Evaluation of morpho-agronomic and nutrient variability in germplasm collection of *Corchorus* spp. in South Africa

by

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ABSTRACT

South Africa is known as food secure at a national level producing enough food coupled with the ability to import food if necessary to meet food and nutritional requirements of its population. However, the same cannot be said at a household level. Several rural households are still suffering from hunger, malnutrition, and poverty. One of the ways in which rural households can alleviate food insecurity and malnutrition is to increase the consumption of neglected indigenous vegetables such as *Corchorus* species. *Corchorus* has been reported to be rich in nutrients such as beta-carotene, Fe, Zn, Ca, Mg and could play an important role by providing relish with considerable amounts of nutrients to poor households. Its use as a food crop needs to be promoted and for this to be achieved there is a need to understand genetic background and generate knowledge for cultivation through plant breeding and precision agronomic studies. This study was therefore, undertaken to assess the genetic diversity and identify potential parents for future use in a *Corchorus* breeding program in the country. Eleven *Corchorus* accessions were sourced from Agricultural Research Council (ARC) and World Vegetable Centre (WVC) Taiwan, were evaluated in a field for morpho-agronomic and nutritional traits using a randomised complete block design with three replications across two cropping seasons in 2015/2016 and 2016/2017. The evaluated *Corchorus* accessions showed significant ($P < 0.05$) differences among them in both morphological and nutritional traits evaluated. Qualitative morphological characters were highly diversified in leaf colour, leaf shape, stem colour, pod and seed colour based on Shannon Weaver diversity index analysis. The multivariate analysis was used to determine the patterns of diversity among accessions. Morpho-agronomic traits for the first two principal components (PCs) accounted for 78.46% of the total variance and identified days to 50% flowering, plant height, canopy width, leaf length-leaf width ratio, leaf length, number of branches, stem diameter, pod diameter, number of pods, and number of seeds per pod as the most contributors to variation in the germplasm. Whereas for the nutritional traits the first two principal components accounted for 70.93% of the total variance with K, Ca, Mg, P, Fe, Mn, B, and Cu contributing most to variability. High level of variability was observed from the principal component biplot and cluster analysis among accessions. The ARC and WVC accessions were clustered into two different groups based on morphological traits.

Genetic parameters were estimated for morphological quantitative and nutritional traits and showed considerable amount of genetic variability. High genotypic coefficient of variation (GCV), broad sense heritability (H^2), and genetic advance (GA) were computed for plant height (59.2%, 89.2%, and 115.1), number of leaves per plant (39.7%, 74.7%, and 70.6), pod length (49.0%, 97.0% and 99.4), number of pods (144.4%, 80.1%, and 266.3), number of seeds per pod (54.1%, 88.4%, and 104.9), calcium (22.3%, 82.1%, and 41.6), magnesium (34.4%, 89.3%, and 66.9), and phosphorus (25.8%, 75.8% and 41.3), respectively, which permits effective selection. This study revealed sufficient genetic variability both for qualitative and quantitative morphological and nutritional characters among the *Corchorus* accessions, which could be exploited for the improvement in the *Corchorus* breeding programme in South Africa.

DECLARATION

I, Sweetbird Phindile Dube, declare that the dissertation, which I hereby submit for Master of Agronomy degree at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institute.

SIGNATURE:
DATE:

DEDICATION

I dedicate this piece of work to my daughter, Andiswa Sikhulile Ngema for her understanding that I am away to study for her to have a better future.

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ABBREVIATIONS

ALVs- African leafy vegetables

ANOVA-Analysis of variance

ARC-VOP- Agricultural Research Council-Vegetable and Ornamental Plants

ARC-ISCW- Agricultural Research Council-Institute of Soil Climate and Water

CA- Cluster analysis

DNA- Deoxyribonucleic acid

FAO- Food and Agriculture Organisation of the United Nations

GA-Genetic advance

GCV-Genotypic coefficient of variation

GHS-General household survey

GV- Genotypic variance

ILVs-Indigenous leafy vegetables

IPGR- International Plant Genetic Resources Institute

KZN- KwaZulu-Natal

LSD- Least significant differences

NWU- North West University

PCA- Principal Component Analysis

PC1- First Principal Component

PC2- Second Principal Component

PCV- Phenotypic coefficient of variation

PV- Phenotypic variance

SA- South Africa

SSA- Sub Saharan Africa

TLVs-Traditional leafy vegetables

UP- University of Pretoria

UZ-University of Zululand

WVC-World Vegetable Center

WV- Wild vegetable

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CHAPTER 1

INTRODUCTION

Indigenous leafy vegetables (ILVs) refer to plant species that originated in a certain region but also include those that were introduced to a region long time ago and are now recognised as native to that area (Jansen van Rensburg et al. 2007). The leafy part of the vegetable together with young tender stems, fruits, and flowers are consumed as a relish (Voster et al. 2007; Lewu and Mavengahama 2010; Jansen van Rensburg et al. 2014).

Sub-Saharan Africa (SSA) is estimated to contain about one thousand plant species consumed as leafy vegetables (Odhav et al. 2007). However, Flyman and Afolayan (2006) considered this as an underestimate since local people utilise many undocumented plants for consumption purposes. South Africa is blessed with great plant biodiversity and more than a hundred different ILV species are reported in South Africa alone (Dweba and Mearns 2011). Many of these species have health benefits and are rich in nutrients with the potential to alleviate hunger and malnutrition in the country (Njeme et al. 2014). *Amaranthus, Corchorus, Cucurbita, Solanum, Moringa* and *Cleome* species are among popularly consumed ILVs in South Africa (Jansen van Rensburg et al. 2007; Smith and Eyzaguirre 2007).

Food insecurity has arisen in recent years as a global crisis following the climate change and rapid population growth. About 814 million people in developing countries are malnourished and about 204 million of this population is from Sub-Saharan Africa (Labadarios et al. 2005; Godfray et al. 2010). The agricultural sector is required to produce large amounts of food, feed, and biofuel on limited crop and land resources for the predicted human population of nine billion by 2050 (Godfray et al. 2010). Food prices will increase gradually in future (Trostle 2008; Altman et al. 2009), forcing underprivileged families to assign a larger percentage of their salary to food. This, in turn, may result in these families following a less diverse and poor quality diet as they try to adapt to the situation (Drewnowski and Specter 2004; Altman et al. 2009).

South Africa is known to be a food secure country producing enough food (Mavengahama et al. 2013), with the capability to import food if necessary to meet the basic nutritional requirements of its population (Lewu and Mavengahama 2010). However, Altman et al. (2009) reported that although South Africa is known to be food secure at the national level, it is not secure at the household level. Several rural households are still suffering from hunger, malnutrition, and poverty. However, indigenous vegetables could play a huge role in alleviating these conditions by providing relish rich in nutrients to resource-poor households (Jansen van Rensburg et al. 2007).

Despite the highlighted potential role of ILVs as food crops, their utilisation is declining at an increasing rate as a result of neglect and absence of proper selection and cultivation methods to increase production and productivity (Odhav et al. 2007; Shiundu and Oniang'o 2007). In addition, ILVs are receiving little attention in breeding and agronomy programs and are considered as minor crops by agricultural researchers (Masuka et al. 2012). Most studies seem to focus on exotic vegetables which are already popular and some of which are fully domesticated (Flyman and Afolayan 2006). The traditional knowledge of ILV uses is usually understood only by the elders (Voster et al. 2007; Lewu and Mavengahama 2010). The youth prefer western foodstuffs promoted by the media and they associate ILVs to poverty (Voster et al. 2007).

In addition to the above, the genetic base of indigenous plant diversity is extremely endangered, mainly due to the replacement of indigenous vegetables by improved or exotic vegetables such as spinach (*Beta vulgaris var. cicla*), cabbage (*Brassica oleracea var. capitata*), and kale (*Brassica oleracea var. sabellica*) (Shiundu and Oniang'o 2007). Furthermore, utilisation of indigenous vegetables without conservation and wise use is a major threat to loss of biodiversity and could result in genetic erosion (Flyman and Afolayan 2006).

Corchorus is an ILV with potential to contribute to rural household food security in South Africa. *Corchorus* species are reported to contain nutrients and vitamins essential to assist in the maintenance of human health, particularly for children who are often susceptible to malnutrition and ailments (Legwaila et al. 2011). According to Ndlovu and Afolayan (2008), *Corchorus* is rich in nutrients and vitamins such as zinc, iron, copper, calcium, magnesium, and beta-carotene. With malnutrition being a critical global problem, resulting in poor health and affecting a large number of children and women in developing countries (Labadarios et al. 2005), *Corchorus* as ILV could play a huge role in addressing this issue. High levels of iron and folate in vegetables could be useful in the prevention of anemia which is the outcome of insufficient intake of iron and zinc (Nesamvuni et al. 2001; Mamabolo et al. 2006; Maunder and Meaker 2007).

Corchorus species grow in the wild or as weeds in cultivated land but has also been semi-domesticated in some parts of South Africa. When cultivated, it requires little effort and can withstand harsh climatic conditions (Flyman and Afolayan 2006; Jansen van Rensburg et al. 2007). It often grows well in areas where cultivation of exotic vegetables is difficult (Jansen van Rensburg et al. 2004).

In genetic diversity analysis, morphological descriptors are still imperative when selecting materials to be utilised in breeding programs, regardless of the benefits of new techniques such as molecular markers. In order to identify superior parental lines, the first step is to gather basic information based on morphological variability of the available crop species (Adebola and Morakinyo 2006).

Corchorus species are reported to be genetically diverse but it is not known to what extent they are different (Choudhary et al. 2016) and also the nutritional composition reported in the literature for the same *Corchorus* species differ markedly (Uusiku et al. 2010). The aim of this study was, therefore, to characterise the available accessions of *Corchorus* kept in the ARC genebank and investigate heritability of its morpho-agronomic and nutritional traits. This characterization, which is part of pre-breeding programme, is expected to generate basic information that can result in the genetic improvement of the accessions evaluated, leading to the selection of the best lines for the development of new cultivar(s) for the traits of interest in South Africa.

1.1 Justification for the study

The available *Corchorus* accessions in the ARC genebank have not been fully characterized in order to identify suitable parental lines for further plant breeding studies. *Corchorus* is touted as a highly nutritious indigenous leafy vegetable and has a potential to contribute to household food security but it remains under-researched. Very little information is available on its genetic diversity and it is therefore difficult to improve the traits of interest through breeding techniques. However, if *Corchorus* and other indigenous vegetables are to compete well with improved vegetables such as cabbage, spinach, and kale and have a significant influence on household food security in South Africa, then there is a need to produce cultivars with higher yield, higher nutritional content and tolerance to biotic and abiotic stress through breeding.

1.2 Problem statement

Corchorus is a highly nutritious indigenous leafy vegetable with the potential to improve food and nutritional security of the resource poor. However, limited information exists about morphological and nutritional variation studies among different *Corchorus* species and heritability of these traits which then makes it difficult to improve for traits of preference. Having this background information, it is an important step in genetically improving this crop to contribute to food and nutritional security in the country.

1.3 Research aim, hypotheses, and objectives

1.3.1 Aim

The aim of the study was to characterize the germplasm collection of *Corchorus* accessions available from the Agricultural Research Council (ARC) of South Africa's genebank using phenotypic and nutritional traits as well as genetic parameters.

1.3.2 Hypotheses

- Different accessions of the same *Corchorus* species grown under same environmental conditions will differ in morphology and the heritability of various traits.
- The nutritional composition and its heritability will differ among *Corchorus* accessions and it will be possible to identify accessions with superior nutrient content.

1.3.3 Objectives

- To characterise the available germplasm of *Corchorus* accessions and investigate genetic parameters of morpho-agronomic traits.
- To assess the nutritional (P, K, Ca, Mg, Fe, B, Zn, Cu and Mn) content and its heritability estimates in *Corchorus* accessions.

CHAPTER 2

LITERATURE REVIEW

2.1 Indigenous leafy vegetables and their consumption

Indigenous leafy vegetables (ILVs) are defined as plant species that are native to a particular region or those species that were intentionally or accidentally introduced to that region for an extended period and have become part of the region's dietary culture (Jansen van Rensburg et al. 2007). Indigenous leafy vegetables are also known as traditional leafy vegetables (TLVs), African leafy vegetables (ALVs) or wild vegetables (WV) (Jansen van Rensburg et al. 2007; Odhav et al. 2007; Schönfeldt and Pretorius 2011). In South Africa they are known by different vernacular languages (Table 2.1), the Nguni tribe refers to them as *imifino* while Sothos collectively refer to them as *morogo* (Jansen van Rensburg et al. 2007; Van Der Hoeven et al. 2013; Njeme et al. 2014; Van Jaarsveld et al. 2014). There are more than a thousand plant species in Sub-Saharan Africa (SSA) consumed as leafy vegetables (Odhav et al. 2007), with South Africa (SA) alone having more than a hundred species (Dweba and Mearns 2011). Indigenous leafy vegetables are well known for their considerable amount of nutrients and other health benefits essential for humans (Njeme et al. 2014).

The soft, tender leaves, fresh shoots and succulent stems of ILVs are harvested from the wild and cooked as a relish to accompany stiff porridge (phuthu or pap) (Jansen van Rensburg et al. 2004; Lewu and Mavengahama 2010). To improve taste and aroma other ingredients are added, such as oil, onions, tomatoes, peanuts and even spices (Darkwa and Darkwa 2013; Jansen van Rensburg et al. 2014). Preparation methods of ILVs differ depending on the taste of an individual. The boiling method uses a considerable amount of water, with the first boiling water which may be replaced with fresh water to reduce the bitter taste of the vegetable depending on the species. Steaming method utilises very little water and it does not take long, especially in an instance of very young and fresh plants. However, Van Averbeke et al. (2007) and Funke (2011) suggested that the optimal cooking methods still need to be tested since some preparation methods are reported to deplete the nutritional content of the ILV, especially the boiling method which can last for an extended period.

Table 2.1: Indigenous leafy vegetables generally consumed in South Africa

Scientific name	Family	Common name	Zulu name	Pedi/Sotho name
Amaranthus hybridus	Amaranthaceae	Cockscomb	Imbuya	Thebe
Bidens pilosa	Asteraceae	Black jack	Uqadolo	Monyane
Cleome gynandra	Capparaceae	Cat’s whiskers	Ulude	Lerotho
Chenopodium album	Chenopodaceae	Fat hen	Imbilikicana	Umbicana
Corchorus olitorius	Malvaceae	Jute mallow	Igushe	Thelele
Cucumis melon	Cucurbitaceae	Muskmelon	Ikhabe	Phara
Momordica balsamina	Cucurbitaceae	Balsam apple	Umkaka	-
Solanum nigrum	Solanaceae	Black nightshade	Umsobo	Umsobo muxe
Vigna unguiculata	Fabaceae	Cowpea	Imbumba	Monawa

Source: Odhav et al. 2007; Uusiku et al. 2010; Mavengahama et al. 2013

Indigenous leafy vegetables are reported to be very rich in nutrients and vitamins essential for the appropriate maintenance of human health (Table 2.2), particularly for children and elders who are often susceptible to malnutrition and ailments (Kwenin et al. 2011; Acho et al. 2014). They contain considerable amounts of minerals such as zinc, iron, copper, manganese, magnesium, potassium, phosphorus, sodium and beta-carotene (Odhav et al. 2007; Ndlovu and Afolayan 2008; Van Jaarsveld et al. 2014). The ILVs have been reported to be highly nutritious or comparable with some of commonly consumed vegetables such as spinach, cabbage, lettuce, and kale (Flyman and Afolayan 2006; Afolayan and Jimoh 2009; Legwaila et al. 2011).

The ILVs are seasonal crops, have a short shelf life, and are easily perishable (Gockowski et al. 2003; Masarirambi et al. 2010). It is therefore very important to preserve them when they are still available in abundance, particularly in summer (Mepba et al. 2007). Sun drying as a preservation method is the most popular among people. It involves harvesting and drying of fresh leaves in the sun or cooking the leaves first then drying them in the sun (Sobukola et al. 2007; Kiremire et al. 2010). Other methods involve blanching and freezing of leaves (Onayemi and Badifu 1987; Mepba et al. 2007; Van Averbeke et al. 2007). These methods can help in the preservation of the vegetables for future use, especially in winter.

Table 2.2: Mineral nutrient content of some indigenous leafy vegetables in South Africa

Accessions	Ca	Mg	P	K	Na	Fe	Zn	Vit. A	Reference
	mg/100 g								
ILVS									
Amaranthus hybridus	44.2	231.2	34.9	54.2	7.4	13.5	3.8	3.3	Akubugwo et al. 2007
Bidens pilosa	1358	658	508	-	290	17	22	5.8	Nesamvuni et al. 2001; Odhav et al. 2007
Cleome gynandra	232	76	138	374	15	2.1	1.0	8.7	Van Jaarsveld et al. 2014
Corchorus olitorius	1159	572.0	554	1669	-	27.4	0.6	6.4	Frison et al. 2006; Acho et al. 2014
Ipomoea Batatas leaves	28.44	340	37.3	4.5	4.2	16	0.1	0.7	Antial et al. 2006
Momordica balsamina	271	159	78.2	839	15.2	16.3	1.6	-	Steyn et al. 2001
Solanum nigrum	4421	667	239	3084	61	49.6	4.2	-	Afolayan and Jimoh 2009
Vigna unguiculata	176	4.8	303	1280	102	2.6	5.1	5.7	Iqbal et al. 2006
Exotic vegetables									
Cabbage	92	55	57	192	57	0.6	-	1.2	Legwaila et al. 2011; Xiao et al.2016
Kale	187	45	68	246	38	32	-	5.1	Xiao et al. 2016
Spinach	1400	0.2	400	3840	4.0	3.10	0.37	-	Meilmann et al. 2017
RDA*	800	120	800	1400	300	10	10	-	National Research Council 1989

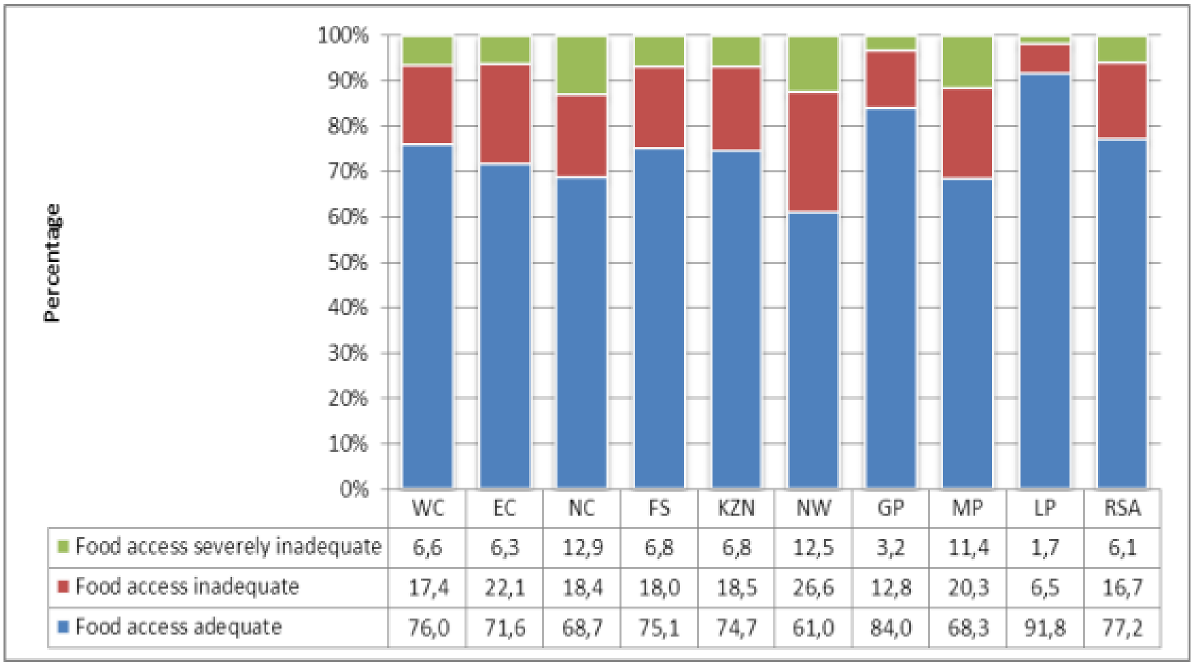
*** RDA-recommended daily allowance**

2.2 Indigenous leafy vegetables and food security

Food security “is a situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life” (FAO 2009). Food insecurity results in a poor diet as people consume anything in order to fill up their stomach which then results to malnutrition. Malnutrition refers to both undernutrition and overnutrition. An extreme consumption of energy is referred to as overnutrition, whereas undernutrition is associated with deficiencies in micronutrient intake (Ndlovu and Afolayan 2008).

Hunger and undernutrition are both consequences of insufficient food consumption but their meaning varies. Hunger is an outcome of insufficient food intake for a sustained period (Jacobs 2009), while undernutrition refers to inadequate intake of micronutrients, essential minerals and vitamins by a human (Labadarios et al. 2005; Faber and Wenhold 2007; Odhav et al. 2007). Malnutrition is a critical global problem, resulting in poor health in almost all developing countries (Labadarios et al. 2005).

Although South Africa is considered a food secure country, there are many people that still suffer from hunger and malnutrition and mostly micronutrient malnutrition at a household level (Mavengahama et al. 2013). General household survey (GHS 2015) indicated the difference in food security among different provinces in South Africa (Figure 2.1). North West province had the highest percentage of households with inadequate access to food (39.1%), followed by Mpumalanga (31.7%), Northern Cape (31.3%) and Eastern Cape (28.4%). Limpopo and Gauteng province had the lowest percentages of 8.2% and 16%, respectively. However, these figures are expected to increase due to an increase in population, lack of employment and economic breakdown in South Africa (Stats 2015). The recent downgrading of South Africa’s economy to junk status will further put a strain on the economy and the poor will be affected the most (News24 2017).



	WC	EC	NC	FS	KZN	NW	GP	MP	LP	RSA
🟩 Food access severely inadequate	6,6	6,3	12,9	6,8	6,8	12,5	3,2	11,4	1,7	6,1
🟥 Food access inadequate	17,4	22,1	18,4	18,0	18,5	26,6	12,8	20,3	6,5	16,7
🟦 Food access adequate	76,0	71,6	68,7	75,1	74,7	61,0	84,0	68,3	91,8	77,2

*WC-Western Cape; EC-Eastern Cape; Northern Cape; Free State; KZN-KwaZulu-Natal; NW-North west; GP-Gauteng province; MP-Mpumalanga; LP-Limpopo; RSA-Republic of South Africa.

Figure 2.1: Household food adequacy by province (Source: Statistics SA 2015).

Surveys have reported that many rural households usually have enough staple food to last them throughout the month but struggle with relish.This food gap can therefore be filled by ILVs which could assist by providing a source of relish with considerable amounts of nutrients (Mavengahama et al. 2013). Although relishes are consumed in small quantities, they can still play an important role in managing hunger and malnutrition in poor household food security (Modi et al. 2006; Afolayan and Jimoh 2009).

Corchorus is among well-known ILVs that have a potential to improve household food security in South Africa. The vegetable is rich in minerals and vitamins essential for the proper maintenance of human health (Legwaila et al. 2011). *Corchorus olitorius* was reported to be rich in zinc, iron, copper, calcium, magnesium, and beta-carotene and can thus play a huge role in addressing malnutrition (Ndlovu and Afolayan 2008). In addition, to its nutritional importance, rural households can harvest and sell *Corchorus* during its abundance on the local markets in order to generate supplementary cash for household necessities such as food and school fees (Nguni and Mwila 2007; Oladele 2011; Mavengahama et al. 2013).

2.3 Threats to indigenous leafy vegetables

The genetic base of Africa’s species diversity is gradually destroyed, mainly as an outcome of the introduction of exotic species replacing indigenous varieties. This is regarded as the main cause of genetic erosion in plants all over the world (Shiundu and Oniang'o 2007).

The population of ILVs is declining at a drastic rate due to several reasons. Indigenous leafy vegetables are not often cultivated but harvested in the wild (Jansen van Rensburg et al. 2007). The utilisation without cultivation is among the threats to loss of biodiversity (Lewu and Mavengahama 2010). Harvesting without cultivation is considered as exploitative and unmanageable in an environment of increasing population density (Mavengahama et al. 2013). Integrating of ILVs in cropping systems can assist in dealing with this utilisation approach (Mavengahama et al. 2013; Bvenura and Afolayan 2015). The decline in the availability of the ILVs is also as the result of chemical elimination by farmers in their fields, the crop may sometimes not be able to germinate in that area again (Mavengahama et al. 2013).

Traditional knowledge of ILV uses is usually contained and understood by the older generation (Voster et al. 2007; Lewu and Mavengahama 2010). The young generation has less interest in indigenous vegetables, they relate them to poverty and prefer western foodstuff promoted by the media (Voster et al. 2007). The knowledge on ILVs may be lost in the future and never regained. This social stigma attached to ILVs as poor people’s food needs to be addressed, some people are now ashamed to consume these vegetables as they do not want to be labeled poor (Voster et al. 2007). According to Masuka et al. (2012) researchers also have an impact on the loss of biodiversity, they regard indigenous vegetables as minor crops and prefer to study exotic vegetables some of which are fully domesticated. The diversity of ILVs needs to be considered and documented. Diversity studies are essential as a primary step in the breeding of species for desirable characteristics (Benor et al. 2012).

2.4 Origin, botanical description, cultivation and genetic aspects of *Corchorus* species

2.4.1 Botanical description

Corchorus species are annual branched herbs, and depending on the variety, can grow up to 4 m tall (Figure 2.2). The stem can be green, red or a mixture of red and green. Leaves are lanceolate, ovate, palmate, and serrate at the margin with distinct hair-like teeth at the base. They are acute at the apex and pubescent. Some leaves have purple margins depending on the species. Leaves can grow up to 14 cm long and 10 cm broad (Benor et al. 2012). Petioles are 2 - 3 cm long, varies from green, red, light red and light green in colour (Osawaru et al. 2012; Islam 2013; Choudhary et al. 2016; Loumerem and Alercia 2016). Flowers are bisexual and bilaterally symmetrical, with numerous stamens and a single hairy pistil. They are yellow in colour with five petals, obovate, obtuse and are pollinated by insects (Edmonds 1990; Osawaru et al. 2012). Pods are cylindrical, straight or slightly curved capsules with 3 to 6 locules. Mature pods can grow up to 9 cm long and 4 to 10 mm in girth, they are green when fresh and dark brown at maturity (Benor et al. 2012). Seeds are green, black, greyish black, and brown to blackish brown and are usually irregular in shape (Halford 1995; Osawaru et al. 2012).

Figure 2.2: Vegetative part of *Corchorus olitorius* species (Anonymous)

2.4.2 Classification and origin

Corchorus species are commonly known as jute, wild jute or wild okra (Jansen van Rensburg et al. 2007). They are mainly distributed in warm climatic regions of the world (Choudhary et al. 2016; Maity et al. 2012). *Corchorus* genus is extremely variable in its natural distribution, genetic diversity and center of origin (Sinha et al. 2011).

Classification of the genus *Corchorus* is being disputed with different authors classifying it under different families. It was previously classified under the family Tiliaceae. The Tiliaceae family is now combined with Malvaceae (Table 2.3), as based on molecular evidence of the chloroplast genes (Whitlock et al. 2003; Benor et al. 2012). However, recent reports are classifying the genus *Corchorus* under the Sparmanniaceae family (Benor et al. 2012). *Corchorus* genus is highly variable but all species are characterized by high-fiber content (Kumar et al. 2014).

Table 2.3: *Corchorus* taxonomy

Rank	**Scientific name and common name**
Kingdom	Plantae- plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Spermatophyta – Seed plants
Class	Magnoliopsida – Dicotyledons
Subclass	Dilleniidae
Order	Malvaleves
Family	Malvaceae
Genus	*Corchorus L.- Corchorus*

Source: Islam 2013

Several authors have reported Africa as the center of diversity and source of origin due to high species diversity of *Corchorus* on the continent and for the reason that both wild and cultivated type, *Corchorus capsularis and Corchorus olitorius* are distributed throughout Africa (Edmonds 1990). About 170 species of *Corchoru*s are documented by Index Kewensis, a publication that aims to register all botanical names for seed plants at the rank of species and genera. Out of 170 species, only 50 to 60 have been validated and are distributed all over the warm areas of the world (Edmonds 1990), with the majority of the diversity reported in Africa, Asia, and Australia (Figure 2.3). Africa comprises of the majority of the world *Corchoru*s species with 16 reported in South Africa alone (Chakraborty and Palit 2009; Maity et al. 2012).

Figure 2.3: Distribution of *Corchorus* in Africa, Asia, Australia, and North and South America (Source: Sinha et al. 2011).

2.4.3 Ecological requirements

Corchorus species prefers warm, humid conditions, and performs well in areas with a rainfall of about 1000 to 2000 mm per annum. A high day temperature of 30°C and night temperature of 25°C favours the production of *Corchorus,* whereas temperatures below 15°C and above 42°C, retard growth. *Corchorus* grows in a wide range of soils, although it prefers rich, well-drained loam soil and cannot tolerate waterlogged conditions. The species performs well in soil with a pH ranging from 4.5 to 8.2 (Jansen van Rensburg et al. 2007; Maity et al. 2012).

2.4.4 Cultivation

In South Africa, *Corchorus* species are usually harvested from the wild and not cultivated, but there are some regions where they are semi-domesticated (Jansen van Rensburg et al. 2007). When cultivated, *Corchorus* species are generally propagated by seeds (Emongor et al. 2004). Seeds are sown directly in the field, either by broadcasting or by line sowing. However, due to seed dormancy germination of untreated seed is poor (Adebooye et al. 2005).

The impermeable seed coat of *Corchorus* seeds has been reported to be the major cause of dormancy (Mavengahama and Lewu 2012). There are a number of methods that can be used to break dormancy, such as heat treatment, and chemical and mechanical scarification (Baskin and Baskin 2004; Baskin et al 2000). Highest germination percentage of 80% and 90% was observed in *Corchorus olitorius* after steeping the seeds for 10 seconds in 80°C hot water and 10 seconds in boiling water, respectively (Velempini et al. 2003; Mavengahama and Lewu 2012; Mohammadi et al. 2012). Sulphuric acid treatment is also a reliable method of breaking the impermeable seed coat in several plant species (Keogh and Bannister 1992; Baskin et al. 2000). However, Emongor et al. (2004) reported a decrease in germination capacity of *Corchorus tridens* after exposing it to sulfuric acid for more than 30 minutes. Unfortunately, chemical treatment can be expensive and dangerous at household level which then leaves heat treatment as the best option.

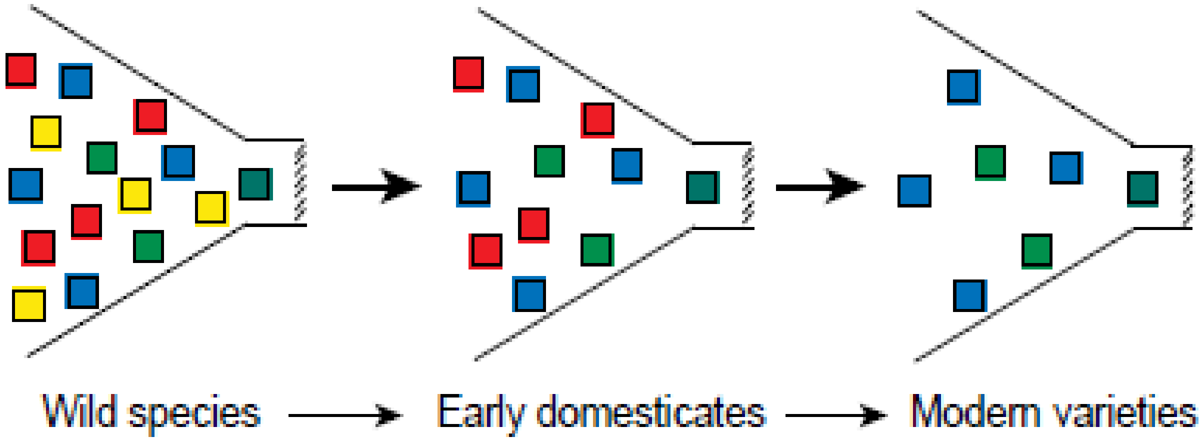
Alternatively, to direct sowing in the fields, seedlings are often produced in seed trays and used as propagation material when cultivating *Corchorus*. Good yields have been obtained from crops established from seedlings (Maity et al. 2012).

2.5 Plant genetic diversity

Genetic diversity refers to the variation of heritable characteristics present in a population of the same organisms (Swingland 2001). Diversity permits the organisms or species to adapt and evolve in response to changing environments and natural selection pressures (Allard 1988; Fu 2015). An outcome of genetic differences may be evident as differences in DNA sequence, biochemical characteristics, physiological properties and morphological characters (Grondona et al. 1997). Genetic variation may also occur in organisms through gene and chromosome mutations, and in species with sexual reproduction, it can be broadened through the population by recombination (Govindaraj et al. 2015). There are numerous natural and artificial processes that influence genetic diversity in plants and the following are some processes:

2.5.1 Domestication

Domestication is mainly a process by which genetic alterations in wild species are carried out through a selection process enforced by a human, based on his/her desire (Reif et al. 2005; Ross-Ibarra et al. 2007). There is no documented evidence on how plants were domesticated, however, it is believed that strong selection pressure applied by humans on the genetic diversity found in the wild caused a rapid and radical change in plant species (Sonnante et al. 1994; Tanksley and Mccouch 1997). Initially, there was high variability in plant species in the wild but it is now lost as a result of domestication and selection (Figure 2.4) (Tanksley and Mccouch 1997). Selection is usually based on the unique phenotypes of an organism, which may include increased plant height, large fruit sizes and desirable colours (Diamond 2002; Pickersgill 2007).



*Different coloured blocks represent gene variations

Figure 2.4: Allelic variations of genes initially found in the wild but now lost (Tanksley and Mccouch 1997)

2.5.2 Plant breeding

Plant breeding is the practice in which plant breeders alter the characteristics of plant species with the purpose of improving them. Breeders use their specialized skills to improve yield and yield attributes, adaptation, and drought tolerance in which modern agriculture is focused on (Finlay and Wilkinson 1963).

The increase in human population places the agricultural sector under pressure to produce more food on limited land. In response to this, new cultivars need to be produced with the ability to achieve high yields with low chemical application and with genetic diversity required to maintain yield stability under changing climatic conditions (Tester and Langridge 2010). Tanksley and Mccouch (1997) pointed out that “ironically, it is the plant breeding process itself that threatens the genetic base on which breeding depends since new varieties are usually derived from crosses among genetically related modern varieties, genetically more variable, but less productive, primitive ancestors are excluded.”

2.5.3 Mutation

Mutation refers to an unexpected heritable change in the genetic material not caused by recombination or segregation (Acquaah 2009). Mutation is a source of all new genetic diversity in plants (Burdon and Silk 1997). It results when there are abnormalities in replication of DNA or other storages of genetic information in a cell (Jablonka and Lamb 2006). Mutations can have positive, neutral, or harmful impacts on species, but it does not always imply something negative (Osawaru et al. 2012).

2.5.4 Migration

The movement of genetic diversity within plants is generally referred to as migration (Myers et al. 2004). Migration of plants depends on pollen and seed dispersal, and movement of vegetative propagules, such as suckers or rhizomes, in asexually reproducing plants (Cain et al. 1998). Migration, also known as gene flow, occurs both with the advancing front of a population when it is colonising new regions, and when genes of two or more populations mix through pollen and seed dispersal (Hughes et al. 1994; Vellend et al. 2003).

2.5.5 Selection

Natural selection occurs when there are differences in fitness and survival among species and genetic basis for the differences (Ross-Ibarra et al. 2007). Selection is well known for affecting genetic diversity and resulting in populations becoming better adapted to the environment (Piepho et al. 2008). It occurs in the phenotype of a species. With time all the better adapted species will survive and reproduce offspring that have inherited characteristics that are more adaptive (Denison et al. 2003; Glaszmann et al. 2010).

2.6 Importance of morphological characterization in plants

Morphological characterization of plant species is principally applied to make a comprehensive investigation of genetic diversity in germplasm collection and contributes valuable information for breeding programs and conservation strategies (Idris and Saad 2001; Borokini et al. 2010; Benor et al. 2012). According to Perry and Bettencourt (1995), characterization is an evaluation of traits that are highly heritable, visually accessible and equally expressed in all environments. Morphological characterization is a primary requirement for the selection of valuable attributes in crop improvement programs. Knowing the morphological variability of a species is the first step before it can be genetically improved (Adebola and Morakinyo 2006), hence the information obtained from characterization should be documented and made available to both germplasm collectors and breeders (Idris and Saad 2001; Ghosh et al. 2013).

Characterization involves the description of plant material, genotypes or species in a germplasm collection. Through a thorough characterization and evaluation, the authenticity, homonyms, and duplicates in germplasm collection are validated. Characterization assists in the identification of potential lines that can be further evaluated to produce cultivars (Idris and Saad 2001; Galović et al. 2006). Genetic diversity, the relationship among species and heritability estimates of certain characters can be obtained through morphological characterization (Gerrano et al. 2015). Characterization data may include plant height, leaf shape, flower colour and other traits (Bar-Hen et al. 1995). Characterization also assists in removing unnecessary or duplication of material filling up genebank space (Engels and Visser 2003).

Morphological characterization may contain a morphological descriptor lists which is used to describe the phenotype of the plant (Osawaru et al. 2012). Descriptor lists developed by the International Plant Genetic Resources (IPGR) and World Vegetable Center (WVC) provide an efficient means of data scoring in morphological characterization. Herbarium specimens are excellent documentation of variation and are used as reference after the material has been removed from the field. Herbarium specimens may include hard-pressed mounted plants, seeds, fluid preserved flowers and fruits, microscope slides and frozen DNA extractions (Shenoy et al. 2007). In a process of characterization, pictures are taken to taxonomically classify the species and record attributes showing variation (Osawaru et al. 2012).

Agronomic characterization is part of morphological characterization where the emphasis is given on performance characteristics and helps in the utilisation of germplasm. Traits taken to account during agronomic evaluation differ according to species mostly between perennial and annual plants (Idris and Saad 2001). However, in most crops and indigenous leafy vegetables, agronomic traits may include days to emergence, days to 50% flowering, yield and yield components, and uniformity of characteristics (K'opondo 2011).

2.7 The use of heritability to estimate genetic diversity

Heritability is the proportion of the observed variation in a progeny that is inherited (Acquaah 2009). Heritability of individual characteristics determines the effectiveness with which genetic variability can be exploited by selection (Zecevic et al. 2010; Ogunniyan and Olakojo 2014). It provides an indication as to how certain characteristics will respond to selection (Nyadanu and Dikera 2014).

There are two types of heritability estimate, broad sense, and narrow sense heritability. The broad sense heritability refers to the ratio of genetic variance to phenotypic variance (Nyquist and Baker 1991; Holland et al. 2003; Acquaah 2009). It is statistically represented as $H^2 = V_G/V_P$. Where H^2 represents broad sense heritability of the trait, V_P the total phenotypic variance, and V_G the genetic variance. Whereas narrow sense heritability is defined as a ratio of additive genetic variance to the total phenotypic variance

(Holland et al. 2003; Acquaah 2009). It is statistically represented as $h^2 = V_A/V_P$. Where h^2 represents narrow sense heritability of the trait, V_A the additive variance, and V_P the phenotypic variance.

Heritability plays a predictive role in breeding programme, expressing the reliability of phenotype as a guide to its breeding value (Nyadanu and Dikera 2014). It is the phenotypic value which can be measured directly, while breeding values of characters are derived from suitable analyses (Mohsin et al. 2009). Breeding value decides on the amount of the phenotype that would be transmitted to the offspring (Akhtar et al. 2011; Al-Tabbal and Al-Fraihat 2011; Denton and Nwangburuka 2011).

High heritability estimates together with high genetic advance permit the best condition for selection of parental lines for the traits of interest (Mohsin et al. 2009; Khan et al. 2015). Genetic advance (GA) is a predictable response to selection. When estimating the GA, the effectiveness of heritability will increase showing the degree of genetic gain for a character acquired under a certain selection pressure (Zecevic et al. 2010).

CHAPTER 3

Morphological characterization of *Corchorus* accessions in the Agricultural Research Council of South Africa

3.0 Abstract

Corchorus is an ILV with potential to contribute to rural household food security in South Africa. The *Corchorus* species has been reported to be rich in nutrients such as beta-carotene, Fe, Zn, Ca, and Mg. Phenotypic characterization of the available germplasm collection is very important as it permits the breeders to identify and select superior parental lines for utilization in future *Corchorus* development in the country. Eleven *Corchorus* accessions obtained from the ARC-VOP were evaluated in a field for morpho-agronomic traits using a randomised complete block design with three replications across two seasons. Qualitative morphological characters were highly diversified in leaf colour, leaf shape, stem colour, pod and seed colour based on Shannon Weaver diversity index analysis. *Corchorus* accessions showed significant ($P<0.05$) differences in all the quantitative traits evaluated. The first two principal components (PCs) accounted for 78.46% of the total variance. The cluster grouped the accessions into two different groups based on their genetic similarity. Genetic parameters were estimated for morphological quantitative traits and showed considerable amount of genetic variability. High genotypic coefficient of variation (GCV), broad sense heritability (H^2), and genetic advance (GA) were computed for plant height (59.2%, 89.2%, and 115.1), number of leaves per plant (39.7%, 74.7%, and 70.6), pod length (49.0%, 97.0% and 99.4), number of pods per plant (144.4%, 80.1%, and 266.3), and number of seeds per pod (54.1%, 88.4%, and 104.9), respectively, which permits effective selection. This study revealed sufficient genetic variability in *Corchorus* accessions which can be exploited for crop improvement.

3.1 Introduction

South Africa is blessed with wide biodiversity, having a large number of well adapted indigenous leafy vegetables (ILVs) (Dweba and Mearns 2011). The ILVs have a potential to contribute to household food security, especially for underprivileged families (Bvenura and Afolayan 2015). However, researchers, policy makers, and farmers are neglecting the potential of ILVs in contributing to food security and are more focused on well-developed and domesticated vegetables (Mwai et al. 2007).

Corchorus species are among several other ILVs that grow in the wild as weeds in South Africa (SA). It is not formally cultivated as a commercial crop but semi-domesticated in some rural parts of SA (Jansen van Rensburg et al. 2007). Around the world, *Corchorus* species are cultivated for their fiber content, following after cotton (Ghosh et al. 2013) but in South Africa, the leaves and tender shoots are harvested and consumed as a vegetable in a form of a relish (Mavengahama et al. 2013). *Corchorus* leaves are also traditionally used to treat several illnesses around the world (Mensah et al. 2008).

Corchorus species are very rich in nutrients and minerals (Zeghichi et al. 2004; Ndlovu and Afolayan 2008; Choudhary et al. 2013; Islam 2013). They contain considerable quantities of beta-carotene, calcium, iron, and zinc (Ndlovu and Afolayan 2008; Choudhary et al. 2013). *Corchorus olitorius* has been reported to be more nutritious when compared to the widely cultivated and commercialised species such as cabbage (Ndlovu and Afolayan 2008; Njeme et al. 2014).

Corchorus species are genetically diverse but it is not known to what extent they are different and also on the improvement of foliage yield (Choudhary et al. 2013). Hence, these research gaps need to be addressed in future breeding programs (Jansen van Rensburg et al. 2007; Choudhary et al. 2013).

Despite the benefits of molecular markers, knowing the morphological variability of a species is the first step before it can be genetically improved for the traits of interest in the breeding programme (Adebola and Morakinyo 2006; Osei et al. 2010). The aim of the study was thus to morphologically characterize the available germplasm of *Corchorus* accessions with the specific aim of identifying superior lines exhibiting desirable traits.

3.2 Materials and Methods

3.2.1 Plant material

Four of the *Corchorus* accessions used in this study were sourced from the ARC and seven from the WVC genebank (Table 3.1). All the ARC accessions were from South Africa, but no other information is available on them except that SA001, SA002 and SAA3 were collected in University of Zululand, KwaZulu-Natal (KZN) and SA004 was collected in Sekhukhune, Limpopo.

Table 3.1: Eleven *Corchorus* accessions evaluated in this study

Accessions no.	Centre	Origin
SA001	ARC	UZ
SA002	ARC	UZ
SA003	ARC	UZ
SA004	ARC	Sekhukhune
AV001	WVC	-
AV002	WVC	-
AV003	WVC	-
AV004	WVC	-
AV005	WVC	-
AV006	WVC	-
AV007	WVC	-

*ARC-Agricultural Research Council; WVC-World Vegetable Centre (Taiwan); UZ-University of Zululand

3.2.2 Study site

The study was carried out in the open field on the premises of the Agricultural Research Council, Vegetable and Ornamental Plants, Roodeplaat research farm, Pretoria. Roodeplaat is situated just outside Pretoria in the Gauteng province of South Africa at an altitude of 1159 m above sea level and at 25°59’ S and 28°35’ E. The average minimum and maximum temperatures for season 1 were 15.8°C and 31.3°C, respectively and for season 2 were 15.4°C and 28.9°C, respectively. The site received a total of 89.79 and 105 mm of rainfall in season 1 and 2, respectively (ARC-ISCW 2017).

3.2.3 Treatments and experimental design

The morpho-agronomic evaluation was performed on 11 *Corchorus* accessions using a randomized complete block design with three replications. Each accession represented a treatment. Each experimental plot had four rows, 3 m in length with an inter-row spacing of 50 cm and intra-row spacing of 30 cm.

3.2.4. Experimental management

The experimental field was ploughed and harrowed with a tractor before the experiment started. Seedlings for each accession were germinated in trays in Hygromix® growth medium. Four weeks after emergence seedlings were transplanted to the field. The experiment was carried out over two seasons, November 2015 to April 2016 and November 2016 to April 2017. Soil fertility status was determined from a composite soil sample collected from the experimental area to a depth of 30 cm before both planting seasons (Table 3.2). However, no fertilisers and chemicals (insecticides, fungicides etc.) were applied throughout the seasons. Plants were kept weed free during the experiment by hand weeding. The experiment was conducted under rainfed conditions, meteorological data is presented in table 3.3. However, supplemental overhead irrigation was applied when it was deemed necessary when plants started to show signs of wilting.

Table 3.2: Chemical characteristics of the field trial soil

Chemical property	**2015/16**	**2016/17**
P (mg kg^{-1})	72.5	40.1
K (mg kg^{-1})	117	113
Ca (mg kg^{-1})	1056	1283
Mg (mg kg^{-1})	419	494
Na (mg kg^{-1})	69.6	81.3
pH (H_20)	7.1	7.6

*P-phosphorus; K-potassium; Ca-calcium; Mg-magnesium; Na-sodium; pH-potential hydrogen

Table 3.3: Weather conditions at ARC-VOP farm

Month	Temperature Average max	Temperature Average min	Relative humidity (%) Average	Rainfall (mm) Total
Season 1				
November 2015	31.77	13.95	42.21	29.72
December 2015	33.88	18.09	50.87	60.2
January 2016	31.67	17.63	55.68	135.13
February 2016	32.46	17.82	57.03	49.53
March 2016	29.35	15.61	61.35	204.47
April 2016	28.41	11.79	59.66	59.66
Season 2				
November 2016	29.4	15.49	61.62	175.51
December 2016	30.14	17.39	61.74	67.57
January 2017	29.36	17.24	64.47	131.83
February 2017	28.74	17.37	65.48	140.98
March 2017	29.36	13.68	60.95	32.01
April 2017	26.55	11.3	63.11	82.54

*Source: Agricultural Research Council-Institute of Soil, Climate and Water (ARC-ISCW 2017)

3.3 Data collection

Morpho-agronomic characterization was conducted using a descriptor for *Corchorus* adapted from Ghosh et al. (2013). Ten plants from each accession were randomly selected from the two inner rows per plot and tagged for data collection.

3.3.1 Qualitative traits

Qualitative data were recorded during 50% flowering stage from the marked plants in each plot for eleven qualitative traits. To avoid variances due to growth, qualitative trait evaluations were done on the same day. The following traits were recorded: branching habit, stem, leaf, leaf vein, petiole, pod, seed and flower colour, and leaf and pod shape. Cotyledon colour was measured at germination. Descriptors for each qualitative traits are listed in Table 3.4.

Table 3.4: Qualitative traits descriptors used for characterization of *Corchorus* accessions in the study

Trait	Code	Description
Cotyledon colour	CC	Light green (1), Green (2)
Flower colour	FC	Yellow (1), White (2)
Branching habit	BH	No effective branching (1), medium primary branches (2), strong primary and secondary branches (3)
Leaf colour	LC	Light green (1), Dark green (2), Green (3)
Leaf vein colour	LVC	Green (1), Red (2)
Petiole colour	PC	Light green (1), Green (2), red (3), Light red (4)
Leaf shape	LS	Ovate (1), Lanceolate (2), Palmate (3)
Pod colour	POC	Green (1), Green with red stripes (2), Red (3), Light brown (4)
Pod shape	PS	Straight (1), Curved (2)
Stem colour	STC	Light green (1), Dark green (2), Red (3), Light red (4), Dark red (5)
Seed colour	SC	Brown (1), Green (2), Black (3), Grayish black (4), Dark brown (5)

*Numbers in bracket shows descriptors codes

Source: (Ghosh et al. 2013).

3.3.2 Quantitative traits

All quantitative traits apart from days to 50% flowering were taken at harvest maturity. Traits measured and counted are listed in Table 3.5.

Table 3.5: Quantitative traits descriptors used for characterization of *Corchorus* accessions in the study

Traits	Code	Descriptors
Days to 50% flowering	DF	Number of days from seed germination to 50% flowering
Plant height	PH	Plant height at maturity measured from the base of the plant to the tip of the main stem using a meter ruler (cm)
Canopy width	CW	Canopy width at maturity measured at widest-point (cm)
Leaf length	LL	Length of a matured lamina from the proximal end of the mid-vein to the distal end, excluding the petiole (cm)
Leaf width	LW	Length of matured leaf at widest-point (cm)
Leaf length/leaf width ratio	LL/LW ratio	The ratio of the leaf blade length to the leaf width LL/LW
Number of pods per plant	NPP	Total number of pods per plant
Pod length	PL	Pod length (cm)
Pod diameter	PD	Diameter of the pod at the widest point (mm)
Stem diameter	SD	Diameter of the plant base at soil level (mm)
Number of leaves	NL	Total number of leaves per plant
Number of branches	NB	Total number of branches per plant
Number of seeds	NS	Total number of seeds per pod
Fresh mass	FM	Total fresh mass per plant (g)
Dry mass	DM	Total dry mass per plant (g)

Source: (Ghosh et al. 2013).

3.4 Data analysis

Qualitative traits were given scores using *Corchorus* descriptors (Ghosh et al. 2013). Shannon-Weaver diversity index (H’) was used to calculate phenotypic frequency of alleles controlling each qualitative trait as described by Shannon and Weaver (1949) as follows:

$$H' = 1 - \sum_{i=1}^{n} pi \ln pi$$

Where:
H’: Diversity index
n: Total number of phenotypic classes of traits
Pi: Proportion of accessions in the i^{th} class of n-class character

Quantitative data were subjected to ANOVA using the Genstat statistical software (12th edition, version 12.2; VNS International Ltd. 2010). The least significant differences (LSD) test was applied for pair-wise comparisons of the means of traits. Differences were accepted as significant at $p<0.05$. Multivariate analysis was perfomed to identify the most significant descriptors in capturing the morphological variation in the germplasm using XLSTAT statistical computer package (2017.06.5). Principal component analysis (PCA) and cluster analysis (CA) were used to discriminate and group accessions respectively. Pearson’s correlation coefficients were used to decide on the relationship between selected traits.

3.4.1 Estimate of genetic parameters

An estimation of broad sense heritability was calculated as described by Allard (1960) as

$$\mathrm{H}^2 = \frac{\delta^2\mathrm{g}}{\delta^2\mathrm{p}} \times 100$$

Where H^2 represent broad-sense heritability, δ^2g genotypic variance, δ^2p phenotypic variance. Genotypic and phenotypic variances were obtained from the ANOVA table according to Comstock and Robinson (1952) as cited by Al-Tabbal and Al-Fraihat (2011) using:

$$\delta^2 g = \frac{Mg1 - Mg2}{rs}, \quad \delta^2 p = \frac{Mg1}{rs}$$

Where *r* =replication, *s* = season, *M*g1 =mean squares for genotype, *M*g2 =mean square for accessions by season interaction. Trait grand mean (*x*) values were used for genetic parameter analysis to estimate the genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) according to Singh and Chaudhury (1979).

$$\mathrm{GCV} = \frac{\sqrt{\delta^2 g}}{x} \times 100$$

$$\mathrm{PCV} = \frac{\sqrt{\delta^2 p}}{x} \times 100$$

The genetic advance was estimated according to Farshadfar et al. (2013) as follows:

$$\mathrm{GA} = \left(\frac{i.\delta^2 g}{\sqrt{\delta^2 p}}\right) * 100/\times$$

Where i= 2.06 is standard selection differential at 5% selection intensity.

3.5 Results

3.5.1 Qualitative traits

To avoid variances due to growth, qualitative trait evaluations were done on the same day. The two seasons had no significant effect on the qualitative traits, but the *Corchorus* accessions exhibited variation in all the different traits evaluated and measured. The most distinctive variations in leaf, stem, pod and seed characteristics for some of the lines are presented in Figures 3.1 – 3.4

Leaf attributes

A total of 72.7% of the accessions in the current study were observed to be green in colour, 18.2% was light green, and 9.1% was dark green in colour (Table 3.6 and Figure 3.1). Most of the accessions (72.7%) had light red petiole colour and the rest of the accessions had red (9.1%), green (9.1%) and light green (9.1%) colours. A total of 72.7% of the accessions had a lanceolate leaf shape, 18.2% were ovate shaped, and 9.1% palmate shaped.

There was no variation in the cotyledon colour among the accessions with all (100%) showing a green colour. Similarly, the leaf vein colour of all accessions was green. There was also no variation in flower colour with all accessions having yellow flowers.

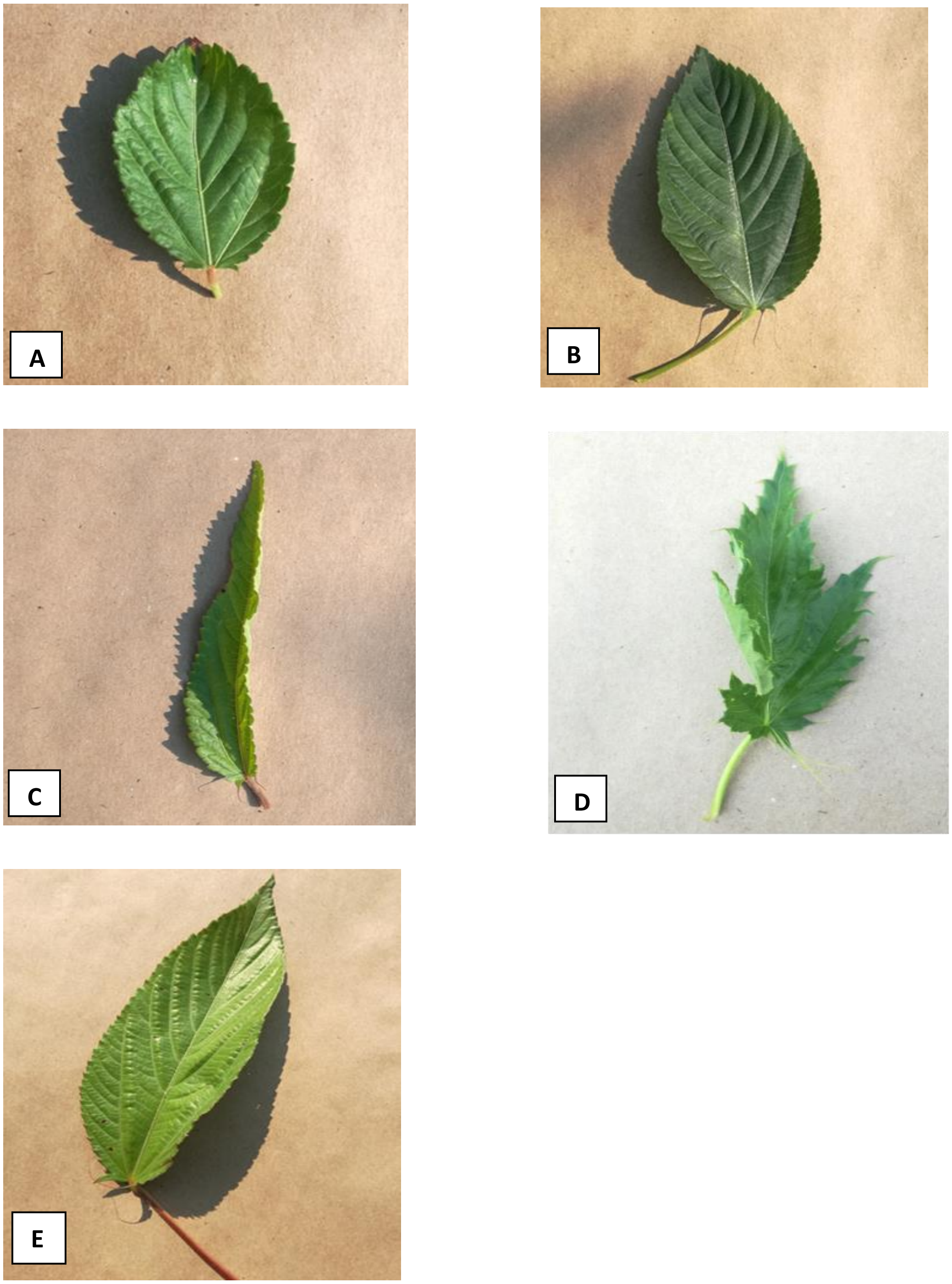


Figure 3.1: Variation in leaf blade colour, leaf shape as well as petiole colour of some of the *Corchorus* accessions- (A) SA002: green leaf, ovate, light red petiole; (B) AV002, dark green leaf, ovate, green petiole, C: SA004: light green leaf, lanceolate, light red petiole, D: AV007: green leaf, palmate, light green petiole, E: AV006, green leaf, lanceolate shape and red petiole.

Stem, pod and seed colour

The accessions stem colour was differentiated into 45.5% red, 27.3% light red, 9.1% dark red, and 18.2% light green (Table 3.6 and Figure 3.2). Variation was also observed in pod colour, with 63.6% of the accessions having green pods with red stripes, 18.2% having red pods, 9.1% dark brown pods and 9.1% green pods (Figure 3.3). About 81.8% of the accessions portrayed curved a pod shape, whereas 18.2% displayed a straight shape. An amount of 54.6% of the accessions were observed to have green seeds, 18.2% were dark brown, 9.1% greyish black, 9.1% black, and 9.1% brown (Figure 3.4).

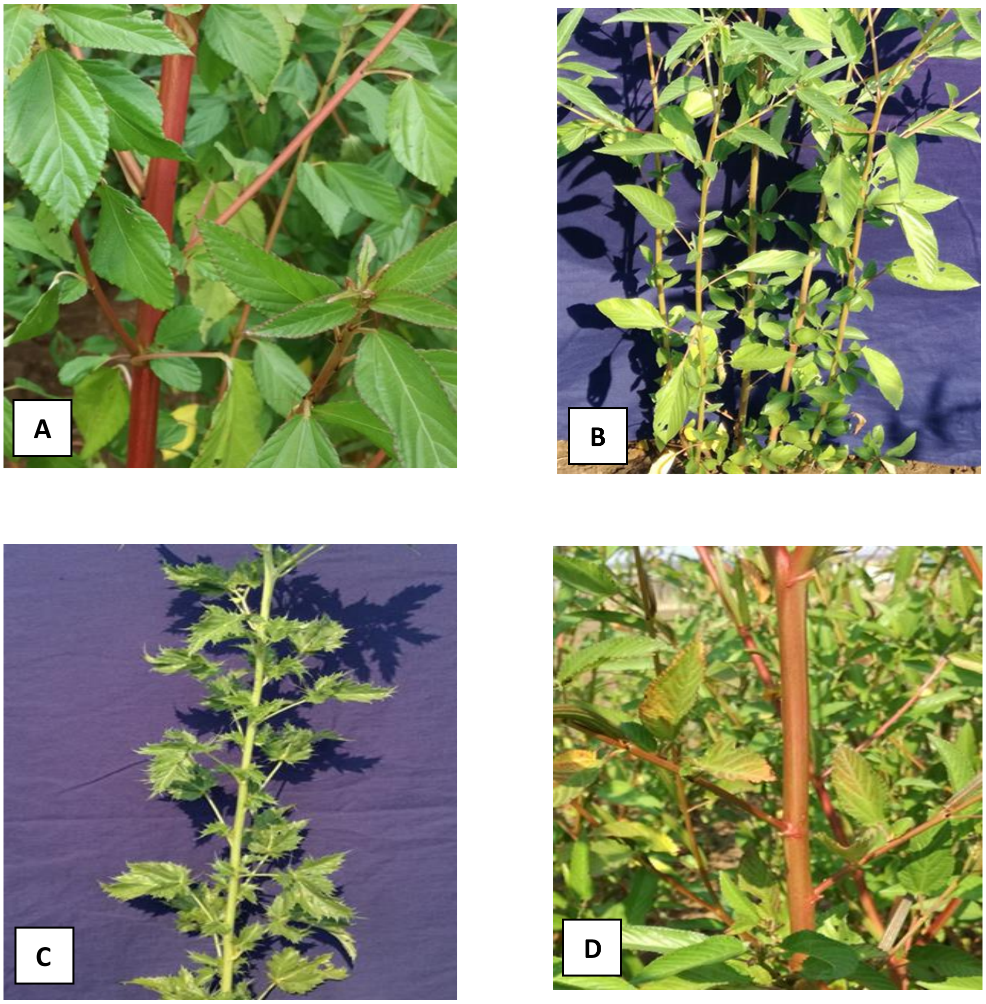


Figure 3.2: Variation in stem colour of some of the *Corchorus* accessions - (A) AV006: dark red stem; (B) AV001: light red stem; (C) AV007: light green stem, (D) SA003: red stem.

Figure 3.3: Variation in pod colour and shape of some of the *Corchorus* accessions - (A) AV007: green pod, curved (B) SA001: dark red pod, straight (C) SA002: light brown pod, curved (D) AV002: green with red stripes pod, curved.

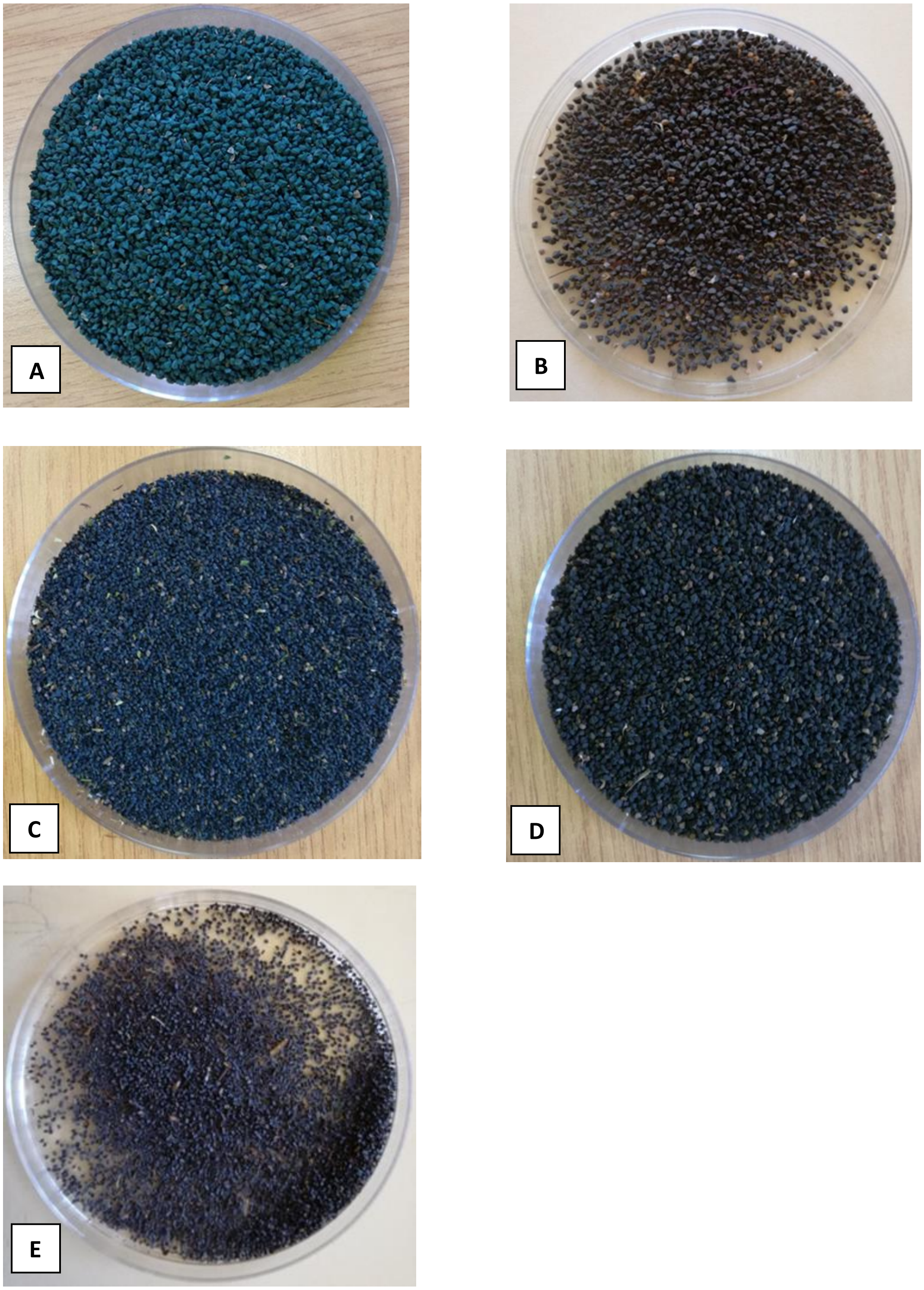


Figure 3.4: Variation in seeds of some of the *Corchorus* accessions- (A) AV002: green seeds, (B) AV006, brown seeds, (C) SA002, greyish black seeds, (D) SA003, black seeds, (E) SA001, dark brown seeds.

Growth pattern

There were two types of branching habit observed with 63.6% of the accessions having a primary branching habit, while 36.4% had both a primary and secondary branching habit (Table 3.6). All the WVC accessions showed primary branching habit, whereas ARC accessions produced both primary and secondary branching habit.

Table 3.6: Morphological descriptors of 11 *Corchorus* accessions

Acc	CC	LC	LVC	PC	STC	POC	SC	LS	BH	FC	PS
SA001	Green	Light green	Green	Light red	Light red	Red	Dark brown	Lanceolate	Primary & Secondary	Yellow	Straight
SA002	Green	Green	Green	Light red	Light red	Dark brown	Greyish black	Ovate	Primary & Secondary	Yellow	curved
SA003	Green	Green	Green	Light red	Red	Green & red stripes	Black	Lanceolate	Primary & Secondary	Yellow	curved
SA004	Green	Light green	Green	Light red	Light red	Red	Dark brown	Lanceolate	Primary & Secondary	Yellow	straight
AV001	Green	Green	Green	Light red	Red	Green & red stripes	Green	Lanceolate	Primary	Yellow	curved
AV002	Green	Dark green	Green	Green	Light green	Green & red stripes	Green	Ovate	Primary	Yellow	curved
AV003	Green	Green	Green	Light red	Red	Green & red stripes	Green	Lanceolate	Primary	Yellow	curved
AV004	Green	Green	Green	Light red	Red	Green & red stripes	Green	Lanceolate	Primary	Yellow	curved
AV005	Green	Green	Green	Light red	Red	Green & red stripes	Green	Lanceolate	Primary	Yellow	curved
AV006	Green	Green	Green	Red	Dark red	Green & red stripes	Brown	Lanceolate	Primary	Yellow	curved
AV007	Green	Green	Green	Light green	Light green	Green	Green	Palmate	Primary	Yellow	curved

*CC-cotyledon colour; LC-leaf colour; LVC-leaf vein colour; PC-petiole colour; STC- stem colour; POC-pod colour; SC-seed colour; LS-leaf shape; BH-branching habit; FC-flower colour; PS-pod shape.

3.5.2 Diversity index

The Shannon-Weaver diversity index estimate for qualitative traits exhibited diversity in most of the traits, although other traits such as cotyledon, leaf vein, and flower colour showed no diversity (Table 3.7). The index was high for seed colour (0.83), followed by the stem colour (0.81), and pod colour (0.71). The lowest diversity was observed in pod shape (0.39). Leaf colour and leaf shape exhibited same index of 0.57 and branching habit with 0.50, these values were slightly lower than the average diversity index which was 0.63. All traits that showed high levels of polymorphism (H'>0.50) could be used to differentiate among accessions.

Table 3.7: Shannon-Weaver diversity index of qualitative traits for *Corchorus* accessions

Qualitative traits	**Shannon-Weaver (H')**
Cotyledon colour	0.00
Leaf colour	0.57
Leaf vein colour	0.00
Leaf shape	0.57
Petiole colour	0.63
Flower colour	0.00
Pod colour	0.71
Pod shape	0.39
Seed colour	0.83
Stem colour	0.81
Branching habit	0.50
Average diversity index	0.63

3.5.3 Quantitative traits

Days to 50% flowering

Significant (P<0.05) differences were observed among the studied accessions for days to 50% flowering (Table 3.8). The highest number of days for 50% flowering was observed in accession AV007, flowering in 77 days, followed by AV004 flowering in 62.2 days.

Accession that flowered early was SA002, flowering at 35.8 days. It was observed that all the ARC accessions flowered earlier than the WVC accessions.

Plant height

Corchorus accessions studied showed significant ($P<0.05$) differences in plant height (Table 3.8). Accession AV006 was the tallest of all accessions with a height of 184.9 cm, whereas accession SA004 was the shortest of the accessions with a height of 48.0 cm with the average of 120.0 cm.

Canopy width

Significant ($P<0.05$) differences were observed among the studied accessions for canopy width (Table 3.8). Accession SA001 had the widest plant canopy of 69.3 cm while accession AV007 had narrowest canopy width of 31.7 cm and mean of 48.5 cm. The short accessions had broad plant canopy compared to taller accessions.

Leaf traits

There were significant ($P<0.05$) differences among evaluated accessions for number of leaves per plant, leaf length, leaf width and leaf length-width ratio (Table 3.8). Leaves are an important part of *Corchorus* species since they are the main part of the vegetable consumed. The number of leaves per plant ranged from 115.1 for accession SA003 to 238.5 for accession AV006 with a mean of 157.3. Leaf length was 6.7 cm for SA004 and 9.4 cm for AV006. Leaf width ranged from 1.8 cm for accession SA001 to 5.8 cm for accession SA004 with the mean of 2.9 cm, whereas leaf length-width ratio ranged from 1.27 to 4.47 for AV007 and SA001, respectively.

Pod traits

Significant variation ($P<0.05$) was also recorded in pod length, pod diameter, number of pods per plant, and number of seeds per pod (Table 3.8). Accession AV005 and AV003 had the longest pods of 8.4 cm while the shortest was SA004 with 3.0 cm. Pod diameter varied from 0.4 mm for SA004 to 6.6 mm for AV007. Accessions from the ARC had the highest number of pods per plant compared to WVC accessions. Accession SA001 had more pods than the rest of the accessions having 285.5 pods per plant, whereas AV006 recorded the least number of pods of 13.8 pods per plant. Accession AV004 had the highest number of seeds per pod having 212 seeds per pod while SA004 recorded the least number of seeds per pod of 76.1 with the mean of 145.0 seeds per pod. Majority of the WVC accessions had the higher number of seeds per pod than the ARC accessions.

Stem diameter

Significant differences ($P<0.05$) were observed among the studied accessions for stem diameter (Table 3.8). Accession AV007 had the thickest stem of 18.3 mm while SA004 had slender stems with a diameter of 5.6 mm. All the tall accessions had thicker stems (wider diameter) which is good for support.

Number of branches per plant

The number of branches per plant showed significant variation ($P<0.05$) among accessions (Table 3.8). Accession AV006 recorded 24.9 branches being the highest. The accession with the least number of branches was SA004 recording 7.5 branches per plant with the mean of 16.58.

Fresh and dry mass

Significant variation ($P<0.05$) was observed in fresh and dry mass in the evaluated accessions (Table 3.8). Accession with minimum fresh mass was SA004 with 6.7 tonnes ha^{-1} and maximum being AV004 recording 22.2 tonnes ha^{-1}. Dry mass ranged from 2.6 tonnes ha^{-1} to 5.6 tonnes h^{1} for SA004 and AV004, respectively. The average dry weight was 4.3 tonnes ha^{-1}.

Table 3.8: Quantitative traits means for *Corchorus* accessions evaluated under field conditions

Acc.	DF	PH	CW	LL	LW	LL/LW	SD	PL	PD	NL	NB	NPP	NSPP	FM	DM
SA001	38.5e	60.3i	69.3a	8.0de	1.8f	4.5a	6.9g	4.2d	0.8f	198.2b	14.1e	285.5a	84.9f	11.8f	2.8f
SA002	35.8e	77.3h	68.8a	8.3cd	2.3e	4.0b	8.2f	7.0b	1.6e	130.5e	12.8f	149.6c	145.2c	9.8g	3.6e
SA003	52.2d	127.6e	51.9c	8.4bc	2.4de	3.5cd	10.7e	6.0c	4.1d	115.1f	17.3d	73.0d	106.9e	20.3b	5.0c
SA004	36.5e	48.0j	61.3b	6.7g	1.8f	3.7c	5.6h	3.0e	0.4f	118.9f	7.5g	166.9b	76.1f	6.7h	2.6f
AV001	61.2b	116.5g	40.8e	7.8e	3.4b	2.3g	14.3c	7.2b	5.3b	202.2d	18.0d	60.7f	181.7b	15.1e	4.3d
AV002	56.8c	157.7c	37.5f	7.9de	2.9c	2.8f	15.5b	8.3a	5.0bc	142.1d	17.0d	24.1h	132.5d	12.4f	3.9e
AV003	59.3c	133.0d	47.7d	8.5bc	2.7cd	3.2e	12.0d	8.4a	5.0bc	126.7e	14.9e	50.7g	179.1b	14.3e	4.4d
AV004	62.2b	122.0f	42.3e	7.3f	2.7cd	2.7f	13.6c	8.0a	4.9c	170.1c	23.7b	59.6f	212.0a	22.2a	5.9a
AV005	57.8c	132.7d	45.9d	8.8b	3.4b	2.6f	12.6d	8.4a	5.1bc	117.5f	19.9c	67.8e	176.4b	17.3d	4.1d
AV006	54.8d	184.9a	36.3f	9.4a	2.9c	3.3de	16.2b	7.3b	4.8c	238.5a	24.9a	13.8j	201.0a	18.9c	5.1bc
AV007	77.0a	170.5b	31.7g	7.3f	5.8a	1.3h	18.3a	4.5d	6.6a	170.1c	12.3f	16.7i	99.3e	21.1b	5.6ab
Mean	53.8	121.0	48.5	8.03	2.9	3.09	12.18	6.58	3.95	157.3	16.58	88.03	145.0	15.5	4.3
P value	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
LSD	5.8	3.8	3.9	0.7	0.52	0.41	1.3	0.7	0.5	10.0	1.6	6.8	17.0	1.5	0.8
CV %	6.5	1.9	4.8	4.9	10.8	8.0	6.3	6.8	7.90	3.9	5.9	4.7	7.1	5.9	11.5

*Acc-accessions; DF-days to 50% flowering; PH-plant height; CW-canopy width; LL-leaf length; LL/LW ratio; SD-stem diameter; PL-pod length; PD-pod diameter; NL-number of leaves per plant; NB-number of branches per plant; NPP-number of pods per plant; NSPP-number of seeds per pod; FM-fresh mass per plant; DM-dry mass per plant. Means with different letters within a column are significantly different at $p<0.05$ according to Fisher's LSD.

3.6 Correlation among traits

A strong positive correlation was observed between days to 50% flowering and the following traits, plant height (r^2= 0.79), leaf width (r^2= 0.85), stem diameter (r^2= 0.90), pod diameter (r^2= 0.95), fresh mass (r^2= 0.77) and dry mass (r^2= 0.79) (Table 3.9). Pod length was positively correlated with number of seeds per pod (r^2= 0.84). The longer the pod the more were the number of seeds inside (Table 3.9). Furthermore, number of branches were positively correlated with fresh mass (r^2= 0.68) and dry mass (r^2= 0.62). Canopy width was positively correlated to number of pods per plant (r^2= 0.90). Shorter accessions were spread having a larger canopy and produced more pods. A positive correlation was also observed between number of seeds per pod and pod diameter (r^2= 0.53). Pods that were thick had more seeds inside whereas thin pods had few seeds inside (Table 3.9).

A strong negative correlation was observed between days to 50% flowering and the following traits, leaf length-width ratio (r^2= -0.95), canopy width (r^2= -0.90), and number of pods per plant (r^2= -0.79) (Table 3.9). It was observed that all accessions that flowered early produced more pods than those that flowered late. Another strong negative correlation was observed between plant height and canopy width (r^2= -0.90), and also plant height and number of pods per plant (r^2= -0.88). Accessions that were taller had a narrow canopy and produced less number of pods whereas shorter accessions such as SA001 had a broad canopy and yielded more pods per plant (Table 3.8, Table 3.9). A moderate correlation was recorded between number of leaves and number of branches (r^2= 0.48) (Table 3.9).

Table 3.9: Correlation table for 16 quantitative traits of *Corchorus* accessions evaluated under field conditions

TS	DF	PH	CW	LL	LW	LL/LW	SD	PD	PL	NL	NB	NPP	NSPP	FM	DM
DF	**1**														
PH	**0.79**	**1**													
CW	**-0.90**	**-0.90**	**1**												
LL	-0.01	0.46	-0.10	**1**											
LW	**0.85**	**0.65**	**-0.72**	-0.09	**1**										
LL/LW	**-0.95**	**-0.66**	**0.86**	0.21	**-0.91**	**1**									
SD	**0.90**	**0.94**	**-0.95**	0.22	**0.79**	**-0.83**	**1**								
PD	**0.95**	**0.89**	**-0.92**	0.23	**0.76**	**-0.85**	**0.93**	**1**							
PL	0.36	**0.52**	-0.42	**0.56**	0.04	-0.21	0.46	0.57	**1**						
NL	0.20	0.30	-0.31	0.19	0.18	-0.10	0.40	0.17	-0.04	**1**					
NB	0.38	**0.60**	**-0.51**	**0.57**	0.06	-0.18	**0.54**	**0.53**	**0.68**	0.48	**1**				
NPP	**-0.79**	**-0.88**	**0.90**	-0.25	**-0.60**	**0.75**	**-0.86**	**-0.89**	**-0.58**	-0.05	-0.49	**1**			
NSPP	0.38	0.47	-0.45	0.47	0.07	-0.25	0.47	**0.53**	**0.84**	0.31	**0.81**	**-0.55**	**1**		
FM	**0.77**	**0.71**	**-0.67**	0.23	**0.56**	**-0.57**	**0.70**	**0.76**	0.33	0.27	**0.68**	**-0.60**	0.45	**1**	
DM	**0.79**	**0.76**	**-0.74**	0.16	**0.60**	**-0.64**	**0.77**	**0.81**	0.40	0.25	**0.62**	**-0.76**	0.54	**0.93**	**1**

*TS-traits; DF- days to 50% flowering; PH-plant height; CW-canopy width; LL-leaf length; LW-leaf width; LL/LW ratio; SD-stem diameter; PL-pod length; PD-pod diameter; NL-number of leaves per plant; NB-number of branches per plant; NPP-number of pods per plant; NSPP-number of seeds per pod; FM-fresh mass; DM-dry mass.

3.7 Principal component analysis (PCA)

The patterns of diversity were determined using multivariate analysis among accessions. Principal component analysis grouped 15 quantitative traits into 15 principal components, which accounted for the entire genetic variability among the 11 *Corchorus* accessions (Table 3.10). The first two principal components contributed 78.46% of the genetic variability. The first principal component (PC1) had an eigenvalue of 9.18 and accounted for 61.17% of genetic variability. Traits that contributed most to the variability among accessions both negative and positive loadings in the PC1 were days to 50% flowering (0.92), plant height (0.93), canopy width (-0.94), pod diameter (0.97), stem diameter (0.96), dry mass (0.87), fresh mass (0.82), number of seeds per pod (0.61, pod length (0.57), number of branches (0.65), leaf width (0.73), and number of pods per plant (-0.90).

The second principal component (PC 2) had an eigenvalue of 2.59 and accounted for 17.29% of genetic variability. The phenotypic attributes that played the main role in the variability among accessions were leaf width (-0.58), pod length (0.62), leaf length (0.74), number of seeds per pod (0.64), and number of branches (0.66).

The number of leaves per plant (0.87) was the main contributing factor in the third principal component with an eigenvalue of 1.19 and accounted for 7.95% of the total genetic variability among accessions. Whereas the fourth PC had an eigenvalue of 0.72 and accounted for 4.82% of variability with leaf length, fresh and dry mass contributing 0.42, -0.36, and -0.37, respectively.

The principal component variable circle also illustrate the correlation between traits. Traits that are grouped together are positively correlated, whereas those that are far apart are negatively correlated (Figure 3.5). Variables on the first and second quadrants, which are days to 50% flowering, stem diameter, fruit diameter, plant height, fresh and dry mass, leaf length, fruit length, and number of branches are positively correlated. However, the variables on the first and second quadrants are negatively correlated to the third and fourth quadrant, for example, number of pods and plant height (-0.88). Variables circle can be confirmed by the correlation table (Table 3.9).

Table 3.10: Principal component analysis (PCA) for quantitative traits of 11 *Corchorus* accessions

Traits	**PC 1**	**PC 2**	**PC 3**	**PC 4**	**PC 5**
Eigenvalue	9.18	2.59	1.19	0.72	0.65
Variability %	61.17	17.29	7.95	4.82	4.30
Cumulative %	61.17	78.46	86.41	91.23	95.54
Factor loading					
DF	**0.92**	-0.36	-0.02	-0.07	0.03
PH	**0.93**	0.06	0.00	0.27	0.18
CW	**-0.94**	0.17	-0.01	-0.12	0.13
LL	0.28	**0.74**	-0.03	**0.42**	**0.40**
LW	**0.73**	**-0.58**	0.06	0.15	0.07
LL/LW	**-0.81**	**0.52**	0.09	0.00	0.18
SD	**0.96**	-0.12	0.09	0.19	-0.06
PL	**0.57**	**0.62**	-0.45	0.00	-0.20
PD	**0.97**	-0.09	-0.14	0.03	0.00
NL	0.31	0.20	**0.87**	0.18	-0.25
NB	**0.65**	**0.66**	0.22	-0.19	-0.01
NPP	**-0.90**	0.02	0.29	-0.10	0.03
NSPP	**0.61**	**0.64**	-0.08	-0.18	-0.35
FM	**0.82**	0.03	0.20	**-0.36**	0.36
DM	**0.87**	0.00	0.09	-0.37	0.20

* PC1-5: Principal components 1-5; DF- days to 50% flowering; PH-plant height; CW-canopy width; LL/LW ratio; leaf length; LW-leaf width; LL/LW-leaf length/leaf width ratio; SD-Stem diameter; PL-pod length; PD-pod diameter; NL-number of leaves per plant; NB-number of branches per plant; NPP-number of pods per plant; NSPP-number of seeds per pod; FM-fresh mass; DM-dry mass.

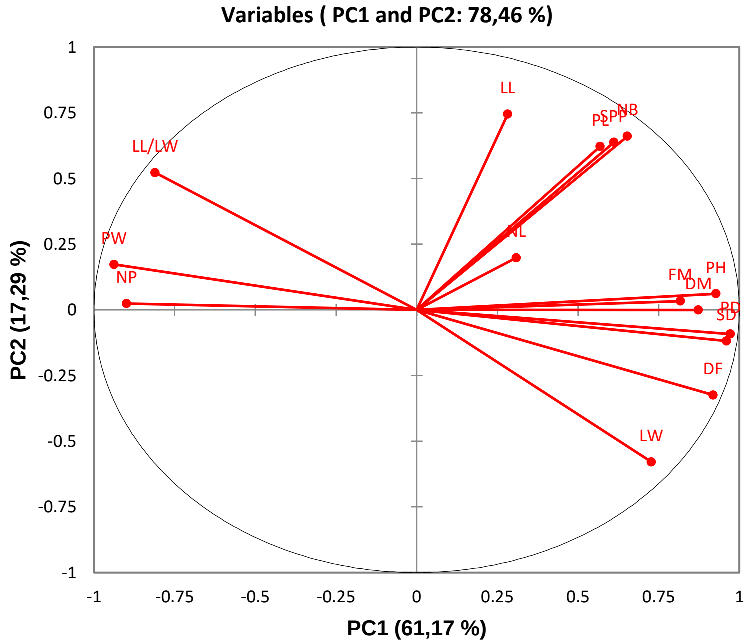


Figure 3.5: Principal component variable circle of 15 phenotypic traits of *Corchorus* accessions

In the observation principal component accessions are scattered in a way that is easy to visualize the distance among them (Figure 3.6). Accessions are grouped in different quadrants based on their similarity in phenotypic attributes. The biplot clearly indicates the link between accessions (Figure 3.7). Accessions in the first and second quadrant, AV003, AV005, AV006, AV004, AV002, and AV001 are associated by tallest plant, high number of branches and pods per plant, wider stem and pod diameter, high fresh and dry mass. Whereas, accessions in the opposite side, SA001, SA002, and SA004 are associated by early flowering, widest plant canopy, high leaf length-leaf width ratio and number of pods per plant. Accession AV007 shared most characteristics with its fellow WVC accessions but slightly differed with regards to days to 50% flowering and leaf width.

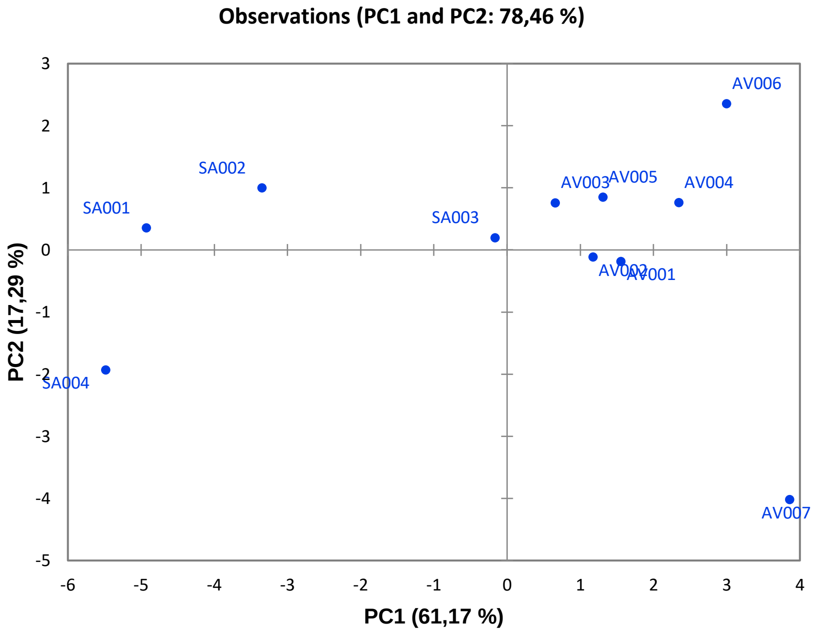


Figure 3.6: Principal component of 11 *Corchorus* accessions in the biplot

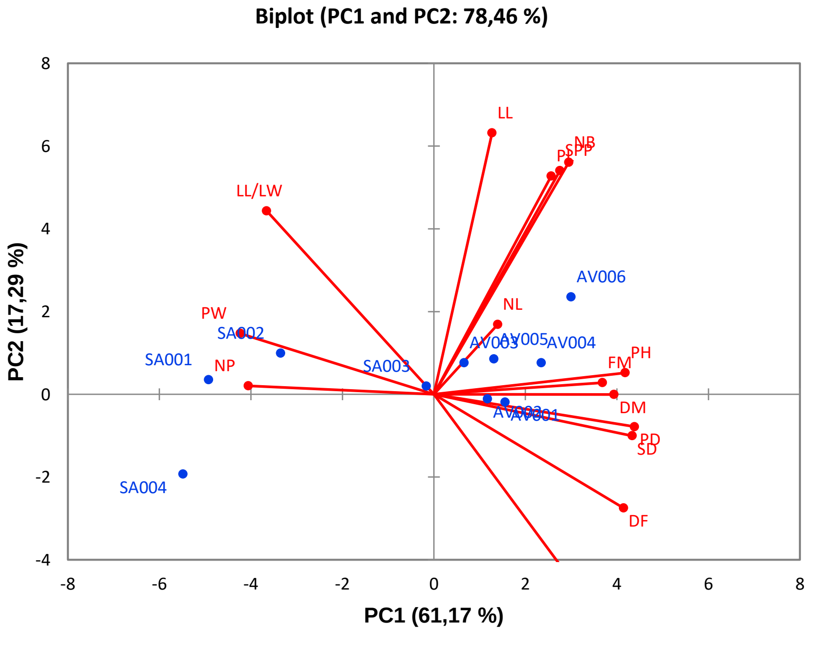


Figure 3.7: Principal component biplot showing variation of *Corchorus* accessions by phenotypic traits

3.8 Cluster analysis

A cluster analysis dendrogram clustered the 11 *Corchorus* accessions into two major clusters at 0.91 euclidean distance based on the 15 phenotypic traits evaluated (Figure 3.8). Cluster I was subdivided into subcluster A which contained SA002 and SA004 accessions and a singleton. Singletons are those accessions that are placed separately from the rest of the accessions in a cluster. They are more diverse and are to be given special attention during selection because of their superiority over other accessions (Choudhary et al. 2013). These two accessions (SA002 and SA004) were more similar based on the highest number of pods per plant, shortest plant height with larger canopy, early flowering and low fresh and dry mass. The singleton, SA001 was more diverse than the other accessions but shares some similar characteristics with the accessions in the same cluster. SA001 flowered early, exhibited lowest number of seeds per pod, less number of branches per plant, and lowest fresh and dry mass. However, it recorded the highest number of leaves per plant, widest plant canopy width and highest number of pods per plant.

The cluster II was subdivided into two main subclusters A and B. Subcluster A contained accessions SA003, AV003, AV005, AV001 and AV004 whereas subcluster B consisted of accessions AV007, AV002 and AV006. Cluster II was grouped together based on the tallest plants, larger stem diameter, less number of pods and highest fresh and dry mass.

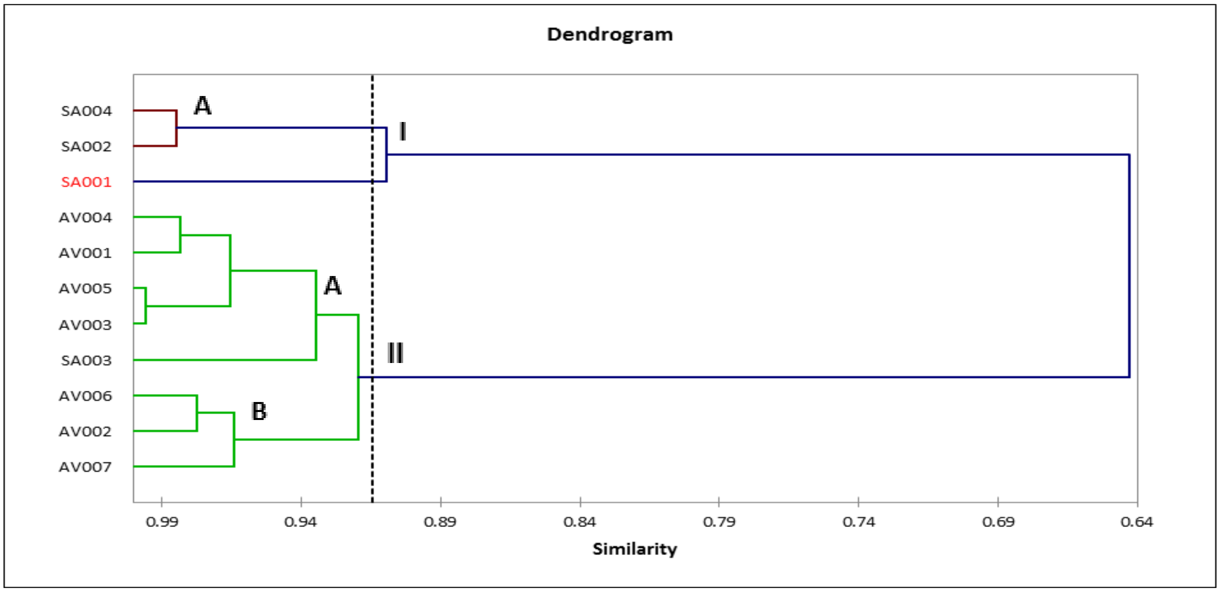


Figure 3.8: Hierarchical cluster analysis dendrogram displaying relationship among 11 *Corchorus* accessions using quantitative traits

3.9 Genetic parameters

All the accessions evaluated in this study showed high variability with high significant differences in morphological traits (Table 3.11).The phenotypic variance was higher than the genotypic variance in all traits. High phenotypic variance values of 20178.6, 6958.9, 5747.2, 5202.1 and 502.2 were recorded for the number of pods per plant, number of seeds per pod, plant height, number of leaves per plant, and canopy width, respectively. Whereas for genetic variances the values were 16161.9, 6150.7, 5124.0, 3887.5 and 262.5 for number of pods per plant, number of seeds per pod, plant height, number of leaves per plants, and canopy width, respectively (Table 3.12).

There were less differences between genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) (Table 3.12). The highest values for PCV were recorded in number of pods (161.4%), fruit diameter (89.6%), leaf width (64.3%), plant height (62.9%), number of seeds per pod (57.5%), stem diameter (57%), fresh mass (55.6%), number of branches per plant (52.1), pod length (49.8%), canopy width (46.2%), and number of leaves per plant (45.9%) and dry mass (42.5%). The GCV recorded the following values in number of pods (144.4%), pod diameter (88.7%), leaf width (61.5%), plant height (59.2%), number of seeds per pod (54.1%), stem diameter (52.2%), pod length (49%), number of branches per plant (44.8%), and fresh mass (40.8%).

In this study, all the evaluated traits exhibited high level of broad-sense heritability. The pod diameter had the highest heritability estimate (98%), followed by pod length (97%), days to 50% flowering (95.5%), leaf width (91.2%), plant height (89.2%), number of seeds per pod (88.39%), stem diameter (84.08%), number of pods per plant (80.1%), number of leaves per plant (74.7%), leaf length (72.8%), dry mass (72.4%), number of branches (71.4%), fresh mass (53.3%) and canopy width (52.3%) (Table 3.12). Genetic advance (GA) ranged from 25.3 to 266.3 with number of pods recording the highest (266.3), followed by pod diameter (180.9), leaf width (120.9), plant height (115.1), and stem diameter (98.6). Leaf length recorded the lowest GA of 25.3 (Table 3.12).

Table 3.11: Analysis of variance for 15 quantitative traits of 11 *Corchorus* accessions evaluated in the field for combined seasons

Traits	Season	Rep.	Accessions	S*A	Residual	CV%	GM
df	1	2	10	10	42		
DF	946.5*	15.6	117.3**	5.3^{ns}	12.4	6.5	53.8
PH	25750.5**	37.7	11494.5**	1246.5**	5.3	1.9	121.0
CW	626.8**	14.2	1004.4**	479.4**	5.5	4.8	48.5
LL	7.2**	1.1	3.7**	1.0**	0.2	4.9	8.0
LW	4.2**	0.2	7.1**	0.6**	0.1	10.8	2.9
LL/LW	2.8**	0.1	4.6**	1.0**	0.1	8.0	3.1
SD	141.4**	9.2	96.4**	15.4**	0.6	6.3	12.2
FL	3.3**	0.2	21.4**	0.6**	0.2	6.8	6.6
FD	6.6**	0.9	25.1**	0.5**	0.1	7.8	4.0
NL	26008.3**	293.1	10404.7**	2629.7**	36.9	3.9	157.3
NB	423.6**	10.1	154.9**	44.4**	1.0	5.9	16.6
NPP	8033.4**	24.8	40357.3**	7348.5**	17.2	4.7	88.0
NSPP	27585.0**	298.2	13917.8**	1616.5**	106.1	7.1	145.0
FM	1102.1**	1.9	148.6**	69.3**	0.8	5.9	15.5
DM	78.6**	1.2	6.7**	1.8**	0.2	11.5	4.3

*CV-coefficient of variation; GM-grand mean; S*A- season by accessions; *-significant; ** -highly significant; ns- not significant; DF- days to 50% flowering; PH-plant height; CW-canopy width; LL-leaf length; LW-leaf width; LL/LW ratio; SD-stem diameter; PL-pod length; PD-pod diameter; NL-number of leaves per plant; NB-number of branches per plant; NPP-number of pods per plant; NSPP-number of seeds per pod; FM-fresh mass; DM-dry mass.

Table 3.12: Genetic parameters for quantitative traits in 11 *Corchorus* accessions

Variables	δ^2g	δ^2p	GCV	PCV	H^2	GA
DF	56.0	58.7	13.9	14.2	95.5	28.0
PH	5124.0	5747.2	59.2	62.7	89.2	115.1
CW	262.5	502.2	33.4	46.2	52.3	49.8
LL	1.3	1.8	14.4	16.9	72.9	25.3
LW	3.2	3.5	61.5	64.3	91.2	120.9
LL/LW	1.8	2.3	43.3	49.1	77.8	78.7
SD	40.5	48.2	52.2	57.0	84.1	98.6
FL	10.4	10.7	49.0	49.8	97.0	99.4
FD	12.3	12.5	88.7	89.6	98.0	180.9
NL	3887.5	5202.4	39.7	45.9	74.7	70.6
NB	55.3	77.5	44.8	52.1	71.4	78.0
NPP	16161.9	20178.6	144.4	161.4	80.1	266.3
NSPP	6150.7	6958.9	54.1	57.5	88.4	104.7
FM	39.6	74.3	40.8	55.8	53.3	34.2
DM	2.4	3.3	36.2	42.5	72.4	63.4

*δ^2g-genotypic variance; δ^2p-phenotypic variance; GCV-genotypic coefficient of variation; PCV-phenotypic coefficient of variation; H^2 –broad sense heritability; GA-genetic advance; DF- days to 50% flowering; PH-plant height; CW-canopy width; LL-leaf length; LW-leaf width; LL/LW ratio; SD-stem diameter; PL-pod length; PD-pod diameter; NL-number of leaves per plant; NB-number of branches per plant; NP-number of pods per plant; NSPP-number of seeds per pod; FM-fresh mass; DM-dry mass.

3.10 Discussion

Morphological characterization of species is an important step in crop improvement programme, it permits breeders to identify and select superior lines for further crop advancement (Adebola and Morakinyo 2006). In this study, 11 *Corchorus* accessions were evaluated in the field for morpho-agronomic traits and revealed great variability in morpho-agronomic traits measured, counted and scored during the characterization.

Morphological characters were highly variable in leaf shape, leaf, petiole, stem, fruit, and seed colour. Indigenous leafy vegetable leaves are the main part of the vegetable consumed (Tallantire and Goode 1975). The fresh green leaves are harvested and consumed as relish in Africa (Nguni and Mwila 2007; Ekesa et al. 2009; Masarirambi et al. 2010; Jansen van Rensburg et al. 2014) which then makes the leaf colour an important trait when characterising ILVs for consumption purposes. About five of the accessions had green leaves, three had light green, one had dark green, and one had leaves with red margins. Ghosh et al. (2013) also reported light green and dark green leaf colour among 63 *Corchorus* accessions. The majority of the accessions leaves were lanceolate in shape, whereby out of eleven accessions, eight were lanceolate with two ovate and one palmate. The Leaf characteristics observed from this study are comparable to those recorded by Adebo et al. (2015). The seed colour is among the most informative phenotypic traits in the classification and characterization of *Corchoru*s species (Benor et al. 2012; Ghosh et al. 2013). High variability in seed colour was observed, six of the accessions produced green seeds, two dark brown, one greyish black, one black, and one brown. These results are aligned with those obtained by Ghosh et al. (2013).

The analysis of variance exhibited highly significant differences ($P<0.05$) among the accessions in days to 50% flowering, plant height, canopy width, leaf length, leaf width, leaf length-leaf width ratio, petiole length, stem and pod diameter, pod length, number of leaves, branches and pods per plant, number of seeds per pod, and fresh and dry mass. These results are in accordance with those reported by Benor et al. (2012) who reported significant differences among species of *Corchorus olitorius* for quantitative traits. Gichimu et al. (2009) also recorded significant variation among wild and cultivated species of watermelon. Oyekale et al. (2014) reported significant positive variation in 11 quantitative traits of *Corchorus olitorius* species and Ahmed et al. (2016) reported significant variation in agro-morphological traits for 32 rice genotypes. This indicates the existence of wide genetic diversity among the species and potential for exploiting the observed genetic diversity for the improvement of the crop (Gerrano et al. 2014).

Plant height ranged from 48.0 to 184.9 cm with the mean value of 120 cm. Similar range was also observed by Sinha et al. (2006) in *Corchorus aestuans* with plant height ranging from 75 to 130 cm with a mean of 104.5 cm. Mir et al. (2008) reported 151 to 349 cm with a mean of 261 cm in *Corchorus capsularis.* However, Das and Kumar (2012) reported relatively greater range in plant height, ranging from 275 cm to 444.33 cm with the mean of 353.23. This huge difference in plant height could be the results of few accessions evaluated in the current study.

A strong positive correlation was observed between plant height and stem diameter, the wider the stem the taller the plant grew. Oboh (2007) also reported a positive correlation between plant height and stem diameter (r^2=0.65) in *Amaranthus hybridus* species. This relationship indicates strong support and vigour of the plant (Adeyinka and Akintade 2015). Another strong positive correlation was observed between days to 50% flowering and plant height, number of seeds per pod and pod diameter, number of branches and fresh and dry mass. Ghosh et al. 2013, similarly found a positive correlation between days to 50% flowering and plant height, and stem diameter and plant height in *Corchorus* species. The positive correlation between and among the traits is an indication that selecting and improving the primary desirable traits would have a positive effect on the secondary traits in the breeding programme (Gerrano et al. 2015).

A strong negative correlation was observed between plant height and canopy width. Accessions from WVC were the tallest with narrower canopy, whereas ARC accessions were the shortest with wider canopy. The variation in number of leaves per plant ranged from 115.1 cm to 238.5 with the mean 157.3. This was relatively higher than the findings by Adebo et al. (2015) who reported the highest mean being 85.16 for number of leaves per plant. Another strong negative correlation was observed between number of pods per plant and days to 50% flowering. All the ARC accessions flowered earlier than the WVC accessions, with SA002 flowering at 35.8 and AV007 flowering at 77 days. These results are within the range reported by Adebo at el. (2015) who recorded flowering in 30, 54.84, 65.55, and 76.53 days in *Corchorus olitorius*. However, their findings in number of pods per plant in relation to early flowering are in contrast with this study. They recorded less number of pods for early flowering (30 day) with the mean of 41.70 pods per plant whereas in this study high number of pods per plant was observed in early flowering accessions (35.8 days) with the mean of 88.03 pods per plant. Masuka et al. (2012) reported similar relationship between early flowering and high pod production in *Cleome gynandra* accessions. Which suggests further investigation into this relationship.

The principal component analysis explained the genetic diversity of the evaluated *Corchorus* accessions. Principal component analysis measures the contribution of each component to total variance, while each factor loading specifies the amount of contribution of every trait with each principal component associated with that trait (Nachimuthu et al. 2014). Each trait was regarded as an important contributor to the variability in a component if its factor loading had total value ≥0.40, irrespective of the plus or minus sign (Nsabiyera et al. 2012; Nachimuthu et al. 2014). The first two components accounted for 78.46% of the total variance. PC1 accounted for 61.17% and PC2 for 17.29% of variation. These results are similar to the findings by Denton and Nwangburuka (2012) who reported similar finding with the first two PCs accounting for 80.45% variation with PC1 and PC2 accounted for 56.80% and 23.60%, respectively, among 15 *Corchorus olitorius* species.

The high level of similarity was observed from the principal component biplot among accessions. Seven of the accessions were clustered together in relation to their traits in the first and second quadrants and are regarded as closely related. However, accessions SA001, SA002, SA003, and AV007 were isolated from the rest of the accessions and are considered to be more diverse and could be used for *Corchorus* improvement. Gerrano et al. (2015) reported similar findings among 32 amaranthus genotypes. They also reported that genotypes that were close to each other were genetically similar, whereas those that were far from each other were reported to be different.

The cluster analysis demonstrated the existence of diversity among the 11 *Corchorus* accessions for the morphological traits studied. The clustering pattern shows that accessions from WVC were genetically distant from the ARC accessions and can be used to improve one another. However, accessions from the same research centre showed high level of similarity and were grouped in a same cluster. This is described as similarity due to locational adaptation (Lewu et al. 2007).

The genetic parameters such as genetic variances and heritability are very important in selection for superior parental lines in breeding programme (Alvi at et al. 2003). The 11 *Corchorus* accessions displayed high variability in genetic parameters. Relatively high genotypic and phenotypic variance values were obtained for plant height, canopy width, number of leaves, number of pods per plant, and number of seeds per pod. This is an indication that the genotype could be reflected by the phenotype and these traits could be selected and utilised in the breeding programme (Nyadanu and Direka 2014).

Danquah and Ofori (2012) categorised phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) as high (>20%), medium (10-20%) and low (<10%). High values of PCV and GCV suggest sufficient genetic variability to facilitate improvement through selection of the desirable trait (Shukla et al 2006). There were very small differences between GCV and PCV, with PCV slightly higher than GCV in all traits. According to Alvi et al. (2003) the low differences in GCV and PCV indicates that there is low environmental effect in the development of these traits. These results are in conformity with the findings by Shukla et al. (2006), Denton and Nwangburuka (2012) who reported small differences between GCV and PCV.

The heritability estimates provide an indication of which trait could be transmitted from parent to an offspring (Usman et al. 2014; Khan et al. 2015). However, effectiveness of selection does not only depend on heritability but also on genetic advance. A combination of high GCV, high broad-sense heritability (H^2), and genetic advance (GA) is the best for selection (Tefera et al. 2003). Johnson et al. (1955) categorized heritability estimate as low (0–30%), moderate (30–60%), and high ≥60%. When the GA and heritability are high for certain trait it is mainly due to an additive gene effect (Shukla et al. 2006). In this present study high GCV, H^2, and GA were recorded for plant height (59.2%, 89.2%, and 115.1), number of leaves per plant (39.65%, 74.7%, and 70.6), pod length (49%, 97% and 99.4), number of pods per plant (144.4%, 80.1%, and 266.3), and number of seeds per pod (54.1%, 88.4%, and 104.9), respectively. These findings suggest the effect of additive genes in the inheritance of these traits. Similar results have also been reported by Immanuel et al. (2011) for grain yield and yield trait in 21 rice genotype and Denton and Nwangburuka (2012) for 6 yield and yield related traits in 15 *Corchorus* species.

3.11 Conclusion

This present study revealed high genetic variability both for qualitative and quantitative morphological characters among the *Corchorus* accessions for crop improvement. High levels of dissimilarity were observed between accessions from the two different research centers (ARC and WVC) which indicates the potential for genetic improvement through selection and cross breeding.

CHAPTER 4

Variability in Mineral Nutrient Content of *Corchorus* Accessions from the Agricultural Research Council of South Africa

4.0 Abstract

Indigenous leafy vegetables (ILVs) are an underutilised source of minerals and vitamins in South Africa, however, they could play a huge role in addressing the issue of poverty, food insecurity and mulnutrition. *Corchorus* is reportedly a highly nutritious ILV, which is cooked and eaten as relish by some rural communities in Africa. The vegetable is very rich in calcium, iron, and zinc. The leaves of *Corchorus* accessions were analysed for their nutritive value (phosphorus, potassium, calcium, magnesium, iron, zinc, boron, cupper and manganese). There were significant ($P<0.05$) differences among the *Corchorus* accessions studied with regards to mineral nutrient composition. The average nutrient composition for accessions were 575, 2093, 2278, 358.6, 55.3, 2.7, 0.7, and 6.8 mg/100g for P, K, Ca, Mg, Fe, B, Zn, Cu, and Mn, respectively. The first two principal components accounted for 70.93% of the total variance with PC1 accounting for 43.98% and PC2 for 26.95% of variation with K, Ca, Mg, P, Fe, Mn, B, and Cu contributing most to variability. High levels of similarity were observed in cluster II with 8 of the accessions grouped together. High heritability coupled with high genetic advance was found for Ca (82.1% and 41.6), Mg (89.3% and 66.9), P (75.8% and 41.3), and B (73.3% and 22.4), respectively, which indicate that these traits are governed by additive type of gene action; hence selection may be effective for improvement of these traits.

4.1 Introduction

South Africa is regarded as a food secure country but there are many households that still suffer from hunger and malnutrition, and mostly micronutrient malnutrition (Mavengahama et al. 2013). The majority of the population suffering from food and nutritional insecurity in South Africa resides in rural areas and informal settlements (Oldewage-Theron et al. 2006). These families rely on pension, child support grants and informal jobs for source of income and the money is often not enough to provide for all their needs (Aliber 2003). These households are usually unable to provide their families with food that comprises of sufficient nutrients for proper development of the body (Njeme et al. 2014).

Micronutrient deficiency, which is an outcome of the diet that lacks sufficient nutrients is predominant in South Africa (Labadarios et al. 2005). The diet of most underprivileged South Africans largely consists of starch and lacks vitamins and minerals which are usually obtained from fruits and vegetables when a healthy diet is followed (Jansen Van Rensburg et al. 2004; Altman et al. 2009). Low consumption of vegetables and fruits is an international problem resulting to malnutrition and mortality in mostly children and the elderly (Labadarios et al. 2005).

Indigenous leafy vegetables are an underutilised source of minerals and vitamins in South Africa (Jansen Van Rensburg et al. 2004; Uusiku et al. 2010; Njeme et al. 2014). They can play a huge role in addressing the issue of poverty, food insecurity and mulnutrition (Uusiku et al. 2010). Indigenous leafy vegetables have been reported to contain considerable quantities of minerals and vitamins (Jansen Van Rensburg et al. 2004; Kamga et al. 2013). *Corchorus* is reportedly a highly nutritious ILV, which is cooked and eaten as relish by some rural communities in Africa (Mavengahama and Lewu 2012). The vegetable is very rich in calcium, iron, and zinc and these are micronutrients that are usually absent in most South African's daily diet (Jansen van Rensburg et al. 2004). Ndlovu and Afolayan (2008) reported higher nutrient content in *Corchorus olitorius* when compared to cabbage. Several other observations agree to ILVs being highly nutritious or comparable with commonly consumed vegetables such as spinach, cabbage, lettuce, and kale (Flyman and Afolayan 2006; Afolayan and Jimoh 2009; Legwaila et al. 2011). This emphasises the potential of ILVs to contribute to household food security and to reduce nutrient deficiencies. This study was therefore undertaken to evaluate the nutritional composition and heritability of nutritional traits of 11 *Corchorus* accessions that are kept at the ARC genebank in South Africa and identify lines with superior nutrient content to use in a *Corchorus* crop improvement programme.

4.2 Materials and Methods

4.1.1 Preparation of samples and determination of mineral nutrient content

The *Corchorus* leaves were collected at harvest maturity from the same trial as reported in chapter 3. Thirty three leaf samples were collected representing 11 accessions each replicated 3 times. Leaves were washed with de-ionised water and oven dried at 60^{o}C (Labcon incubator, Model LTIE). They were then ground into a fine powder using a laboratory grinder (Ultra centrifugal Mill ZM 200), sieved and stored in air-tight containers under room temperature prior to analysis. The phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), boron (B), cupper (Cu) and manganese (Mn) content were analysed using an atomic absorption spectrophotometer after acid digestion of the samples.

4.1.2 Data analysis

Data were subjected to ANOVA using the Genstat statistical software (12th edition, version 12.2; VNS International Ltd. 2010). The least significant differences (LSD) test was applied for pair-wise comparisons of the means of traits. The differences were accepted as significant at $p<0.05$. Multivariate analyses were conducted to identify the most significant descriptors in capturing the nutritional variation in the germplasm using XLSTAT statistical computer package (Version 2017.06.05). Principal component analysis (PCA) and cluster analysis (CA) were used to discriminate and group accessions, respectively. Pearson’s correlation coefficients were used to decide on the relationship between selected traits. Genetic parameters were calculated using relevant formulas as described in chapter 3.

4.3 Results

There were significant ($P<0.05$) differences among the *Corchorus* accessions studied with regards to mineral nutrient composition (Table 4.1). The variation observed among these accessions suggests a wide range of diversity in nutrient levels and offers potential genetic material to improve the mineral nutrient composition through breeding.

The accession AV007 had the highest P content of 714.2 mg/100g and followed by SA003 with 662.4 mg/100g and the average of 575 mg/100g. Accession with the lowest P content was SA002 with 388.9 mg/100g. Potassium content ranged from 1830 (SA001) to 2405

(AV006) mg/100g with the mean of 2093 mg/100g. The mineral Ca content among the accessions studied ranged from 2632 (AV005) to 1720 (SA004) mg/100g with the mean of 2278 mg/100g. The WVC accessions tend to have higher and more uniform Ca contents than the ARC accessions. Accession AV005 recorded the highest Ca content. Accession AV007 recorded the highest level of Mg of 445 mg/100g followed by AV004 recording 427.1 mg/100g and AV001 with 413.5 mg/100g. The lowest Mg content was found in SA004 with 225.9 mg/100g and the mean of 358.6 mg/100g. All the ARC accessions recorded lower Mg content than the WVC accessions.

The accession SA002 outperformed the rest of the accessions recording 87.7 mg/100g of Fe followed by SA004 with 79.6 mg/100g and AV007 recording the lowest Fe content of 29.4 mg/100g. The mean Fe content was 55.3 mg/100g. Two of the ARC accessions, SA002 and SA004 excelled in Fe content and can be used to improve the iron content in other accessions. The SA accessions tend to have higher Fe contents than the WVC accessions in general. The mean Zn content recorded in this experiment was 2.7 mg/100g. The accession SA003 recorded the highest Zn content of 3.4 mg/100g followed by AV006 recording 3.1 mg/100g, whereas accession AV007 recorded the lowest Zn content of 2.3 mg/100g. Foliar Boron content among accessions varied from 5.3 for (SA003) to 7.2 (SA001) mg/100g in this study with a mean of 6.5 mg/100g.

Accession SA002 had the highest amount of Cu of 0.9 mg/100g which was closely followed by accessions AV001, AV005 and SA003 all having a similar Cu content of 0.8 mg/100g. Accession SA001 recorded the lowest amount of copper of 0.5 mg/100g and the mean was 0.7 mg/100g. Over all the range of this element was not very big. The best performer for Mn was SA002 with 8.7 mg/100g followed by AV005 with 7.6 mg/100g. The accession with the lowest Mn was SA001 recording 5.6 mg/100g and the mean Mn content of 6.8 mg/100g. Apart from SA002, the SA accessions had the lowest Mn contents.

Table 4.1: Mineral nutrient content (mg/100g) of *Corchorus* accessions

Acc.	P	K	Ca	Mg	Fe	B	Zn	Cu	Mn
SA001	514.8bc	1830d	1985def	243.3f	62.4abc	5.3e	2.8bc	0.5c	5.6d
SA002	388.9d	2252ab	2566ab	288.5e	87.7a	7.1ab	2.5c	0.9a	8.7a
SA003	662.4a	2239abc	2066cde	339.0d	56.2bcd	7.2a	3.4a	0.8a	5.8d
SA004	422.7cd	1859cd	1720f	225.9f	79.6ab	5.7e	2.4c	0.6bc	5.9d
AV001	641.5a	2106bc	2446ab	413.5ab	43.5cde	6.4cd	2.5c	0.8a	7.0bc
AV002	608.1ab	2059bcd	2591a	411.0ab	39.0cde	6.4d	2.6bc	0.7ab	6.9c
AV003	607.6ab	2147ab	2347abc	395.2bc	41.3cde	6.5cd	2.5c	0.7ab	7.0bc
AV004	618.3ab	2023bcd	2590a	427.1ab	63.5abc	6.2abc	2.7bc	0.7ab	7.4bc
AV005	617.3ab	2079bcd	2632a	396.2bc	40.3cde	6.6bcd	2.8bc	0.8a	7.6b
AV006	513.0bc	2405a	1866ef	359.6cd	65.4abc	6.5cd	3.1ab	0.7ab	7.1bc
AV007	714.2a	2021bcd	2246bcd	445.0a	29.4e	6.8abc	2.3c	0.7ab	6.2d
Mean	575	2093	2278	358.6	55.3	6.5	2.7	0.7	6.8
P value	<.001**	0.002	<.001	<.001**	<.001**	<.001**	0.006	0.008	<.001**
LSD	163.3	364.1	453.4	58.0	37.0	0.74	0.75	0.2	0.9
CV	1.2	10.6	12.1	9.8	40.6	6.9	17.1	17.8	8.8

*Acc-accessions; Ca-calcium; Mg-magnesium; Mn-manganese; Fe-iron; Zn-zinc; B-boron; Cu-copper; P-phosphorus; K-potassium; LSD-Least significant difference; CV- coefficient of variation. Means with different letters within a column are significantly different at p<0.05 according to Fisher's LSD.

4.4 Correlation among traits

The correlation matrix revealed many significant (P<0.05) positive and negative correlations among nutrients in this study (Table 4.2). A positive correlation was observed between boron and copper (r^2=0.85), magnesium and phosphorus (r^2=0.79), calcium and manganese (r^2=0.69), boron and potassium (r^2=0.68), copper and potassium (r^2=0.68), manganese and copper (r^2=0.66), calcium and magnesium (r^2=0.62), and calcium and copper (r^2=0.60).

A moderate positive correlation was observed between magnesium and boron (r=0.54), magnesium and potassium (r^2=0.51), magnesium and copper (r^2=0.50). A very strong negative significant correlation was observed between phosphorus and iron (r^2=-0.88) and magnesium and iron (r^2=-0.76).

Table 4.2: Correlation matrix (r^2 values) for the 9 mineral nutrient traits of the tested *Corchorus* accessions

TS	K	Ca	Mg	P	Fe	Mn	Zn	B	Cu
K	**1**								
Ca	0.12	**1**							
Mg	0.29	**0.62****	**1**						
P	-0.01	0.29	**0.79****	**1**					
Fe	0.07	-0.35	**-0.76****	**-0.88****	**1**				
Mn	**0.51****	**0.69****	0.27	-0.30	0.21	**1**			
Zn	**0.46****	-0.24	-0.10	0.12	0.10	-0.20	**1**		
B	**0.68****	**0.48****	**0.54****	0.33	-0.09	**0.49****	0.26	**1**	
Cu	**0.68****	**0.60****	**0.50****	0.22	-0.11	**0.66****	0.23	**0.85****	**1**

*TS-traits; Ca-calcium; Mg-Magnesium; Mn-Manganese; Fe-iron; Zn-zinc; B-boron; Cu-copper; P-phosphorus; K-potassium, *** =** significant;**** =** highly significant; ns = not significant**.**

4.5 Principal component analysis (PCA)

The principal component analysis grouped the nine nutrient traits into nine principal components, which accounted for the entire genetic variability among the 11 *Corchorus* accessions (Table 4.3). The first three principal components (PCs) contributed 88.60% of the genetic variability. The first principal component (PC1) had an eigenvalue of 3.96 and accounted for 43.98% of genetic variability. Nutritional traits that contributed the most to variation in the PC1 were copper (0.87), boron (0.84), magnesium (0.83), calcium (0.75), potassium (0.62), manganese (0.60), phosphorus (0.53), and iron (-0.46).

The second principal component (PC2) had an eigenvalue of 2.43 and accounted for 26.95% of genetic variability. Factor loadings that contributed more to variability in the second principal component were iron (0.84), phosphorus (-0.79), manganese (0.59), potassium (0.52), and manganese (-0.48). The third principal component had an eigenvalue of 1.59 and accounted for 17.68% of genetic variability. The following are the factor loadings that contributed the most in this principal component, zinc (0.88), potassium (0.41), calcium (-0.53), and manganese (-0.50). The rest of the principal components had factor loadings <40 and eigenvalue <1.

The principal component biplot distinguished the *Corchorus* accessions based on their nutritional traits explained by the PC1 and PC2 (Figure 4.1; Table 4.3). Accessions in the first quadrant, SA002, SA003, AV006 were associated by high manganese, copper, boron, zinc and potassium content. Accessions in the second quadrant AV001, AV002, AV003, AV004, AV005, and AV007 were associated by high calcium, magnesium, and phosphorus content. Accessions SA001 and SA004 had high Fe content but relatively low in B, Mn, and Mg content. The nutritional traits grouped in the right quadrants were positively correlated with each other and negatively correlated with the opposite side (Figure 4.1, Table 4.2). The principal component scattered the accessions in a way that is easy to visualise the distance among them (Figure 4.2).

Table 4.3: Principal component analysis (PCA) for mineral nutrient traits of *Corchorus* accessions

	Eigenvectors (Principal Component)				
Trait	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Eigenvalue	3.96	2.43	1.59	0.43	0.34
Variability %	43.98	26.95	17.68	4.76	3.83
Cumulative%	43.98	70.93	88.60	93.36	97.19
		Factor loading			
K	**0.62**	**0.52**	**0.41**	0.37	-0.18
Ca	**0.75**	-0.04	**-0.53**	-0.36	-0.12
Mg	**0.83**	**-0.48**	-0.07	0.16	-0.05
P	**0.53**	**-0.79**	0.28	-0.05	0.10
Fe	**-0.46**	**0.84**	-0.04	-0.09	0.20
Mn	**0.60**	**0.59**	**-0.50**	0.03	-0.19
Zn	0.11	0.23	**0.88**	-0.34	-0.19
B	**0.84**	0.27	0.20	-0.01	0.39
Cu	**0.87**	0.35	0.07	-0.09	0.11

* PC1-5: Principal components 1-5; Ca-calcium, Mg-Magnesium; Mn-Manganese; Fe-iron; Zn-zinc; B-boron; Cu-copper; P-phosphorus; K-potassium.

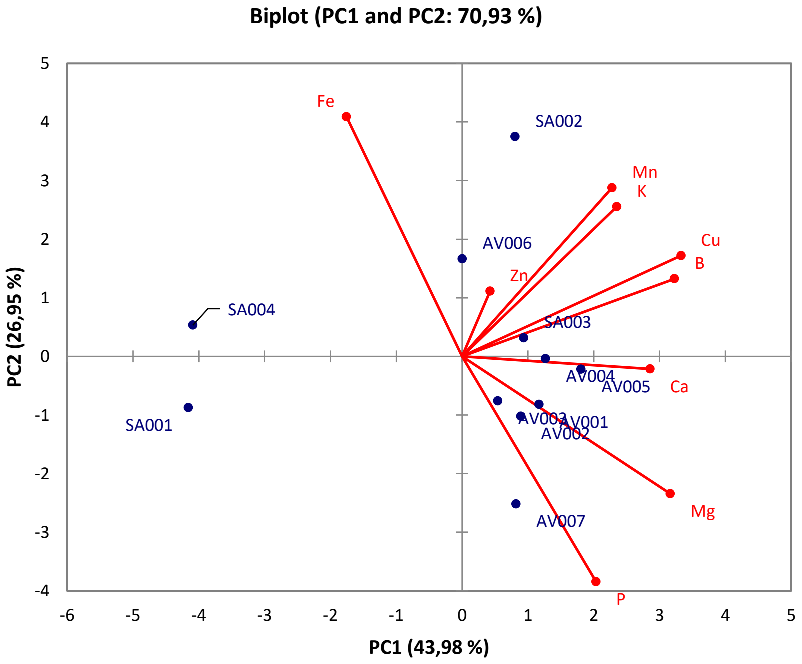


Figure 4.1: Principal component biplot showing variation of *Corchorus* accessions by mineral nutrient traits

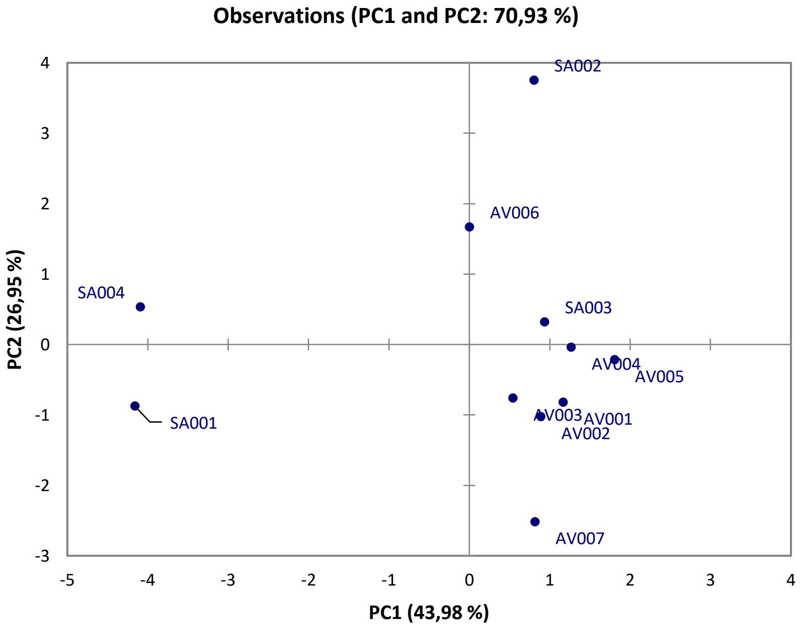


Figure 4.2: Principal component of 11 *Corchorus* accessions in the biplot

4.6 Cluster analysis

The dendrogram clustered the 11 *Corchorus* accessions into two major clusters at 0.993 Euclidean distance based on the nine nutritional traits evaluated. Accessions separated into two main clusters, I and II. Accessions grouped within a cluster are said to be closely related (Figure 4.3). Cluster I was divided into subcluster A and a singleton. Subcluster A contained accessions SA003 and SA004 and these two were characterised by relatively low mineral of calcium, magnesium, and manganese content but relatively high iron, and phosphorus content. The singleton AV006 is considered to be more diverse and richer in mineral nutrients than other accessions in the same cluster and was high in potassium, phosphorus, cupper, iron, manganese and zinc content. Cluster II contained 8 accessions with 6 from WVC and two from ARC. Cluster II was subdivided into subclusters, A, B and a singleton. Subcluster A contained accessions AV001, AV003, AV007 and SA001 and these accessions were associated by relatively high copper, boron, iron, manganese, phosphorus and potassium mineral content. Whereas subcluster B contain AV002, AV004, and AV005 were associated by relatively low boron, iron, potassium, and zinc mineral content. Singleton SA002 was relatively low in mineral phosphorus but outstandingly high in mineral calcium, iron, manganese, boron, copper, and potassium content.

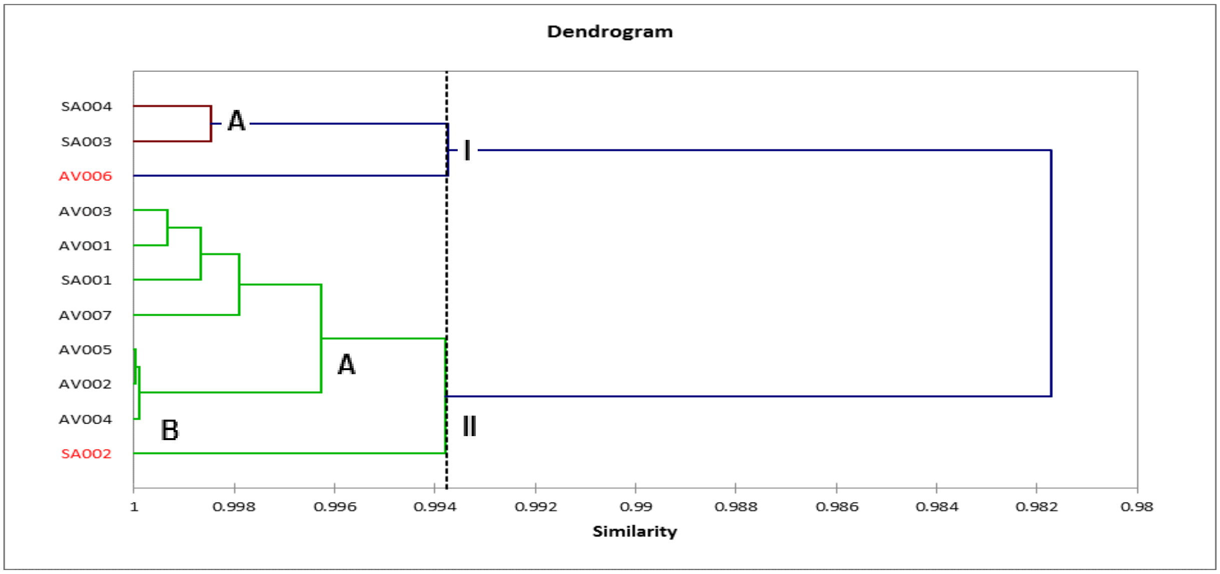


Figure 4.3: Hierarchical cluster analysis dendrogram displaying relationship among 11 *Corchorus* accessions with regards to mineral nutrient traits

4.7 Genetic parameters

Genetic parameters showed considerable amount of genetic variability in nutritional traits. Phenotypic variance values were greater than the genotypic variance for all traits (Table 4.4). The highest phenotypic variances were recorded in calcium (314679), potassium (84584.5), phosphorus (29066), and magnesium (17069).

The differences between genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were small. The highest values for PCV were recorded for mineral iron (57.4%), magnesium (36.4%), phosphorus (29.7%), calcium (24.6%), manganese (23.7%), and copper (21.4%). Whereas GCV recorded values for magnesium, phosphorus, and calcium of 34.4%, 25.8% and 22.3%, respectively. The broad sense heritability (H^2) ranged from 9.5 to 89.3% with magnesium having the highest percentage (89.3%), followed by calcium (82.5%), phosphorus (75.8%), and boron (73.1%). For genetic advance (GA) magnesium recorded 66.9 followed by calcium (41.6), and phosphorus (41.3).

Table 4.4: Estimates of genetic parameters for mineral nutrient of *Corchorus* accessions

Source of variance	df	P	K	Ca	Mg	Fe	B	Zn	Cu	Mn
Season	1	137682**	1834233**	294429*	48793**	8.3^{ns}	2.0564*	2.6002*	0.27885**	8.5680**
Replication	2	19810	116868	380927	1444	2622.6	0.4978	1.4765	0.03339	1.6128
Accessions	10	58132**	169169*	629352**	34137**	2008.3**	1.8790**	1.2099**	0.04470*	5.1552**
S*A	10	14081^{ns}	132919*	112957^{ns}	3655*	1818.3*	0.5126*	0.6343*	0.03662*	2.8034**
Residual	42	9821	48821	75718	1239	502.6	0.2020	0.2094	0.01549	0.3600
CV (%)		17.2	10.6	12.1	9.8	40.6	6.9	17.1	17.8	8.8
GM		575	2093	2278	359	55	6.5	2.679	0.7	6.849
δ^2g		22026	18125	258197.5	15241	95	0.68	0.29	0.00404	1.2
δ^2p		29066	84584.5	314679	17069	1004.2	0.93	0.60	0.02235	2.7
GCV		25.8	6.4	22.3	34.4	17.6	12.7	20.0	9.1	16.1
PCV		29.7	13.9	24.6	36.4	57.4	14.8	28.7	21.4	23.7
H^2		75.8	21.4	82.1	89.3	9.5	73.1	48.3	18.1	46.2
GA		41.3	6.13	41.6	66.9	11.2	22.4	28.6	8.0	22.5

*CV-coefficient of variation; S*A-season by accessions; GM-grand mean; δ^2g-genotypic variance; δ^2p-phenotypic variance; GCV-genotypic coefficient of variation; PCV-phenotypic coefficient of variation; H^2 –broad sense heritability; GA-genetic advance; *-significant; ** -highly significant; ns- not significant; Ca-calcium; Mg-magnesium; Mn-manganese; Fe-iron; Zn-zinc; B-boron; Cu-copper; P-phosphorus; K-potassium.

4.8 Discussion

The analysis of variance exhibited significant differences ($p<0.05$) among the *Corchorus* accessions studied with regard to mineral composition. Ndlovu and Afolyan (2008); Choudhary et al. (2013); and Acho et al. (2014) similarly reported the significant differences in nutrient composition in *Corchorus* species.

The average nutrient composition for accessions were 575, 2093, 2278, 358.6, 55.3, 2.7, 0.7, and 6.8 mg/100g for P, K, Ca, Mg, Fe, B, Zn, Cu, and Mn, respectively. Calcium was the major mineral present in *Corchorus* accessions followed by potassium and phosphorus. These results suggest higher amounts of Ca, K, P, Fe, and Zn in *Corchorus* accessions than previously reported in studies by Frison et al. (2006) and Acho et al. (2014). The accessions AV002, AV004, and AV005 showed high levels of Ca of 2591, 2590, and 2632 mg/100g, respectively. Acho et al. (2014) reported lower calcium content of 1159.1 mg/100g in *Corchorus olitorius*. Calcium, together with phosphorus are associated with growth and maintenance of bones, teeth and muscles in humans (Turan et al. 2003).

Anaemia is a worldwide problem which is mostly the outcome of iron deficiency. Iron is an essential component of body systems involved in the utilization of oxygen. In children iron deficiency results in poor physical and mental development (Arivalagan et al. 2013; Abbaspour et al. 2014). Zinc is also a very important mineral during the period of rapid growth and development (Frassinetti et al. 2006). Accessions that showed high levels of Fe and Zn were SA001, SA002, and SA004. Accessions SA004 had zinc content of 3.4 mg/100g comparable to that of leaves of *Amaranthus hybridus* of 3.8 mg/100g reported by Akubugwo et al. (2007). However, iron content values for all the accessions in this present study were noticeably higher than those reported by Van Jaarsveld et al. (2014) in *Corchorus* species, Antial et al. (2006) in *Ipomoea batatas* leaves, and Iqbal et al. (2006) in *Vigna unguiculata*. High levels of iron in *Corchorus* can be beneficial for the prevention of anaemia (Choudhary et al. 2013). The increase in utilisation of *Corchorus* species can play a huge role in addressing the micronutrient deficiencies in the country and ultimately contribute towards the fight against hidden hunger.

The correlation matrix revealed positive significant ($p>0.05$) correlation between P and Mg, K and B, Ca and Mn, and B and Cu. However, a negative and highly significant correlation was observed between magnesium and Iron (r^2-=0.79), meaning that as magnesium increase, iron decreases and vice versa. These results are in contrast to findings by Kehinde et al. (2015) who found a strong significant and positive correlation between Mg and Fe ($r^2 = 0.97$) in bush okra. Negative relationships among nutrients impose a challenge to breeders when trying to improve a particular nutrient in species without taking into account the associated effect on other nutrients (Burlingame et al. 2009).

The patterns of diversity were determined using multivariate analysis among accessions. The first two principal components accounted for 70.93% of the total variance with PC1 accounting for 43.98% and PC2 for 26.95% of variation. In the first principal component, K, Ca, Mg, P, Fe, Mn, B, and Cu contributed most to variability. These results are similar to the findings by Arivalagan et al. (2013) where the first two PCs explained 63.17% of variation with PC 1 accounting for 42.27% where P, Mg, and Cu contributed the most to variability in *Solanum melongena*.

The high levels of similarity were observed in cluster II with 8 of the accessions grouped together. Accessions AV006 and SA002 were placed separately in a different cluster and are considered to be more diverse, hence they can be used as parental lines in further breeding programs. Accessions placed separately in a different cluster are considered superior and diverse to other accessions (Choudhary et al. 2013).

Genetic parameters were estimated and showed considerable amounts of genetic variability in mineral nutrient content among the tested accessions. When heritability estimate of a particular trait is high but having low genetic advance that trait is considered governed by a non-additive gene, whereas if heritability and genetic advance are high a trait is governed by an additive gene action, hence substantial gain can be achieved through selection (Mohsin et al. 2009; Khan et al. 2015). This present study revealed high heritability and genetic advance in Ca (82.1% and 41.6), Mg (89.3% and 66.9), P (75.8% and 41.3), and B (73.3% and 22.4), respectively. This suggests the effect of an additive gene in the inheritance of these traits. The nutritional variation among genotypes suggests a wide range of diversity in the accessions with regards to nutrient levels and offers potential genetic material to improve the nutrient composition through breeding (Burlingame et al. 2009).

4.9 Conclusion

There was a wide variation in mineral composition among accessions in the present study. The vegetable exhibited high mineral nutrient content and accessions SA002, AV001, AV004, and AV006 were identified as good source of all minerals studied, hence could become parental lines in further improvement of the germplasm.

CHAPTER 5
General discussion and recommendations

5.1 General discussion

Limited information currently exists on the levels and patterns of genetic diversity, as well as utilisation of *Corchorus* species in South Africa. Hence, *Corchorus* accessions obtained from the ARC and WVC were characterised using agro-morphological and nutritional traits so as to identify suitable lines to be utilised further in breeding programs for developing high yielding and nutrient rich varieties of *Corchorus*.

Morphological characters were highly variable in qualitative traits including leaf colour, leaf shape, stem colour, pod and seed colour and these traits could be used to distinctively identify cultivars in a breeding programme. There were significant differences among the accessions in quantitative traits such as plant height, canopy width, leaf length, leaf width, leaf length-leaf with ratio, petiole length, stem and pod diameter, pod length, number of leaves, branches and pods per plant, number of seeds per pod, and fresh and dry mass which suggests a wide range of diversity in the accessions and offers potential genetic material to improve *Corchorus*. Accessions from the WVC flowered later than the ARC accessions and produced high number of leaves and these are the important traits in ILVs and should be considered during selection.

The principal component analysis identified days to 50% flowering, plant height, canopy width, leaf length-leaf width ratio, number of branches, stem diameter, pod diameter, and number of pods as the main contributors to morphological variation in the germplasm. Likewise mineral magnesium, manganese, calcium, boron, and copper were the main contributors to variation in mineral nutrient content. There was a high level of dissimilarity observed among the accessions in both morphological and nutritional traits which shows the potential for genetic improvement of the crop through selection. However, high level of similarity was observed between accessions from the same research centre for most of the attributes. Majority of WVC accessions were grouped in one cluster in both morphological and nutritional traits.

High heritability estimates and genetic advance were observed in plant height, number of leaves per plant, pod length, number of pods per plant, number of seed per pod, and mineral calcium, magnesium, and phosphorus suggesting the effect of an additive gene in the inheritance of these traits.

The study identified five accessions namely, SA001, SA002, AV004, AV006, and AV007 that were unique and superior from the rest of the accessions for desirable traits such as days to 50% flowering, number of leaves per plant, leaf length-leaf width ratio, number of pods per plant, and number of seeds per pod. Accessions SA002, AV001, AV004, and AV006 were identified as accessions with good mineral composition, especially in Ca, Fe, Mg and Zn. An increased utilisation of *Corchorus* could help reduce micronutrient deficiency prevalent in South Africa.

5.2 Recommendations

This experiment should be conducted under diverse environmental conditions, across seasons for further confirmation of observed results in the current study. Molecular characterization of *Corchorus* accessions is also recommended to support this present work by classifying differences that are not due to environmental conditions. *Corchorus* accessions with lowest genetic similarity and traits of interest can be selected and used in crop improvement for higher yield and superior nutrient content varieties. The establishment of a breeding programme for *Corchorus* at ARC-VOP is also recommended so that the morphological characterization is moved to the actual breeding process.

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APPENDICES

Appendix 1: Analysis of variance (ANOVA) tables for the morphological and nutritional traits

1.1: ANOVA table for days to 50% flowering

Source of variation	d.f.	s.s.	m.s.	v.r.	P value
Rep	2	31.18	15.59	1.26	
Season	1	9465.15	946.52	76.62	<.001**
Accessions	10	117.33	117.33	9.50	0.004*
A.S	10	53.33	5.33	0.43	0.923^{ns}
Residual	42	518.82	12.35		
Total	65	10185.82			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.2: ANOVA table for plant height (cm)

Source of variation	d.f.	s.s.	m.s.	v.r.	P value
Rep	2	75.307	37.653	7.18	
Season	1	25750.445	25750.445	4907.26	<.001**
Accessions	10	114944.677	11494.468	2190.50	<.001**
A.S	10	12464.580	1246.458	237.54	<.001**
Residual	42	220.391	5.247		
Total	65	153455.400			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.3: ANOVA table for canopy width (cm)

Source of variation	d.f.	s.s.	m.s.	v.r.	P value
Rep	2	28.361	14.180	2.58	
Season	1	626.842	626.842	114.05	<.001**
Accessions	10	10043.610	1004.361	182.74	<.001**
A.S	10	4794.130	479.413	87.23	<.001**
Residual	42	230.833	5.496		
Total	65	15723.776			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.4: ANOVA table for leaf length (cm)

Source of variation	d.f.	s.s.	m.s.	v.r.	P value
Rep	2	2.1561	1.0781	6.93	
Season	1	7.1610	7.1610	46.00	<.001
Accessions	10	36.7069	3.6707	23.58	<.001
A.S	10	9.9828	0.9983	6.41	<.001
Residual	42	6.5377	0.1557		
Total	65	62.5446			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.5: ANOVA table for leaf width (cm)

Source of variation	d.f.	s.s.	m.s.	v.r.	P value
Rep	2	0.38088	0.19044	1.93	
Season	1	4.21549	4.21549	42.81	<.001
Accessions	10	70.63678	7.06368	71.73	<.001
A.S	10	6.30718	0.63072	6.41	<.001
Residual	42	4.13572	0.09847		
Total	65				

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.6: ANOVA table for leaf length-leaf width ratio

Source of variation	d.f.	s.s.	m.s.	v.r.	P value
Rep	2	0.09092	0.04546	0.75	
Season	1	2.78186	2.78186	46.02	<.001
Accessions	10	46.17076	4.61708	76.38	<.001
A.S	10	10.36823	1.03682	17.15	<.001
Residual	42	2.53888	0.06045		
Total	65	61.95064			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.7: ANOVA table for stem diameter (mm)

Source of variation	d.f.	s.s.	m.s.	v.r.	P value
Rep	2	18.3322	9.1661	15.32	
Season	1	141.2995	141.2995	236.16	<.001
Accessions	10	963.5516	96.3552	161.04	<.001
A.S	10	154.1513	15.4151	25.76	<.001
Residual	42	25.1297	0.5983		
Total	65	1302.4643			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.8: ANOVA table for pod length (cm)

Source of variation	d.f.	s.s.	m.s.	v.r.	P value
Rep	2	0.3850	0.1925	0.96	
Season	1	3.3233	3.3233	16.54	<.001
Accessions	10	214.4157	21.4416	106.73	<.001
A.S	10	6.3714	0.6371	3.17	<.001
Residual	42	8.4378	0.2009		
Total	65	232.9331			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.9: ANOVA table for pod diameter (mm)

Source of variation	d.f.	s.s.	m.s.	v.r.	P value
Rep	2	1.80808	0.90404	9.31	
Season	1	6.57407	6.57407	67.68	<.001
Accessions	10	250.56253	25.05625	257.96	<.001
A.S	10	4.94104	0.49410	5.09	<.001
Residual	42	4.07958	0.09713		
Total	65	267.96532			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.10: ANOVA table for number of leaves per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	P value
Rep	2	586.28	293.14	7.95	
Season	1	26008.26	26008.26	705.42	<.001
Accessions	10	104046.92	10404.69	282.21	<.001
A.S	10	26297.39	2629.74	71.33	<.001
Residual	42	1548.51	36.87		
Total	65	158487.36			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.11: ANOVA table for number of branches per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	P value
Rep	2	20.1296	10.0648	10.55	
Season	1	423.5733	423.5733	444.05	<.001
Accessions	10	1549.1596	154.9160	162.41	<.001
A.S	10	443.6873	44.3687	46.51	<.001
Residual	42	40.0630	0.9539		
Total	65	2476.6129			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.12: ANOVA table for number of pods per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	P value
Rep	2	49.55	24.77	1.44	
Season	1	8033.37	8033.37	465.95	<.001
Accessions	10	403572.51	40357.25	2340.80	<.001
A.S	10	73484.99	7348.50	426.23	<.001
Residual	42	724.11	17.24		
Total	65	485864.53			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.13: ANOVA table for number of seeds per pod

Source of variation	d.f.	s.s.	m.s.	v.r.	P value
Rep	2	596.4	298.2	2.81	
Season	1	27585.0	27585.0	260.02	<.001
Accessions	10	139177.8	13917.8	131.19	<.001
A.S	10	16165.2	1616.5	15.24	<.001
Residual	42	4455.7	106.1		
Total	65	187980.1			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.14: ANOVA table for fresh mass (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	P value
Rep	2	3.8029	1.9014	2.31	
Season	1	1102.0105	1102.0105	1339.73	<.001
Accessions	10	1486.2307	148.6231	180.68	<.001
A.S	10	693.2775	69.3277	84.28	<.001
Residual	42	34.5476	0.8226		
Total	65	3319.8692			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.15: ANOVA table for dry mass (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	P value
Rep	2	2.4578	1.2289	5.06	
Season	1	78.4800	78.4800	323.44	<.001
Accessions	10	66.6502	6.6650	27.47	<.001
A.S	10	18.4645	1.8465	7.61	<.001
Residual	42	10.1910	0.2426		
Total	65	176.2436			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.16: ANOVA table for potassium (K)

Source of variation	d.f.	s.s	ms	v.r	P value
Rep	2	233736	116868	2.39	
Season	1	1834233	1834233	37.57	<.001**
Accessions	10	1691688	169169	3.47	0.002*
A.S	10	1329190	132919	2.72	0.011*
Residual	42	2050499	48821		
Total	65	7139345			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.17: ANOVA table for calcium (Ca)

Source of variation	d.f.	s.s	ms	v.r	P value
Rep	2	761853	380927	5.03	
Season	1	294429	294429	3.89	0.055*
Accessions	10	6293516	629352	8.31	<.001**
A.S	10	1129568	112957	1.49	0.176^{ns}
Residual	42	3180155	75718		
Total	65	11659521			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.18: ANOVA table for magnesium (Mg)

Source of variation	d.f.	s.s	ms	v.r	P value
Rep	2	2888	1444	1.17	
Season	1	48793	48793	39.38	<.001**
Accessions	10	341369	34137	27.55	<.001**
A.S	10	36546	3655	2.95	0.007*
Residual	42	52038	1239		
Total	65	52038			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.19: ANOVA table for phosphorus (P)

Source of variation	d.f.	s.s	ms	v.r	P value
Rep	2	39620	19810	2.02	
Season	1	137682	137682	2.02	<.001**
Accessions	10	581320	58132	5.92	<.001**
A.S	10	140809	14081	1.43	0.199^{ns}
Residual	42	412498	9821		
Total	65	1311929			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.20: ANOVA table for iron (Fe)

Source of variation	d.f.	s.s	ms	v.r	P value
Rep	2	5245.3	2622.6	5.22	
Season	1	8.3	8.3	0.02	0.898^{ns}
Accessions	10	20082.6	2008.3	4.00	<.001
A.S	10	18182.6	1818.3	3.62	0.002
Residual	42	21110.5	502.6		
Total	65	64629.3			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.21: ANOVA table for manganese (Mn)

Source of variation	d.f.	s.s	ms	v.r	P value
Rep	2	3.2257	1.6128	4.48	
Season	1	8.5680	8.5680	23.80	<.001**
Accessions	10	51.5517	5.1552	14.32	<.001**
A.S	10	28.0336	2.8034	7.79	<.001**
Residual	42	15.1194	0.3600		
Total	65	106.4984			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.22: ANOVA table for zinc (Zn)

Source of variation	d.f.	s.s	ms	v.r	P value
Rep	2	2.9530	1.4765	7.05	
Season	1	2.6002	2.6002	12.41	0.001*
Accessions	10	6.3432	1.2099	3.03	<.001**
A.S	10	12.0991	0.6343	5.78	0.006
Residual	42	8.7965	0.2094		
Total	65	32.7919			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.23: ANOVA table for boron (B)

Source of variation	d.f.	s.s	ms	v.r	P value
Rep	2	0.9956	0.4978	2.46	
Season	1	2.0564	2.0564	10.18	0.003*
Accessions	10	18.7898	1.8790	9.30	<.001**
A.S	10	5.1256	0.5126	2.54	0.017*
Residual	42	8.4854	0.2020		
Total	65	35.4529			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value

Appendix 1.24: ANOVA table for copper (Cu)

Source of variation	d.f.	s.s	ms	v.r	P value
Rep	2	0.06678	0.03339	2.16	
Season	1	0.27885	0.27885	18.00	<.001**
Accessions	10	0.44701	0.04470	2.89	0.008*
A.S	10	0.36620	0.03662	2.36	0.025*
Residual	42	0.65062	0.01549		
Total	65	1.80946			

*** =** significant; **** =** highly significant; ns = not significant; P value- probability value