those of F1 hybrids were significantly higher. Generally, very low values, ranging from 0.16 ± 0.00 % to 0.32 ± 0.01 % and 0.07 ± 0.00 mg/100 ml to 0.14±0.00 mg/100ml emerged for TTA and vitamin C contents respectively.

Overall, hybrid line A11 x A2 recorded highest moisture content (83.88±00.12 %) although not significantly different from values obtained by A2, A7, A11, A10 x A11. A7 x A12 produced highest pH (2.99±0.00) and ash (2.28±0.06 %) contents, though was not significantly different from 2.27±0.02 % obtained by A11 x A2. Also, A11 x A2 scored highest values for protein (13.13±0.00 %) and TTA (0.32±0.01 %). On the other hand, A12 x A3, A9 and A4 x A12 registered lowest values with respect to moisture, protein and TTA respectively, while A2 came out with least pH and also shared least ash content with A10 x A11. In like manner, A4 and A10 x A7 shared least amount of vitamin C.

Table 6.2 Proximate composition of leaves of 19 lines of roselle (*H. sabdariffa* var *sabdariffa*).

Parent/F1 Offspring	Moisture (%)	Ash (%)	pH	Protein (%)	TTA (%)	Vit C (mg/100ml)
A2	83.61±0.75 ^{a}	1.00±0.00 ^{j}	2.54±0.01 ^{l}	8.75±0.00 ^{ef}	0.28±0.00 ^{c}	0.12±0.04 ^{ab}
A4	80.91±1.55 ^{bc}	2.00±0.08 ^{cde}	2.61±0.00 ^{j}	5.54±0.51 ^{ij}	0.20±0.01 ^{f}	0.07±0.00 ^{c}
A5	82.39±1.40 ^{ab}	1.55±0.00 ^{i}	2.60±0.01 ^{k}	7.29±0.51 ^{g}	0.26±0.00 ^{d}	0.09±0.04 ^{bc}
A7	83.58±1.11 ^{a}	1.60±0.09 ^{i}	2.88±0.00 ^{d}	6.42±0.50 ^{h}	0.24±0.01 ^{e}	**0.14±0.00 ^{a}**
A9	80.49±0.68 ^{bc}	1.73±0.14 ^{fgi}	2.88±0.00 ^{d}	4.38±0.00 ^{k}	0.24±0.00 ^{e}	**0.14±0.00 ^{a}**
A10	82.62±00.15 ^{ab}	1.68±0.32 ^{fgi}	2.79±0.01 ^{g}	4.38±0.00 ^{k}	0.20±0.01 ^{f}	**0.14±0.00 ^{a}**
A11	83.66±0.46 ^{a}	2.02±0.03 ^{cde}	2.83±0.01 ^{e}	9.63±0.00 ^{cd}	0.21±0.01 ^{f}	**0.14±0.00 ^{a}**
A3 x A7	81.99±1.01 ^{ab}	1.67±0.03 ^{gi}	2.74±0.00 ^{i}	6.42±0.50 ^{h}	0.25±0.01 ^{de}	0.12±0.04 ^{ab}
A4 x A12	82.38±0.87 ^{ab}	1.87±0.03 ^{def}	2.89±0.01 ^{c}	8.17±0.50 ^{f}	0.16±0.00 ^{g}	0.09±0.04 ^{bc}
A4 x A1	81.92±1.07 ^{abc}	1.98±0.14 ^{cde}	2.75±0.00 ^{h}	4.96±0.50 ^{jk}	0.26±0.00 ^{d}	**0.14±0.00 ^{a}**
A4 x A11	81.75±1.92 ^{abc}	2.08±0.06 ^{c}	2.61±0.01 ^{j}	5.84±0.51 ^{hi}	0.26±0.00 ^{d}	**0.14±0.00 ^{a}**
A7 x A12	81.44±1.11 ^{bc}	**2.28±0.06 ^{a}**	**2.99±0.00 ^{a}**	10.5±0.00 ^{b}	0.30±0.01 ^{b}	**0.14±0.00 ^{a}**
A10 x A7	81.43±2.16 ^{bc}	2.05±0.09 ^{cd}	2.79±0.00 ^{g}	9.04±0.51 ^{de}	0.25±0.00 ^{de}	0.07±0.00 ^{c}
A10 x A11	83.70±0.27 ^{a}	1.00±0.00 ^{j}	2.80±0.00 ^{f}	6.13±0.00 ^{hi}	0.24±0.00 ^{e}	**0.14±0.00 ^{a}**
A11 x A2	79.83±0.49 ^{c}	2.27±0.02 ^{a}	2.90±0.01 ^{b}	**13.13±0.00 ^{a}**	**0.32±0.01 ^{a}**	**0.14±0.00 ^{a}**
A11 x A5	81.16±0.36 ^{bc}	1.93±0.06 ^{cde}	2.79±0.00 ^{h}	8.17±0.50 ^{f}	0.17±0.00 ^{g}	**0.14±0.00 ^{a}**
A11 x A7	83.58±1.11 ^{a}	1.60±0.09 ^{i}	2.88±0.00 ^{d}	6.42±0.50 ^{h}	0.24±0.01 ^{e}	**0.14±0.00 ^{a}**
A11 x A9	82.45±1.67 ^{ab}	1.83±0.14 ^{efg}	2.83±0.01 ^{e}	9.92±0.50 ^{bc}	0.17±0.00 ^{g}	**0.14±0.00 ^{a}**
A12 x A3	**83.88±00.12 ^{a}**	1.53±0.144 ^{i}	2.60±0.00 ^{k}	9.04±0.51 ^{de}	0.24±0.03 ^{e}	**0.14±0.00 ^{a}**
Average	82.11±1.47	1.94±0.75	2.79±0.15	7.57±2.30	0.23±0.05	0.13±0.03
Los	***	***	***	***	***	***
CV (%)	1.80 %	38.59 %	5.25 %	30.38 %	19.37 %	23.10 %

TTA = Total Titratable Acidity Vitamin C = Ascorbic acid pH = Hydrogen ion concentration

Bolded and underlined values represent highest and least values respectively. ± sd = Standard deviation.
Values in same column with same alphabets are not significantly different according to Duncan‟s multiple range test.
LoS, *** and CV represent level of significant, significant at $P \leq 0.001$ and total coefficient of variation respectively.

6.3.2 Proximate composition of calyces of roselle (*H. sabdariffa* var *sabdariffa* L.).

Table 6.3 displays proximate composition of calyces of 16 lines of roselle. Significant variations ($p \leq 0.05$) were recorded for all the parameters investigated (Appendix 9.3.2). Moisture and ash contents ranged from 10.97±0.40 % to 16.31± 0.12 % and 8.74± 0.11 % to 6.99±0.00 % respectively.

pH values of parental lines and the F1 hybrids were higher than recorded for control varieties (2.44±0.01 and 2.46±0.01), ranging from 2.50±0.01(A1) to 2.68±0.01(A7) and 2.50±0.01 (A4 x A1) to 2.73±0.01 (A9 x A7) respectively. Protein contents of F1 offspring were generally higher than those of parental accessions and control varieties. Total Titratable Acidity (TTA) values for all parental lines and F1 hybrids were significantly lower than were obtained by control varieties (0.75±0.12 and0.76±0.02). Vitamin C contents were equally low but varied significantly from 0.72±0.00 mg/100 ml to 4.57±0.42 mg/100 ml.

Overall, hybrid lines A11 x A2 and A9 x A7 gave the best moisture (10.97±0.40) and pH (2.73±0.01) respectively, while A11 x A1 gave highest ash (16.31± 0.12 %) and protein (15.77±1.50 %) values and also shared significantly higher vitamin C contents with parental line A1 and control 2.

Table 6.3: Proximate composition of calyces of roselle (*H. sabdariffa* var *sabdariffa* L.).

Parent/F1 Offspring	Moisture (%)	Ash (%)	pH	Protein (%)	TTA (%)	Vit C (mg/100ml)
A1	15.06±1.49 [fg]	7.68±0.17 [bc]	2.50±0.01 [k]	9.93±1.33 [bc]	0.64±0.01 [b]	4.33±0.00 [a]
A7	14.36±0.11 [g]	6.45±0.12 [defg]	2.68±0.01 [c]	6.13±0.00 [fg]	0.47±0.01 [f]	1.20±0.42 [c]
A10	13.27±0.06 [gh]	5.99±0.01 [efghi]	2.66±0.0 [d]	4.67±0.50 [gh]	0.46±0.01 [f]	<u>0.72±0.00 [d]</u>
A11	15.58±0.23 [ef]	6.72±0.23 [def]	2.61±0.01 [f]	7.00±0.88 [f]	0.47±0.01 [f]	2.10±0.00 [b]
A13	16.07±0.06 [de]	5.85±0.11 [ghi]	2.58±0.00 [h]	5.25±0.00 [gh]	0.50±0.01 [e]	1.20±0.42 [c]
A4 x A1	18.74±0.20 [ab]	5.29±0.17 [ij]	2.55±0.00 [i]	9.07±1.32 [cd]	0.52±0.02 [de]	1.86±0.40 [b]
A9 x A7	16.09±0.10 [de]	<u>4.26±0.64 [k]</u>	**2.73±0.01 [a]**	5.25±0.00 [gh]	<u>0.40±0.01 [g]</u>	1.40±0.00 [c]
A10 x A7	18.31±0.03 [b]	5.36±0.46 [hij]	2.66±0.01 [d]	<u>4.38±0.00 [h]</u>	0.46±0.01 [f]	1.44±0.00 [c]
A11 x A1	15.10±0.03 [f]	**8.74± 0.11 [a]**	2.71±0.00 [b]	**15.77±1.50 [a]**	0.52±0.01 [de]	4.33±0.00 [a]
A11 x A2	<u>10.97±0.40 [i]</u>	6.19±0.17 [defgh]	2.51±0.00 [j]	8.50±0.52 [d]	0.52±0.02 [de]	1.40±0.00 [c]
A11 x A7	16.43±0.17 [cd]	5.92±0.24 [fghi]	2.59±0.00 [g]	6.10±0.00 [fg]	0.53±0.01 [d]	1.40±0.00 [c]
A11 x A9	17.10±0.14 [c]	4.97±0.64 [jk]	2.62±0.01 [e]	5.25±0.00 [gh]	0.50±0.01 [e]	1.40±0.00 [c]
A11 x A13	12.47±0.14 [h]	8.21± 0.06 [ab]	2.72±0.01 [a]	10.50±1.56 [b]	0.59±0.01 [c]	2.10±0.00 [b]
Control 1	**19.41±0.20 [a]**	6.79± 1.39 [de]	2.46±0.01 [l]	5.84±0.51 [fgh]	0.75±0.12 [a]	2.16±0.00 [b]
Control 2	16.31± 0.12 [de]	6.99±0.00 [cd]	<u>2.44±0.01 [m]</u>	7.0±0.00 [f]	**0.76±0.02 [a]**	**4.57±0.42 [a]**
Mean	15.69 ± 2.28	6.36±1.25	2.60±0.09	7.38±3.01	0.539778	2.11±1.24
Los	***	***	***	***	***	***
CV (%)	14.5131%	19.63 %	3.57 %	40.83%	19.16 %	58.69 %

Control 1 and 2 represent local and imported varieties sold on open market at Madina.

TTA= Total Titratable Acidity   Vitamin C = Ascorbic acid   pH = Hydrogen ion concentration

Bolded and <u>underlined</u> values represent maximum and minimum values respectively. Means in same Column with same Alphabets are not significantly different according to Duncan‟s multiple range test.
LoS, ***, and CV represent level of significant, significant at P ≤ 0.001 and coefficient of variation respectively.

6.3.3 Concentration of essential elements in calyces of roselle (*Hibiscus sabdariffa* var *sabdariffa* L.).

Table 6.4 displays concentrations of five essential elements in calyces of six local landrace cultivars of roselle, 8 F1 hybrids and two control varieties.

Table 6.4: Concentration of five essential elements in calyces of roselle (*Hibiscus sabdariffa* var *sabdariffa* L.).

Parent/F1 Offspring	Calcium (mg/100g)	Magnesium (mg/100g)	Sodium (mg/100g)	Potassium (mg/100g)	Iron (mg/100g)
A1	960.70±1.73 ^{d}	3.82±0.07 ^{b}	49.47±5.77 ^{b}	1958.02±0.02 ^{i}	11.55±0.21 ^{c}
A7	116.47±1.53 ^{c}	2.46±0.33 ^{gh}	36.47±2.30 ^{e}	2250.03±0.01 ^{g}	8.27±0.16 ^{d}
A9	560.63±1.16 ^{k}	2.63±0.49 ^{e}	31.31±4.45 ^{h}	2315.42±0.03 ^{fg}	2.87±0.03 ^{m}
A10	160.40±1.00 ^{m}	2.65±0.01 ^{e}	45.73±1.15 ^{c}	2288.01±0.03 ^{e}	7.25±0.32 ^{f}
A11	720.10±1.00 ^{h}	2.52±0.01 ^{fg}	38.87±1.15 ^{d}	2324.02±0.03 ^{f}	2.73±0.11 ^{n}
A13	800.80±1.00 ^{f}	2.54±0.40 ^{f}	32.73±1.15 ^{g}	2728.04±0.03 ^{b}	4.97±0.22 ^{h}
A4 x A1	936.70±1.73 ^{e}	**4.30±0.88 ^{a}**	31.13±1.15 ^{h}	1229.62±0.02 ^{k}	4.03±0.22 ^{j}
A9 x A7	640.80±0.01 ^{i}	2.53±0.20 ^{f}	27.55±5.6 ^{i}	2737.81±0.02 ^{b}	3.93±0.19 ^{k}
A10 x A7	1224.60±1.73 ^{b}	2.61±0.01 ^{e}	21.00±1.97 ^{l}	1408.06±0.02 ^{j}	5.35±0.39 ^{g}
A11 x A1	800.60±2.00 ^{f}	2.45±0.53 ^{hi}	35.37±5.67 ^{f}	**3284.04±0.01 ^{a}**	**19.45±0.02 ^{a}**
A11 x A2	800.67±1.53 ^{f}	2.40±0.06 ^{ij}	33.15±8.39 ^{g}	2262.08±0.04 ^{f}	3.47±0.38 ^{l}
A11 x A7	320.73±1.53 ^{l}	2.47±0.26 ^{gh}	39.43±0.76 ^{d}	2428.08±0.32 ^{c}	11.94±0.03 ^{b}
A11 x A9	800.60±2.65 ^{f}	2.38±0.20 ^{j}	34.99±0.15 ^{f}	2335.59±0.01 ^{d}	2.43±0.12 ^{o}
A11 x A13	**1360.70±1.0 ^{a}**	3.27±0.46 ^{c}	22.36±2.43 ^{k}	2250.06±0.03 ^{g}	3.47±0.40 ^{l}
Control 1	720.50±3.61 ^{g}	2.51±0.01 ^{fgh}	25.67±1.15 ^{j}	2242.10±0.03 ^{h}	7.99±0.30 ^{e}
Control 2	624.20±1.00 ^{j}	2.81±0.01 ^{d}	**55.50±5.20 ^{a}**	2336.06±0.02 ^{d}	4.13±0.12 ^{i}
Average	787.08±3.02	2.77±5.40	35.03±91.85	3284.04±39.40	6.49±44.69
CV (%)	38.41%	19.49%	26.2138%	22.86%	68.88%
RDI	500 – 1200	320 – 420	1400 – 2500	2000 – 2500	27

Control1and 2 represent local and imported varieties sold on open market at Madina.

±sd = standard deviation, CV = Coefficient of variation, RDI = recommended daily intake (Dietary Reference, 1991).Values in the same column with the same superscripts are not significantly different ($P < 0.05$) according to the Duncan"s Multiple range test. Highest and lowest values are **Bolded** and underlined respectively.

Apart from calcium (Ca) and potassium (K), concentrations of other elements of all accessions were lower than the recommended daily intake values, but significant differences ($p \leq 0.05$) were observed among the accessions with respect to levels of all five elements(Appendix 9.4.1).

Ca contents varied from 560.63±1.16 mg/100 g to 1360.70±1.0 mg/100 g, with A11 x A13 and A9 registering highest and lowest values respectively. Hybrid line A4 x A1 gave significantly highest concentration of Magnesium (Mg) (4.30±0.88 mg/100 g), compared to highest among parental lines A1 (3.82±0.07 mg/100 g) and controls (2.81±0.01 mg/100 g), while A11 x A9 gave the least (2.34±0.06 mg/100 g).

Values recorded for sodium contents varied from 21.00±1.97 mg/100 g to 55.50±5.20 mg/100 g with A10 x A7 and Control 2 scoring lowest and highest values respectively. With respect to composition of K, values ranged from 1229.62±0.02 mg/100 g to 3284.04±0.01 mg/100 g with A4 x A1 and A11 x 13 recording lowest and highest values respectively. Hybrid line A11 x A1 recorded the highest concentration of iron (19.45±0.02 mg/100 g) compared to A1 (11.55±0.21 mg/100 g) and Control 1 (7.99±0.30 mg/100 g) which emerged highest among parental lines and control varieties, while A3 x A7 scored the least value 1.68±0.06 mg/100 g with respect to iron content.

6.3.4 Concentration of five essential elements in leaves of roselle (*Hibiscus sabdariffa* var *sabdariffa* L.).

Table 6.5 displays concentration of five essential elements of leaf samples of seven parental lines of roselle and 12 F1 hybrids.

Table 6.5: Concentration of five essential elements in leaves of roselle.

Parent/F1 Offspring	Calcium (mg/100g)	Magnesium (mg/100g)	Sodium (mg/100g)	Potassium (mg/100g)	Iron (mg/100g)
A2	1280.20±1.00^{b}	2.65±0.52^{b}	15.73±1.15^{i}	**1641.08±0.01^{a}**	3.66±0.26^{o}
A4	480.30±2.00^{i}	2.47±0.26^{ij}	11.60±2.00^{l}	1041.42±0.02^{r}	2..82±0.11^{r}
A5	800.40±1.73^{e}	2.46±0.35^{j}	18.60±3.46^{ef}	1261.43±0.02^{h}	3.84±0.05^{q}
A7	640.50±3.00^{fg}	2.34±0.13^{k}	16.60±2.00^{h}	1040.29±0.06^{s}	13.85±0.98^{b}
A9	960.30±2.65^{d}	2.45±0.04^{j}	13.00±2.00^{k}	1281.45±0.05^{g}	4.61±0.06^{k}
A10	800.33±2.08^{e}	2.50±0.10^{ghi}	16.67±1.15^{h}	1440.21±0.04^{d}	5.34±0.34^{g}
A11	480.40±1.00^{i}	2.54±0.01^{de}	18.93±2.31^{e}	1421.86±0.05^{e}	4.4.7±0.09^{l}
A3 x A7	800.47±3.21^{e}	2.53±0.12^{def}	15.60±2.00^{i}	1181.00±0.01^{n}	1.68±0.06^{s}
A4 x A12	480.77±1.53^{h}	2.56±0.02^{d}	25.60±2.00^{b}	1201.44±0.01^{l}	8.17±0.02^{e}
A4 x A1	800.20±1.00^{e}	2.46±0.01^{j}	16.40±2.00^{h}	1221.42±0.01^{i}	4.04±0.23^{n}
A4 x A11	320.20±1.00^{j}	2.59±0.02^{c}	15.80±2.00^{i}	1201.42±0.04^{m}	4.10±1.2^{m}
A7 x A12	**1440.20±1.00^{a}**	2.64±0.02^{b}	18.67±3.06^{ef}	1580.81±0.02^{b}	**15.59±0.01^{a}**
A10 x A7	640.33±1.53^{fg}	2.48±0.01^{hij}	17.67±2.31^{g}	1041.81±0.02^{q}	6.03±0.21^{f}
A10 x A11	640.43±1.53^{fg}	2.51±0.01^{fgh}	18.20±2.00^{f}	1320.87±0.01^{f}	8.52±3.1^{d}
A11 x A2	1280.47±4.04^{b}	**2.72±0.13^{a}**	**32.93±1.15^{a}**	1580.69±0.05^{c}	13.25±0.34^{c}
A11 x A5	480.53±3.51^{hi}	2.34±0.06^{k}	18.87±3.06^{e}	1201.88±0.01^{k}	3.54±0.15^{p}
A11 x A7	640.20±1.00^{g}	2.52±0.01^{efg}	21.20±5.29^{d}	1081.28±0.03^{p}	4.72±4.5^{j}
A11 x A9	640.63±1.53^{f}	2.53±0.01^{def}	13.67±1.15^{j}	1221.05±0.26^{j}	4.89±1.67^{h}
A12 x A3	1120.33±1.53^{c}	2.47±0.01^{ij}	22.33±3.06^{c}	1101.22±0.73^{o}	4.76±0.21^{i}
Average	775.16±3.65	2.51±0.91	17.40±57.41	1266.45±18.41	9.89±2.12
CV (%)	39.54%	3.63%	32.7293%	14.5442%	13.54%
RDI	500 – 1200	320 – 420	1400 – 2500	2000 – 2500	27

± sd = standard Control1and 2 represent local and imported varieties sold on open market at Madina.

± sd = standard deviation, CV = Coefficient of variation, RDI = recommended daily intake (Dietary Reference, 1991). Values in the same column with the same superscripts are not significantly different (P < 0.05) according to the Duncan"s Multiple range test. Highest and smallest values are **Bolded** and underlined respectively.

Significant differences ($p \leq 0.05$) were recorded among the accessions with respect to levels of all five essential elements investigated (Appendix 9.3.2).

Calcium (Ca) contents were generally within the range of the recommended daily intake (RDI) value with A7 x A12 and A4 x A11 registering highest (1440.20±1.00 mg/100 g) and least (320.20±1.00 mg/100 g) values respectively. With reference to RDI value, concentration of magnesium of all the accessions was low ranging from 2.34±0.06 mg/100 g (A11 x A5) to highest of 2.72±0.13 mg/100 g (A11 x A2). Similarly, sodium contents were lower than the RDI value. A11 x A2 and A7 scored highest (13.00±1.15 mg/100 g) and least (13.00±2.00 mg/100 g) values respectively for sodium content.

Concentration of potassium in all studied accessions was also lower than the RDI value, with A2 and A7 scoring highest (1641.08±0.01mg/100g) and least (1040.29±0.06 mg/100 g) values respectively. Compared with the RDI value, iron contents were generally low. Hybrid line A7 x A12 recorded an outstanding amount (15.59±0.01 mg/100g), compared to highest among the parents A7 (13.85±0.98 mg/100g), whileA3 x A7 gave the least (1.64±0.06 mg/100g).

6.3.5 Correlation between five essential elements detected in leaves and calyces of roselle.

Table 6.6 displays degree of association among five essential elements detected in leaf of roselle. Calcium (Ca) exhibited very weak association with Iron (Fe), potassium (K) and magnesium (Mg), but shared moderate and negative association with sodium (Na). Fe shared very strong positive and negative association with Mg and K respectively. K showed strong positive relationship with Mg, but was weak and negatively associated with Na, while Mg and Na shared very weak and negative relationship.

Table 6.6: Correlation matrix of five essential elements detected in leaves of roselle (*H. sabdariffa* var *sabdariffa*).

	Calcium	Iron	Potassium	Magnesium	Sodium
Calcium					
Iron	**0.1136**ns 0.4135				
Potassium	**-0.0791**ns 0.5695	**-0.3477**** 0.0100			
Magnesium	**0.0722**ns 0.6037	**0.4088**** 0.0021	**0.3486*** 0.0098		
Sodium	**-0.2300*** 0.0942	**0.0422**ns 0.7617	**-0.0594**ns 0.6694	**-0.0215**ns 0.8773	

Below each correlation coefficient (**bolded**) is P-value (underlined). *, **, represent significant at $P \leq 0.05$, 0.01 respectively; ns represents not significant at $P \leq 0.05$.

Table 6.7 displays degree of association among five essential elements detected in calyx samples of roselle. Calcium (Ca) shared strong positive and negative association with Magnesium (Mg) and sodium (Na) respectively, but was weakly and negatively correlated to iron (Fe) and potassium (K). Fe exhibited very strong positive relationship with K, but was weakly association with Mg and Na. K was weakly associated to Mg and Na, but Mg and Na shared very strong negative association with each other.

Table 6.7: Correlation matrix for five essential elements detected in calyces of roselle (*H. sabdariffa* var *sabdariffa*).

	Calcium	Iron	Potassium	Magnesium	Sodium
Calcium					
Iron	**-0.1901**^{ns} 0.1955				
Potassium	**-0.1167**^{ns} 0.4294	**0.6036***** 0.0000			
Magnesium	**0.3981**** 0.0051	**-0.1088**^{ns} 0.4618	**0.0085**^{ns} 0.9541		
Sodium	**-0.4292**** 0.0023	**0.1108**^{ns} 0.4534	**-0.1924**^{ns} 0.1902	**-0.5355***** 0.0001	

Below each correlation coefficient **(bolded)** is P-value (underlined). *, **, *** represent significant at $P \leq 0.05$, 0.01, 0.001 respectively; ns represents not significant at $P \leq 0.05$.

6.4 Discussions

6.4.1 Proximate composition of leaves and calyces of roselle ((*Hibiscus sabdariffa* var *sabdariffa* L.).

The analyses reveal that, moisture contents of the studied leaf samples are generally high, but lower than those of most indigenous leafy vegetables, including "Kontomire" (*Xanthosoma sagittifolia*) "Bokoboko" (*Talinum triangulare*), "Aleefu" (*Amaranth cruentus*) and sweet potato (*Impomoea batatas*) leaf (Kwenin, *et al*., 2011; Assibey-Berko and Tavie, 1999). Similarly, documented values of moisture contents for exotic vegetables cultivated in Ghana, such as cabbage (*Brassica oleracea var capitata*), cauliflower (*Brassica oleracea*), lettuce (*Lactuca sativum*) and spinach (*Spinacia oleracea*) (Roe*et al*., 2013; Hanif *et al*., 2006), are higher than was obtained for roselle in this study.

With respect to moisture contents of calyx samples, values obtained in this studyare comparable to findings of earlier workers (Buah, 2013; Abdallah *et* al., 2011), but lower than those reported by Adanlawo and Ajibade, (2006). Calyces of hybrid line A11 x A2 recorded the best moisture content which differed significantly from the best among the parental lines as well as control varieties.

Generally, high moisture content of a food sample is an indication of greater proportion of water which is associated with proliferation of spoilage microbes under ambient conditions (Chartterjea and Shinde, 1998). Therefore, low moisture contents of studied samples suggest that roselle may be relatively less perishable than most leafy vegetables cultivated and consumed in Ghana.

This could be useful especially, in rural areas where facilities for preservation such as refrigeration or irradiation are not available. However, high moisture of leaf samples indicate that drying of leaves of roselle should be considered to increase the shelf life during longer storage.

With respect to ash contents, calyx samples of hybrid lines gave significantly higher values compared to those recorded by the parental and the control varieties with calyces of A11 x A1 and A11 x A13 scoring highest. These values are within the range of findings of Buah, (2013), Abdallah *et al*., (2011) and Adanlawo and Ajibade, (2006), but are substantially higher than those reported earlier (Fasoyiro *et al*., 2005).

Similarly, ash contents of leaf samples examined in this study are generally higher than those of most local leafy vegetables such as "Bokoboko" (*Talinum triangulare*) and bitter leaf (*Vernonia amydalina*) (Asaolu *et al*., 2012; Seidu *et al*., 2012), as well as those recorded by Roe *et al*., 2013 for major exotic vegetables utilised in Ghana. High ash content is an indication of high proportion of minerals. Hence hybrid lines with high ash contents such as A11 x A1 and A11 x A13, A7 x A12 and A11 x A2 could be targeted as sources of higher concentration of essential elements.

The analyses further reveal that, pH values of F1 offspring were generally higher than those of parental lines and control varieties with calyces of hybrid line A9 x A7 and leaves of A7 x A12 registering overall highest.

Astringency and acidity of beverages of roselle have long been recognized due to low pH of the calyces (Mgaya *et al*., 2014; Sagayo-Ayerdi *et* al., 2007), which is a major challenge to acceptability of the drinks by people who are sensitive to high acidity. Consequently, calyces of roselle lines with high pH offer good prospects for producing beverages with low acidity to meet the demand of consumers who are sensitive to high acidity, such as patients with stomach ulcer.

Values obtained for protein contents of calyx samples are higher than those reported by earlier authors (Buah, 2013; Abdallah *et al*., 2011 and Atta *et al*,. 2010). Likewise, those of leaf samples are comparatively higher than protein contents of most local and exotic leafy vegetables, earlier investigated by Kwenin, *et al*., (2011) in Ghana and Roe *et al*., (2013) in the United Kingdom respectively. Corresponding to results obtained for ash and pH contents, most F1 hybrids recorded higher amounts of protein than was obtained by their respective parents, with A11 x A1 and A11 x A2 recording highest amount which was significantly different from highest among parental and control varieties.

High amount of protein in roselle is very crucial considering that some rural folks in West Africa consume the calyces as substitutes for meat (Lykke *et al*., 2002; McLean, 1973). This means that any improvements in protein contents of roselle will certainly enhance the nutritional status of these vulnerable groups who usually lack sufficient amounts of this vital nutrient in their diets.

Contrary to findings of several studies conducted elsewhere, attributing high vitamin C and malic acid contents to roselle (Mgaya *et al*., 2014; Abdallah *et al*., 2011; Fasoyiro *et al*., 2005), very low amounts of these nutrients were recorded in both calyx and leaf samples analysed in this study. The values of vitamin C obtained for roselle in this study are also lower than those recorded for most vegetables cultivated and utilised in Ghana (Roe *et al*., 2013; Kwenin, *et al*., 2011).

This may suggest a general deficiency of desirable genes for these traits in the roselle germplasm resource utilised for the analyses. However, treatment and storage conditions prior to the analyses could have influenced the outputs of TTA and vitamin C as suggested by Mgaya *et al*., (2014).

Vitamin C is required to prevent scurvy and also aids in the formation of folic acid derivatives, which is essential for DNA synthesis and effective absorption of iron. Though most of it is retained during cooking, high amounts are required to meet dietary needs since as water-soluble it is frequent lost from body (Chatterjea and Shinde, 1998).

However, being acids, high titratable acidity (Malic acid) and vitamin C (Ascorbic acid) contribute to the low pH and sour taste of fruit beverages (Sánchez and Fernandez, 2003) of which roselle is not an exception (Mgaya *et al*., 2014). Therefore, low amount of these nutrients obtained in this study would reduce sourness of the „„sobolo drink‟‟ and thus enhance the taste.

6.4.2 Composition of essential elements in leaves and calyces of roselle (*Hibiscus sabdariffa* var *sabdariffa* L.).

The analyses reveal significant difference among the studied accessions with respect to levels of accumulation of the assayed elements. This offers a mixture of variants from which selection for further hybridisation could be carried out towards development of new varieties with improved nutritional contents.

Generally, F1 hybrids obtained higher concentration of the examined elements (calcium, potassium, sodium and iron) than was recorded by their corresponding parents. Interactions between genes for mineral uptake and accumulation inherited from the parents could have accounted for the higher concentration of these elements in F1 progenies, and thus underscores contribution of hybridisation towards attainment of high elemental composition.

With respect to calcium, values recorded in this study were higher than those reported by Adanlawo and Ajibade, (2006), and also compare with findings of Mgaya *et al*., (2014). With limited research conducted on nutritional attributes of roselle, there is a dearth of information on elemental composition of leaves of roselle. However, compared with documented values of calcium contents of common vegetables utilized in Ghana (Ahiakpa *et al*., 2014; Roe *et al*., 2013; Kwenin, *et al*., 2011; Assibey-Berko and Tavie, 1999), values recorded for roselle in this study are significantly higher.

Calcium plays major structural roles as constituents of bones and teeth offering protection against osteoporosis. Considering its vital role in the human body, calcium content in food samples cannot be underrated.

High amount of calcium obtained for roselle in the study means that, selections made from the accessions studied could meet the dietary calcium needs of consumers. In this regard, calyces and leaves of F1 hybrids A11 x A13 and A7 x A12 respectively, would be most suitable candidates since they produced the highest values for calcium.

The concentrations of Magnesium (Mg) recorded for all accessions were comparatively lower than the RDI value. However, compared to result of similar studies conducted elsewhere, values obtained in this study are considerably higher (Mgaya *et al*., 2014; Atta *et al*., 2010 and Adanlawo and Ajibade, 2006).

The analyses also reveal that magnesium contents of leaf samples of F1 hybrids are higher than those of parental varieties. However, values recorded for magnesium in this study are generally higher than those of major local and exotic vegetables utilised in Ghana including Okra (*Abelmoschus* spp (L.) (Ahiakpa *et al*., 2014; Roe *et al*., 2013; Asaolu *et al*., 2012; Seidu *et al*., 2012; Hanif *et al*., 2006).

Mg is responsible for the activation of more than 300 enzymes in the body which is essential for normal physiological functions of the body such as maintenance of muscle and nerve function (Faryadi, 2012). Mg deficiency is one of the major factors in many severe illnesses such as heart attack, heart disease, anxiety, depression, fatigue and diabetes. People with Mg deficiency are always tired, irritable, nervous and also experience regular stiffness in the muscles (Faryadi, 2012). Dietary surveys indicate that high intake of Mg is associated with a lower risk of stroke (Ascherio *et al*., 1998).

In Ghana and across West Africa the leaves of roselle are used as substitute for okra which is predominantly used to prepare potherb (Amusa, 2004). Significantly higher concentration of essential elements recorded among leaf samples evaluated in this study means that roselle could be developed as a major crop to meet the recommended dietary intake.

Concentrations of sodium in all accessions evaluated in this study were lower than the RDI value, as well as those recorded by earlier investigators (Atta *et al.*, .2010 and Adanlawo and Ajibade, 2006) who also studied elemental composition of roselle calyx extracts. However, values recorded among the leaf samples were higher than those reported for Okra (*Abelmoschus* spp L.) (Ahiakpa *et al.*, 2014) as well as those of most indigenous leafy vegatables and exotic vegetables cultivated and utilised in Ghana such as cabbage (*Brassica oleracea var capitata*) and lettuce (*Lactuca sativum*) (Roe *et al.*, 2013; Asaolu *et al.*, 2012; Hanif *et al.*, 2006).

Contrary to low Na contents observed among the studied accessions, potassium contents were generally high, in agreement with values reported by earlier workers (Mgaya *et al.*, 2014) who also studied composition of essential elements in calyx extracts of roselle. The potassium values recorded in the leaf samples are also significantly higher than those recorded by other researchers for major leafy vegetables utilised in Ghana (Roe *et al.*, 2013; Kwenin, *et al.*, 2011; Assibey-Berko and Tavie, 1999; Hanif *et al.*, 2006) as well as values reported by Ahiakpa *et al.*, 2014 for Okra (*Abelmoschus* spp L.).

Apart from being required for bone and teeth development, K and Na are all important for the normal muscle function, the transmission of nerve impulses, energy metabolism and utilisation of B-group vitamins (Paul and Southgate, 1988). They are also involved in regulation of blood sugar levels which helps to control blood pressure and thus prevents and manages hypertension, cardiovascular disease, diabetes, and joint pains (Faryadi, 2012). The high potassium and low sodium content of roselle lines observed in this study would provide a good potassium-sodium balance in the human body, which could be very crucial in management of hypertension and diabetes (Walsh, 2003).

Although, iron (Fe) contents of all accessions investigated in present study were lower than RDI, the value of hybrid line A7 x A12 is closer and also significantly higher than those of most leafy indigenous and exotic vegetables cultivated in Ghana (Roe *et al*., 2013; Kwenin, *et al*., 2011; Hanif *et al*., 2006).

According to Ghana Demographic Health Survey, (2004), children and women, especially those of reproductive age and pregnant women living in rural communities are most vulnerable to iron deficiency. Fe deficiency may contribute to maternal morbidity through effects on immune function with increased susceptibility or severity of infections, poor work capacity and performance (Nestel *et al.*, 2006). High amounts of Fe observed in this study, indicates that promotion of consumption of roselle could be very critical in tackling deficiency of this mineral particularly among rural dwellers.

6.4.3 Correlation between five essential elements detected in leaves and calyces of roselle.

In recent years, there has been a growing interest on the part of breeders to develop new varieties of crops which combine desirable agronomic attributes such as high yield and disease resistance with high elemental composition (Nestel *et al*., 2006; Gregorio, 2002). Adequate knowledge of association between major essential elements is paramount in determining appropriate strategies to adopt in breeding.

Results of the correlation analyses between the five essential elements detected in this study reveal that targeting high magnesium through breeding would simultaneous lead to improvement of potassium and calcium contents in the leaves and calyces of roselle respectively due to very strong positive association between these pairs of elements. These outcomes are desirable as these elements are required in relatively large amount in the diets of humans (Roe *et al*., 2013; Paul and Southgate, 1988). Similarly, strong positive association between iron and potassium detected in the calyces suggest that significant improvement of this pair of elements could be achieved simultaneously through breeding.

However, breeding for high iron content would lead to reduction in concentration of potassium in leaves of roselle due to the negative association with each other.

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CHAPTER SEVEN

7.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

7.1 CONCLUSIONS

On basis of results obtained from the study the following conclusions may be drawn:

1a. Cross-fertilisation among genotypes of *H. sabdariffa* var *sabdariffa* and *H. sabdariffa* var *altissima* was unsuccessful. This may suggest possible existence of crossability barriers between the sub-species of roselle.

1b. Both direct and reciprocal crosses among accessions of var *altissima* were successful, ranging from 30 % (D1 x D5) to 80 % (D3 x D4), a strong indication of high fertility rates among genotypes belonging to this sub-species. Hence, a full diallele design could be utilised for gene transfer among the accessions of this group.

1c. In contrast, most crosses among accessions of *H. sabdariffa* var *sabdariffa* were not successful in either direct or reciprocal direction, which may also suggest possible cross-compatibility barriers among genotypes of this group. Therefore, breeding designs in which genotypes can be used as either male or female parent only, such as North Carolina or partial diallele designs would be suitable for breeding cultivars of *H. sabdariffa* var *sabdariffa*.

1d. Crossability success among accessions of *H. sabdariffa var sabdariffa* was generally low, ranging from 0.00 % to 43.33 %. Accessions A3 and A11 are the most compatible male and female parents respectively.

1e. Crossability success of roselle is high during early hours of the day, but decreases steadily in subsequent hours, with the best time for crossing being 6 am – 7 am.

f. The qualitative traits, red pigmentation and narrowly-lobed leaf structure are dominant to green pigmentation and broad-lobed leaf structure respectively.

2.a. Most of the accessions of *H. sabdariffa* var *sabdariffa* investigated in this study share close genetic association, with eight pairs of entries being possible duplicates.

2b.Variations in fresh calyx weight, fresh fruit weight, leaf length and petiole length contributed most to the total variability among accessions of *H. sabdariffa* var *sabdariffa*, on basis of the outcome of the quantitative analysis and PCA.

2c. Strong positive association between leaf and calyx parameters suggests that component breeding may lead to significant improvement in these major yield attributes of roselle.

3a. Significant variation exists among the six parental varieties of *H. sabdariffa* var *altissima* with regards to the seven quantitative traits studied.

3b Among the parental cultivars of *H. sabdariffa* var *altissima*, D2, D3, D4 and D6 exhibited good general combining ability for major yield traits and thus possess inherent genetic potential to produce superior offspring.

3c. Six pairs of crosses, namely D2 x D5, D5 x D2, D2 x D4, D6 x D5,D3 x D6and D6 x D4 emerged as most promising in specific cross combinations for major yield and fibre parameters, days to first flowering, number of fruits per plant, plant height and stem basal diameter.

3d. F1 hybrids expressed significant positive mid-parent and better-parent heterosis for all the measured characteristics which shows that significant improvements in these characteristics are achievable through intra-specific hybridisation.

3e. Both additive and non-additive gene effects control inheritance of key traits of roselle. Hence, a breeding approach which could mop up the fixable genes (additive genes) and also maintain considerable heterozygosity for exploiting the dominant gene action such as recurrent selection would be suitable for simultaneous improvement of the useful traits of roselle.

4a. For calyx samples, F1 hybrids performed better with respect to all parameters investigated apart from total titratable acidity (TTA) and Na contents. Similarly, with the exception of vitamin C and K contents, leaf sample of F1 hybrids obtained significantly higher values with respect to all other studied parameters than were recorded by their corresponding parents.

4b. Among the elements detected in the calyces, Mg shared strong positive association with Fe and K. With respect to elements detected in the leaf samples, Mg was positively correlated to Ca, while Fe and K exhibited strong positive association with each other suggesting that simultaneous improvement in these elements could be achieved by targeting only one of each pair during a breeding programme.

7.2 RECOMMENDATIONS FOR FUTURE RESEARCH

On basis of results obtained in these studies and previous achievements of breeding of roselle, the following recommendations are to be considered future breeding work;

1. Ascertaining specific barriers to crossability between accessions of var *altissima* and var *sabdariffa* would aid transfer of useful traits between them; hence any research in this regard is recommended.

2. Further studies on inheritance of qualitative traits stretching to the F2 or even F3 generations should be carried out, preferably using molecular markers to fully understand the pattern of segregation with appropriate ratios. Use of molecular markers would also conclusively confirm results of morphological characterisation of the accessions of*H. sabdariffa* var *sabdariffa*.

3. Calyx and leaf parameters made significant positive contribution to the overall genetic divergence observed among accessions of *H. sabdariffa* var *sabdariffa* which shows that, they would be potentially effective in differentiating genotypes of roselle. Hence high priority should be given to these traits in future breeding work.

4. Apart from the outstanding performance for major yield traits, calyces of F1 hybrid A11 x A1, A11 x A13, and A11 x A2 performed better on most of the proximate parameters and also hold high concentrations of calcium, magnesium and potassium. Similarly, among the leaf samples, hybrids line A7 x A12 performed better in most of the parameters investigated. Therefore, high priority should be given to these hybrid lines in selections to develop standard varieties.

5. Proximate and elemental analyses should be expanded to include other essential elements such as zinc, phosphorus, copper, vanadium and selenium as well as other nutritional composition such as fibre and carbohydrate to complement the documented nutritional information of the crop.

6. Further screening of the F1 hybrids is also recommended to identify genotypes with superior phytochemical contents (such as anthocyanin, polyphenols), as this will complement the proximate and elemental analyses.

7. Backcrossing of superior F1 hybrids to the parents would help to mop up genes for other useful traits such as high anthocyanin within the roselle germplasm resource towards further improvement of the crop.

8. Demand for roselle fibre is likely to increase as a result of the rising interest in natural, biodegradable fibres. Hence hybrid lines of *H. sabdariffa* var *altissima* which exhibited high heterosis for major yield traits (D2 x D4, D6 x D5, D5 x D2, D3 x D6 and D5 x D2) should be selected and improved through evaluation to develop a standard variety or varieties with enhanced yield and fibre attributes for the local industry.

9. Accessions D2, D3, D4 and D6 exhibited good general combining ability for major yield traits. Therefore, they could be utilized in future breeding programme for genetic improvement of roselle.

9.0 APPENDICES

Appendix 9.1a: ANOVA for PH of accessions of var *sabdariffa*.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	22130.3	13	1702.33	32.31	0.0000
Within groups	1475.33	28	52.6905		
Total	23605.6	41			

Appendix 9.1b: ANOVA for DF of accessions of var *sabdariffa*.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	2434.4	13	187.262	45.73	0.0000
Within groups	114.667	28	4.09524		
Total	2549.07	41			

Appendix 9.1c: ANOVA Table for FCW of accessions of var *sabdariffa*.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	10419.8	13	801.52	106.20	0.0000
Within groups	211.321	28	7.54719		
Total	10631.1	41			

Appendix 9.1d: ANOVA for FFW of accessions of var *sabdariffa*.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	4505.98	13	346.614	35.09	0.0000
Within groups	276.571	28	9.87753		
Total	4782.55	41			

Appendix 9.1e: ANOVA for LL of accessions of var *sabdariffa*.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	264.324	13	20.3326	7.67	0.0000
Within groups	74.24	28	2.65143		
Total	338.564	41			

Appendix 9.1f: ANOVA for LW of accessions of var *sabdariffa*.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	92.7846	13	7.13727	10.05	0.0000
Within groups	19.1667	27	0.709877		
Total (Corr.)	111.951	40			

Appendix 9.1g: ANOVA for NBPP of accessions of var *sabdariffa*.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	462.063	13	35.5433	16.32	0.0000
Within groups	60.9867	28	2.1781		
Total (Corr.)	523.05	41			

Appendix 9.1h: ANOVA for NFPP of accessions of var *sabdariffa*.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	75.5095	13	5.80842	5.31	0.0001
Within groups	30.6467	28	1.09452		
Total (Corr.)	106.156	41			

Appendix 9.1i: ANOVA for PL of accessions of var *sabdariffa*.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	8429.62	13	648.432	17.38	0.0000
Within groups	1044.67	28	37.3095		
Total	9474.29	41			

Appendix 9.2a: ANOVA for DF of accessions of var *altissima*.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	27.32	5	5.464	1.59	0.2358
Within groups	41.2	12	3.43333		
Total	68.52	17			

Appendix 9.2b: ANOVA for LL of accessions of var *altissima*.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	109.611	5	21.9222	16.44	0.0001
Within groups	16.0	12	1.33333		
Total	125.611	17			

Appendix 9.2c: ANOVA for LW of accessions of var *altissima*.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	36.0783	5	7.21567	3.61	0.0317
Within groups	23.9667	12	1.99722		
Total	60.045	17			

Appendix 9.2d: ANOVA for NFPP of accessions of var *altissima*.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	448.944	5	89.7889	1.53	0.2528
Within groups	704.667	12	58.7222		
Total	1153.61	17			

Appendix 9.2e: ANOVA for PH of accessions of var *altissima*.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	1200.94	5	240.189	0.63	0.0006
Within groups	4573.33	12	381.111		
Total	5774.28	17			

Appendix 9.2f: ANOVA for PL of accessions of var *altissima*.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	98.6133	5	19.7227	6.57	0.0037
Within groups	36.0467	12	3.00389		
Total	134.66	17			

Appendix 9.3.1a: ANOVA for MOISTURE of ACCESSION (LEAVES)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	30.8023	18	1.71124	148.42	0.0000
Within groups	0.438133	38	0.0115298		
Total	31.2404	56			

Appendix 9.3.1b: ANOVA Table for ASH of ACCESSION (LEAVES)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	76.4098	18	4.24499	3.56	0.0005
Within groups	45.3554	38	1.19356		
Total	121.765	56			

Appendix 9.3.1c: ANOVA for pH of ACCESSION (LEAVES)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	290.59	18	16.1439	109.37	0.0000
Within groups	5.60893	38	0.147604		
Total	296.199	56			

Appendix 9.3.1d: ANOVA for PROTEIN of ACCESSION (LEAVES)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	1.19599	18	0.0664441	4734.14	0.0000
Within groups	0.000533333	38	0.000014035		
Total	1.19653	56			

Appendix 9.3.1e: ANOVA for TTA of ACCESSION (LEAVES)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.0347643	18	0.00193135	5.45	0.0000
Within groups	0.0134645	38	0.00035433		
Total	0.0482288	56			

Appendix 9.3.1f: ANOVA for VIT C of ACCESSION (LEAVES)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.110793	18	0.00615517	73.09	0.0000
Within groups	0.0032	38	0.000084210		
Total	0.113993	56			

Appendix 9.3.2a: ANOVA for MOISTURE of ACCESSION (CALYCES)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	62.2426	14	4.4459	20.98	0.0000
Within groups	6.35633	30	0.211878		
Total	68.5989	44			

Appendix 9.3.2b: ANOVA for ASH of ACCESSION (CALYCES).

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	222.786	14	15.9133	90.98	0.0000
Within groups	5.24727	30	0.174909		
Total	228.033	44			

Appendix 9.3.2 c: ANOVA for pH of ACCESSION (CALYCES)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	379.539	14	27.1099	41.72	0.0000
Within groups	19.4921	30	0.649738		
Total	399.031	44			

Appendix 9.3.2 d: ANOVA for PROTEIN of ACCESSION (CALYCES)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.379187	14	0.0270848	1108.01	0.0000
Within groups	0.000733333	30	0.000024444		
Total	0.37992	44			

Appendix 9.3.2 e: ANOVA for TTA of ACCESSION (CALYCES)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	65.8998	14	4.70713	103.40	0.0000
Within groups	1.3657	30	0.0455232		
Total	67.2655	44			

Appendix 9.3.2 f: ANOVA for VIT C of ACCESSION (CALYCES)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.466298	14	0.033307	227.09	0.0000
Within groups	0.0044	30	0.000146667		
Total	0.470698	44			

Appendix 9.4.1a: ANOVA for Ca of Accession (calyces)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	1366.75	15	91.1168	754.03	0.0000
Within groups	3.86687	32	0.12084		
Total	1370.62	47			

Appendix 9.4.1b: ANOVA for Mg of Accession (calyces)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	4.29445	15	2.86296	98866493.01	0.0000
Within groups	92.6667	32	2.89583		
Total	4.29445	47			

Appendix 9.4.1c: ANOVA for Fe of Accession (calyces)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	1.33995	15	8.93302	1269.63	0.0000
Within groups	0.2252	32	0.0070375		
Total	1.33995	47			

Appendix 9.4.1d: ANOVA for K of Accession (calyces)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	93881.3	15	6258.75	112910.21	0.0000
Within groups	1.7738	32	0.0554313		
Total	93883.1	47			

Appendix 9.4.1e: ANOVA for Na of Accession (calyces)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	5.2611	18	2.92283	666117.84	0.0000
Within groups	168.0	38	4.42105		
Total	5.2611	56			

Appendix 9.4.2a: ANOVA for Ca of Accession (leaves)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	396038.	15	26402.6	1782.85	0.0000
Within groups	473.893	32	14.8092		
Total	396512.	47			

Appendix 9.4.2b: ANOVA for Mg of Accession (leaves)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	184328.	18	10240.4	1677.31	0.0000
Within groups	232.0	38	6.10526		
Total (Corr.)	184560.	56			

Appendix 9.4.2c: ANOVA for Na of Accession (leaves)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	45.5891	18	2.53273	94.71	0.0000
Within groups	1.0162	38	0.0267421		
Total	46.6053	56			

Appendix 9.4.2d: ANOVA Table for K of Accession (leaves)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	962744.	18	53485.8	3796153.03	0.0000
Within groups	0.5354	38	0.0140895		
Total	962745.	56			

Appendix 9.4.2e: ANOVA Table for Fe of Accession (leaves)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	1.89998	18	1.05554	14568015096.29	0.0000
Within groups	0.0275333	38	0.000721		
Total	1.89998	56			