UNIVERSITY OF ZULULAND

Agro-morphological, nutritional variability and heritability studies of *Lagenaria siceraria* landraces in northern KwaZulu-Natal, South Africa

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DECLARATION

The research described in this thesis was carried out in the Department of Botany at the University of Zululand, KwaDlangezwa, under the supervision of Dr. N.R. Ntuli and Dr. S. Mavengahama. These studies have not otherwise been submitted in any form for any degree or diploma at any University. Where use has been made of work of others, it is duly acknowledged in the text.

Lungelo Given Buthelezi

I certify that the above statement is correct.

Dr N.R. Ntuli

ABSTRACT

Lagenaria siceraria (Molina) Standley of the Cucurbitaceae family is one of many underutilised ancient cultigens with great economic potential. Its nutritious tender shoots, flowers, fruits, and seeds are of culinary use and widely consumed in rural communities as a vegetable relish. It has abundant nutrients and minerals essential for human health. However, research on morphological and nutritional variation among *L. siceraria* landraces from South Africa is very marginal. Therefore, the objective of this study was to characterise variability in growth, yield and nutritional composition among *L. siceraria* landraces from northern KwaZulu-Natal. *L. siceraria* landraces with various fruit and seed morphology, collected from different agro-ecological areas of northern KwaZulu-Natal were grown in a randomised complete block design with three replications over two summer seasons. Seedling, vegetative and reproductive traits were compared among landraces, while pulp of the mature fruit was analysed for nutrient composition. Analysis of variance, correlation, principal component analysis, cluster analysis and heritability estimates were conducted on morphological traits and nutrient content. Landraces with different fruit and seed morphology, from different areas varied significantly in seedling, vegetative and reproductive traits as well as nutrient content. Significant positive correlations were mainly recorded among reproductive traits and also among the majority of nutrients. The first five and three informative principal components were responsible for 74.393% and 80.270% of the total variability in morphological traits and nutrient composition, respectively. First components (PC1) with 26.635% and 42.076% variability were positively associated with most of morphological traits and nutrients, respectively. In morphological trait and nutrient analyses, biplot and dendrogram grouped landraces mainly according to fruit and seed morphology and then their origin. High heritability estimates were recorded among fruit and seed traits as well as among various nutrients. Therefore, this study can be the foundation for strategic improvement, direct production or conservation of the *Lagenaria siceraria* using these landraces.

CONFERENCE PRESENTATIONS

Buthelezi LG, Mavengahama S and Ntuli NR. 2018. Morpho-agronomic characterization of *Lagenaria siceraria* landraces from northern KwaZulu-Natal, South Africa. South African Association of Botanists, Hatfield campus, University of Pretoria, 09 - 12 January 2018.

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DEDICATION

I dedicate this dissertation to my mother, Thabisile Zondi, for the moral support, her prayers, her belief in me and words of encouragement when obstacles seemed greater than my abilities. To my mentor and father, Sandile Mathews Buthelezi, for being a constant reminder that anything is possible in life, if you believe in yourself first. To my son, Alwande Mnotho Buthelezi, for being my ultimate motivation throughout my studies and for understanding that I must be away from home most of the time for a better future. To my supervisor, Dr N.R Ntuli, for being an endless source of inspiration throughout this study.

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ABBREVIATIONS

%:	Percentage
°:	Degrees
°C:	Degrees Celsius
100SM:	100 seed mass
AAS:	Atomic absorption spectroscopy
ADF:	Acid detergent fibre
Al:	Aluminium
ANOVA:	Analysis of variance
AOAC:	Association of official analytical chemists
B:	Boron
b:	Breath
Ca:	Calcium
CLA:	Cotyledonous leaf area
cm:	Centimetres
Cu:	Copper
CV:	Coefficient of variation
DAE:	Days after emergence
DAP:	Days after planting
DAS:	Days after sowing
DAT:	Days after transplant
DMSO:	Dimethylsulfoxide
DSI:	Dundee smooth isodiametric shaped
DSM:	Dry shoot mass
E:	East
ECV:	Environmental coefficient of variation
Fe:	Iron
FL:	Fruit length
FM:	Fruit mass
FRT:	Fruit rind thickness

FSM:	Fresh shoot mass
FSR:	Flower sex ratio
FW:	Fruit width
FY/P:	Fruit yield per plot
g L^{-1}:	Grams per litre
g:	Grams
GA:	Genetic advance
GCV:	Genotypic coefficient of variation
GM:	Grand mean
GP:	Germination percentage
H^2:	Broad sense heritability estimate
ha:	Hectares
HCl:	Hydrochloric acid
K:	Potassium
kg/ha:	Kilograms per hectare
kg:	Kilograms
kg^{-1}:	Per kilogram
KRI:	Khangelani rough isodiametric shaped
KSC:	Khangelani smooth-curvilinear shaped
KSP:	Khangelani smooth-pear shaped
KZN:	KwaZulu-Natal
LAN:	Limestone Ammonium Nitrate
LG:	Leaf growth
LR:	Landraces
LSD:	Least Significant Difference
m:	Meters
M:	Moles
mg cm^{-2}:	Milligrams per square centimetre
mg kg^{-1}:	Milligrams per kilogram
mg/100g:	Milligrams per hundred grams
mg:	Milligrams

Mg:	Magnesium
mL:	Millilitres
mm:	Millimetres
mm^2:	Square millimetres
Mn:	Manganese
MSC:	Mbazwana smooth-curvilinear shaped
N:	Nitrogen
Na:	Sodium
NDF:	Neutral detergent fibre
NF/P:	Number of fruits per plot
nm:	Nanometres
NOB:	Number of branches
NOL:	Number of leaves
NPF/p:	Number of pistillate flowers per plot
NPK:	Nitrogen, Phosphorus and Potassium
NqRC:	Nquthu rough curvilinear shaped
NqSC:	Nquthu smooth semi-curvilinear shaped
NRB:	Ndumo rough rind with brown seeds
NRC:	Ndumo rough rind with creamy seeds
NSC:	Nquthu smooth-curvilinear shaped
NSF/p:	Number of staminate flowers per plot
NSRC:	Nquthu semi-rough curvilinear shaped
NSRP:	Nquthu semi-rough pear shaped
P value:	Probability value
P:	Phosphorus
PC:	Principal Component
PCA:	Principal Component Analysis
PCV:	Phenotypic coefficient of variation
PL:	Pedicel length
Pro:	Protein
RCBD:	Randomised Complete Block Design

RRP:	Rorke's Drift rough-pear shaped
RSP:	Rorke's Drift smooth-pear shaped
S:	South
SG:	Shoot growth
SH:	Seedling height
SL:	Seed length
SMC:	Shoot moisture content
SW:	Seed width
t/ha:	Tonnes per hectare
TBL:	Tendril basal length
TCC:	Total chlorophyll content
TLL:	Tendril longer lobe length
TSHL:	Tendril shorter lobe length
TSM:	Total seed mass
V_A:	Additive variance
V_D:	Dominance variance
V_E:	Environmental variance
V_G:	Total genetic variance
V_{GE}:	Genetic and environmental interaction variance
V_I:	Interaction
VL:	Vine length
V_P:	Total phenotypic variance
w:	Width
Zn:	Zinc
$\delta^2 g$:	Genotypic variance
$\delta^2 p$:	Phenotypic variance

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Chapter 1

1. Introduction

Lagenaria siceraria (Molina) Standley is commonly known as the bottle gourd and identified as the white-flowered gourd belonging to the Cucurbitaceae family (Jain *et al.* 2017). It is an annual herbaceous and monoecious plant with a prostrate growing habit (Panigrahi *et al.* 2018; Guran *et al.* 2015). It has tender fleshy fruits at juvenile stage and hardens with maturity; with the seeds embedded within the fruit pulp (Xaba and Croeser 2011).

L. siceraria has promising economic potential as it is used for various purposes, such as for food, medicine, decoration and for household implements (Mashilo *et al.* 2016). In many parts of South Africa the very young tender fruits of the bottle gourd are sliced and cooked as vegetables and the vining shoots are cooked like spinach (Xaba and Croeser 2011). The leaves are consumed as a vegetable condiment and added fresh to maize porridge as a relish (Jain *et al.* 2017). In West Africa the mature seeds of the bottle gourd are roasted and crushed to a paste used to thicken stews (Chimonyo and Modi 2013). In Botswana, Zimbabwe and South Africa the oil extracts from mature bottle gourd seeds are an ideal alternative for vegetable oil (Chimonyo and Modi 2013).

Developing countries like South Africa, suffering from inadequate food supply and malnutrition, especially in the rural areas, could benefit greatly from *L. siceraria* as it is a good source of protein and energy supplements which can contain up to 45% of essential oil and 35% of protein (Chimonyo and Modi 2013). It is also a source of carbohydrates, minerals and soluble dietary fibre (Milind and Satbir 2011; Panigrahi *et al.* 2018). *L. siceraria* possesses high choline content, which is a micronutrient essential for maintaining vital human organs such as the liver and normal brain development (Rahman 2003; Patel *et al.* 2017).

L. siceraria is found virtually throughout the African continent and is very popular in West African countries such as Nigeria and Côte d'Ivoire (Koffi *et al.* 2009). The *L. siceraria* is also produced in large quantities in Asian countries such as India, Sri Lanka, Indonesia, and Malaysia (Barot *et al.* 2015). It is also popular in Southern African countries such as South Africa, Namibia, Zimbabwe, Botswana and Swaziland (Chimonyo and Modi 2013). It is hardly found in the wild unless it has escaped from the cultivation fields (Chimonyo and Modi 2013; Xaba and Croeser 2011). In South Africa the bottle gourd populations are concentrated mostly in Limpopo, Northern Cape, Gauteng and KwaZulu-Natal (Xaba and Croeser 2011). *L. siceraria* is largely used for decorative purposes, and for making utensils but it has been replaced by exotic species as a food source, overlooked and underutilised by communities due to their lack of knowledge about its economic and nutritional importance (Sithole *et al.* 2015).

Geographic origins are responsible for great variability in terms of morphological characterisation and nutritional elements, thus different landraces of the *L. siceraria* from different origins distinctively vary in the nutritional content of essential elements (Sithole *et al.* 2015). The morphological diversity of *L. siceraria* landraces is affected by the genotypic variation among landraces and the area of origin (Mlandenovic *et al.* 2012; Sharma and Sengupta 2013; Panigrahi *et al.* 2018).

Phenotypic descriptors are characteristics of a plant species used to differentiate the morphological structures of plants within the same or similar family, such as: vine length, leaf size, chlorophyll content, fruit mass, fruit shape, fruit size, seed size, seed shape and seed texture (Mlandenovic *et al.* 2012; Koffi *et al.* 2009). The phenotypic descriptors traditionally have proven to be a useful tool in determining variation among the *L. siceraria* landraces (Koffi *et al.* 2009), but molecular markers are a more accurate and complementary tool to phenotypic descriptors (Mashilo *et al.* 2016).

1.1 Problem statement

Lagenaria siceraria plays an important role as a leafy vegetable in northern KwaZulu-Natal. It is also used to make household implements and decorative articles for rural communities. Although some research has been conducted on the medicinal aspects of the *L. siceraria*, it is still very much under-researched. It is also considered as a poor man's crop and is underutilised despite its impressive nutritional composition and potential as a food source in developing countries like South Africa where protein and energy malnutrition are still a problem in the rural communities. *L. siceraria* could play an important role in supplying essential nutrients lacking in the diets of most rural communities. One of the important steps in promoting the use of *L. siceraria* is to understand the extent of its genetic and nutritional diversity, as well as the heritability of important morphological and nutritional traits.

An understanding of genetic diversity and heritability of traits will enable the pre-breeding of *Lagenaria* to identify desirable traits which can be used in the selection of breeding lines to be improved for yield and nutritional composition. Presently, little is known about the genetic variability of *L. siceraria* in South Africa in general and KZN in particular. Most of the planting materials used by the farmers are uncharacterised landraces with no pedigree and uniformity. Therefore, there is the possibility of characterising these landraces according to the similarities in their morphological and nutritional traits as well as identifying landraces with desirable traits for possible genetic improvement and direct production.

1.2 Aim of the study

The aim of the study was to determine diversity in agro-morphological traits, nutritional composition, genetic advance and heritability among *Lagenaria siceraria* landraces from northern KwaZulu-Natal.

1.3 Research questions

The research questions of the study were as follows:

➢ Do *L. siceraria* landraces from northern KwaZulu-Natal vary in their agro-morphological traits, estimated genetic advance and heritability?

➢ Is there any variation in nutritional composition, genetic advance and heritability among *L. siceraria* landraces?

➢ Do *L. siceraria* landraces differ in estimated genetic advance and heritability of their agro-morphological traits and mineral content?

1.4 Objectives

The objectives were to:

➢ Characterise landraces of *L. siceraria* from northern KwaZulu-Natal using morphological finger prints.

➢ Analyse nutritional variability of *L. siceraria* landraces.

➢ Estimate genetic advance and heritability of agro-morphological traits and mineral content among *L. siceraria* landraces.

1.5 Research hypotheses

H_0 = *L. siceraria* landraces from northern KwaZulu-Natal do not vary in their agro-morphological traits.

H_1 = Wide variation exists in agro-morphological traits of *L. siceraria* landraces from northern KwaZulu-Natal.

H_0 = Fruit nutritional and mineral content does not differ among *L. siceraria* landraces.

H_1 = *L. siceraria* landraces differ in their fruit nutritional and mineral content.

H_0 = Estimated genetic advance and heritability of agro-morphological traits and mineral content do not differ among *L. siceraria* landraces.

H_1 = Estimated genetic advance and heritability of agro-morphological traits and mineral content differ among *L. siceraria* landraces.

1.6 Structure of the dissertation

Chapter 1 focuses on the general introduction, problem statement, general aim of the study, research questions, objectives and hypotheses. **Chapter 2** has a literature review on the variation among morphological traits and nutritional content of different *Lagenaria siceraria* landraces and other related species within the Cucurbitaceae family. Chapters three and four have their own introduction, materials and methods, results, discussion and conclusion.

Chapter 3 investigates variability in seedling, vegetative and reproductive traits among *L. siceraria* landraces with various fruit and seed morphology sourced from different origins. Fruit pulp of these landraces was further analysed for nutritional composition in **Chapter 4. Chapter 5** presents conclusions and recommendations on variation in seedling, vegetative and reproductive traits as well as nutrient content among *L. siceraria* landraces.

Chapter 2

2. Literature review

2.1 Taxonomy of *L. siceraria*

Lagenaria siceraria (Molina) Standley is a member of the Cucurbitaceae family easily distinguished by its white flowers and great variations in fruit and seed morphology (Kalyanrao *et al.* 2016; Mashilo *et al.* 2017). *L. siceraria* is variously known as the bottle gourd, calabash gourd, kalbas, moraka, segwana and iselwa across different racial groups and tribal communities in South Africa (van Wyk and Gericke 2000). It is an annual monoecious and herbaceous crop with a prostrate growth habit (Morimoto *et al.* 2005). It has large cordate-shaped leaves with five lobes that alternate in orientation and has fine pubescent hairs on both leaf surfaces (Badmanaban *et al.* 2009). *L. siceraria* vines have bifurcate tendrils almost always present on every node (Gorasiya *et al.* 2011). It is a diploid species with a total of 22 chromosomes (Guran *et al.* 2015; Mashilo *et al.* 2017).

The genus *Lagenaria* has five other wild species: *L. abyssinica* (Hook f.) Jeffrey; *L. breviflora* (Benth.) Roberty; *L. guineensis* (G. Don) Jeffrey; *L. rufa* (Gilg.) Jeffrey and *L. sphaerica* (Sonder) Naudin (Mashilo *et al.* 2017). Of all the six species *L. siceraria* is the only one cultivated (Morimoto *et al.* 2005; Mashilo *et al.* 2017). It is found throughout the African continent and Asia, where its genetic variation originated (Yetisir *et al.* 2008; Jha *et al.* 2017). Archaeologists proclaim that it has been utilised by mankind for at least 12 000 years (Yetisir *et al.* 2008).

2.2 Uses of *L. siceraria*

L. siceraria is used as a vegetable where the leaves, young shoots, and flowers are consumed as spinach and may be added to maize porridge as a relish (Chimonyo and Modi 2013; Sithole *et al.* 2016). Its seeds are roasted and ground to powder to thicken

stew as a condiment, and the seed kernel is used by the livestock feed industry as it is very rich in oils and proteins (Sithole *et al.* 2016; Mashilo *et al.* 2017).

Leaves and fruit pulp are used as a laxative or mixed with sugar to cure jaundice (Rahman *et al.* 2008). Fruit pulp also treats mental health disorders, cures stomach-aches and stimulates proper functioning of the liver (Rahman 2003; Mashilo *et al.* 2017), as well as treating night blindness related illness (Harika *et al.* 2012). The fruit pulp is used as an antidote against certain poisons and may be externally applied as a bandage for cooling fevers or high body temperatures (Harika *et al.* 2012). The fruit pulp also possesses cardioprotective and anticancer properties (Barot *et al.* 2015). Dried fruit rinds are widely used for serving and storing beverages and as musical instruments (Xaba and Croeser 2011; Sithole *et al.* 2016). The *L. siceraria* is also used as a rootstock for *Citrullus lanatus* (watermelon) against soil-borne diseases and the effect of low soil temperature (Chimonyo and Modi 2013; Mashilo *et al.* 2017).

2.3 Landraces

A landrace is a crop that is traditionally domesticated and given a name where it locally originates or predominates (Mlandenovic *et al.* 2012). There are two types of landraces, namely, primary and secondary landraces. A primary landrace is a crop that has developed desired traits through repeated propagation over several generations by informal maintainers (Zeven 1998; Cardoso and Maxted 2014). It is also subdivided into autochthonous and allochthonous primary landraces. An autochthonous landrace is a crop that develops traits in a specific region and is maintained in that region through the grower's selection. An allochthonous landrace is a crop that develops desired adaptive traits from a different region than its origin (Cardoso and Maxted 2014).

A secondary landrace is a crop that has been developed in the modern sector for commercial purposes and seed saving, and which may be genetically different from the original bred material (Zeven 1998).

2.4 Variation among *L. siceraria* landraces

The *L. siceraria* landraces have a broad variation on their fruit and seed traits, so distinctly that they can be easily identified by these traits (Chimonyo and Modi 2013; Muralidharan *et al.* 2017). Traits are morphological characteristics in which a landrace may show variations from or similarities to other landraces (Koffi *et al.* 2009). Variation in *L. siceraria* landraces is morphologically described by seedling, vegetative and reproductive traits (Uddin *et al.* 2014; Rambabu *et al.* 2017; Rani and Reddy 2017).

2.4.1 Seedling traits of *L. siceraria*

The germination rate of different *L. siceraria* landraces varies according to fruit shape and seed size. The germination percentage of isodiametric-shaped fruits ranges from 54.50 – 68.33% (Yao *et al.* 2013). Elongated to pear-shaped fruit landraces have a range of 49.00 – 72.50% of germination percentage (Yao *et al.* 2013). Larger seeded landraces possess a superior germination rate with seedlings showing high vigour, thus producing more competitive seedlings than smaller seeded landraces (Chimonyo and Modi 2013). Larger seeds can provide energy for a longer period at a more rapid rate than smaller seeds, hence they produce vigorously growing seedlings (Chimonyo and Modi 2013). The seedling height of *L. siceraria* landraces generally ranges from 22 – 56 mm, with a mean of 39 mm (Yetisir *et al.* 2008). Isodiametric-shaped fruit landraces tend to have tall seedlings ranging from 66.85 – 81.68 mm. The seedling height of elongated to pear-shaped landraces ranges from 57.67 – 80.72 mm (Yao *et al.* 2013).

Cotyledonous leaf area is highly affected by the seed size, where large-seeded landraces produce seedlings with larger cotyledons (Chimonyo and Modi 2013). Cotyledon leaf length ranges from 25.6 – 49.8 mm while cotyledon leaf width ranges from 15.8 – 29.8 mm, and total cotyledonous leaf area ranges from 42.9 – 148.2 mm^2 (Mashilo *et al.* 2016).

2.4.2 Stem and leaf traits variation in *L. siceraria*

As a member of the Cucurbitaceae family, *L. siceraria* displays a great variation in its vegetative structures, ranging from compact, single stem bushes to rambling multi-stem vines (Uddin *et al.* 2014). Larger landraces of *L. siceraria* with less dense leaf foliage have a larger leaf area (97 540 mm^2) and longer vine length (6.8 m) compared to the vastly dense leaf foliage of smaller landraces with a smaller leaf area (76 120 mm^2) and shorter vine length (4.8 m) (Uddin *et al.* 2014).

The number of branches varies significantly across different landraces, ranging from 9.42 – 34.1 per plant (Mashilo *et al.* 2016). Shoot and leaf growth is part of *L. siceraria* vegetative traits that have significant variations. Shoot and leaf growth is highly affected by the orientation and direction in which the plants are facing or are propagated onto the field (Sharma and Tomar 2016). The sun rises from the east and sets in the west, providing congenial growth conditions with maximum solar radiation to the plants (Sharma and Tomar 2016). Landraces propagated from an east to west direction have a greater leaf area of 10425.17 cm^2 compared to the plants propagated from a north to south direction with a leaf area of 8727.33 cm^2 (Sharma and Tomar 2016). The average leaf growth of *L. siceraria* is estimated to be 298.9% over a ten day interval (Sharma and Tomar 2016).

L. siceraria has a high number of leaves, similar to other plant species belonging to the Cucurbitaceae family such as *Citrullus lanatus* (watermelon) which has a mean of 36.33 leaves per plant (Rahaman *et al.* 2018). Landraces with longer vines are less branched and have bigger leaves and thus will have the least number of leaves present per plant compared to the ones with shorter vines, more branches, and smaller leaves, which have a high number of leaves per plant (Koffi *et al.* 2009).

Younger leaves are frequently three-lobed, while the mature leaves have five shallow to deep lobes with narrow to wide sinuses (Carle and Loy 1996). The leaf width of mature *L. siceraria* landraces ranges from 165.3 – 310.5 mm, with the leaf length ranging from

128.1 – 181.2 mm (Mashilo *et al.* 2016). The *L. siceraria* leaf texture also varies from velvety to leathery texture with the presence or absence of fine hairs on the lower surface of leaves (Stephens 1994; Abd-El Maksoud and Nassar 2013).

2.4.3 Total chlorophyll content and tendril morphology variation of the *L. siceraria* landraces

The chlorophyll content of *L. siceraria* landraces is greatly affected by environmental factors such as water availability, soil moisture content, and temperature. (Sithole *et al.* 2015). At leaf juvenile stage the chlorophyll content is higher than at leaf maturity stage (Sithole *et al.* 2015). The total chlorophyll content of *Lagenaria siceraria* landraces varies significantly from 35.6 – 55.6% across different genotypes (Uddin *et al.* 2014). According to Koffi *et al.* (2009), tendril formation and appearance do not differ significantly in landraces with large and small seeds. Both, larger and smaller landraces in terms of fruit sizes have roughly the same tendril formation date, averaging 30.34 days after seedling emergence (DAE) (Koffi *et al.* 2009). A variation in the tendril morphology is not significant as all landraces have bifurcate tendril lobes (Sithole *et al.* 2015). Tendrils are always present in *L. siceraria* landraces and exist in one tendril type which is coiled (Kalyanrao *et al.* 2016).

2.4.4 Fresh and dry mass as well as moisture content of shoots

L. siceraria shoots have a high-water content of 96.1 g/100 g (Husna *et al.* 2014). Shoot fresh and dry mass, as well as moisture content, are largely impacted by environmental factors. Therefore, these would vary from season to season due to permissible growing conditions such as water availability, soil moisture content, and temperature (Mohammed *et al.* 2017). Under optimum water availability at 40 days after planting (40 DAP), *L. siceraria* landraces have a high shoot fresh mass ranging from 15.6 – 17.1 g and shoot dry mass varying from 2.55 – 2.81 g (Sithole *et al.* 2015). In minimal water availability at juvenile stage (40 DAP), *L. siceraria* landraces have a low shoot fresh mass that varies from 6.0 – 9.1 g and shoot dry mass range of 0.94 and 1.36 g (Sithole

et al. 2015). However, at maturity stage (60 DAP), the shoot fresh mass ranges from 333.3 – 374.8 g and the shoot dry mass ranges from 64.1 – 64.7 g, with a shoot moisture content ranging from 80.8 – 82.7% (Mohammed *et al.* 2017).

2.4.5 Staminate and pistillate flowers of *L. siceraria* landraces

L. siceraria landraces are monoecious with both pistillate and staminate flowers occurring on the same plant where staminate and pistillate flowers occur on the longer and shorter pedicels, respectively (Koffi *et al.* 2009). Staminate flowers open first at approximately 45 days after seedling emergence (DAE) and pistillate flowers follow 50 DAE (Uddin *et al.* 2014). Flowering is intensely affected by photoperiodism, where short days with long nights (winter) promote staminate flower formation with less yield and long days with short nights (summer) promote pistillate flower formation with a greater yield (Chimonyo and Modi 2013).

The number of staminate and pistillate flowers per plant vary from 85.1 – 380 and 2 – 16, respectively (Mashilo *et al*. 2016). Like most cucurbits, the sex ratio of *L. siceraria* is high with a ratio of 5.5:1 resulting in higher chances of pollination and fruit formation (Sharma and Tomar 2016). The flower of *L. siceraria* can grow up to 101.6 mm in diameter with their distinct white attractive colour which is mostly pollinated by hawk moths and bees at night and during the early hours of the day (Koffi *et al.* 2009).

2.4.6 Fruit variation of the *L. siceraria* landraces

Fruit colour is a noticeable qualitative trait of the *L. siceraria*, varying from light green to dark green across various landraces (Mashilo *et al*. 2016). Fruit texture also varies from very rough to smooth, with rough fruits being the most common (Sharma and Sengupta 2013). Fruit shapes are used to differentiate between different *L. siceraria* landraces and can variously be elongated, isodiametric, curvilinear, conical pyriform or pear-shaped (Chimonyo and Modi 2013). Amongst fruit shapes, curvilinear-shaped and elongated shaped fruits are the most common types. The elongated curvilinear shaped

fruits are the least common fruit shape of *L. siceraria* landraces (Mlandenovic *et al.* 2011).

Fruit yield is largely affected by the flower sex ratio and environmental factors, where favourable conditions lead to greater fruit yield that ranges from 5.22 – 43.71 t/ha (Deepthi *et al.* 2016). *Lagenaria siceraria* landraces also differ in their fruit mass, which ranges from 1.35 – 5.08 kg (Kalyanrao *et al.* 2016). Different shapes account for a vast variation in fruit length and width (Morimoto *et al.* 2005). Fruit length displays a great variation from 95.1 – 377.9 mm (Mashilo *et al.* 2016). Fruit width also varies from 50 – 400 mm (Koffi *et al.* 2009). Pedicel length ranges from 0.53 – 24.08 cm, while fruit rind thickness ranges from 1.2 – 4.3 mm (Mashilo *et al.* 2016).

2.4.7 Seed variation among *L. siceraria* landraces

Landraces of *L. siceraria* with bigger fruit sizes have larger seeds and landraces with smaller fruit sizes have smaller seeds embedded within the fruit pulp (Mlandenovic *et al.* 2011). Seed morphology varies greatly in size, shape and seed lines as well as the seed coat surface texture (Sivaraj and Pandravada 2005).

Seeds range from flat and irregular to semi-cylindrical and oblong in shape (Chimonyo and Modi 2013). Seed colour varies greatly from light brown to dark brown (Mashilo *et al.* 2016). From 36 landraces, 94.4% of *L. siceraria* landraces have prominent seed lines and 94% have slightly rough seed texture, suggesting that landraces with smooth seed texture have no prominent seed lines (Mashilo *et al.* 2016). In *L. siceraria* landraces seed length varies from 1.13 – 1.88 cm and seed width ranges from 0.5 – 0.67 cm (Mashilo *et al.* 2016). The 100-seed mass from one fruit can vary between 9.80 – 29.84 g; variations are dependent on seed shape and size (Mlandenovic *et al.* 2011).

2.5 The nutritional composition of *L. siceraria*

The *L. siceraria* landraces are one of many essential and native crops to Africa, especially Southern Africa which are underutilised by households despite them probably having been one of the first cultigens domesticated in ancient times (Sithole *et al.* 2015). They are herbaceous with various nutritional mineral elements found in the shoots, leaves, fruits and seeds such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), zinc (Zn), copper (Cu), manganese (Mn) and iron (Fe) which vary across different landraces of the crop (Sithole *et al.* 2015).

The edible fleshy parts and fruit pulp of *L. siceraria* contain carbohydrates, minerals, proteins, soluble dietary fibres and fats which help to maintain and build up the human body (Rahman 2003). The nutritional content of the *L. siceraria* across different landraces recorded high mean values of moisture content 90.25%, fibre content 4.70%, protein (0.62 g/100g), fat (0.02 g/100g) and carbohydrates (3.39/100 g) (Rahman 2003; Ahmed *et al.* 2016). In parts per million (ppm), mineral elements of different *L. siceraria* accessions ranged from Cu (0.56 – 3.50), Mg (3.22 – 6.61), Zn (0.67 – 2.15), Fe (1.33 – 5.02), Na (0.35 – 4.67), K (2.34 – 9.95) and Ca (19.87 – 26.01) (Aliu *et al.* 2012).

2.6 Genetic diversity and heritability of *L. siceraria*

The fruit and seed morphology greatly influence the genetic diversity amongst landraces of the *L. siceraria* (Mlandenovic *et al.* 2012). The genetic diversity of *L. siceraria* landraces of different origins remains unchanged and results in homozygosity which is maintained by inbreeding (Mlandenovic *et al.* 2012). This is, however, a result of human selection for desired traits, which explains the pear-shaped and elongated-shaped landraces such as the African gourd, Japanese siphon, calabash, and Chinese gourd's popularity amongst other landraces since they are more usable and possess the desired fruit shape (Mlandenovic *et al.* 2012).

Genetic identities of landraces regulated by inbreeding from self-pollination show very minute variation compared to those that are exposed to cross-pollination (Mlandenovic *et al.* 2012). Each morphological trait of the *L. siceraria* has a heritability estimate unique to it (Sharma and Sengupta 2013). The estimated value of heritability measures the similarities of the phenotypic and breeding value (Acquaah 2007). Breeding value is the genotypic value of heritable genetic material that can be passed on from one generation to another (Sharma and Sengupta 2013).

The heritability of desirable traits results from gene advancement and the presence of additive genes (Sharma and Sengupta 2013). The additive gene effect is brought about by selection where landraces of *L. siceraria* with desired traits are conserved, which prolongs the presence of the dominant genes, in most cases leading to homozygosity and permanent genetic prints as well as advancement (Acquaah 2007; Sharma and Sengupta 2013).

According to Acquaah (2007), heritability may be estimated by narrow or broad sense. Broad sense is a genetic fraction where phenotypic variance can be attributed to variance of genotypic values and is expressed as $H^2 = V_G/V_P$. Narrow sense is the fraction where the genetic variance can be partitioned into the variance of additive genetic effects, namely dominance genetic effects and epistatic genetic effects, that are expressed as $H^2 = V_A/V_P$. However, broad sense heritability estimate expression is more relevant and is presented in percentages by multiplying by 100 hence,

$H^2 = V_G/V_P \times 100$

where:

V_G = Total genetic variance

V_P = Total phenotypic variance

The phenotypic traits are a result of the interactions between the genotype and environment, thus changing the environment could possibly alter some genes or even lead to the loss of some desired traits inherited from previous generations (Acquaah 2007). V_P (the total phenotypic variance) is the desired investigated and measured

traits, which in this study are: germination percentage; seedling height; cotyledonous leaf area; shoot growth; leaf growth; vine length; number of branches; number of leaves; total chlorophyll content; fresh shoot mass; dry shoot mass; shoot moisture content; tendril basal length; tendril lobe length; number of staminate flowers; number of pistillate flowers; flower sex ratio; number of fruits per plot; fruit yield per plot; fruit mass, length, width, pedicel length and rind thickness; seed length, width, 100 seed mass and total seed mass as well as the nutritional composition of *L. siceraria* landraces. This variance is the sum of genetic variation amongst individuals and environmental factors which yield a proportion of recorded variables that can correlate to genetic variation expressed as $V_P = V_G + V_E + V_{GE}$

where:

V_P = total phenotypic variance of the segregating population, V_G = genetic variance, V_E = environmental variance, and V_{GE} = variance associated with the genetic and environmental interaction (Acquaah 2007).

V_G, the total genetic variance, is the sum of additive variance, dominance variance, and interaction between genes expressed as:

$V_G = V_A + V_D + V_I$

where:

V_A = additive variance, V_D = dominance variance and V_I = interaction (Acquaah 2007; Conner and Hartl 2001). Additive genetic variance is the primary cause of morphological similarities amongst individuals because only the additive effect of genes is passed on directly from parent to offspring, making it easier to identify and distinguish the variation within a given population and estimate the heritability value (Conner and Hartl 2001).

2.7 Conclusion

This chapter reviewed the taxonomy and uses of *L. siceraria*. Variation in seedling, vegetative and reproductive traits as well as nutritional content among landraces was also reviewed. The *L. siceraria* landraces are well documented on medicinal and household uses. However, they are marginally documented on their agronomic and nutritional traits as well as their heritability studies, particularly landraces from northern

KwaZulu-Natal, South Africa, thus the rationale to conduct the study. Heritability and genetic diversity in morphological and nutritional traits among landraces were outlined. The following chapter (Chapter 3) focuses on the variation in seedling, vegetative and reproductive traits among *L. siceraria* landraces.

Chapter 3

Characterisation of *Lagenaria siceraria* landraces from northern KwaZulu-Natal using morphological traits

3.1 Introduction

The *Lagenaria siceraria* (Molina) Standley, commonly known as bottle gourd is an important member of the Cucurbitaceae family. It is an annual herbaceous and monoecious plant that is easily distinguished by its white flowers and has a prostrate growing pattern. (Muralidharan *et al.* 2017). The *L. siceraria* is believed to be of African and Asian origin. In many parts of South Africa the very young tender fruits of the bottle gourd are sliced and cooked as vegetables and the vining shoots are cooked like spinach (Xaba and Croeser 2011 and Sithole *et al.* 2015). In West Africa the mature seeds are roasted and crushed to a paste used to thicken stew (Chimonyo and Modi 2013). In Botswana, Zimbabwe and South Africa the oil extracts from mature bottle gourd seeds are an ideal alternative to vegetable oil (Chimonyo and Modi 2013).

The *L. siceraria* shows great diversity in germination percentage, cotyledonous leaf area, seedling height, shoot growth, leaf growth, vine length, number of branches, number of leaves, shoot fresh mass, shoot dry mass, and shoot moisture content (Kumar 2016; Muralidharan *et al.* 2017; Uddin *et al.* 2014). The landraces with larger seeds tend to have a higher germination percentage than those with smaller seeds, thus producing a very vigorously growing seedling with bigger cotyledonous leaves and greater seedling height (Chimonyo and Modi 2013). More variations in the vegetative traits are notable as landraces with greater shoot growth and vine length tend to have bigger and fewer leaves, less branching and have a higher shoot moisture content than those with an inferior shoot growth and vine length (Koffi *et al.* 2009; Mlandenovic *et al.* 2011). The landraces with an inferior shoot growth and vine length tend to have smaller but many leaves, be highly branching and have a lower shoot moisture content (Uddin *et al.* 2014; Kumar 2016).

The shape of *L. siceraria* fruit varies between pear-shape, conic, oval, isodiametric, discus-shaped and curvilinear (Mlandenovic *et al.* 2011; Mashilo *et al.* 2017). The fruit colour also varies from light green to dark green as well as the texture of the fruit which can be smooth, semi-rough or rough (Mashilo *et al.* 2016; Mashilo *et al.* 2017). Seeds are one of the major differentiating traits among the *L. siceraria* landraces (Sivaraj and Pandravada 2005). There are three types of seeds, siceraria, asiatica, and intermediate type. These vary in seed colour ranging from creamy brown to dark brown, and they can either be smooth or leathery in texture (Mlandenovic *et al.* 2012). Seed margins and lines also add to the variations, as some landraces have smooth margins to wavy margins, and a range from the presence of prominent seed lines to the absence of seed lines (Mlandenovic *et al.* 2012). Landraces with bigger fruits produce the heaviest seeds (Muralidharan *et al.* 2017).

Although *L. siceraria* landraces have various utensil, medicinal and food use, they are still underutilised in South Africa, including northern KwaZulu-Natal. Studies in the morphological diversity among these landraces are still marginal in the country. Therefore, a study on their morphological diversity will benefit the future breeding of these landraces. The objective of this study was to characterise *L. siceraria* genotypes of different origins using morphological traits.

3.2 Materials and Methods

3.2.1 Seed source and field layout

Landraces were collected from six (6) different areas in northern KwaZulu-Natal: Khangelani (29.0106° S, 31.2211° E), Ndumo (26.9342° S, 32.2824° E), Mbazwana (27.4937° S, 32.5882° E), Rorke's Drift (28.3492° S, 30.5351° E), Nquthu (28.2195° S, 30.6746° E), and Dundee (28.1650° S, 30.2343° E) (Table 3.1). These landraces were named according to their area of origin, fruit texture and fruit shape. They showed great diversity among each other in their fruit and seed morphology (Figures 3.1 and 3.2).

Fourteen different genotypes of *L. siceraria* landraces were cultivated in the same field and evaluated using a randomised complete block design (RCBD) generated by GenStat 15th edition software. The field experiment was conducted over two summer seasons, September 2016 – January 2017, and September 2017 – January 2018. The field was divided into 14 equal sized plots with 3 replicates per landrace which amounted to 42 plots in total. Each plot was 9 m^2 with 2 m inter-row spacing and 1 m intra-row spacing housing 16 plants per plot giving a total of 672 plants in a 1050 m^2 plot of land (Table 3.2). At transplanting, fertilizer NPK 2:3:4(30) at a rate of 40 g/m^2 was band placed below the seedlings in 10 – 15 cm deep pits and the field was irrigated to field capacity. At 31 days after transplanting (DAT), nitrogen fertilizer (Limestone Ammonium Nitrate (LAN (28)) of the same rate (40 g/m^2) was band placed around each plant. Plants were irrigated adequately depending on the amount of rainfall and temperature received. Weeding and insecticides applications were performed when necessary.

3.2.2 Data collection

Seedling traits were measured at 31 days after sowing (DAS) while still growing in the plug trays. Vegetative traits were recorded at vining stage (vines with a minimum of six leaves) at 38 days after transplanting (DAT). Shoot and leaf growth percentages were determined from 38 – 45 DAT. Flower traits were recorded at 60 DAT. Fruit and seed traits were measured at harvest (125 DAT). Seedling, vegetative, fruit and seed traits were measured from nine plants (replicates) per landrace. Fruit yield traits were measured per plot with three replicates.

Table 3.1: Description of landraces according to their origin as well as fruit and seed morphology

LR	Area	Fruit colour	Fruit texture	Fruit shape	Seed type	Seed colour	Seed texture	Seed size	Seed line	Seed shape
KSP	Khangelani	Pale green	Smooth	Pear	Asiatica	Brown	Leathery	Large	Present	Slightly oblong to rectangular
KSC	Khangelani	Pale green	Smooth	Curvilinear	Asiatica	Brown	Leathery	Large	Present	Slightly oblong to rectangular
KRI	Khangelani	Green	Rough	Isodiametric	Siceraria	Dark brown	Leathery	Large	Present	Slightly oblong to rectangular
NRB	Ndumo	Dark green	Rough	Cylindrical	Siceraria	Dark brown	Smooth	Small	Absent	Oblong
NRC	Ndumo	Dark green	Rough	Cylindrical	Siceraria	Creamy brown	Smooth	Small	Absent	Oblong
MSC	Mbazwana	Pale green	Smooth	Curvilinear	Asiatica	Brown	Leathery	Large	Present	Slightly oblong to rectangular
RSP	Rorke's Drift	Pale green	Smooth	Pear	Asiatica	Light brown	Leathery	Large	Present	Rectangular
RRP	Rorke's Drift	Pale green	Rough	Pear	Asiatica	Light brown	Leathery	Large	Present	Rectangular
NqRC	Nquthu	Pale green	Rough	Curvilinear	Intermediate	Light brown	Leathery	Medium	Present	Slightly oblong
NSRP	Nquthu	Pale green	Semi-rough	Pear	Intermediate	Brown	Leathery	Medium	Present	Slightly oblong
NqSC	Nquthu	Pale green	Smooth	Semi-Curvilinear	Asiatica	Light brown	Leathery	Medium	Present	Slightly oblong
NSC	Nquthu	Pale green	Smooth	Curvilinear	Asiatica	Light brown	Leathery	Medium	Present	Slightly oblong
NSRC	Nquthu	Green	Semi-rough	Curvilinear	Intermediate	Brown	Leathery	Medium	Present	Slightly oblong
DSI	Dundee	Dark green	Smooth	Isodiametric	Siceraria	Dark brown	Smooth	Large	Present	Oblong

LR-Landraces – from Khangelani area with smooth fruit texture, (KSP) pear-shaped; (KSC) curvilinear shape; and (KRI) rough isodiametric shaped fruits; from Ndumo area (NRB) with rough fruits with brown seeds and (NRC) rough fruits with creamy seeds; from Mbazwana area (MSC) with smooth cylindrical-shape; from Rorke's Drift area (RSP) with smooth pear-shaped and (RRP) rough pear-shaped; from Nquthu area (NqRC) with rough curvilinear shape,(NSRP) with semi-rough pear-shaped, (NqSC) and (NSC) with smooth curvilinear shaped and (NSRC) semi-rough curvilinear shaped and from Dundee area (DSI) with smooth isodiametric shape

Table 3.2: Field layout of 14 *L. siceraria* landraces

14	13	12	11	10	9	8	7	6	5	4	3	2	1
9	5	13	1	7	8	11	3	14	2	6	4	10	12
11	3	4	6	14	2	7	10	12	8	1	13	9	5

Landraces from Khangelani area with smooth, (1=KSP) pear-shaped; smooth, (2=KSC) curvilinear shape; and (3=KRI) rough isodiametric shape fruits; from Ndumo area (4=NRB) with rough fruits with brown seeds and (5=NRC) rough fruits with creamy seeds; from Mbazwana area (6=MSC) with smooth cylindrical-shape; from Rorke's Drift area (7=RSP) with smooth pear-shaped and (8=RRP) rough pear-shaped; from Nquthu area (9=NqRC) with rough curvilinear shape,(10=NSRP) with semi-rough pear-shaped, (11=NqSC) and (12=NSC) with smooth curvilinear shaped and (13=NSRC) semi-rough curvilinear shaped and from Dundee area (14=DSI) with smooth isodiametric shape
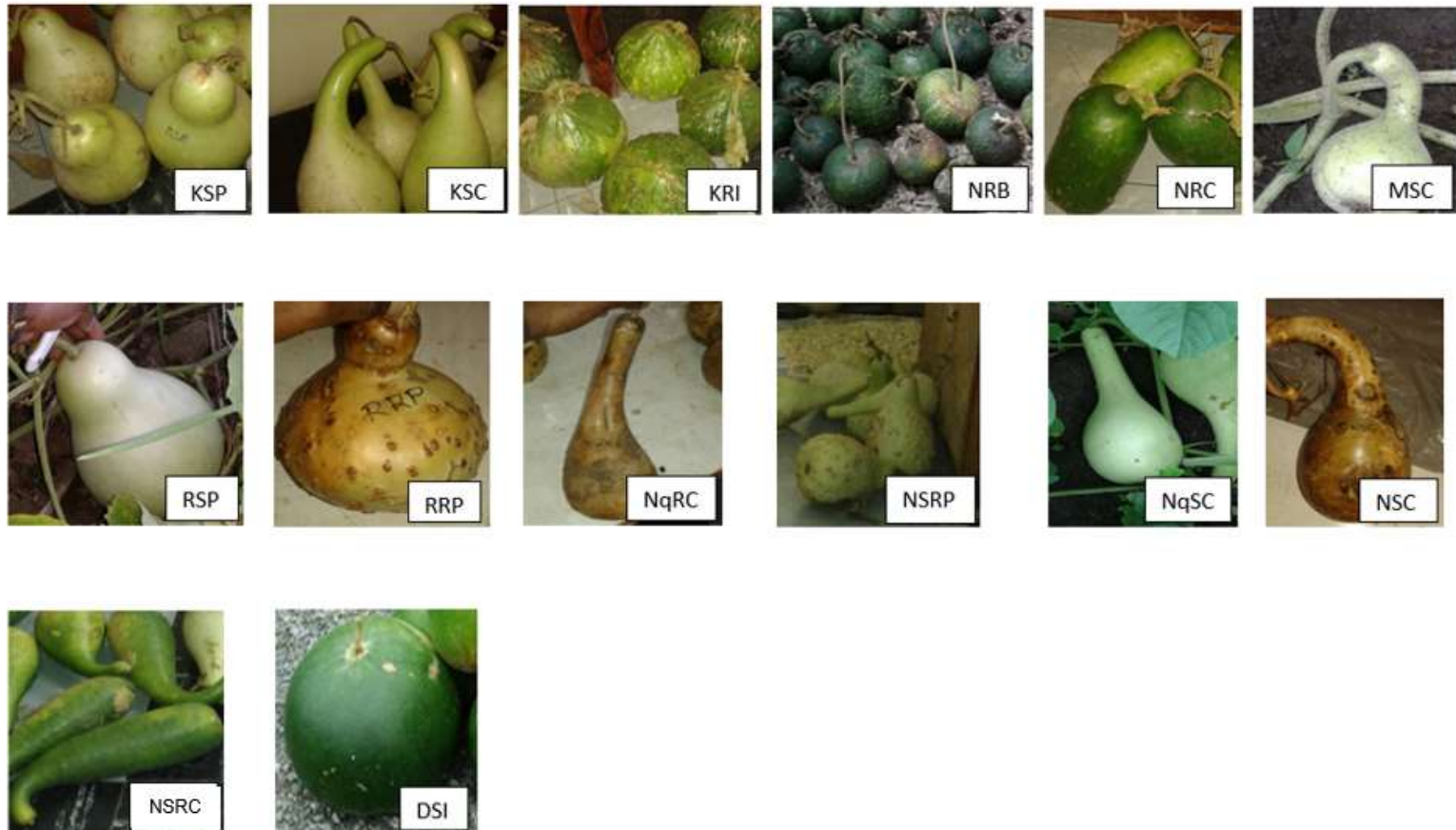
Figure 3.1: Variation in fruit traits among 14 *L. siceraria* landraces from northern KwaZulu-Natal, South Africa (see Table 3.1)

Figure 3.2: Variation in seed morphology among 14 *L. siceraria* landraces from northern KwaZulu-Natal, South Africa (see Table 3.1).

(a) Seedling traits

The germination percentage was calculated from the 80 seeds (per landrace) sown onto the plug trays using the following formula: GP (%) = (number of germinated seeds / Total number of seeds sown) × 100. The seedling height (mm) and cotyledonous leaf area (mm^2) of seedlings were measured at the first true leaf stage using a 300-millimeter ruler. Height was measured from the ground surface of the plug tray to the apical growth tip of the uniform seedlings for each landrace. Cotyledonous leaf area was measured on one cotyledonous leaf in nine uniform seedlings using the formula: Leaf area (mm) = length (mm) × width (mm).

(b) Shoot and leaf growth

The apical point of the desired vine was marked with prestik just below the first true leaf. At 38 DAT, the initial shoot length (from the base of the prestik towards the apical tip of the leaf) and initial leaf area (length × breadth) of the first true leaf was measured. At 45 DAT the final shoot length was measured from the base of the initial point towards the apical end of the shoot. Shoot and leaf growth was calculated using the formula: Growth (%) = ((final - initial) ÷ initial) × 100.

(c) Stem traits

Vine length (m) was measured using a fibreglass measuring tape, five-meter string, and prestik. The string was used (due to its property of being flexible) to trace the vine length from the stem base to the apex leaf. The measuring fibreglass tape measured the length of the string in relation to vine length, which was recorded as vine length. On the same vines measured for length, the number of branches and leaves was counted and recorded.

(d) Total chlorophyll content

The chlorophyll content was extracted on the third leaf from the apex. About 100 mg of living leaf tissue was emptied into 7 ml of dimethylsulfoxide (DMSO) in volumetric tubes (Stein and Braga 2010). The solution was incubated in a water bath at 65°C for 60 minutes (Stein and Braga 2010). After extraction, 3 ml of pure dimethylsulfoxide solvent was added to make 10 ml extract for adequate absorbance reading at a wavelength of 645 and 663 nm (Stein and Braga 2010). As per Hiscox and Tsraelstam (1979) total chlorophyll content was calculated using Anorn's equation:

Total chlorophyll (g l^{-1}) = 0.0202 × A_{645} + 0.00802 × A_{663}

(e) Tendril traits

The tendril traits (mm) were measured from nine plants across all three plots (3 plants per plot). Mature tendrils were collected within the first nine internodes of the selected primary vines. The tendril basal length and lengths of bifurcate lobes were measured using a string. The length of the string tracing the basal and lobe lengths was measured with the fiber glass measuring tape and recorded as basal and lobe lengths.

(f) Shoot fresh and dry mass and shoot moisture content

Shoots from the third leaf towards the apex leaf on the vine were collected and had their fresh mass (g) determined using an analytical scale. These shoots were dried in an oven at 65°C for 24 hours to obtain the shoot dry mass. The shoot moisture content (SMC) was determined using the formula described by Reeb and Milota (1999) as:

$$SMC = \frac{Fresh\ mass - dry\ mass}{Fresh\ mass} \times 100$$

(g) Number of staminate flowers, number of pistillate flowers and flower sex ratio

At tailspin formation, the number of staminate and pistillate flowers from three selected plants per plot for each of the three replicate plots (total of nine plants per landrace) was recorded. Following Uddin *et al.* 2014, the flower sex ratio was calculated using the formula: Flower sex ratio (FSR) = (number of staminate flowers ÷ number of pistillate flowers).

(h) Fruit yield and agronomic traits at harvest

The number of fruits per plot for all landraces with three replicates was recorded at harvest 125 DAT. Fruit mass was measured using the analytical weighing scale in kilograms (kg) and fruit size was measured using a vernier caliper to measure the length (mm) and width (mm) of the fruits. Following Oloyede *et al.* 2013, fruit yield per hectare was calculated using the following formula:

$$\text{Fruit yield (kg/ha)} = \frac{\text{fruit mass /plot (kg) x 10 000m}^2}{\text{Area of plot (m}^2) \times 1\text{kg}}$$

The pedicel length (mm) was measured using a string which was measured by the fiber glass measuring tape and recorded as pedicel length. Uniform fruits were cut longitudinally and had rind thickness (mm) measured using a vernier caliper.

(i) Seed size and mass

At harvest, the seeds were removed from the fruit pulp and air dried at room temperature (25°C) for 7 days. A vernier caliper was used to measure the seed length (mm) and width (mm). Hundred seed mass and total seed mass from the nine uniform fruits was measured using the analytical weighing scale in grams (g) for each of the 14 landraces.

3.2.3 Data analysis

Data were subjected to ANOVA using the GenStat 15th edition. ANOVA was combined for both growing seasons. Means were separated using Tukey's LSD at the 5% significance level. Correlations and principal component analysis (PCA) were implemented to determine multi-character variation. Cluster analysis through biplot and dendrogram was conducted to study the similarities and dissimilarities of closely related landraces.

3.2.4 Estimation of variance components

The phenotypic, genotypic and environmental variances and coefficient of variation were calculated according to the formula described by Burto and Devane (1953) and cited by Singh *et al.* 2017 as follows:

Environmental variance ($\delta^2 e$) = MSE

Genotypic variance ($\delta^2 g$) = $-\dfrac{MSG - MSE}{r}$

Phenotypic variance ($\delta^2 p$) = $\delta^2 g + \delta^2 e$

where MSG is mean square due to genotype, MSE is mean square of error (environmental variance) and (*r*) is the number of replications.

Phenotypic coefficient of variation (PCV) = $\sqrt{(\delta^2 p)}/x \times 100$

Genotypic coefficient of variation (GCV) = $\sqrt{\dfrac{\delta^2 g}{x}} \times 100$

where

$\delta^2 p$ = phenotypic variation

$\delta^2 g$ = genotypic variation

X = Grand mean of the character studied.

Estimation of heritability in broad sense: Broad sense heritability (h^2), expressed as the percentage of the ratio of the genotypic variance ($\delta^2 g$) to the phenotypic variance ($\delta^2 p$), was calculated using the following formula (Allard 1960):

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

Genetic advance (GA) was estimated as per the formula given by Allard (1960) and cited by Meena *et al.* 2015:

$$GA = k \times \frac{\sqrt{\delta^2 p \times \delta^2 g}}{\delta^2 p}$$

where:

GA = expected genetic advance

$\delta^2 p$ = phenotypic variation

$\delta^2 g$ = genotypic variation

k= the standard selection differential at 5% selection intensity (k = 2.063).

3.3 Results

Lagenaria siceraria landraces differed significantly (P<0.05) in their seedling, vegetative, reproductive and yield traits. Significant variation was recorded among morphologically similar landraces from different areas, as well as landraces with different fruit traits but from the same area. The study assessed morphological variability, correlation coefficiency, principal component analysis, cluster analysis as well as genetic parameters amongst the *L. siceraria* landraces.

3.3.1 Seedling traits

Germination percentage varied significantly among *L. siceraria* landraces at 31 days after sowing (Table 3.3). Landrace DSI from Dundee had the highest germination percentage at 95% whereas the landrace RRP from Rorke's Drift had the least at 67.5%, with an average of 82.5%.

Table 3. 3: Variation in seedling traits (31 DAS), shoot and leaf growth (38-45 DAT) and other vegetative traits (45DAT) among *L. siceraria* different landraces

LR	GP	SH	CLA	SG	LG	VL	NOB	NOL	TCC	TBL	TLL	TSHL	FSM	DSM	SMC
KSP	80.0 e	41.9 bcd	1322 de	1029 b	6548 bc	2.4 ef	5.3 cd	24.8 de	16.2 a	53.1 bcde	171.2 gh	130.8 bcd	57.0 b	9.9 c	82.7 a
KSC	82.5 de	48.8 a	1306 def	1256 a	9566 a	3.1 c	5.5 c	39.4 a	13.5 abc	59.4 b	185.0 fgh	108.8 e	59.5 a	12.5 a	79.0 de
KRI	75.0 f	37.3 def	1111 efg	959 bc	7264 b	3.0 cd	8.4 a	41.4 a	14.5 abc	56.9 bc	189.6 efgh	115.6 de	56.5 bc	11.8 b	79.1 de
NRB	90.0 b	22.5 h	887 g	827 d	5176 cde	3.0 cd	7.6 b	26.9 bcd	15.3 ab	28.8 g	162.4 h	88.1 f	39.4 ef	8.1 e	79.4 cde
NRC	80.0 e	40.9 bcd	880 g	596 f	3726 e	3.7 ab	7.8 ab	31.3 b	14.7 abc	53.8 bcde	176.0 gh	104.8 ef	35.1 f	8.7 d	75.2 g
MSC	80.0 e	37.8 def	1444 bcd	694 ef	4673 de	3.4 bc	4.4 de	26.3 cd	14.3 abc	45.0 ef	214.8 cdef	135.6 bc	56.4 bc	10.5 c	81.3 a
RSP	75.0 f	35.0 efg	1049 fg	962 bc	3888 e	2.4 f	4.9 de	25.8 cd	13.8 abc	69.1 a	267.0 a	143.5 b	59.1 a	12.5 a	78.9 e
RRP	67.5 g	30.9 g	1017 g	740 e	6325 bcd	2.4 f	4.6 de	27.3 bcd	16.6 a	75.0 a	267.6 a	129.8 bcd	58.1 ab	11.7 b	80.0 c
NqRC	90.0 b	33.5 fg	1118 efg	1084 b	6287 bcd	2.3 f	7.1 b	25.8 cd	12.7 bc	54.1 bcde	256.1 ab	167.5 a	54.1 c	10.5 c	80.5 bc
NSRP	90.0 b	43.1 bc	1627 abc	412 g	4620 de	4.0 a	7.4 b	39.6 a	11.7 c	50.0 cdef	226.0 bcd	141.1 b	52.7 cd	9.7 c	81.5 a
NqSC	82.5 de	44.4 ab	1443 bcd	1098 b	4002 e	2.2 f	5 cde	21.0 ef	15.8 ab	54.8 bcd	238.4 abc	144.1 b	59.0 ab	12.1 ab	79.4 d
NSC	85.0 c	31.4 g	1422 cd	893 cd	5448 bcde	2.6 def	4.6 de	17.6 e	13.5 abc	57.5 bc	219.6 cde	121.1 cde	59.2 ab	12.1 ab	76.4 f
NSRC	82.5 de	39.4 cde	1726 a	970 bc	1518 f	3.8 ab	7.5 b	38.5 a	15.0 abc	45.9 def	182.5 fgh	113.8 de	51.1 d	9.9 c	80.6 b
DSI	95.0 a	43.1 bc	1695 ab	925 bc	4893 cde	2.9 cde	4.8 de	29.9 bc	16.1 a	43.0 f	201.0 defg	111.5 de	56.8 bc	11.0 c	80.6 b
GM	82.5	37.4	1288	889	5281	2.9	6.1	29.7	14.4	53.3	211.3	125.4	53.9	10.8	79.6
P value	0.404	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
LSD	13.92	4.87	268.8	47.83	1866.8	0.54	0.72	4.41	0.003	9.2	33.38	20.00	1.62	0.54	0.76
CV%	29.5	13.1	21.0	54.2	35.6	18.5	12.0	15.0	24.1	17.5	15.9	16.1	1.8	3.0	0.6

LR-Landraces – from Khangelani area with smooth fruit texture, (KSP) pear-shaped; (KSC) curvilinear shape; and (KRI) rough isodiametric shaped fruits; from Ndumo area (NRB) with rough fruits with brown seeds and (NRC) rough fruits with creamy seeds; from Mbazwana area (MSC) with smooth cylindrical-shape; from Rorke's Drift area (RSP) with smooth pear-shaped and (RRP) rough pear-shaped; from Nquthu area (NqRC) with rough curvilinear shape,(NSRP) with semi-rough pear-shaped, (NqSC) and (NSC) with smooth curvilinear shaped and (NSRC) semi-rough curvilinear shaped and from Dundee area (DSI) with smooth isodiametric shape. GP-Germination percentage, CLA-Cotyledonous leaf area (mm), SH-Seedling height (mm), SG-Shoot growth (%) and VL-Vine length (m), LG-Leaf growth (%), NOB- Number of branches, NOL-Number of leaves, TCC-Total chlorophyll content (g/mL X10^{-3}), SFM-Shoot fresh mass (g), SDM-Shoot dry mass (g), SMC-Shoot moisture content (%), TBL-Tendril basal length (mm), TLL-Tendril longer lobe length (mm), TSHL-Tendril shorter lobe length (mm). Mean values in the same column with different letter (s), differ significantly at p<0.05 according to Tukey's LSD.

Great diversity on germination percentage was noted in landraces from different origins but sharing similar fruit morphological traits. Germination of seeds of landrace NRB from Ndumo was significantly superior to landrace KRI from Khangelani though they both had rough fruits. Similarly, landrace KSP from Khangelani had its seeds germinating significantly better than RSP from Rorke's Drift with both landraces having smooth pear-shaped fruits. Landrace NSC from Nquthu had a greater germination percentage than landrace MSC from Mbazwana and they both had smooth curvilinear fruits.

Landraces from Khangelani varied in their germination percentage. Landraces KSP and KSC with smooth fruit rinds had a higher germination percentage than KRI with rough fruit rind. Landraces from Ndumo with brown seeds (NRB) germinated better than those with cream seeds (NRC). Again, in pear-shaped landraces from Rorke's Drift, those with smooth rind (RSP) had a higher germination percentage than landraces with rough fruit rind (RRP). Landraces from Nquthu with rough curvilinear fruits (NqRC) had a significantly higher germination percentage than landraces with smooth curvilinear fruits (NqSC).

Landrace KSC from Khangelani had the tallest seedlings at 48.8 mm while landrace NRB from Ndumo had the shortest at 22.5 mm, with an average seedling height of 37.4 mm (Table 3.3). The seedling height of landrace KSP from Khangelani was higher than that of landrace RSP from Rorke's Drift, even though they both had smooth pear-shaped fruits. Landraces KSC and MSC both had smooth curvilinear-shaped fruits. However, landrace KSC from Khangelani had taller seedlings than landrace MSC from Mbazwana. Landrace NSRP of Nquthu origin produced taller seedlings than landrace RRP of Rorke's Drift origin even though both landraces had semi-rough to rough pear-shaped fruits. Landraces NRC from Ndumo and NqRC from Nquthu both had rough fruits; however, NqRC had a greater seedling height. Landrace DSI from Dundee area had taller seedlings than landrace KRI from Khangelani, as both landraces had isodiametric-shaped fruits.

In landraces from Khangelani, seedlings of KSP and KSC were taller than those of landrace KRI. Ndumo Landrace NRC produced significantly superior seedling height than landrace NRB. Landraces NqSC and NSC are both from Nquthu with smooth fruit rind. However, landrace NqSC with semi-curvilinear shaped fruit had significantly taller seedlings than landrace NSC with curvilinear shaped fruit.

Landrace NSRC had the largest cotyledonous leaf area at 1726 mm^2 while landrace NRC had the smallest at 880 mm^2, with an average cotyledonous leaf area of 1289 mm^2 (Table 3.3). Landrace KSP from Khangelani had significantly larger cotyledonous leaves than RSP from Rorke's Drift, with both landraces having smooth pear-shaped fruits. Landrace NSRP from Nquthu area had bigger cotyledonous leaves than RRP from Rorke's Drift, but both had semi-rough to rough pear-shaped fruits. Landrace DSI from Dundee had superior cotyledonous leaves to KRI from Khangelani area but both landraces had isodiametric-shaped fruits. Landraces from Nquthu bearing curvilinear-shaped fruits differed. Landrace NSRC had larger cotyledonous leaf area than landraces NqSC, NSC and NqRC.

3.3.2 Shoot and leaf growth

Landrace KSC had the most vigorous shoot growth, recording 1256%, and landrace NSRP had the least vigorous shoot growth at 412%, with the average being 889%. Landrace KSC from Khangelani had a greater shoot growth than landraces MSC from Mbazwana and NSC from Nquthu, with all three landraces having smooth curvilinear-shaped fruits. Landrace KSP from Khangelani had a higher shoot growth than landrace NSRP from Nquthu with both landraces having pear-shaped fruits. Shoots of landraces with rough fruits, NqRC from Nquthu grew faster than NRC from Ndumo.

Ndumo landraces having rough fruits showed significant variations, where shoots of landrace NRB grew more vigorously than NRC. In Rorke's Drift landraces, RSP had a significantly higher shoot growth than RRP, with both landraces having pear-shaped

fruits. In Nquthu landraces, NqSC with smooth semi-curvilinear fruits had a higher shoot growth than NSC with smooth curvilinear-shaped fruits.

Landrace KSC from Khangelani had the highest leaf growth at 9566% and landrace NSRC from Nquthu had the lowest leaf growth at 1518%. The average leaf growth was 5281% (Table 3.3). Landrace KSP from Khangelani had a superior leaf growth to RSP from Rorke's Drift, with both landraces having smooth pear-shaped fruits. Landrace KSC from Khangelani had a higher leaf growth than landrace NqSC from Nquthu, with both landraces possessing smooth curvilinear fruits. Nquthu landraces, NqRC had significantly faster-growing leaves than NSRC, with both landraces possessing rough curvilinear-shaped fruits.

3.3.3 Stem traits

Landrace NSRP had the longest vines reaching 4.0 m and landrace NqRC had the shortest vines at 2.3 m, with an average of 2.9 m (Table 3.3). Landrace KSC from Khangelani had longer vines than Nquthu landraces NqSC and NSC, with all three landraces having smooth pear-shaped fruits. Landrace NSRP from Nquthu had longer vines than RRP from Rorke's Drift, with both landraces possessing semi-rough to rough pear-shaped fruits. Smooth curvilinear landraces, MSC from Mbazwana had a longer vine length than NqSC from Nquthu. More variations were noted in landraces with rough fruits as NRC from Ndumo had a longer vine length than landrace NqRC from Nquthu.

Regarding landraces from Khangelani, KSC and KSP with a smooth rind texture were found to be different as KSC had a longer vine length. Landraces from Ndumo with rough fruit rinds differed significantly as NRC had a longer vine length than NRB. Landraces from Nquthu with rough curvilinear-shaped fruits also differed significantly as NSRC had a longer vine length than NqRC.

Landrace KRI recorded the highest number of branches at 8.4 while landrace MSC had the least number of branches at 4.4, with an average of 6.1 branches. Landrace MSC

from Mbazwana had a larger branch number than landraces KSC from Khangelani, NqSC, and NSC both from Nquthu, with all four landraces possessing smooth curvilinear-shaped fruits. Landrace NSRP from Nquthu had more branches than RRP from Rorke's Drift, with both landraces having rough pear-shaped fruits. Landraces from Nquthu, NqRC, NSRP, and NSRC with rough fruit rinds had a significantly higher number of branches than those with a smooth fruit rind, namely NqSC and NSC.

The landrace KRI from Khangelani had the most numerous leaves per plant at 41.5 while landrace NSC from Nquthu had the least number of leaves at 17.6, with an average of 29.7 leaves (Table 3.3). Landrace KSC from Khangelani had more leaves than landraces MSC from Mbazwana. NqSC and NSC (both from Nquthu) possessed smooth semi-curvilinear and curvilinear-shaped fruits, respectively. Landrace NSRP from Nquthu had more leaves than RRP from Rorke's Drift, with both landraces having rough pear-shaped fruits. Landrace KRI from Khangelani had a superior number of leaves to DSI from Dundee, with both landraces having isodiametric-shaped fruits. In landraces from Nquthu, NSRC had more leaves than NqRC, even though they both have semi-rough and rough textured curvilinear-shaped fruits.

3.3.4 Total Chlorophyll content

Landraces RRP, recording 16.6 g/ml, had the highest total chlorophyll content while NSRP had the lowest at 11.7 g/ml, with an average of 14.5 g/ml (Table 3.3). Landrace RRP from Nquthu drift had a greater total chlorophyll content than NSRP from Nquthu, with both landraces having rough pear-shaped fruits.

3.3.5 Tendril traits

Landrace RRP, at 75 mm, had the longest tendril basal length and NRB had the shortest at 28.4 mm, with an average of 53.3 mm (Table 3.3). Landrace RSP from Rorke's Drift had longer basal lengths than KSP from Khangelani, with both landraces displaying smooth pear-shaped fruits. Landraces KSC from Khangelani, and NqSC and

NSC both from Nquthu, had longer basal lengths than MSC from Mbazwana, with all four landraces having smooth curvilinear and semi-curvilinear shaped fruits. Rough pear-shaped landraces, RRP from Rorke's Drift and NSRP from Nquthu differed as RRP had longer basal lengths than NSRP.

Landrace KRI from Khangelani had longer tendril basal lengths than DSI from Dundee, with both landraces portraying isodiametric-shaped fruits. Landrace NqRC from Nquthu had longer basal lengths than NRB from Ndumo, with both landraces having rough fruits. Landraces from Ndumo with rough fruits, NRC and NRB, differed significantly as NRC had longer tendril basal lengths than landrace NRB. In Nquthu landraces, NSC had a superior tendril basal length to landrace NSRC, with both landraces having curvilinear-shaped fruits.

Landrace RRP at 267.6 mm had the longest longer lobe length while NRB had the shortest at 162.4 mm, with an average of 211.2 mm (Table 3.3). Landrace RSP from Rorke's Drift had a longer, longer lobe length than KSP from Khangelani, with both landraces displaying smooth pear-shaped fruits. NqSC from Nquthu had a superior longer lobe length to that of KSC from Khangelani, with both landraces having smooth semi-curvilinear and curvilinear-shaped fruits. Landrace RRP from Rorke's Drift had a superior longer lobe length to NSRP from Nquthu, with both landraces having rough pear-shaped fruits. Landrace NqRC from Nquthu had a longer, longer lobe length than Ndumo landraces NRB and NRC, with all three having rough fruits.

Landrace NqRC recording 167.5 mm had the longest shorter lobe length whereas NRB had the shortest at 88.1 mm, with an average of 125.4 mm (Table 3.3). Landraces with smooth semi-curvilinear and curvilinear-shaped fruits differed significantly as MSC from Mbazwana had a longer, shorter lobe length than landraces KSC from Khangelani and NqSC from Nquthu. Nquthu landraces with smooth curvilinear-shaped fruits varied in their tendril shorter lobe lengths. NqSC had longer shorter lobe length than NSC.

3.3.6 Shoot fresh and shoot dry mass and shoot moisture content

Landrace KSC had the highest shoot fresh mass at 59.5 g whereas NRC had the lowest at 35.1 g, with a mean of 53.9 g (Table 3.3). Landrace RSP from Rorke's Drift had a greater shoot fresh mass than KSP from Khangelani, with both landraces having smooth pear-shaped fruits. Landraces KSC from Khangelani and MSC from Mbazwana both had smooth curvilinear-shaped fruits, however, KSC had a greater shoot fresh mass than MSC. Landrace RRP from Rorke's Drift recorded a higher shoot fresh mass than NSRP from Nquthu, with both landraces having rough pear-shaped fruits. Landrace NqRC from Nquthu had a greater shoot fresh mass than Ndumo area landraces (NRB and NRC), with all three landraces having rough fruits.

Landraces KSC and RSP had the highest shoot dry mass at 12.5 g while KRI had the lowest at 8.1 g, with a mean of 10.8 g (Table 3.3). Landrace KSP from Khangelani had a higher shoot dry mass than RSP from Rorke's Drift, both had smooth pear-shaped fruits. Landrace KSC from Khangelani had a greater shoot dry mass than MSC from Mbazwana, with both landraces displaying smooth curvilinear-shaped fruits. Landrace RRP from Rorke's Drift recorded a greater shoot dry mass than NSRP from Nquthu, with both landraces having rough pear-shaped fruits.

Ndumo area landraces with rough fruits also showed great diversity as NRC had a greater shoot dry mass than NRB. In Rorke's Drift pear-shaped landraces, RSP had a higher shoot dry mass than RRP. Landraces from Nquthu with smoothly textured fruits NqSC and NSC had a higher shoot dry mass than landraces with roughly textured fruits NSRP, NSRC, and NqRC.

Landrace KSP with 82.7% had the highest shoot moisture content and NRC had the lowest at 75.2%, with the average of 79.6% (Table 3.3). Smooth curvilinear and semi-curvilinear shaped landraces differed. MSC from Mbazwana exhibited a superior shoot moisture content to landraces KSC from Khangelani and NSC and NqSC, both from Nquthu. Landrace KSP from Khangelani had a superior shoot moisture content to RSP

from Rorke's Drift, with both landraces having smooth pear-shaped fruits. Semi-rough to rough pear-shaped landraces, NSRP from Nquthu, had a greater shoot moisture content than RRP from Rorke's Drift. Landraces with rough fruits, NqRC from Nquthu, had a superior shoot moisture content to NRC from Ndumo.

In Khangelani area landraces, KSP had a higher shoot moisture content than KSC, with both landraces having a smooth rind texture. In Ndumo landraces with rough fruits, NRB had a superior shoot moisture content to NRC. In Rorke's Drift landraces with pear-shaped fruits, RRP noted a higher shoot moisture content than RSP.

3.3.7 Number of staminate flowers, number of pistillate flowers and flower sex ratio

Landrace KRI had the highest number of staminate flowers per plot at 21.0 whereas NRB had the lowest at 7.7, with a mean of 12.9 (Table 3.4). Landraces NqSC and NSC (statistically equivalent) from Nquthu had a higher number of staminate flowers than KSC from Khangelani and MSC from Mbazwana (statistically equivalent), with all four landraces having smooth semi-curvilinear to curvilinear-shaped fruits. Landraces from Nquthu (NSRC and NqRC), with semi-rough to rough curvilinear fruits, differed. Landrace NSRC had more staminate flowers than NqRC.

Table 3. 4: Diversity in flower, fruit and seed traits among _L. siceraria_ landraces

LR	NSF/P	NPF/p	FSR	NF/p	FY/p	FM	FL	FW	PL	FRT	SL	SW	100SM	TSM
KSP	13.3 cde	18.3 ab	0.7 def	15.0 b	82500 a	4.95 a	417.9 b	347.1 b	78.6 cde	7.5 b	15.8 hi	8.5 e	21.6 a	86.8 b
KSC	8.3 g	14.3 bcd	0.6 ef	15.7 b	25763 bc	1.48 e	349.2 de	244.2 d	71.0 defg	3.9 f	17.4 ef	8.5 e	16.0 cd	52.3 d
KRI	21.0 a	18.7 ab	1.0 cd	44.3 a	71919 ab	1.46 ef	230.7 j	258.2 d	62.9 fg	8.0 a	15.5 i	8.0 ef	15.2 cd	53.1 d
NRB	7.7 g	7.3 e	1.2 bc	25.3 ab	29500 bc	1.05 fg	197.9 k	219.2 e	75.0 def	7.0 c	14.0 j	6.5 h	12.5 ef	31.7 f
NRC	9.0 fg	9.7 de	1.0 cd	23.3 ab	24889 bc	0.96 g	242.1 ij	174.4 g	82.1 cd	6.4 d	16.6 fgh	7.8 f	12.3 ef	29.5 f
MSC	9.3 fg	10.0 de	1.5 a	14.0 b	17111 c	1.11 efg	380.7 c	226.1 e	80.5 cde	4.3 f	16.4 ghi	7.1 g	14.2 de	41.2 e
RSP	11.0 efg	7.7 e	1.3 bc	11.0 b	62334 abc	5.11 a	340.0 def	372.1 a	59.3 g	6.0 d	20.3 b	14.0 b	21.6 a	101.1 a
RRP	12.3 def	8.0 e	1.5 a	15.7 b	47174 abc	2.71 c	335.7 ef	290.7 c	64.4 fg	4.0 f	18.0 de	13.0 c	18.6 b	86.4 b
NqRC	10.7 efg	7.0 e	1.6 a	22.7 ab	32523 bc	1.29 efg	368.9 cd	193.2 f	87.9 bc	4.0 f	23.0 a	14.9 a	16.4 c	64.6 c
NSRP	13.7 cde	13.3 cd	1.1 cd	31.7 ab	35186 abc	1.00 g	283.6 gh	165.6 g	74.1 def	4.0 f	16.9 fg	7.8 f	16.9 bc	53.8 d
NqSC	15.7 bcd	18.7 ab	0.9 d	30.3 ab	36064 abc	1.08 efg	272.9 h	203.0 f	69.3 efg	3.0 g	17.0 fg	12.0 d	11.8 f	30.1 f
NSC	19.0 ab	20.0a	0. 4 g	23.0 ab	27856 bc	1.09 efg	266.4 hi	227.9 e	78.6 cde	3.3 g	18.8 cd	12.0 d	11.6 f	30.3 f
NSRC	17.0 bc	18.0 abc	1.0 cd	23.7 ab	51278 abc	1.96 d	467.1 a	197.1 f	115.0 a	4.0 f	18.4 cd	11.9 d	16.9 bc	55.6 d
DSI	12.3 def	13.3 cd	1.0 cd	20.3 ab	67345 ab	3.19 b	311.0 fg	283.1 c	96.1 b	5.0 e	19.1 c	4.0 i	13.2 ef	41.1 e
GM	12.9	13.2	1.1	22.6	43674	2.0	318.9	243.0	78.2	5.0	17.6	9.0	15.6	54.0
P value	<.001	<.001	<.001	0.452	0.182	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
LSD	3.89	4.97	0.62	24.9	48197.0	0.42	3.03	1.58	1.23	0.41	0.95	0.54	1.91	6.31
CV%	18.1	22.6	34.1	66.0	66.0	20.8	9.6	6.6	15.9	8.4	5.4	5.7	12.3	11.7

LR-Landraces – from Khangelani area with smooth, (KSP) pear-shaped; smooth, (KSC) curvilinear shape; and (KRI) rough isodiametric shape fruits; from Ndumo area (NRB) with rough fruits with brown seeds and (NRC) rough fruits with creamy seeds; from Mbazwana area (MSC) with smooth cylindrical-shape; from Rorke's Drift area (RSP) with smooth pear-shaped and (RRP) rough pear-shaped; from Nquthu area (NqRC) with rough curvilinear shape,(NSRP) with semi-rough pear-shaped, (NqSC) and (NSC) with smooth curvilinear shaped and (NSRC) semi-rough curvilinear shaped and from Dundee area (DSI) with smooth isodiametric shape. NSF/p-Number of staminate flowers, NPF/p-Number of pistillate flowers, FSR-Flower sex ratio, NF/P-Number of fruits per plot, FY/P-Fruit yield per plot (kg/ha), FM-Fruit mass (kg), FL-Fruit length (mm), FW-Fruit width (mm),FRT-Fruit rind thickness (mm), PL-Pedicel length (mm), SL-Seed length (mm), SW-Seed width (mm), 100SM-100 Seed mass (g) and TSM-Total seed mass (g). Mean values in the same column with different letter (s), differ significantly at p<0.05 according to Tukey's LSD.

Landrace NSC at 21.0 noted the greatest number of pistillate flowers per plot while NqRC had the least at 7.0, with an average of 13.2 (Table 3.4). Landraces KSP from Khangelani and RSP from Rorke's Drift differed as KSP had more pistillate flowers and both landraces had smooth pear-shaped fruits. Landraces NqSC and NSC from Nquthu had a superior number of pistillate flowers to MSC from Mbazwana, with all three landraces displaying smooth semi-curvilinear and curvilinear-shaped fruits.

Semi-rough and rough pear-shaped landraces, NSRP from Nquthu and RRP from Rorke's Drift differed from NSRP, having more pistillate flowers than RRP. Nquthu area landraces varied, NSC had more pistillate flowers than NqSC, with these landraces having smooth curvilinear and semi-curvilinear-shaped fruits, respectively. Landraces NSRC and NqRC both have semi-rough and rough curvilinear-shaped fruits, however, NSRC had more pistillate flowers than NqRC.

Landraces NqRC had the highest flower sex ratio at 1.6 and NSC had the lowest at 0.4, with an average of 1.1 (Table 3.4). Landraces with smooth pear-shaped fruits, KSP from Khangelani and RSP from Rorkers, drift differed significantly, with KSP having a higher flower sex ratio. Landrace MSC from Mbazwana had a superior flower sex ratio to NqSC from Nquthu, with both landraces having smooth curvilinear and semi-curvilinear shaped fruits, respectively. Landrace KSC from Khangelani had a higher flower sex ratio than NSC from Nquthu, with both landraces also displaying smooth curvilinear-shaped fruits. Landraces with rough and semi-rough pear-shaped fruits, RRP from Rorke's Drift and NSRP from Nquthu varied. RRP had a higher flower sex ratio than NSRP.

In Rorke's Drift landraces, RRP recorded a higher flower sex ratio than RSP, with both landraces displaying pear-shaped fruits. Nquthu landraces with rough and semi-rough curvilinear-shaped fruits, NqRC and NSRC differed significantly, NqRC had a higher flower sex ratio. In Nquthu landraces with smooth semi-curvilinear and curvilinear-shaped fruits, NqSC had a higher flower sex ratio than NSC.

3.3.8 Fruit yield and agronomic traits at harvest

Landrace KRI had the greatest number of fruits per plot at 44.3 whereas landrace RSP at 11.0 had the least number of fruits per plot, with the average of 22.6 (Table 3.4). Landraces from Khangelani showed variations in the number of fruits per plot. KRI recorded more fruits per plot than both KSP and KSC. Landrace KSP from Khangelani, measuring 82500 kg/ha, had the highest fruit yield per plot and MSC had the lowest at 17111 kg/ha, with a mean of 43674 kg/ha.

The landrace RSP at 5.1 kg had the heaviest fruit mass whereas NRC had the lightest at 1.0 kg, with an average of 2.0 kg (Table 3.4). Landraces with rough and semi-rough pear-shaped fruits (RRP from Rorke's Drift and NSRP from Nquthu) differed significantly, as RRP had heavier fruits compared to NSRP. Landraces from Nquthu (NSRC and NqRC) with semi-rough and rough curvilinear-shaped fruits respectively differed significantly, with NSRC recording heavier fruits than NqRC.

Landrace NSRC had the longest fruit length at 447.0 mm while NRB had the shortest at 198.0 mm, with an average of 319.0 mm (Table 3.4). KSP from Khangelani and RSP from Rorke's Drift both had smooth pear-shaped fruits, but KSP had longer fruits than RSP. Smooth curvilinear-shaped landraces differed as landrace MSC from Mbazwana had longer fruits than KSC from Khangelani. Landraces with rough and semi-rough pear-shaped fruits differed, RRP from Rorke's Drift had longer fruits than NSRP from Nquthu.

Of the Ndumo landraces with rough fruits NRC had longer fruits than NRB. In Nquthu landraces, NSRC had longer fruits than NqSC, with both landraces having curvilinear-shaped fruits. Also, in Nquthu landraces, NSRC had a superior fruit length to NqRC, with both landraces possessing rough curvilinear-shaped fruits.

Landrace RSP had the widest fruits at 372.1 mm and NSRP had the narrowest at 165.6 mm, with the average of 243.0 mm (Table 3.4). Landrace RSP from Rorke's Drift had wider fruits than KSP from Khangelani, with both landraces having smooth pear-shaped fruits. Smooth curvilinear-shaped landraces, KSC from Khangelani, MSC from Mbazwana and NSC from Nquthu, differed. KSC had wider fruits than both MSC and NSC. Landrace RRP from Rorke's Drift had wider fruits than NSRP from Nquthu, with both landraces displaying rough pear-shaped fruits.

In Ndumo landraces with rough fruit rinds, NRB had wider fruits than NRC. Rorke's Drift landraces with pear-shaped fruits differed, with RSP having wider fruits than landrace RRP. In Nquthu landraces, NSC had wider fruits than landrace NqSC, with both landraces having smooth curvilinear-shaped fruits.

Landrace NSRC had the longest pedicel at 115.0 mm while RSP had the shortest at 59.3 mm, with an average of 78.0 mm (Table 3.4). Landraces KSP from Khangelani and RSP from Rorke's Drift with smooth pear-shaped fruits differed, with KSP having a longer pedicel than RSP. Nquthu landraces with rough curvilinear-shaped fruits, NqRC and NSRC, differed as NSRC had a superior pedicel length to NqRC.

Landrace KRI had the thickest fruit rind at 8.0 mm and NqSC had the thinnest at 3.0 mm, with an average of 5.0 mm (Table 3.4). Smooth pear-shaped landraces, KSP from Khangelani and RSP from Rorke's Drift, varied; KSP had a thicker fruit rind than RSP. Landraces KSC from Khangelani and MSC from Mbazwana had thicker fruit rinds than NqSC and NSC from Nquthu, with all four landraces displaying smooth curvilinear-shaped fruits. Ndumo area landraces with rough fruit rinds varied, as NRB had a thicker fruit rind than NRC.

3.3.9 Seed length, width, and mass

Landrace NqRC had the longest seed length at 23.0 mm while NRB had the shortest at 14.0 mm, with an average of 17.6 mm (Table 3.4). Landrace RSP from Rorke's Drift had

longer seeds than KSP from Khangelani with both landraces displaying smooth pear-shaped fruits. Landraces with smooth curvilinear shaped fruits varied, as NSC had longer seeds than KSC from Khangelani, NqSC from Nquthu and MSC from Mbazwana.

Landraces with rough pear-shaped fruits (RRP from Rorke's Drift and NSRP from Nquthu) varied as RRP had longer seeds than NSRP. Ndumo area landraces with rough fruit rinds differed, as NRC had longer seeds than NRB. Nquthu landraces also varied, as landrace NSC had longer seeds than landraces NqSC, with both landraces having smooth curvilinear and semi-curvilinear shaped fruits.

Landrace NqRC had the widest seeds at 14.9 mm and DSI had the narrowest at 4 mm, with the average of 9.7 mm (Table 3.4). Landrace RSP from Rorke's Drift had wider seeds than KSP from Khangelani, with both landraces displaying smooth pear-shaped fruits. Landraces with smooth curvilinear-shaped fruits differed as NqSC and NSC from Nquthu had wider seeds than MSC from Mbazwana. Landrace MSC from Mbazwana had wider seeds than KSC from Khangelani, with both landraces having smooth curvilinear-shaped fruits. Landraces with rough pear-shaped fruits (RRP from Rorke's Drift and NSRP from Nquthu) varied. RRP had wider seeds than NSRP.

In Ndumo landraces, NRC had wider seeds than NRB, with both landraces having rough fruit rinds. Nquthu landraces, NqRC had wider seeds than NSRC and both had rough curvilinear-shaped fruits. Landrace RSP had the heaviest 100 seed mass per fruit at 21.6 g, whereas NSC had the lightest at 11.6 g, with the average of 15.6 g (Table 3.4). Landraces KSC from Khangelani and MSC from Mbazwana had heavier 100 seed mass than landraces NqSC and NSC from Nquthu, with all four landraces having smooth curvilinear-shaped fruits.

Landrace RSP had the maximum total seed mass per fruit at 101.1 g and NRC had the minimum at 29.5 g, with the average of 54.1 g (Table 3.4). Landrace RSP from Rorke's Drift had a superior total seed mass to KSP from Khangelani, with both landraces having smooth pear-shaped fruits. Landraces with smooth curvilinear-shaped fruits

varied, as KSC from Khangelani had a greater total seed mass than MSC from Mbazwana. Landrace MSC from Mbazwana had a greater total seed mass than NqSC and NSC from Nquthu, with all three landraces having smooth curvilinear-shaped fruits. Rough pear-shaped landraces differed, as RRP from Rorke's Drift had a superior total seed mass to NSRP from Nquthu. Landraces with rough curvilinear-shaped fruits; NqRC and NSRC from Nquthu differed, as NqRC had a higher total seed mass than NSRC.

3.3.10 Correlation among the traits of the *L. siceraria* landraces

The number of branches showed strong positive correlation with vine length, number of leaves, number of fruits per plot (Table 3.5). Shoot try mass showed strong positive correlation with shoot fresh mass and tendril basal length. Tendril longer lobe length had a strong positive correlation with tendril basal length and tendril shorter lobe length. All tendril traits and seed length showed strong positive correlation with seed width; tendril longer and shorter lobe lengths were also positively correlated with seed length, while tendril basal length was positively correlated with total seed mass.

Table 3. 5: Correlation among agro-morphological traits of *L. siceraria* landraces

Variables	GP	SH	CLA	SG	LG	VL	NOB	NOL	TCC	SFM	SDM	SMC	TBL	TLL	TSHL
GP															
SH	0.083														
CLA	0.423	0.532													
SG	-0.004	0.193	0.029												
LG	-0.114	0.115	-0.280	0.392											
VL	0.217	0.247	0.296	-0.588	-0.327										
NOB	0.179	-0.114	-0.245	-0.212	-0.115	0.516									
NOL	-0.014	0.399	0.162	-0.118	0.146	**0.678**	**0.614**								
TCC	-0.310	-0.089	-0.096	0.194	-0.101	-0.346	-0.294	-0.270							
SFM	-0.219	0.296	0.440	0.461	0.345	-0.501	**-0.651**	-0.144	-0.025						
SDM	-0.380	0.271	0.155	0.515	0.365	-0.517	-0.566	-0.115	-0.045	**0.857**					
SMC	0.140	0.158	0.449	0.066	0.058	-0.047	-0.140	0.146	0.108	0.381	-0.093				
TBL	**-0.728**	0.144	-0.235	0.139	0.267	-0.418	-0.361	-0.075	-0.021	0.517	**0.701**	-0.199			
TLL	-0.280	-0.139	-0.046	-0.036	-0.060	-0.478	-0.438	-0.354	-0.205	0.516	0.551	0.059	**0.653**		
TSHL	-0.076	0.129	0.143	0.072	-0.036	-0.403	-0.267	-0.292	-0.355	0.513	0.347	0.364	0.433	**0.775**	
NSF/P	-0.160	0.057	0.377	0.100	-0.128	-0.076	0.118	0.082	0.020	0.393	0.364	-0.038	0.194	0.051	0.099
NPF/p	0.022	0.439	0.565	0.300	0.017	0.023	-0.030	0.092	0.106	0.375	0.296	0.016	-0.014	-0.335	-0.116
FSR	-0.152	-0.387	-0.303	-0.306	-0.203	-0.035	0.089	-0.007	-0.022	-0.142	-0.209	0.313	0.032	0.495	0.411
NF/p	0.155	0.011	0.049	-0.132	-0.011	0.234	**0.673**	0.401	-0.172	-0.174	-0.102	-0.134	-0.229	-0.213	-0.119
FY/p	-0.201	0.124	0.112	0.256	0.033	-0.296	-0.003	0.140	0.427	0.317	0.171	0.392	0.187	-0.066	0.025
FM	-0.293	0.064	-0.019	0.249	-0.007	-0.424	-0.403	-0.173	0.368	0.358	0.231	0.350	0.356	0.165	0.163
FL	-0.138	0.307	0.451	0.297	-0.143	-0.002	-0.290	0.062	0.070	0.390	0.113	0.565	0.164	0.113	0.369
FW	-0.430	-0.061	-0.165	0.338	0.238	-0.560	-0.510	-0.229	0.411	0.477	0.423	0.241	0.425	0.181	0.064
PL	0.504	0.132	0.557	0.030	-0.494	0.409	0.189	0.133	0.071	-0.243	-0.422	0.175	-0.513	-0.357	-0.148
FRT	-0.176	-0.211	-0.505	-0.052	0.141	0.023	0.434	0.209	0.257	-0.388	-0.366	0.002	-0.189	-0.507	-0.395
SL	0.140	0.019	0.132	0.281	-0.085	-0.342	-0.227	-0.217	-0.334	0.362	0.388	-0.017	0.400	**0.664**	**0.639**
SW	-0.393	-0.239	-0.189	0.298	-0.124	-0.493	-0.142	-0.338	-0.181	0.323	0.416	-0.116	**0.622**	**0.694**	**0.624**
100SM	-0.438	0.076	-0.067	0.118	0.119	-0.199	-0.125	0.155	-0.003	0.352	0.165	0.519	0.484	0.278	0.385
TSM	-0.515	-0.045	-0.176	0.155	0.148	-0.367	-0.214	0.036	0.062	0.412	0.288	0.426	**0.609**	0.446	0.435

Table 3. 5: Correlation among agro-morphological traits of *L. siceraria* landraces

(Continued)

Variables	NSF/P	NPF/p	FSR	NF/p	FY/p	FM	FL	FW	PL	FRT	SL	SW	100SM
NPF/p	**0.781**												
FSR	-0.390	**-0.784**											
NF/p	**0.613**	0.419	-0.144										
FY/p	0.419	0.314	-0.115	0.082									
FM	-0.042	-0.046	0.010	-0.532	**0.768**								
FL	-0.033	0.088	0.123	0.544	0.232	0.437							
FW	-0.010	-0.037	-0.025	-0.490	**0.702**	**0.916**	0.254						
PL	0.052	0.169	-0.049	-0.062	-0.022	-0.124	0.499	-0.371					
FRT	-0.034	-0.077	0.005	0.189	0.543	0.364	-0.262	0.4076	-0.227				
SL	0.003	-0.240	0.275	-0.305	0.001	0.197	0.396	0.077	0.234	-0.472			
SW	0.203	-0.088	0.217	-0.171	-0.053	0.136	0.312	0.094	-0.129	-0.396	**0.637**		
100SM	-0.062	-0.153	0.225	-0.419	0.575	**0.780**	0.597	**0.665**	-0.185	0.247	0.226	0.351	
TSM	-0.048	-0.244	0.315	-0.439	0.576	**0.799**	0.518	**0.732**	-0.278	0.206	0.339	0.461	**0.966**

Values >0.6, deemed to be significantly correlated. Variables - GP-Germination percentage, CLA-Cotyledonous leaf area (mm), SH-Seedling height (mm), SG-Shoot growth (%) and VL-Vine length (m), LG-Leaf growth (%), NOB- Number of branches, NOL-Number of leaves, TCC-Total chlorophyll content (g/mL X10^{-3}), FSM-Shoot fresh mass (g), DSM-Shoot dry mass (g), SMC-Shoot moisture content (%) , TBL-Tendril basal length (mm), TLL-Tendril longer lobe length (mm), TSHL-Tendril shorter lobe length (mm), NSF/p-Number of staminate flowers, NPF/p-Number of pistillate flowers, FSR-Flower sex ratio, NF/P-Number of fruits per plot, FY/P-Fruit yield per plot (kg/ha), FM-Fruit mass (kg), FL-Fruit length (mm), FW-Fruit width (mm), FRT-Fruit rind thickness, PL-Pedicel length (mm), SL-Seed length (mm), SW-Seed width (mm), 100SM-100 Seed mass (g) and TSM-Total seed mass (g).

Fruit mass strongly correlated positively with fruit width. Hundred seed mass strongly correlated positively with total seed mass and they both positively correlated with fruit yield per plot, fruit mass, length, and width. Germination percentage correlated negatively with tendril basal length. The number of branches negatively correlated strongly with shoot fresh mass. The number of pistillate flowers per plot negatively correlated strongly with flower sex ratio.

3.3.11 Principal component analysis (PCA)

The first five informative principal components (PC1 – 5) were responsible for 74.4% cumulative variability, with each principal component having an eigenvalue greater than 1.0 (Table 3.6). The first principal component (PC1), with 26.6% of the total variation, was positively associated with shoot fresh mass, shoot dry mass, tendril basal length, tendril longer lobe length, tendril shorter lobe length, fruit mass, fruit width, seed width, 100 seed mass and total seed mass. However, it was negatively correlated with vine length and number of branches.

The second principal component (PC2), with 13.9% of the total variability, was positively correlated with cotyledonous leaf area and the number of pistillate flowers per plot. Fruit rind thickness was negatively correlated with PC2. The third principal component (PC3), responsible for 13.8% of the total variability, was positively associated with fruit yield per plot and fruit rind thickness but negatively correlated with tendril longer lobe length. Shoot moisture content, fruit length and pedicel length correlated positively with the fourth principal component (PC4), responsible for 11.5% of total variability. The number of branches and number of leaves correlated positively with the fifth principal component (PC5), responsible for 8.6% of the total variability.

Table 3.6: Loadings of the variables for the first five principal components

Variables	PC1	PC2	PC3	PC4	PC5
GP	-0.499	0.340	-0.107	0.316	-0.282
SH	0.049	0.563	0.309	0.121	0.120
CLA	-0.031	**0.843**	0.197	0.398	-0.071
SG	0.412	0.277	0.245	-0.214	-0.263
LG	0.215	-0.080	0.177	-0.428	0.034
VL	**-0.696**	0.101	0.065	0.384	0.349
NOB	**-0.620**	-0.201	0.087	0.064	**0.625**
NOL	-0.322	0.076	0.346	0.199	**0.673**
TCC	0.134	-0.243	0.464	-0.064	-0.497
SFM	**0.751**	0.522	0.107	-0.146	0.000
SDM	**0.685**	0.411	-0.026	-0.479	0.041
SMC	0.291	0.116	0.296	**0.630**	0.063
TBL	**0.752**	-0.018	-0.144	-0.346	0.316
TLL	**0.679**	0.060	**-0.627**	-0.047	0.172
TSHL	**0.608**	0.243	-0.464	0.198	0.266
NSF/P	0.082	0.532	0.301	-0.362	0.378
NPF/p	-0.060	**0.706**	0.542	-0.313	0.046
FSR	0.166	-0.473	-0.489	0.431	0.212
NF/p	-0.505	0.219	0.131	-0.392	0.552
FY/p	0.436	-0.076	**0.752**	0.091	0.176
FM	**0.713**	-0.264	0.481	0.258	-0.129
FL	0.490	0.294	0.135	**0.677**	-0.030
FW	**0.745**	-0.327	0.483	-0.038	-0.193
PL	-0.346	0.405	0.060	**0.641**	-0.196
FRT	-0.144	**-0.660**	0.613	-0.075	0.166
SL	0.524	0.274	-0.510	0.238	0.055
SW	**0.612**	0.082	-0.494	-0.094	0.220
100SM	**0.711**	-0.262	0.289	0.397	0.340
TSM	**0.820**	-0.321	0.178	0.278	0.298
Eigenvalue	7.724	4.035	3.999	3.325	2.490
Variability %	26.635	13.914	13.791	11.465	8.587
Cumulative %	26.635	40.550	54.340	65.806	74.393

PC1-5: Principal components 1-5. Values >0.6 are deemed to be significantly correlated. Variables- agronomic traits; GP-Germination percentage, CLA-Cotyledonous leaf area, SH-Seedling height, SG-Shoot growth and VL-Vine length, LG-Leaf growth , NOB- Number of branches, NOL-Number of leaves, TCC-Total chlorophyll content, SFM-Shoot fresh mass, SDM-Shoot dry mass, SMC-Shoot moisture content, TBL-Tendril basal length, TLL-Tendril longer lobe length, TSHL-Tendril shorter lobe length, NSF/p-Number of staminate flowers, NPF/p-Number of pistillate flowers, FSR-Flower sex ratio, NF/P-Number of fruits per plot, FY/P-Fruit yield per plot, FM-Fruit mass, FL-Fruit length, FW-Fruit width, PL-Pedicel length, FRT-Fruit rind thickness, SL-Seed length, SW-Seed width, 100SM-100 Seed mass and TSM-Total seed mass.

Based on the observations from the biplot, landraces with similar morphological attributes were grouped into three clusters, I, II, and III using PC1 and PC2 (Figure 3.3). The first cluster (I), included landraces NqRC, KSP, RSP, and RRP. Landrace

NqRC correlated positively with both PC1 and PC2. Landraces KSP, RSP, and RRP correlated positively with PC1 but negatively with PC2.
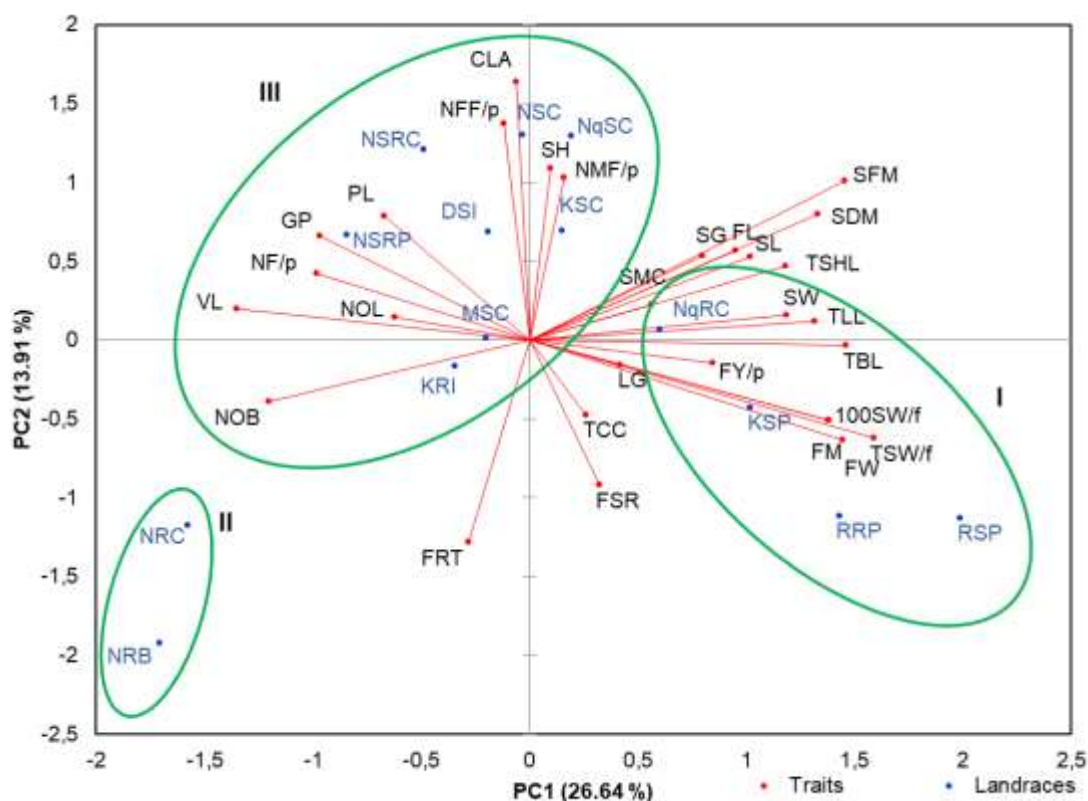
Figure 3.3: Biplot of *L. siceraria* landraces and agronomic traits.

The blue marker indicates landraces from Khangelani area with smooth, (KSP) pear-shaped; smooth, (KSC) curvilinear shape; and (KRI) rough isodiametric shape fruits; from Ndumo area (NRB) with rough fruits with brown seeds and (NRC) rough fruits with creamy seeds; from Mbazwana area (MSC) with smooth cylindrical-shape; from Rorke's Drift area (RSP) with smooth pear-shaped and (RRP) rough pear-shaped; from Nquthu area (NqRC) with rough curvilinear shape,(NSRP) with semi-rough pear-shaped, (NqSC) and (NSC) with smooth curvilinear shaped and (NSRC) semi-rough curvilinear shaped and from Dundee area (DSI) with smooth isodiametric shape. Red marker indicates traits GP-Germination percentage, CLA-Cotyledonous leaf area, SH-Seedling height, SG-Shoot growth and VL-Vine length, LG-Leaf growth , NOB- Number of branches, NOL-Number of leaves, TCC-Total chlorophyll content, SFM-Shoot fresh mass, SDM-Shoot dry mass, SMC-Shoot moisture content, TBL-Tendril basal length, TLL-Tendril longer lobe length, TSHL-Tendril shorter lobe length, NSF/p-Number of staminate flowers, NPF/p-Number of pistillate flowers, FSR-Flower sex ratio, NF/P-Number of fruits per plot, FY/P-Fruit yield per plot, FM-Fruit mass, FL-Fruit length, FW-Fruit width, FRT-Fruit rind thickness, PL-Pedicel length, SL-Seed length, SW-Seed width, 100SM-100 Seed mass and TSM-Total seed mass.

The second cluster (II), included landraces NRB and NRC, with both correlating negatively with PC1 and PC2. The third cluster (III) Included landraces KSC, NqSC, NSRC, NSC, DSI, NSRP, MSC and KRI. Landraces NqSC and KSC correlated positively with PC1 and PC2. Landraces NSRC, NSC, DSI, NSRP, and MSC

correlated positively with PC2 but negatively with PC1. Landrace KRI correlated negatively with both PC1 and PC2.

3.3.12 Cluster analysis

Hierarchical cluster analysis of landraces based on the similarities of agronomic traits was conducted using the complete linkage method and a dendrogram was constructed as shown in (Figure 3.4). The cluster analysis results agree with those displayed by the PC analysis (Figure 3.3). Landraces were assembled into two major clusters (Cluster-I and Cluster-II) at approximately 0.65 similarity.

Cluster-I consisted of landraces with smooth and rough pear-shaped fruits with NqRC relating with similar fruit and seed traits (KSP, RSP, RRP, and NqRC). These landraces associated with a high fruit yield per plot, heavier and wider fruits, and heavier seeds (Figure 3.4). It was further divided into minor clusters (M1 and M2). M1 consisted of KSP from Khangelani. M2 was further divided into sub-clusters (SC1 and SC2). SC1 grouped Rorke's Drift landraces with pear-shaped fruits, RSP and RRP. SC2 was made up of NqRC from Nquthu.

Cluster-II was further divided into minor-clusters (M3 and M4). M3 was divided into sub-clusters (SC3 and SC4). These landraces are associated with greater germination percentage, cotyledonous leaf area, vine length, number of leaves, number of pistillate flowers per plot, number of fruits per plot, and pedicel length. SC3 grouped landraces with a smooth fruit rind and predominantly curvilinear-shaped fruits. It was further divided into nano-clusters (N1 and N2), where N1 grouped landraces with smooth fruit rind; KSC from Khangelani, MSC Mbazwana and DSI from Dundee, N2 grouped NqSC and NSC from Nquthu with smooth curvilinear-shaped fruit. SC4 grouped landraces with rough fruit rinds and was further clustered into nano-clusters (N3 and N4). N3 was made up of KRI from Khangelani and N4 grouped NSRP and NSRC from the same origin, Nquthu. M4 grouped landraces from Ndumo with rough fruit rinds (NRB and NRC) (Figure 3.4).
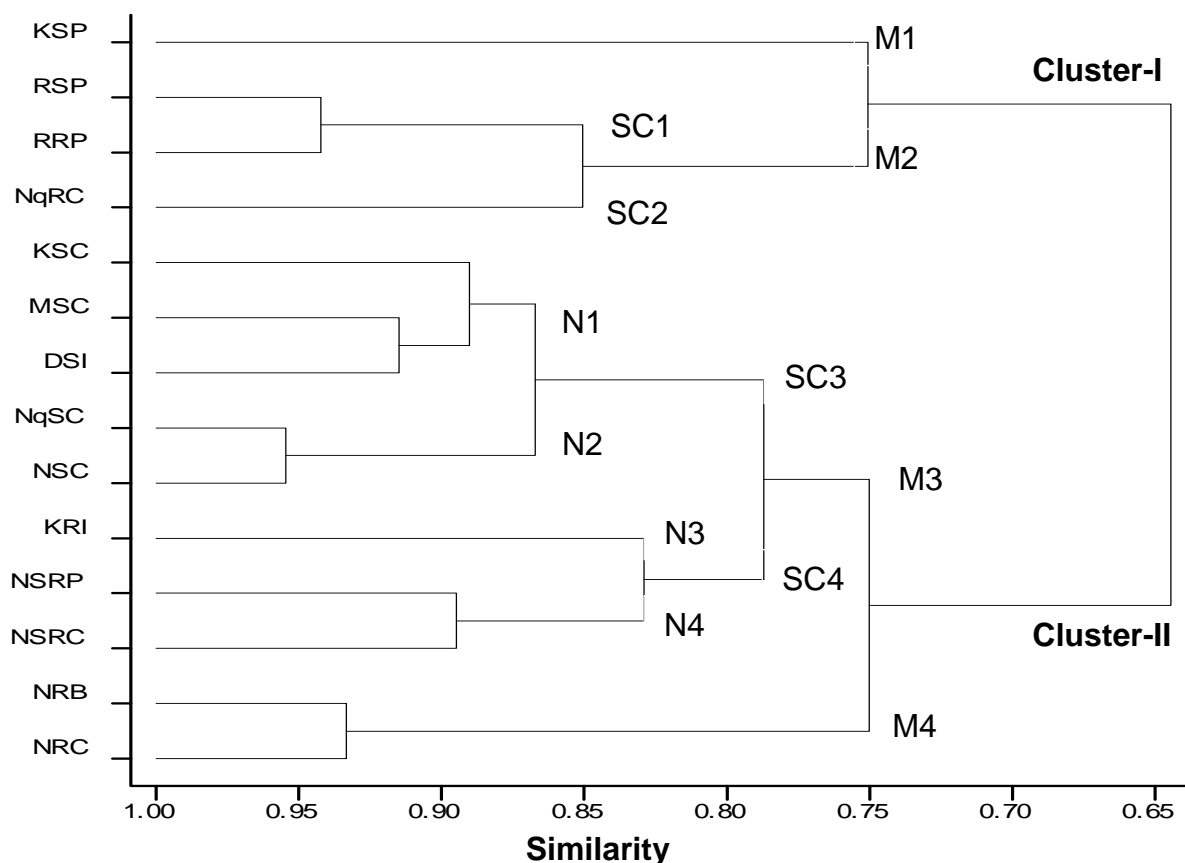
Figure 3.4: Hierarchical cluster showing similarities amongst *L. siceraria* landraces using the complete linkage method.

Landraces from Khangelani area with smooth, (KSP) pear-shaped; smooth, (KSC) curvilinear shape; and (KRI) rough isodiametric shape fruits; from Ndumo area (NRB) with rough fruits with brown seeds and (NRC) rough fruits with creamy seeds; from Mbazwana area (MSC) with smooth cylindrical-shape; from Rorke's Drift area (RSP) with smooth pear-shaped and (RRP) rough pear-shaped; from Nquthu area (NqRC) with rough curvilinear shape,(NSRP) with semi-rough pear-shaped, (NqSC) and (NSC) with smooth curvilinear shaped and (NSRC) semi-rough curvilinear shaped and from Dundee area (DSI) with smooth isodiametric shape.

3.3.13 Genetic parameters

Relatively high genotypic (δ^2g) and phenotypic (δ^2p) variances were obtained for the cotyledonous leaf area, shoot growth, leaf growth, tendril basal length, tendril longer lobe length, tendril shorter lobe length, and total seed mass per fruit. This observation indicates that the phenotypic degree of expression of these agronomic traits is reflective of the genotypic variation present, which is useful to breeders and farmers when implementing artificial selection based on phenotypic attributes. In this study the phenotypic coefficient of variation (PCV) differed significantly, ranging from 0.3% in the total chlorophyll content to 14959.7% for fruit yield per plot (Table 3.7).

Table 3.7: Genetic parameters for agronomic traits of *L. siceraria* landraces

Variables	δ^2g	δ^2e	δ^2p	GM	PCV	GCV	ECV%	H2	GA
GP	2.1	69.3	71.4	70.5	10.1	1.7	9.9	3.0	0.4
SH	46.1	24.1	70.2	37.4	13.7	11.1	8.0	65.7	1.7
CLA	72300	73251	145552	1289	106.3	74.9	75.4	49.7	1.5
SG	195.9	2319	2514.9	88.9	53.2	14.8	51.1	7.8	0.6
LG	3185028	3533042	6718070	5231	246.8	259.9	259.9	47.4	1.4
VL	0.3	0.3	0.6	2.9	4.6	3.3	3.2	52.2	1.5
NOB	2.1	0.5	2.6	6.1	6.6	5.8	2.9	79.6	1.8
NOL	52.2	19.8	72.0	29.7	15.6	13.3	8.2	72.5	1.8
TCC	7.8E-07	1.2E-05	1.3E-05	0.01	0.3	0.1	0.3	6.0	0.5
SFM	56.3	70.3	126.6	53.9	15.3	10.2	11.4	44.5	1.4
SDM	1.9	71.3	73.2	54.9	11.6	1.9	11.4	2.7	0.3
SMC	0.1	72.3	72.3	55.9	11.4	0.3	11.4	0.1	0.1
TBL	116.4	86.5	202.9	53.3	19.5	14.8	12.7	57.4	1.6
TLL	1140	1129	2269	211.2	32.8	23.2	23.1	50.2	1.5
TSHL	363.6	405.4	769.0	125.4	24.8	17.0	17.9	47.3	1.4
NSF/P	14.6	73.3	87.9	56.9	12.4	5.0	11.4	16.6	0.8
NFF/p	20.9	74.3	95.2	57.9	12.8	6.0	11.3	21.9	0.9
FSR	3.8	75.3	79.0	58.9	11.9	2.5	11.3	4.8	0.4
NF/p	2.3	76.3	78.6	59.9	11.5	1.9	11.3	2.9	0.4
FY/p	1.36E+08	77.3	1.36E+08	60.9	14959.7	14959.7	11.3	99.9	2.1
FM	2.0	0.2	2.2	2.0	10.5	10.0	2.95	91.9	2.0
FL	55.9	9.3	65.3	31.9	14.3	13.2	5.4	85.7	1.9
FW	37.8	2.6	40.4	24.3	12.9	12.5	3.2	93.7	2.0
PL	1.9	1.6	3.5	7.8	6.7	4.9	4.5	55.1	1.5
FRT	2.7	0.2	2.8	5.0	7.5	7.3	1.9	93.8	2.0
SL	4.9	0.9	5.8	17.6	5.7	5.3	2.3	84.1	1.9
SW	10.3	0.3	10.6	9.7	10.4	10.3	1.8	97.1	2.0
100SM	10.7	3.7	14.4	15.6	9.6	8.3	4.9	74.2	1.8
TSM	535.2	40.4	575.6	54.1	32.6	31.5	8.6	92.9	2.0

δ^2g-Genotypic variance, δ^2e-Environmental variance, δ^2p-Phenotypic variance, GCV-Genotypic coefficient of variation, PCV-Phenotypic coefficient of variation, H2-Broad sense heritability, GA-Genetic advance, Variables- agronomic traits; GP-Germination percentage, CLA-Cotyledonous leaf area, SH-Seedling height, SG-Shoot growth and VL-Vine length, LG-Leaf growth , NOB- Number of branches, NOL-Number of leaves, TCC-Total chlorophyll content, SFM-Shoot fresh mass, SDM-Shoot dry mass, SMC-Shoot moisture content, TBL-Tendril basal length, TLL-Tendril longer lobe length, TSHL-Tendril shorter lobe length, NSF/p-Number of staminate flowers, NPF/p-Number of pistillate flowers, FSR-Flower sex ratio, NF/P-Number of fruits per plot, FY/P-Fruit yield per plot, FM-Fruit mass, FL-Fruit length, FW-Fruit width, PL-Pedicel length, FRT-Fruit rind thickness, SL-Seed length, SW-Seed width, 100SW-100 Seed mass and TSW-Total seed mass.

The following traits had high (>20%) PCV and GCV values (Mlandenovic *et al.* 2011): cotyledonous leaf area; shoot growth; leaf growth; tendril longer lobe length; tendril shorter lobe length; total seed mass per fruit. The following traits had medium (10–20%) PCV and GCV (Mlandenovic *et al.* 2011): germination percentage; seedling height; number of leaves; shoot fresh mass; shoot dry mass; shoot moisture

content; tendril basal length; number of male flowers per plot; number of pistillate flowers per plot; flower sex ratio; number of fruits per plot; fruit mass; fruit length; seed width. All the remaining traits had low (<10%) phenotypic and genotypic coefficient of variation (Mlandenovic *et al.* 2011). This is indicative of the genetic variability among these traits which can be potentially subjected to manipulation and improvement upon selection (Jain *et al.* 2017).

The agronomic traits in this study were subjected to the estimate of heritability using the broad sense method. The heritability estimate ranged from 0.1% (shoot moisture content) to 99.9% (fruit yield). The highest heritability estimate values (>50%) were recorded, in their descending order, for: fruit yield; seed width; fruit rind thickness; fruit width; total seed mass per fruit; fruit mass; fruit length; seed length; number of branches; 100 seed mass; number of leaves; seedling height; tendril basal length; pedicel length; vine length; tendril longer lobe length.

The highest genetic advance was recorded for fruit yield per plot (2.1) while the lowest was for shoot moisture content (0.1). The following traits, in their descending order, had high genetic advance values (more than one): fruit mass; fruit width; fruit rind thickness; seed width; total seed mass per fruit; fruit length; number of branches; 100 seed mass per fruit; number of leaves; seedling height; tendril basal length; tendril longer lobe length; cotyledonous leaf area; vine length; pedicel length; leaf growth; shoot fresh mass; tendril shorter lobe length.

3.4 Discussion

3.4.1 Seedling traits

Germination percentage of the *L. siceraria* landraces ranged from 67.5 – 95%, with an average of 83%. This range was higher than that of 15 – 85% with an average of 47.2% recorded among *L. siceraria* landraces from Mashonaland east, Zimbabwe and northern KwaZulu-Natal, South Africa by Chimonyo and Modi (2013). This misalignment was probably caused by wide diversity in origin of landraces. All *L. siceraria* landraces in the current study originated from KwaZulu-Natal, but in the study by Chimonyo and Modi (2013), only two landraces were from northern

KwaZulu-Natal and the rest from Mashonaland east. A comparable study on *L. siceraria* landraces from New Delhi, India (Sharma and Tomar 2016) had a higher germination percentage mean (97.4) than that of the current study.

Landraces with similar fruit morphology but different origins differed in their germination percentage. This was evident in landraces with smooth pear-shaped fruits from Khangelani (KSP) and from Rorke's Drift (RSP), as well as smooth curvilinear-shaped fruits from Khangelani (KSC), Mbazwana (MSC) and Nquthu (NSC). Diversity in origin could be the result of wide differences recorded between the phenotypic and genotypic coefficient of variation for germination percentage (Table 3.7). Diverse environmental conditions play a significant role in the expression of traits through wide differences in phenotypic and genotypic coefficient of variation (Jain *et al.* 2017).

Variations were also recorded among landraces of the same origin with varying morphological features. These include landraces from Ndumo area with brown (NRB) and cream (NRC) seeds, as well as those from Nquthu area with semi-curvilinear (NqSC) and curvilinear (NSC) fruits. Similar results were obtained from landraces of Serbian origin with pear-shaped (LAG 74) and cylindrical-shaped (LAG 77) fruits (Mlandenovic *et al.* 2012).

Seedling height ranged from 22.5 – 48.8 mm, with a mean value of 37.4 mm. A similar seedling height range of 22 – 56 mm with an average of 39mm was reported from 182 accessions collected across seven provinces in Turkey (Yetisir *et al.* 2008). A comparable study with landraces from Abidjan, Côte d'Ivoire, had a longer seedling height, ranging from 66.85 – 81.68 mm for isodiametric shaped fruits and 57.67 – 80.72 mm for elongated to pear-shaped fruits (Yao *et al.* 2013). Cotyledonous leaf area ranged from 880 – 1726 mm^2 with an average of 1289 mm^2. *Lagenaria siceraria* accessions from Limpopo, South Africa had a lower cotyledonous leaf area compared to the current study, ranging from 418 – 1484.08 mm^2 with a mean of 868.2 mm^2 (Mashilo *et al.* 2016).

Landraces with larger and heavier seeds (KSP, KSC, RSP, NqSC, NRC, NSRP, NSRC and DSI) produced vigorously growing seedlings, possibly due to the larger

amount of starch and prolonged energy provision to the developing seedling. which increases its competitive ability and better early growth than landraces with smaller sized seeds. Similar findings on the seedling traits among *L. siceraria* landraces were noted where landraces with larger seeds produced the healthiest and most vigorously growing seedlings (Chimonyo and Modi 2013).

Curvilinear-shaped landraces (KSC, MSC, NqRC, NqSC, NSC and NSRC) were determined to be the overall best performing landraces in terms of the seedling traits, thus producing the most vigorously growing and healthy-looking seedlings. According to Koffi *et al.* (2009) the curvilinear shaped fruits are much more desired due to their vigorous growth habit, agronomic traits and flexibility in uses, traits which explain its popularity across different communities through preservation and artificial selection.

3.4.2 Shoot and leaf growth

The shoot growth of the current study ranged from 412 – 1256%, with an average growth of 889%. A study on *Citrullus lanatus* grafted onto the rootstock of *L. siceraria* obtained a lower shoot growth ranging from 180 – 500%, with an average of 332.5% (Yetisir *et al.* 2006). Landraces from northern KwaZulu-Natal, South Africa, propagated from a north to south direction had a leaf growth ranging from 1518 – 9566%, with an average of 5281%. A similar study with landraces from New Delhi, India had a lower leaf growth average of 298.9%, with plants propagated from a north to south and east to west direction (Sharma and Tomar 2016).

3.4.3 Vine length, number of branches and number of leaves

Vine length of landraces from the current study ranged from 2.3 – 4 m, with an average of 2.9 m. A comparable range of 2.8 – 3.2 m, with an average of 3.1 m was recorded among *L. siceraria* landraces from Africa, Asia, and America (Mlandenovic *et al.* 2012). Some of the genotypes used in the study were sourced from Africa. The seeds were germinated under similar temperatures and later transplanted onto the field with similar inter- and intra- plant row spacing and sample size. These factors arguably explain the similarities to the current study. Landraces from Limpopo, South

Africa recorded a higher range of 3.3 – 8.4 m, with a mean of 5.3 m (Mashilo *et al.* 2016). During the growing seasons (2013 – 2014) Limpopo received one of the highest rainfalls (627 mm) in history with an average temperature of 29°C (Manhique *et al.* 2015). These conditions are ideal for optimal growth, hence, the landraces from Limpopo were more vigorously growing compared to the ones in the current study with a lower rainfall range of 299.95 – 350.02 mm (Naidoo *et al.* 2016). A lower range of 1.4 – 3.4 m with an average of 2.2 m was also recorded among *L. siceraria* landraces from Jabalpur during the winter seasons, thus explaining the growth reduction as the *L. siceraria* is a summer crop (Sharma and Sengupta 2013).

Landraces with similar phenotypic traits and of different origins differed significantly, namely those with smooth curvilinear fruits from Khangelani (KSC) and from Nquthu (NqSC), as well as rough pear-shaped fruits from Nquthu (NSRP) and from Rorke's Drift (RRP). A similar outcome of diversity was recorded from *L. siceraria* genotypes with pear-shaped fruits from Serbia (LAG 65) and Nigeria (LAG 84) (Mlandenovic *et al.* 2012).

The number of branches ranged from 4.4 – 8.4, with an average of 6.1. As a result of a higher rainfall a higher range of 9.4 – 34.1, with a mean of 19.2 was recorded for landraces from Limpopo, South Africa (Mashilo *et al.* 2016). Landraces from Andhra Pradesh, India also had a higher range of 10.3 – 20.5, with a mean of 15.6 branches (Rani and Reddy 2017). The higher range could possibly result from the use of more purified genotype generations (F_3 generation) compared to the genotypes randomly collected from different origins in the current study. Landraces from West Bengal, India, recorded a lower number of branches than those of the current study, ranging from 3.9 – 7.8 with a mean of 5.2, possibly because they were not supplemented by any form of fertilizer (Rambabu *et al.* 2017).

Landraces of different origins with similar phenotypic attributes differed significantly in the number of branches. Landraces with smooth curvilinear-shaped fruits, namely KSC from Khangelani, MSC from Mbazwana as well as NqSC and NSC from Nquthu, differed significantly. A comparable trend to the current study was noted for genotypes Arka Bahar and Punjab Barkat with elongated straight shaped fruits from West Bengal, India (Rambabu *et al.* 2017).

The number of leaves ranged from 17.6 – 41.5, with an average of 29.7. *Citrullus lanatus* of the Cucurbitaceae family had a higher average leaf number of 36.33 than the crop of the current study at 45 days after planting. Possible reasons are that *C. lanatus* is from a different genus to *Lagenaria* and its seeds were subjected to mutagens for four hours prior to planting, thus explaining the high number of leaves. Mutagens alter the genetic make-up and enhance the performance of crops above natural background level (Rahaman *et al.* 2018).

3.4.4 Total chlorophyll content

The total chlorophyll content of landraces from KwaZulu-Natal measured with a spectrophotometer ranged from 0.0117 – 0.0166 g/mL, with an average of 0.0145 g/ml. A study on landraces from Arabhavi, India had a lower total chlorophyll content measured with a SPAD unit ranging from 0.46 – 2.39 mg cm^{-2} with a mean of 1.44 mg cm^{-2} (Koppad *et al.* 2016). That study used a non-destructive method compared to the destructive extraction from the current study, hence, the noted significant difference between the two.

3.4.5 Tendril traits

The tendril basal length of the current study ranged from 28.4 – 75 mm with a mean of 53.3 mm. Tendril longer lobe length ranged from 162.4 – 267.6 mm with a mean of 211.2 mm and tendril shorter lobe length ranged from 88.1 – 167.5 mm with a mean of 125.4 mm. A comparable study with landraces from India had a longer tendril length ranging from 190.3 – 364.4 mm, but the basal length and lobe length were combined (Deepthi *et al.* 2016). A similar study, also from India, had a combined tendril length ranging from 241.7 – 308.3 mm with a mean of 273.3 mm (Koppad *et al.* 2016).

3.4.6 Shoot fresh and shoot dry mass and shoot moisture content

Landraces from northern KwaZulu-Natal had a shoot fresh mass ranging from 35.1 – 59.5 g with a mean of 53.9 g. Shoot dry mass ranged from 8.1 – 12.5 g, with an

average of 10.8 g and the shoot moisture content ranged from 75.5 – 82.7%, with a mean of 79.6%. A comparable study with landraces from Dokki, Egypt had a higher shoot fresh mass average of 374.8 g, shoot dry mass average of 64.7 g, and a shoot moisture content of 82.7% (Mohammed *et al.* 2017). The landraces were grown under greenhouse conditions, in a controlled environment with enough nutrients and water for optimal growth, hence, the superior shoot fresh and dry mass and shoot moisture content (Mohammed *et al.* 2017). For the current study, apical shoots with three fresh leaves were used compared to the landraces from Egypt which used four fresh epical leaves, which explains the difference in shoot fresh and dry mass and shoot moisture content.

3.4.7 Number of staminate flowers, number of pistillate flowers and flower sex ratio

The number of staminate flowers from the current study with landraces from northern KwaZulu-Natal ranged from 7.7 – 21.0 with a mean of 12.9, while the number of pistillate flowers ranged from 7.0 – 21.0 with a mean of 13.2. A comparable study with landraces from Pradesh, India had a lower number of staminate flowers, ranging from 5.3 – 10.37 with a mean of 7.4 and pistillate flowers ranging from 6.2 – 15.6 with an average of 10.88 (Jain *et al.* 2017). The flower sex ratio of the current study ranged from 0.4 – 1.6 with a mean of 1.1. A study by Rani and Reddy (2017) on genotypes from Pradesh, India had a lower flower sex ratio than the current study ranging from 0.42 – 0.59 with a mean of 0.48. This difference could possibly have resulted from lower temperatures as the study was conducted in winter (Rani and Reddy 2017). Crops from the Cucurbitaceae family have better flower development in warmer seasons than in colder seasons where flower development is delayed and there is a high fruit abortion rate (Korkmaz and Dufault 2001). This explains why the flower sex ratio of the current study was higher as it was conducted during the warmer, more favourable seasons.

3.4.8 Fruit yield and agronomic traits at harvest

The fruit yield per plot for northern KwaZulu-Natal landraces ranged from 17111 – 82500 kg/ha with a mean of 43674 kg/ha. A similar study with landraces from

Pradesh, India had a higher fruit yield per plot ranging from 33100 – 82000 kg/ha with a mean of 52300 kg/ha (Uddin *et al.* 2014). The landraces from India were sown on bigger plots with a greater plant population of 20 plants per plot, which explains the higher yield per plot compared with the landraces from the current study with 16 plants per plot. The fruit mass in the current study ranged from 1.0 – 5.1 kg with an average of 2.0 kg. A range from 1.55 – 2.73 kg with an average of 2.0 kg was obtained in a similar study by Rani and Reddy (2017). The landraces were also grown using similar horticultural practices, hence the identical fruit mass means with the current study.

The fruit length of the current study ranged from 198.0 – 447.0 mm with a mean of 319.0 mm, fruit width ranged from 165.6 – 372.1 mm with a mean of 243.0 mm, pedicel length ranged from 59.3 – 115.3 mm with a mean of 78.0 mm, and fruit rind thickness ranged from 3.0 – 8.0 mm with a mean of 5.0 mm. A comparable study on *L. siceraria* landraces from Limpopo had shorter fruits ranging from 95.0 – 379.9 mm with a mean of 248.0 mm, narrower fruits at 65.9 – 131.2 mm with an average of 94.8 mm, shorter pedicels ranging from 8.4 – 57.8 mm with a mean of 29.5 mm, and thinner fruit rinds ranging from 1.2 – 4.3 mm with a mean of 2.5 mm (Mashilo *et al.* 2016). A similar study with landraces from Arabhavi, India had landraces with thinner fruit rinds ranging from 1.38 – 2.28 mm with a mean of 1.73 mm (Koppad *et al.* 2016).

Landraces from Limpopo had smaller seeds; hence, landraces with smaller seeds tend produce smaller fruits with vegetative structures that are highly branching with a high number of leaves (Koffi *et al.* 2009; Mashilo *et al.* 2016). This trend was noted during the current study. Landraces in the current study yielded bigger fruit, possibly due to the high rainfall received during the fruit sizing stage, genetic make-up, and environmental effects on the phenotypical attributes such fruit mass and size.

3.4.9 Seed size and mass

Seed length of the current study ranged from 14.0 – 23.0 mm with a mean of 17.6 mm. Seed width ranged from 4.0 – 14.9 mm with a mean of 9.7 mm, and 100 seed mass ranged from 11.6 – 21.6 g with a mean of 15.6 g. A similar study had shorter

seed length ranging from 11.3 – 18.8 mm with a mean of 14.4 mm, narrower seed width ranging from 5.0 – 8.7 mm with a mean of 6.7 mm, and lighter 100 seed mass ranging from 7.6 – 23.9 g with a mean of 13.2 g (Mashilo *et al.* 2016). Landraces with smaller seeds contain less starch than landraces with larger seeds, hence they have a smaller seed mass (Chimonyo and Modi 2013).

3.4.10 Correlation

Correlation measured the similarities and dissimilarities between variables, thus a correlation of (r ≥ 0.60 and r ≥ -0.60) was a strong correlation. This study simulated the studies conducted by Koffi *et al.* 2009 and Muralidharan *et al.* 2017. The number of branches correlated positively with vine length (r = 0.614), number of leaves (r = 0.678), and number of fruits per plot (r = 0.673) (Table 3.5). This suggests that the selection of genotypes with numerous branches will instinctively lead to genotypes with longer vines, many leaves and produce many fruits per plots. The number of branches of *L. siceraria* landraces from Gazipur, Bangladesh, also strongly correlated positively with the number of fruits per plot (r = 0.868) and total fruit yield (r = 0.833) (Sultana *et al.* 2018).

The number of staminate flowers per plot positively correlated with the number of pistillate flowers per plot (r= 0.781) and the number of fruits per plot (r = 0.613). The number of staminate flowers in a similar study on *Cucurbita maxima* from northern KwaZulu-Natal correlated negatively with the number of pistillate flowers (r = -0.371) (Ntuli *et al.* 2017).

Fruit yield per plot strongly correlated positively with fruit mass (r = 0.768) and fruit width (r = 0.702). A comparable study from Raipur, India, concurs with the current study's results as it had fruit mass strongly positively correlated with fruit yield per plot at r = 0.777 (Thakur *et al.* 2017). The landraces were grown during the Kharif season (July – October) which is the monsoon season, well known for high rainfall, which is relevant to the current study as the landraces from KwaZulu-Natal were grown during the summer seasons (October – January) with the highest rainfall of the year.

Hundred seed mass strongly correlated positively with the total seed mass (r = 0.966) and they both positively correlated strongly with the fruit traits of fruit mass (r = 0.780 and r = 0.799) and width (r = 0.665 and r = 0.732). Similar results were obtained for landraces studied in Serbia, with 100 seed mass positively correlating with; total seed mass (r = 0.49) and fruit mass (r = 0.53) (Mlandenovic *et al.* 2012). A similar study with accessions from Côte d'Ivoire had 100 seed mass positively correlating strongly with fruit mass (r = 0.794) (Koffi *et al.* 2009).

Hundred seed mass positively correlated with fruit width (r = 0. 358) in a comparative study with landraces from Gazipur, Bangladesh (Sultana *et al.* 2018). In a similar study on *Cucurbita maxima* landraces from northern KwaZulu-Natal, 100 seed mass strongly correlated positively with total seed mass (r = 0.985) (Ntuli *et al.* 2017).

3.4.11 Principal component analysis

The cumulative variance of 74.4% of the first five components with an eigenvalue of >1.0 is indicative of the amount of influence the identified traits within the designated components have on the phenotypic attributes of the landraces. The principal component biplot was constructed using the first two components, with a total cumulative variance of 40.6%, grouping landraces with similar morphological traits (Koffi *et al.* 2009) (Figure 3.3).

The principal component analysis identified diversity among landraces. It measured the effect each trait had on, or the contribution each made, towards the total cumulative variability (Rahajeng and Rahayuningsih 2017). A factor loading with a total value of ≥ 0.60 was deemed a significant contributor towards the total variability, positively or negatively, due to the high variability among *L. siceraria* genotypes (Yetisir *et al.* 2008 and Rahajeng and Rahayuningsih 2017).

Vine length and number of branches correlated negatively with PC1 in the current study but positively with PC1 in landraces from Limpopo (Mashilo *et al.* 2016). In the same study by Mashilo *et al.* (2016) staminate and pistillate flowers correlated positively with PC1, whereas in this study pistillate flowers positively correlated with PC2 and staminate flowers had insignificant correlations with all PCs. A positive

correlation of fruit width with PC1 in the current study was similar to that recorded in Serbia (Mlandenovic *et al.* 2012) and Kenya (Morimoto *et al.* 2005). Similar positive correlations were also recorded for seed length and seed width in PC1 (Morimoto *et al.* 2005) as in the present study.

3.4.12 Cluster analysis

Cluster-I in a biplot (Figure 3.3) and dendrogram (Figure 3.4) consisted of landraces with Asiatica type seeds that had pear-shaped (KSP, RSP, RRP) and curvilinear-shaped (NqRC) fruits that were either smooth or rough in texture (Table 3.1). The association of *L. siceraria* landraces according to their fruit shape and texture was also recorded in Kenya (Morimoto *et al.* 2005). A comparable study on Cucurbita landraces by Ntuli *et al.* (2017) clustered landraces according to their leaf and fruit colour. A similar study recorded similar results for landraces from India, with Pant Sankar-1, Pant Sankar-2, and Azad Kranti being grouped according to their fruit shape and fruit colour (Kalyanrao *et al.* 2016). These landraces were also associated with having longer tendril bases and lobes, higher fruit yield, heavier and wider fruits, as well as heavier and wider seeds. Landraces RSP and RRP had the longest tendril basal length and tendril longer lobe length while landrace NqRC had the longest tendril shorter lobe length (Table 3.3). These traits correlated positively with PC1, suggesting that they are amongst the most important traits contributing to the total variability (Table 3.6) (Morimoto *et al.* 2005; Mashilo *et al.* 2016).

Cluster-II was further divided into minor-clusters (M3 and M4). M3 was divided into sub-clusters (SC3 and SC4). These landraces are associated with greater germination percentage, cotyledonous leaf area, vine length, number of leaves, number of pistillate flowers per plot, number of fruits per plot and pedicel length. SC3 grouped vigorously growing landraces that have asiatica seed type, smooth fruit rind and dominantly curvilinear-shaped fruits (KSC, MSC, DSI, NqSC and NSC). Similar results were obtained in a study on *L. siceraria* landraces from Kenya, where landraces with the same seed type and fruit morphology were grouped together (Morimoto *et al.* 2005).

Landrace DSI had a superior germination percentage. Landrace KSC had superior seedling height (SH), shoot growth (SG), leaf growth (LG), shoot fresh mass (SFM) and shoot dry mass (SDM). SC3 was further clustered into nano-clusters (N1 and N2) where N1 grouped landraces with smooth fruit rinds from different origins (KSC from Khangelani, MSC from Mbazwana and DSI from Dundee) as well as, N2 grouped NqSC and NSC from the same origin (Nquthu) and same fruit shape (smooth curvilinear-shaped fruit). A similar study on landraces from Chimbwanda (18.19° S, 31.12° E), Mashonaland East, Zimbabwe and Richards Bay (28.19° S, 32.06° E), northern KwaZulu-Natal, South Africa yielded similar clustering of landraces by their origin and fruit morphology (Chimonyo and Modi 2013).

SC4 grouped landraces with rough fruit rinds (KRI, NSRP, NSRC, NRB, and NRC) and intermediate to siceraria seed type. These landraces were highly associated with cotyledonous leaf area (CLA), vine length (VL), number of branches (NOB), number of leaves (NOL), number of staminate flowers per plot (NSF/P), and number of fruits per plot (NF/P). SC4 further clustered into nano-clusters (N3 and N4). N3 represented KRI from Khangelani with isodiametric-shaped fruit while N4 grouped NSRP and NSRC from the same origin, Nquthu (Figure 3.4). Similar studies on the *L. siceraria* landraces had similar results to the current study with different landraces being clustered according to their morphological similarities and area of origin (Koffi *et al.* 2009; Mlandenovic *et al.* 2012). M4 grouped landraces from Ndumo with rough fruit rinds and siceraria type seeds (NRB and NRC). *Lagenaria siceraria* landraces of the same origin with smooth spherical-shaped fruits (S and R) clustered together (Chimonyo and Modi 2013).

3.4.13 Genetic parameters

For improved crop yield genetic improvement is necessary (Yadagiri *et al.* 2017). The background information regarding the expressiveness and size of genetic variation in respect of quantitative traits like yield and its components is essential for executing crop yield improvement (Jha *et al.* 2017). The estimates of mean serve as a basis for eliminating undesirable crosses, whereas genetic variability, GCV, and PCV help to select a potential cross with the traits desirable to breeders and farmers (Yadagiri *et al.* 2017). The size of heritable estimate in breeding material, more

particularly its genetic component, has an immense effect on fixation of a trait and helps in the identification of a genotype by its phenotypic expression (Yadagiri *et al.* 2017).

Genetic advance refers to the improvement in the mean genotypic value of a selected germplasm over parental material. High genetic advance, along with high heritability, indicates that improvement could be made for such characteristics by artificial selection, based on the desirable phenotypic display (Jain *et al.* 2017). The genetic parameters may enable breeders to plan proper breeding programmes and can also make valuable recommendations for future studies.

In general, the phenotypic coefficient was higher than the genotypic coefficient of variability, indicating the strong influence of the environment on the expressiveness of genes in the phenotypic display. Similar findings were recorded in landraces from Pradesh, India (Jain *et al.* 2017). A high to moderate phenotypic coefficient of variation was recorded in the current study for fruit yield per plot (14959.7%), leaf growth (246.8%), cotyledonous leaf area (106.3%), tendril longer lobe length (32.8%) and total seed mass (32.6%). Similar results were recorded on *L. siceraria* landraces from India, where fruit yield and fruit mass had higher phenotypic coefficients (373.29% and 1303.33% respectively) (Singh *et al.* 2014). In another comparable study with landraces from India, high phenotypic coefficients were recorded for yield and fruit mass (71.79% and 39.35% respectively) (Yadagiri *et al.* 2017).

The highest value for broad sense estimate was recorded for the following traits: fruit yield per plot, seed width, fruit rind thickness, fruit width, total seed mass, fruit mass, fruit length, seed length, number of branches, 100 seed mass and number of leaves. Landraces from India had similar broad sense heritability results for traits such as fruit mass (96.20%), fruit width (92.20%), fruit yield (89.60%) and vine length (75. 20%) (Singh *et al.* 2014). Similar findings of high heritability for fruit weight (99.10%), vine length (97.40%) and fruit length (91.40%) were recorded by Yadagiri *et al.* (2017).

The genetic advance of the current study was lower for all traits when compared to studies conducted by Jain *et al.* (2017) and Yadagiri *et al.* (2017) such as vine length

(24.82%), number of branches (30.79%), fruit length (75.27%), fruit width (59.15%), fruit mass (68.00%) and fruit yield (71.10%). The current study had higher genetic advancement compared to genotypes from Pradesh, India in a similar study with; flower sex ratio (0.08), fruit mass (0.61), 100 seed mass (0.66) and yield (1.50) (Rani and Reddy 2017). *L. siceraria* genotypes from Pradesh, India recorded a higher genetic advance for the number of branches and vine length, 5.93 and 4.35 respectively, compared to the current study (Rani and Reddy 2017).

3.5 Conclusion

Landraces DSI, KSP and NSRC had the highest germination percentage, tallest seedlings and broadest cotyledonous leaves, respectively. In vegetative traits, landraces KSC and RSP had the most vigorously growing shoots and leaves, numerous leaves, higher total chlorophyll content, and longest tendrils as well as heavy fresh and dry shoots. In reproductive traits, landraces KRI and RSP had numerous flowers and fruits, high fruit yield, and heavier and wider fruits with thicker rind as well as heavier seeds. Most traits correlated positively with each other, except for few negative correlations. In principal component analysis, most of the morphological traits were positively associated with five informative principal components. The biplot and dendrogram clustered landraces according to their fruit and seed morphology, and then the area of origin. Among genetic parameters, the phenotypic coefficient of variation was higher than the genotypic coefficient variation for most traits. The heritability and genetic advance was significantly high for fruit and seed morphology.

Chapter 4

Nutritional variability among *L. siceraria* landraces from northern KwaZulu-Natal

4.1 Introduction

Lagenaria siceraria of the Cucurbitaceae family is one of the species that are well known for their nutritional content and economical importance (Koffi *et al.* 2009; Ntuli *et al.* 2017). The area of origin, and abiotic factors such as soil type and fertility have significant effects on the mineral content of different cucurbits (Abbey *et al.* 2017). *L. siceraria* is one of the oldest cultigens to be collected, utilised and preserved by mankind, dating back an estimated 12 000 years (Sithole *et al.* 2015). It is regarded as the poor man's crop due to its ability to tolerate and thrive under stressful conditions (Mlandenovic *et al.* 2012; Koffi *et al.* 2009). This species is among the main sources of nutrients and mineral elements for rural communities (Sithole *et al.* 2015).

L. siceraria is neglected in the face of modern agronomic improvements even though it is endowed with essential nutrients and mineral elements to meet the necessary dietary requirements of the average human being (Sithole *et al.* 2015). *L. siceraria* has great potential as most of its plant parts can be of culinary use (Koffi *et al.* 2009). Juvenile and mature fruits, seeds, flowers, leaves, and shoot tips can be consumed as a vegetable relish (Sithole *et al.* 2016).

The fruits of *L. siceraria* accessions contain essential nutrients such as moisture content (90.25%), ash (1.03%), protein (1.25%), lipids (0.12%) and carbohydrates (2.65%) (Aliu *et al.* 2012). It has neutral detergent fibre (NDF) of 21.16 g/100g, acid detergent fibre (ADF) of 15.67 g/100g, and vitamin C of 10.1 mg/100g (Upaganlawar and Balaraman 2009). *L. siceraria* fruits also contain essential mineral elements (mg/100g) such as iron (5.129), copper (0.057), silver (0.682), manganese (0.318), zinc (1.629), cobalt (0.137) and lead (0.5) (Ahmed *et al.* 2016). *Lagenaria siceraria*

also contain phosphorus (187.33), potassium (3356.67), magnesium (146.33), calcium (52.78) and sodium (36.68) (Upaganlawar and Balaraman 2009).

Variability is an essential factor in crop enhancement and characterisation of variability of desired traits is a must for obtaining access to significant alleles for genetic enhancement in nutritional traits (Nagar *et al.* 2018). All traits expressions depend on the genotype and environment, hence the genotypic and phenotypic coefficient of variability was determined for all traits studied (Nagar *et al.* 2018). The heritability estimates and genetic advancements were determined in all traits to establish any possible genetic improvement upon selection of desired nutritional traits (Nagar *et al.* 2018).

Although the morphological variation of *L. siceraria* landraces is receiving some attention from researchers in South Africa, including northern KwaZulu-Natal, the nutritional and mineral element content of these landraces has never been documented. Hence, a study on the nutritional and mineral element content across various genotypes of the *L. siceraria* will be beneficial in selecting parental material for future breeding programmes of these landraces. Therefore this study aimed to determine variability in nutritional and mineral content among *L. siceraria* landraces.

4.2 Materials and Methods

4.2.1 Preparation of samples and determination of mineral and nutrient content

Mature fruits of *Lagenaria siceraria* were collected at 125 days after transplant (DAT) from the same trials as reported in chapter 3. Forty-two fruit samples were collected representing fourteen landraces with three replicates per landrace. Fruits were rinsed with tap water and cut into smaller portions using a clean stainless-steel knife. Fruit pulp was removed from the fruit rind, separated from the seeds, sun-dried for 24 hours and then oven dried at 60°C for 24 hours (Labcon incubator, Model 5016LC). The samples were ground into powder through a 0.84 mm sieve, using a laboratory grinder (Hammer mill SMC).

Milled fruit pulp of *L. siceraria* landraces was assayed for moisture content, ash, lipids, crude protein and fibre according to the Association of Official Analytical Chemists (2000) methods. It was also analysed for both macronutrients (nitrogen (N), phosphorus (P), calcium (Ca), potassium (K), and magnesium (Mg)), and micronutrients (sodium (Na), zinc (Zn), copper (Cu), manganese (Mn), iron (Fe), aluminium (Al) and boron (B) by the Soil Fertility and Analytical Services Laboratory (Manson and Roberts 2000). Sub-samples of fruit pulp material were dried and ashed at 450°C overnight. The ash was dissolved in 1 M HCl. The supernatant was analysed for Al, Ca, Cu, K, Mg, Mn, Na, and Zn by atomic absorption spectroscopy (AAS).

Phosphorus concentrations were determined colorimetrically on a 2 mL aliquot of filtrate using a modification of the Murphy and Riley (1962) molybdenum blue procedure (Hunter 1974). Potassium was determined from the extract directly on a flame photometer. Samples for boron (B) analysis were ashed separately, and boron was determined photometrically by the azomethine H method (Gaines and Mitchell 1979).

4.2.2 Data analysis

Data were subjected to ANOVA using the GenStat 15th edition. ANOVA was combined for both growing seasons. Means were separated using Tukey's LSD at 5% significant level. Correlations and principal component analysis (PCA) were implemented to determine multi-character variation. Cluster analysis through biplot and dendrogram was conducted to study the similarities and dissimilarities of closely related landraces.

4.2.3 Estimation of variance components

The phenotypic, genotypic and environmental variances and coefficient of variation were calculated according to the formula described by Burto and Devane (1953) and cited by Singh *et al.* (2017) as follows:

Environmental variance ($\delta^2 e$) = MSE

Genotypic variance (δ^2g) = $-\dfrac{\text{MSG}-\text{MSE}}{r}$

Phenotypic variance (δ^2p) = δ^2g + δ^2e

where MSG is mean square due to genotype, MSE is mean square of error (environmental variance) and (*r*) is the number of replications.

Phenotypic coefficient of variation (PCV) = $\sqrt{\dfrac{\delta^2 p}{x}} \times 100$

Genotypic coefficient of variation (GCV) = $\sqrt{\dfrac{\delta^2 g}{x}} \times 100$

where

δ^2p = phenotypic variation

δ^2g = genotypic variation

X = grand mean of the character studied.

Estimation of heritability in broad sense: Broad sense heritability (h^2) expressed as the percentage of the ratio of the genotypic variance (δ^2g) to the phenotypic variance (δ^2p), according to Allard (1960), was calculated with the following formula:

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

Genetic advance (GA) was estimated as per formula given by Allard (1960) and cited by Meena *et al.* 2015.

$GA = k \times \sqrt{\dfrac{\delta^2 p \times \delta^2 g}{\delta^2 p}}$

where:

GA = expected genetic advance

δ^2p = phenotypic variation

δ^2g = genotypic variation

k= the standard selection differential at 5% selection intensity (k = 2.063).

4.3 Results

There were significant (P<0.05) differences among the *L. siceraria* landraces studied from northern KwaZulu-Natal with regards to proximate and mineral nutrient composition (Table 4.1 and 4.2).

4.3.1 Proximate composition of *L. siceraria* landraces

(a) Ash content

Landrace DSI had the highest ash content of 32.70 g/100g and landrace NRB had the lowest at 7.53 g/100g, with an average ash content of 19.68 g/100g (Table 4.1). Landrace KSC from Khangelani area recorded a higher ash content than MSC from Mbazwana area with both landraces having smooth curvilinear-shaped fruits. Significant variations were expressed among landraces of the same origin. Rorke's Drift area landraces with pear-shaped fruits varied, where landrace RSP had higher ash content than landrace RRP. In landraces from Nquthu area with curvilinear-shaped fruits landrace NSRC recorded higher ash content than landrace NSC.

(b) Fat content

Landrace KSP produces the highest fat content (2.41 g/100g), while landrace NSC recorded the lowest (0.19 g/100g), with the mean of 0.98 g/100g (Table 4.1). Curvilinear-shaped landraces with different origins varied in their fat content. Landrace KSC from Khangelani area had a superior fat content to landrace NSC from Nquthu area. Further, landrace NqSC from Nquthu area had a higher fat content than landrace MSC from Mbazwana area.

Landraces from Khangelani area showed variations amongst each other, where landrace KSP had significantly higher fat content than both KSI and KSC landraces. Again, landraces from Nquthu area with smooth curvilinear-shaped landraces also varied, as landrace NqSC had superior fat content to landrace NSC.

Table 4.1: Proximate composition of *L. siceraria* landraces (g/100g)

LR	Ash	Fat	ADF	NDF	Pro
KSP	26.62 abc	2.41 a	30.34 a	39.51 a	9.20 a
KSC	29.43 ab	1.19 bcd	24.25 b	28.72 cde	5.36 e
KRI	23.76 abc	1.50 b	23.55 b	31.08 cd	5.91 de
NRB	7.53 f	1.00 bcde	17.96 cdef	21.94 fg	7.32 bc
NRC	12.73 def	1.35 bc	19.38 cde	23.40 efg	9.62 a
MSC	13.29 def	0.87 cdef	15.35 f	20.11 g	7.09 bc
RSP	21.16 bcd	0.44 fg	23.46 b	27.77 def	6.62 cd
RRP	11.51 ef	0.43 fg	17.79 cdef	21.60 g	6.57 cd
NqRC	20.86 bcd	0.79 def	19.42 cd	22.94 efg	5.38 e
NSRP	18.17 cde	0.36 fg	15.80 def	21.91 fg	7.02 bc
NqSC	19.48 cde	1.50 b	20.87 bc	32.35 bcd	7.09 bc
NSC	13.02 def	0.19 g	15.68 ef	19.17 g	6.76 bcd
NSRC	25.22 abc	0.53 efg	28.09 a	37.25 ab	5.69 de
DSI	32.70 a	1.23 bcd	28.08 a	33.74 abc	7.81 b
Mean	19.68	0.984	21.43	27.25	6.961
P value	<.001	<.001	<.001	<.001	<.001
LSD	9.221	0.514	3.731	5.955	1.079
CV%	28.0	31.2	10.4	13.1	9.3

LR- landraces; – from Khangelani area with smooth, (KSP) pear-shaped; smooth, (KSC) curvilinear shape; and (KRI) rough isodiametric shape fruits; from Ndumo area (NRB) with rough fruits with brown seeds and (NRC) rough fruits with creamy seeds; from Mbazwana area (MSC) with smooth cylindrical-shape; from Rorke's Drift area (RSP) with smooth pear-shaped and (RRP) rough pear-shaped; from Nquthu area (NqRC) with rough curvilinear shape,(NSRP) with semi-rough pear-shaped, (NqSC) and (NSC) with smooth curvilinear shaped and (NSRC) semi-rough curvilinear shaped and from Dundee area (DSI) with smooth isodiametric shape. ADF- acid detergent fibre, NDF- neutral detergent fibre and Pro-protein. Mean values in the same column with different letter (s), differ significantly at p<0.05 according to Tukey's LSD.

(c) Acid detergent fibre content

Landrace KSP had the highest ADF content of 30.34 g/100g, while landrace MSC had the lowest at 15.35 g/100g, with an average of 21.43 g/100g. Landrace KSP from Khangelani had a greater ADF content than landrace RSP from Rorke's Drift, with both landraces having smooth pear-shaped fruits. Smooth curvilinear-shaped landraces differed with KSC from Khangelani, recording a higher ADF content than NSC from Nquthu. Landrace NqSC from Nquthu and MSC from Mbazwana both displayed smooth curvilinear-shaped fruits. Landrace NqSC had a superior ADF content to MSC.

Landraces from Khangelani varied significantly as KSP recorded higher ADF content than both KSC and KRI. Landraces RSP and RRP from Rorke's Drift with pear-shaped fruits differed, with RSP having higher ADF content. Landraces from Nquthu with semi-rough to rough curvilinear-shaped fruits varied, with NSRC recording a higher ADF content than NqRC.

(d) Neutral detergent fibre content

Landrace KSP documented the highest NDF content (39.51 g/100g), but NSC had the lowest (19.17 g/100g), with a mean content of 27.25 g/100g. Smooth pear-shaped landraces differed; KSP from Khangelani had higher NDF content than RSP from Rorke's Drift. Landraces with smooth curvilinear-shaped fruits differed in their NDF content. Landraces NqSC from Nquthu and KSC from Khangelani had higher NDF content than MSC from Mbazwana and NSC from Nquthu.

Landraces from Khangelani area also varied, with KSP having higher NDF content than KSC and KRI. Landraces from Rorke's Drift with pear-shaped fruits differed as RSP recorded more NDF content than RRP. The NDF content of landraces from Nquthu with semi-rough to rough curvilinear-shaped fruits varied, landrace NSRC had a superior NDF content than NqRC. Also, in smooth curvilinear-shaped landraces from Nquthu, landrace NqSC recorded higher NDF content than NSC.

(e) Protein content

Landrace NRC recorded the highest protein content (9.62 g/100g); KSC had the lowest (5.36 g/100g), with an average of 6.96 g/100g. Smooth pear-shaped landraces varied in their protein content. Landrace KSP from Khangelani recorded higher protein content than RSP from Rorke's Drift. Smooth curvilinear-shaped landraces of different origins varied. Landraces NqSC and NSC from Nquthu and MSC from Mbazwana had higher protein content than KSC from Khangelani.

Khangelani landraces varied, with KSP having a superior protein content to KSC and KRI. Variations among Ndumo landraces were noted. NRC recorded a higher protein

content than NRB. Nquthu landraces also displayed variations. Landrace NSRP, NqSC and NSC registered a higher protein content than NqRC and NSRC.

4.3.2 Mineral element composition of *L. siceraria* landraces

(a) Calcium

Landrace KSP outperformed all landraces, with 0.44 g/100g of Ca; NSRP recorded the lowest at 0.13 g/100g, with an average of 0.26 g/100g (Table 4.2). Landrace KSP from Khangelani and RSP from Rorke's Drift, both displaying smooth pear-shaped fruits, varied. KSP had higher Ca content than RSP. Smooth curvilinear-shaped landraces differed in their Ca content. Landrace NqSC from Nquthu recorded higher Ca content than both KSC from Khangelani and MSC from Mbazwana.

Khangelani landraces differed; KSP produced higher Ca content than both KSC and KRI. In Rorke's Drift landraces with pear-shaped fruit, RSP outperformed RRP in terms of Ca content. Again, in Nquthu landraces with semi-rough to rough curvilinear-shaped fruits, NSRC outperformed NqRC.

(b) Magnesium

The highest magnesium (Mg) content was noted for landrace KSP at 0.22 g/100g and the lowest was in NqRC and NSRP at 0.08 g/100g, with an average of 0.14 g/100g. Variations in Mg content were noted among smooth pear-shaped landraces, where KSP from Khangelani outperformed RSP from Rorke's Drift.

Table 4.2: Mineral element traits of _L. siceraria_ landraces

	Macronutrient content (g/100g)					Micronutrient content (mg kg^{-1})						
LR	**Ca**	**Mg**	**K**	**P**	**N**	**Na**	**Mn**	**Fe**	**Zn**	**Cu**	**Al**	**B**
KSP	0.44 a	0.22 a	8.62 ab	0.29 a	1.47 a	0.11 a	17.33 abc	106.70 cde	0.00 d	10.67 a	38.93 cde	38.32 a
KSC	0.25 def	0.16 bc	7.83 abc	0.18 cde	0.85 e	0.06 cde	18.33 ab	169.70 bc	82.67 ab	6.33 bc	43.87 c	35.86 ab
KRI	0.21 ef	0.19 ab	7.04 bcd	0.08 g	0.94 de	0.08 bc	11.67 ef	102.70 cde	0.00 d	4.67 bcde	41.87 cd	31.73 b
NRB	0.21 ef	0.15 bcd	2.80 f	0.24 abc	1.17 bc	0.05 def	16.00 abcd	244.00 b	9.67 d	5.67 bc	119.85 b	20.63 c
NRC	0.28 de	0.19 ab	3.83 ef	0.26 ab	1.53 a	0.04 ef	19.00 a	828.70 a	57.00 bc	7.00 b	525.53 a	19.84 c
MSC	0.25 def	0.09 ef	3.78 ef	0.15 ef	1.13 bc	0.05 def	13.67 cdef	86.30 cde	31.33 cd	1.33 fg	34.42 cdef	19.47 c
RSP	0.27 def	0.11 def	5.64 cde	0.16 def	1.05 cd	0.04 ef	9.67 f	29.70 de	21.00 cd	2.67 defg	20.97 cdef	22.53 c
RRP	0.20 fg	0.13 cde	3.69 ef	0.21 bcd	1.05 cd	0.03 f	14.33 bcde	33.70 de	24.67 cd	4.33 bcde	11.01 f	21.68 c
NqRC	0.19 fg	0.08 f	4.13 ef	0.14 efg	0.86 e	0.06 de	10.33 ef	35.30 de	18.33 cd	4.33 bcde	19.47 def	18.80 c
NSRP	0.13 g	0.08 f	3.53 ef	0.14 efg	1.12 bc	0.03 f	12.00 def	37.30 de	22.33 cd	0.33 g	16.05 ef	20.13 c
NqSC	0.35 bc	0.12 cdef	4.81 def	0.15 ef	1.13 bc	0.07 cd	18.00 ab	23.70 e	24.33 cd	2.00 efg	11.09 f	20.41 c
NSC	0.30 cd	0.11 def	3.56 ef	0.17 de	1.08 bcd	0.05 def	18.33 ab	43.30 de	30.33 cd	5.67 bc	11.23 f	20.22 c
NSRC	0.38 ab	0.17 bc	8.21 ab	0.08 g	0.91 de	0.10 ab	12.00 def	131.70 cd	120.33 a	4.00 cdef	39.74 cd	37.22 a
DSI	0.22 ef	0.18 ab	9.69 a	0.11 fgh	1.25 b	0.08 bc	11.33 ef	31.00 de	116.00 a	5.33 bcd	36.82 cde	34.47 ab
Mean	0.26	0.14	5.51	0.16	1.114	0.06	14.43	136.00	39.90	4.60	69.30	25.81
P value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
LSD	0.074	0.043	2.238	0.058	0.172	0.026	4.048	107.15	39.71	2.920	23.43	5.022
CV%	16.8	18.4	24.3	20.9	9.3	24.6	16.8	47.1	59.5	38.0	20.2	11.6

LR-landraces; from Khangelani area with smooth, (KSP) pear-shaped; smooth, (KSC) curvilinear shape; and (KRI) rough isodiametric shape fruits; from Ndumo area (NRB) with rough fruits with brown seeds and (NRC) rough fruits with creamy seeds; from Mbazwana area (MSC) with smooth cylindrical-shape; from Rorke's Drift area (RSP) with smooth pear-shaped and (RRP) rough pear-shaped; from Nquthu area (NqRC) with rough curvilinear shape (NSRP) with semi-rough pear-shaped, (NqSC) and (NSC) with smooth curvilinear shaped and (NSRC) semi-rough curvilinear shaped and from Dundee area (DSI) with smooth isodiametric shape. Ca-calcium; Mg-magnesium; K-potassium; Na-sodium; P-phosphorus; N-nitrogen; Mn-manganese; Fe-iron; Zn-zinc; Cu-copper, Al-aluminium and B-boron. Mean values with different letter (s) within the same column are significantly different at p<0.05 according to Tukey's LSD.

In landraces with smooth curvilinear-shaped fruits, KSC from Khangelani outperformed MSC from Mbazwana as well as NqSC and NSC from Nquthu. In landraces from Khangelani with smooth textured fruits, landrace KSP recorded a superior Mg content to KSC. Nquthu area landraces with semi-rough to rough curvilinear-shaped fruit varied, with landrace NSRC documenting higher Mg content than NqRC. Landrace RRP from Rorke's Drift recorded a higher Mg content than NSRP from Nquthu, but both had semi-rough to rough pear-shaped fruits.

(c) Potassium

The highest potassium (K) content was noted for landrace DSI at 9.69 g/100g and NRB had the lowest at 2.80 g/100g, with an average of 5.51 g/100g. In smooth pear-shaped landraces, RSP from Rorke's Drift recorded a higher K content than KSP from Khangelani. Also in smooth curvilinear-shaped landraces, KSC from Khangelani had higher K content than MSC from Mbazwana. Landrace NSRC from Nquthu area outperformed all other landraces from the same area in terms of K content.

(d) Sodium

Landrace KSP recorded the highest sodium (Na) content (0.11 mg kg^{-1}) but RRP and NSRP recorded the lowest (0.03 mg kg^{-1}), with an average of 0.06 mg kg^{-1}. Landrace KSP from Khangelani had a higher Na content than RSP from Rorke's Drift, with both landraces having smooth pear-shaped fruits.

Landraces from Khangelani expressed diversity, with KSP recording a Na content superior to both KSC and KRI. Semi-rough to rough curvilinear-shaped landraces from Nquthu varied, with NSRC documenting a higher Na content than NqRC.

(e) Phosphorus

Landrace KSP outperformed all landraces with 0.29 g/100g of phosphorus (P); KRI had the least at 0.08 g/100g, with a mean of 0.17 g/100g. Diversity was noted in smooth pear-shaped landraces, where KSP from Khangelani had higher P content than RSP from Rorke's Drift. In semi-rough to rough pear-shaped landraces, RRP from Rorke's Drift recorded a higher P content than NSRP from Nquthu.

(f) Nitrogen

Landrace NRC recorded the highest nitrogen (N) content at 1.53 g/100g while KSC had the lowest at 0.85 g/100g, with an average of 1.11 g/100g. Among smooth pear-shaped landraces, KSP from Khangelani had higher N content than RSP from Rorke's Drift. In smooth curvilinear-shaped landraces, MSC from Mbazwana, NqSC and NSC from Nquthu recorded a higher N content than KSC from Khangelani. Khangelani landraces differed, wherewith KSP recording a superior N content to KSC and KRI. Diversity among Ndumo landraces was noted, with NRC having a higher N content than NRB. Landraces from Nquthu also expressed variations, where NSRP and NSRC with semi-rough fruits documented a superior N content to NqRC with a rough fruit rind.

(g) Manganese

Landrace NRC had the highest manganese (Mn) content at 19.00 mg kg^{-1} and RSP had the lowest at 9.67 mg kg^{-1} with an average of 14.43 mg kg^{-1}. Smooth pear-shaped landraces expressed diversity in their Mn content. Landrace KSP from Khangelani had a higher Mn content than RSP from Rorke's Drift. Smooth curvilinear-shaped landraces varied in their Mn content. KSP from Khangelani, NqSC and NSC from Nquthu recorded a higher Mn content than MSC from Mbazwana. Landraces from Khangelani expressed dissimilarity; KSP and KSC recorded a superior Mn content to KRI. Rorke's Drift landraces showed diversity as RRP recorded a higher Mn content than RSP. Nquthu landraces also expressed variation in their Mn content. Landraces with smooth fruits (NqSC and NSC)

documented a higher Mn content than those with semi-rough to rough fruits (NSRP, NSRC and NqRC).

(h) Iron

Landrace NRC had the highest Iron (Fe) content at 828.70 mg kg^{-1} while NqSC recorded the lowest at 23.70 mg kg^{-1} with an average of 136.0 mg kg^{-1}. Landraces with smooth curvilinear-shaped fruits varied in their Fe content. KSC from Khangelani documented a higher Fe content than NqSC and NSC from Nquthu. Ndumo area landraces expressed diversity, with NRC recording a superior Fe content than NRB. Landraces from Nquthu with semi-rough curvilinear-shaped fruit (NSRC) and smooth semi-curvilinear-shaped fruit (NqSC) differed. NSRC noted a higher Fe content than NqSC.

(i) Zinc

Landrace NSRC recorded the highest Zinc (Zn) content at 120.33 mg kg^{-1}, KSP and KRI both had the lowest at 0 mg kg^{-1} of Zn, the average content being 39.9 mg kg^{-1}. With regard to landraces with smooth curvilinear-shaped fruits KSC from Khangelani, MSC from Mbazwana, and NqSC and NSC from Nquthu differed. Landrace KSC outperformed MSC, NqSC and NSC with a higher Zn content. Variations among landraces from Khangelani were noted. KSC recorded a superior Zn content to KSP and KRI. Ndumo area landraces also differed with NRC, recording a higher Zn content than NRB.

(j) Copper

KSP outperformed all landraces, recording 10.67 mg kg^{-1} of copper (Cu) content while NSRP recorded the lowest at 0.33 mg kg^{-1} with the mean of 4.60 mg kg^{-1}. Variations were noted in smooth pear-shaped landraces. KSP from Khangelani had a higher Cu content than RSP from Rorke's Drift. Smooth curvilinear-shaped landraces showed diversity in their Cu content. KSC from Khangelani and NSC from Nquthu recorded a higher Cu content than MSC from Mbazwana and NqSC from Nquthu. Semi-rough to rough pear-shaped landraces (NSRP from Nquthu and RRP

from Rorke's Drift) differed. RRP recorded a higher Cu content than NSRP. Landraces from Khangelani varied, with KSP documenting a higher Cu content than KSP and KRI. Landraces from Nquthu with a smooth fruit texture varied in their Cu content. NSC recorded a greater Cu content than NqSC.

(k) Aluminium

Landrace NRC recorded the highest aluminium (Al) content at 525.53 mg kg^{-1} while RRP recorded the lowest at 11.01 mg kg^{-1} with an average of 69.35 mg kg^{-1}. Smooth curvilinear-shaped landraces, namely KSC from Khangelani, NqSC and NSC from Nquthu differed in their Al content. KSC recorded a higher Al content than both NqSC and NSC. Ndumo landraces were noted to vary in their Al content. NRC recorded a higher Al content than NRB.

(l) Boron

Landrace KSP had the highest boron (B) content at 38.32 mg kg^{-1} while NqRC recorded the lowest at 18.80 mg kg^{-1} with an average of 25.81 mg kg^{-1}. Landraces KSP, KSC and KRI from Khangelani, NSRC from Nquthu, and DSI from Dundee recorded a significantly higher B content than all other landraces.

4.3.3 Correlation among the nutritional and mineral composition of the *L. siceraria* landraces

Only positive correlations were significant among nutritional and mineral components (Table 4.3). ADF and NDF were positively correlated with each other and they both correlated positively with ash, magnesium, potassium, sodium and boron. NDF further correlated positively with calcium. Magnesium had a positive correlation with fat, potassium, sodium, copper and boron. Potassium strongly correlated with ash, sodium and boron. Sodium was positively correlated with ash, calcium and boron.

Table 4.3: Correlation among the nutritional and mineral composition of the *L. siceraria* landraces

Variables	Ash	Fat	ADF	NDF	Pro	Ca	Mg	K	Na	P	N	Mn	Fe	Zn	Cu	Al
Fat	0.472															
ADF	**0.824**	0.573														
NDF	**0.773**	0.592	**0.945**													
Pro	-0.179	0.350	0.092	0.106												
Ca	0.245	0.428	0.567	**0.654**	0.325											
Mg	0.404	**0.689**	**0.732**	**0.677**	0.442	0.494										
K	**0.925**	0.535	**0.937**	**0.875**	-0.023	0.428	**0.656**									
Na	**0.657**	0.579	**0.826**	**0.880**	0.047	**0.685**	**0.637**	**0.787**								
P	-0.394	0.366	-0.080	-0.141	**0.675**	0.263	0.280	-0.266	-0.158							
N	-0.179	0.350	0.092	0.106	**1.000**	0.326	0.442	-0.022	0.047	**0.674**						
Mn	-0.271	0.435	-0.130	-0.065	0.434	0.422	0.310	-0.164	-0.001	**0.640**	0.434					
Fe	-0.291	0.238	-0.070	-0.132	0.559	0.088	0.422	-0.179	-0.162	0.481	0.559	0.469				
Zn	0.495	-0.042	0.419	0.333	-0.114	0.133	0.250	0.540	0.261	-0.369	-0.114	-0.060	0.137			
Cu	0.221	**0.625**	0.524	0.386	0.437	0.491	**0.774**	0.410	0.488	**0.610**	0.436	0.479	0.369	0.018		
Al	-0.302	0.187	-0.096	-0.157	**0.611**	0.047	0.378	-0.206	-0.206	0.463	**0.611**	0.411	**0.988**	0.119	0.322	
B	**0.806**	0.561	**0.895**	**0.837**	-0.048	0.461	**0.739**	**0.939**	**0.799**	-0.142	-0.047	-0.026	-0.114	0.480	0.520	-0.186

Values ≥0.6 are deemed to be significantly correlated are in bold. Variables-ash, fat, ADF- acid detergent fibre, NDF- neutral detergent fibre and Pro-protein. Ca-calcium; Mg-magnesium; K-potassium; Na-sodium; P-phosphorus; N-nitrogen; Mn-manganese; Fe-iron; Zn-zinc; Cu-copper, Al-aluminium and B-boron.

Boron also correlated positively with ash. Phosphorus and nitrogen correlated positively with each other and they further correlated with protein. Phosphorus correlated positively with manganese and copper. Copper also correlated positively with fat. Aluminium positively correlated with protein, nitrogen and iron.

4.3.4 Principal component analysis (PCA)

The first three informative principal components (PC1 – 3) were responsible for 80.270% cumulative variability, with each principal component having an eigenvalue greater than 1.0 (Table 4.4). The first principal component (PC1), with 42.076% of the total variation was positively associated with ash, fat, ADF, NDF, calcium, magnesium, potassium, sodium, copper and boron.

Table 4.4: Loadings of the variables for the first three principal components

variables	PC1	PC2	PC3
Ash	**0.752**	-0.508	0.134
Fat	**0.731**	0.303	-0.231
ADF	**0.944**	-0.211	0.043
NDF	**0.916**	-0.225	-0.068
Pro	0.252	**0.818**	0.010
Ca	**0.664**	0.212	-0.303
Mg	**0.848**	0.341	0.124
K	**0.904**	-0.361	0.129
Na	**0.870**	-0.214	-0.188
P	0.070	**0.849**	-0.328
N	0.253	**0.818**	0.010
Mn	0.149	**0.699**	-0.204
Fe	0.082	**0.790**	0.526
Zn	0.395	-0.258	**0.721**
Cu	**0.668**	0.494	-0.172
Al	0.039	**0.796**	0.548
B	**0.908**	-0.276	0.053
Eigenvalue	7.153	4.975	1.518
Variability (%)	42.076	29.266	8.928
Cumulative %	42.076	71.342	80.270

PC1-3: Principal components 1-3. Values ≥0.6 are deemed to be significant are in bold. Variables-ash, fat, ADF- acid detergent fibre, NDF- neutral detergent fibre and Pro-protein. Ca-calcium; Mg-magnesium; K-potassium; Na-sodium; P-phosphorus; N-nitrogen; Mn-manganese; Fe-iron; Zn-zinc; Cu-copper, Al-aluminium and B-boron.

The second principal component (PC2), with 29.266% of the total variability was positively correlated with protein, phosphorus, nitrogen, manganese, iron and aluminium. The third principal component (PC3), responsible for 8.928% of the total variability was positively associated with zinc.

4.3.5 Cluster analysis

To further understand the association among nutritional components and *L. siceraria* landraces of different origins a biplot was constructed using PC1 and PC2 of principal component analysis (PCA) (Figure 4.1). Both PC1 and PC2 were positively correlated with fat, protein, calcium, magnesium, phosphorus, iron, aluminium, nitrogen and copper.

Five distinct clusters (I, II, III, IV and V) were realised, where landraces clustered closely to the nutritional components they strongly relate with, either positively or negatively (Figure 4.1). The first cluster (I) consisted of NRC and correlated positively with both PC1 and PC2. The second cluster (II) was made up of KSP, which correlated positively with both PC1 and PC2. The third cluster (III) consisted of (NqSC, KSC, KRI, NSRC and DSI). These landraces correlated positively with PC1 and negatively with PC2. The fourth cluster (IV) grouped landraces NSRC, RRP, MSC, NSRP, NqRC and RSP. These landraces correlated negatively with both PC 1 and PC2 (Figure 4.1). The fifth cluster (V) consisted of NRB, which correlated negatively with PC1 and positively with PC2.
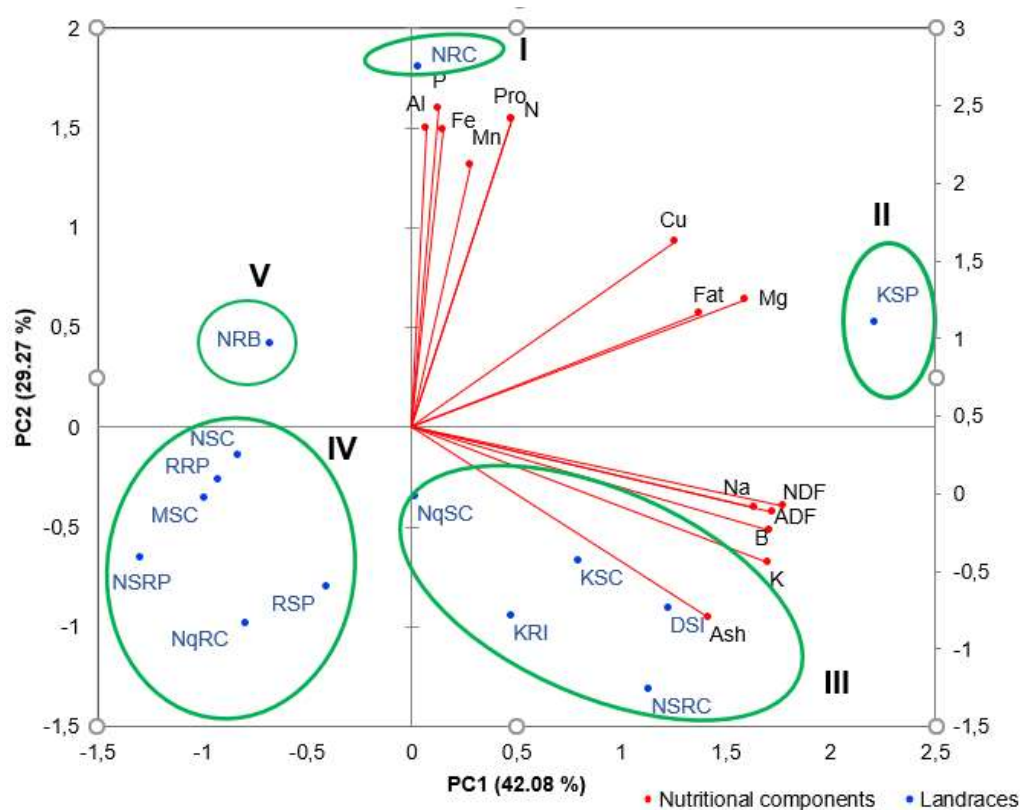
Figure 4.1: Biplot of *L. siceraria* landraces and nutritional components.

The blue marker indicates landraces- from Khangelani area with smooth, (KSP) pear-shaped; smooth, (KSC) curvilinear shape; and (KRI) rough isodiametric shape fruits; from Ndumo area (NRB) with rough fruits with brown seeds and (NRC) rough fruits with creamy seeds; from Mbazwana area (MSC) with smooth cylindrical-shape; from Rorke's Drift area (RSP) with smooth pear-shaped and (RRP) rough pear-shaped; from Nquthu area (NqRC) with rough curvilinear shape,(NSRP) with semi-rough pear-shaped, (NqSC) and (NSC) with smooth curvilinear shaped and (NSRC) semi-rough curvilinear shaped and from Dundee area (DSI) with smooth isodiametric shape. The red marker indicates nutritional components-ash, fat, ADF- acid detergent fibre, NDF- neutral detergent fibre and Pro-protein. Ca-calcium; Mg-magnesium; K-potassium; Na-sodium; P-phosphorus; N-nitrogen; Mn-manganese; Fe-iron; Zn-zinc; Cu-copper, Al-aluminium and B-boron.

The complete linkage method of cluster analysis was used to obtain the similarities of landraces based on their nutritional traits and a dendrogram was constructed as shown in Figure 4.2. Cluster (I) was made up of DSI from Dundee with smooth isodiametric shaped fruits. Cluster (II) grouped landraces from Rorke's Drift (RSP and RRP) and Nquthu (NqSC, NSC, NqRC and NSRP) with pear-shaped and curvilinear-shaped fruits.
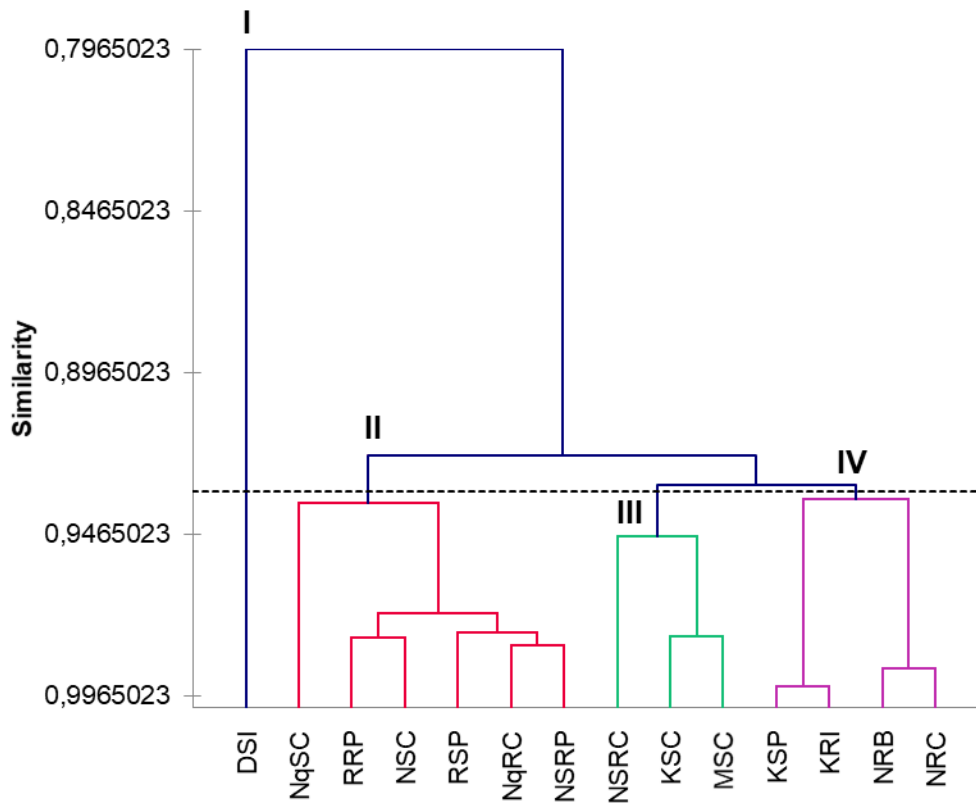
Figure 4.2: Hierarchical cluster showing similarities amongst nutritional traits *L. siceraria* landraces using the complete linkage method.

Landraces- from Khangelani area with smooth, (KSP) pear-shaped; smooth, (KSC) curvilinear shape; and (KRI) rough isodiametric shape fruits; from Ndumo area (NRB) with rough fruits with brown seeds and (NRC) rough fruits with creamy seeds; from Mbazwana area (MSC) with smooth cylindrical-shape; from Rorke's Drift area (RSP) with smooth pear-shaped and (RRP) rough pear-shaped; from Nquthu area (NqRC) with rough curvilinear shape,(NSRP) with semi-rough pear-shaped, (NqSC) and (NSC) with smooth curvilinear shaped and (NSRC) semi-rough curvilinear shaped and from Dundee area (DSI) with smooth isodiametric shape.

Cluster (III) grouped landraces with curvilinear-shaped fruits and further sub-clustered into semi-rough curvilinear-shaped fruit (NSRC from Nquthu) and smooth curvilinear-shaped fruits (KSC from Khangelani and MSC from Mbazwana). Cluster (IV) grouped landraces with rough fruit rinds (KRI from Khangelani, NRB and NRC from Ndumo) with KSP from Khangelani having smooth fruit rind.

4.3.6 Genetic parameters

The phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability, and genetic advancement for nutritional traits of *L. siceraria* landraces from different origins are presented in Table 4.5. The PCV was higher than GCV for all nutritional traits. However, the difference between PCV and GCV was not significant, suggesting that the environment had a big impact on the expression and availability of these traits (Jain *et al.* 2017).

Table 4.5: Genetic parameters for nutritional traits of *L. siceraria* landraces

Variables	$δ^2g$	$δ^2e$	$δ^2p$	GM	PCV	GCV	ECV%	H2	GA
Ash	44.8	30.4	75.2	19.7	19.5	15.1	12.4	59.6	1.6
Fat	0.3	0.1	0.4	1.0	6.6	5.8	3.1	77.6	1.8
ADF	23.0	5.0	28.0	21.4	11.4	10.4	4.8	82.2	1.9
NDF	39.9	12.7	52.6	27.3	13.9	12.1	6.8	75.9	1.8
Pro	1.5	0.4	1.9	7.0	5.2	4.6	2.4	78.0	1.8
Ca	0.0	0.0	0.0	0.3	1.8	1.5	0.9	75.8	1.8
Mg	0.0	0.0	0.0	0.1	1.3	1.1	0.7	71.0	1.7
K	4.7	1.8	6.5	5.5	10.8	9.2	5.7	72.4	1.8
Na	0.0	0.0	0.0	0.1	1.1	0.9	0.6	70.1	1.7
P	0.0	0.0	0.0	0.2	1.7	1.4	0.9	73.3	1.8
N	0.0	0.0	0.0	1.1	2.1	1.8	1.0	78.0	1.8
Mn	9.1	5.9	15.0	14.4	10.2	8.0	6.4	60.9	1.6
Fe	3015.7	4104.0	7119.7	136.0	72.4	47.1	54.9	42.4	1.3
Zn	1371.6	563.7	1935.3	39.9	69.6	58.6	37.6	70.9	1.7
Cu	5.8	3.0	8.8	4.6	13.8	11.2	8.1	65.4	1.7
Al	17933.3	196.3	18129.6	69.3	161.7	160.9	16.8	98.9	2.1
B	56.2	9.0	65.2	25.8	15.9	14.8	5.9	86.2	1.9

$δ^2g$-genotypic variance, $δ^2e$-environmental variance, $δ^2p$-phenotypic variance, GCV-genotypic coefficient of variation, PCV-phenotypic coefficient of variation, H2 –broad sense heritability, GA-genetic advancement, Variables-ash, fat, ADF- acid detergent fibre, NDF- neutral detergent fibre and Pro-protein. Ca-calcium; Mg-magnesium; K-potassium; Na-sodium; P-phosphorus; N-nitrogen; Mn-manganese; Fe-iron; Zn-zinc; Cu-copper, Al-aluminium and B-boron.

The highest GCV (>15%) was recorded for aluminium, followed by zinc, iron and ash. This indicates a relatively high genetic variability of these nutritional traits among *L.*

siceraria landraces of different origins. A similar record was evident for phenotypic coefficient of variation (PCV).

Nutritional traits were subjected to the estimate of heritability using the broad sense method. The heritability estimate ranged from 42.4% (iron) to 98.9% (aluminium). High heritability estimate values (more than 50%) were noted for all nutritional traits except for iron (Fe). Nutrients were then expressed in the following descending order: aluminium; boron; ADF; protein; nitrogen; fat; NDF; calcium; phosphorus; potassium; magnesium; zinc; sodium; copper; manganese and ash. All nutritional components had high genetic advance values of more than one. The highest genetic advance was recorded for aluminium (2.1) while the lowest was for iron (1.3).

4.4 Discussion

4.4.1 Proximate composition of the *L. siceraria* landraces

The analysis of variance revealed significant differences (p<0.05) among the *Lagenaria siceraria* landraces investigated with regards to proximate and mineral composition. The variation recorded among these landraces is indicative of an ample diversity among landraces of different origin even at nutritional level. This provides substantial germplasm for the improvement of mineral nutrient quantity and quality through breeding processes such as biofortification and hybridising. Upaganlawar and Balaraman (2009), Aliu *et al.* (2012), and Ahmed *et al.* (2016) observed similar significant differences in nutrient composition of *L. siceraria* landraces.

The ash content range (7.53 – 32.70 g/100g) and average (19.68 g/100g) of *L. siceraria* landraces from northern KwaZulu-Natal was significantly higher than ranges and averages among *Cucurbita maxima* cultivars from Poland (0.59 – 1.35 g/100g and 1.07 g/100g) (Czech and Stepniowska 2018), and Cameroon (0.3 – 1.3 g/100g and 0.84 g/100g) (Ponka *et al.* 2015). The difference in the genus name and nutritional analysis methods are probable cause for this variation. *Cucurbita maxima* cultivars from

Cameroon were cooked, and this might have denatured and leached out the nutritional content of the vegetables significantly (Mepba *et al.* 2007). The range of ash content of *L. siceraria* fruit pulp from the current study was higher than the range from 2.93 to 4.01 g/100g recorded among *L. siceraria* seeds from Sudan (Mariod *et al.* 2015). Landraces with high ash content indicate the presence of high mineral content, which is essential for human growth and development (Czech and Stepniowska 2018).

The range (0.19 – 2.41 g/100g) and mean (0.98 g/100g) of the fat content of the fruit pulp from the current study was lower than the range of *L. siceraria* seeds from Sudan (24.11 – 26.32 g/100g) (Mariod *et al.* 2015) and Nigeria (38.13 – 43.65 g/100g) (Essien *et al.* 2015). However, *Cucumis sativus* cultivars from Nigeria recorded a lower fat content, varying from 0.12 to 0.30 mg/100g with an average of 0.22 mg/100g (Abbey *et al.* 2017).

ADF and NDF content averages of 21.43 and 27.25 g/100g, respectively, recorded among *L. siceraria* landraces from northern KwaZulu-Natal were higher than the 15.67 and 21.16 g/100g among *L. siceraria* accessions from India (Upaganlawar and Balarama 2009) as well as 14.90 and 17.70 g/100g respectively of *Cucurbita maxima* from Poland (Peksa *et al.* 2016). The high neutral detergent fibre, which is easily digestible, makes the *L. siceraria* ideal to aid against constipation and avoiding haemorrhoids (Barot *et al.* 2015).

The protein content range (5.36 – 9.62 g/100g) and mean of *L. siceraria* landraces from the current study were higher than ranges and averages among the *Cucurbita maxima* from Cameroon (5.36 – 9.62 g/100g and 1.07 g/100g) (Ponka *et al.* 2015) and Poland (0.85 – 1.68 g/100g and 1.26 g/100g) (Czech and Stepniowska 2018). The results of the current study are in the lower range when compared with the reported range and mean value (8.29 – 12.56 g/100g and 9.67 g/100g) in *Cucurbita maxima* accessions consumed in Nigeria (Blessing *et al.* 2009). Protein is important to the human body, as an estimated 45% of the body is made-up of protein (Ponka *et al.* 2015). Protein is

essential for repairing the body, nutrient transport, and the building and functioning of muscles (Ponka *et al.* 2015).

4.4.2 Macronutrients of the *L. siceraria* landraces

The range and mean obtained for Ca (0.13 – 0.44 g/100g and 0.26 g/100g) from the current study proved to be higher than the range and mean reported in *Cucumis sativus* cultivars (0.020 – 0.025 g/100g and 0.022 g/100g) from Nigeria by Abbey *et al.* (2017). A lower range and average of Ca content (0.06 – 0.26 g/100g and 0.17 g/100g) in *Cucurbita maxima* and *Cucurbita moschata* morphotypes from Cameroon was also obtained by Mbogne *et al.* (2015). Calcium is essential for healthy bones and teeth, vital for muscle functioning, boosts the immune system, and regulates blood pressure and clotting (Gharibzahedi and Jafari 2017).

The magnesium (Mg) content of the landraces was between 0.08 – 0.22 g/100g, with an average of 0.14 g/100g. These amounts are very low compared to those reported for some commonly consumed cucurbits such as *Momordica charantia* genotypes (0.25 – 0.36 g/100g with an average of 0.29 g/100g) from Turkey by Karaman *et al.* (2018), as well as *Cucurbita maxima* and *Cucurbita moschata* morphotypes (0.11 – 0.29 g/100g with a mean of 0.18 g/100g) from Cameroon by Mbogne *et al.* (2015). These *Momordica charantia* genotypes were grown in a greenhouse in a controlled environment when compared to the field- grown *L. siceraria* landraces of the current study, thus explaining their superiority resulting from an environment calibrated for optimum results.

The *Cucurbita maxima* and *Cucurbita moschata* morphotypes were grown in soils rich in volcanic ash, which increases the soil fertility drastically, thus explaining the high mineral content present in these morphotypes (Mbogne *et al.* 2015). The *Cucurbita maxima* and *Cucurbita moschata* morphotypes also received 1500 to 2000 mm of annual rainfall (Mbogne *et al.* 2015), which is higher than the annual rainfall received by the *L. siceraria* landraces of the current study at 299.95 – 350.02 mm (Naidoo *et al.*

2016). A similar study on *Cucurbita pepo* from Nigeria by Adnan *et al.* (2017) recorded a lower Mg content, averaging 0.45 mg/100g. Magnesium is vital for protein synthesis and a healthy immune system, and relieves constipation (Gharibzahedi and Jafari 2017).

The Potassium (K) content range and mean value (2.80 – 9.69 g/100g and 5.51 g/100g) was higher than the range and mean value (1.88 – 3.17 g/100g and 2.46 g/100g) reported by Mbogne *et al.* (2015) for *Cucurbita maxima* and *Cucurbita moschata* morphotypes from Cameroon, as well as (1.29 – 2.75 g/100g and 1.78 g/100g) for *Cucurbita maxima* cultivars from Cameroon by Ponka *et al.* (2015).   A study on *L. siceraria* seeds by Mariod *et al.* (2015) obtained a lower K content range and mean (23118 – 33023 mg kg^{-1} and 29385.9 mg kg^{-1}) than the *L. siceraria* fruit pulp of the current investigation. Potassium aids in fluid balance, and regulates blood pressure and waste elimination (Gharibzahedi and Jafari 2017).

The range (0.08 – 0.29 g/100g) and mean (0.17 g/100g) of phosphorus (P) content of the fruit pulp from the current study was lower than the range and mean of *L. siceraria* seeds from Sudan (0.57 – 0.72 g/100g and 0.66 g/100g) recorded by Mariod *et al.* (2015) and *Cucurbita maxima* accessions (0.23 – 0.36 g/100g and 0.29 g/100g) from Cameroon by (Ponka *et al.* 2017).   A similar study on *L. siceraria* varieties from India obtained a higher P content average at 0.19 g/100g (Barot *et al.* 2015). Phosphorus is vital for kidney performance and assists in protein synthesis, cell growth, maintenance and reparation, as well as ATP and energy production (Gharibzahedi and Jafari 2017).

Nitrogen (N) content of *L. siceraria* landraces of the current study from northern KwaZulu-Natal ranged from 0.85 – 1.47 g/100g with a mean of 1.11 g/100g. A comparable study on *Cucurbita pepo* from Slovakia recorded a higher nitrogen content, varying from 2.0 – 7.2 g/100g (Kostalova *et al.* 2010). Nitrogen is essential for boosting the immune system, and fighting infectious agents and malignant tumours (Bogdan *et al.* 2000).

The results show that the range and mean (0.03 – 0.11 mg kg^{-1} and 0.06 mg kg^{-1}) was higher than the range and mean (2.43 – 7.01 mg/100g and 4.29 mg/100g) of *Cucumis sativus* from Nigeria (Abbey *et al.* 2017) and *L. siceraria* landrace seeds (17.64 – 36.70 mg kg^{-1} and 21.98 mg kg^{-1}) from Sudan (Mariod *et al.* 2015). A study on *L. siceraria* varieties from India recorded a lower Na content, with an average of 36.68 mg/100g (Barot *et al.* 2015). Sodium is essential for electrolyte balance, healthy heart functioning, metabolic activities, and nerve transition (Gharibzahedi and Jafari 2017).

4.4.3 Micronutrients *L. siceraria* landraces

The range and mean obtained for manganese (Mn) (9.67 – 19.00 mg kg^{-1} and 14.43 mg kg^{-1}) from the current study proved to be higher than the range and mean for *Cucumis sativus* (0.08 – 0.81 mg/100g and 0.38 mg/100g) from Nigeria documented by Abbey *et al.* (2017) and *Cucurbita maxima* (0.7 – 1.6 g/100gwith and 1.1 mg/100g) from Cameroon (Ponka *et al.* 2017). The average content of Mn was higher than the one reported by Upaganlawar and Balaraman (2009), (0.31 mg/100g) and Ahmed *et al.* (2017) (0.032 mg/100g) on *L. siceraria* from India and Pakistan, respectively. *Cucurbita maxima* from Colombia also recorded a lower Mn content average at 0.02 mg/100g (Leterme *et al.* 2006). Manganese is essential for healthy bone structure development and brain and nervous system functioning, and aids against osteoporosis (Gharibzahedi and Jafari 2017).

The range and mean of iron (Fe) content (23.70 – 828.70 mg kg^{-1} and 136.0 mg kg^{-1}) among *L. siceraria* landraces of the current study proved to be higher than the range and mean reported for *Cucurbita pepo* (1.33 – 5.02 mg kg^{-1} and 2.80 mg kg^{-1}) from Kolova by Aliu *et al.* (2012). The average content of Fe was higher than the one reported by Upaganlawar and Balaraman (2009), (2.33 mg/100g) and Rahman (2003) (0.20 mg/100g) on *L. siceraria* from India. Iron is important for the formation of haemoglobin in red blood cells which transports oxygen from the lungs to the cells throughout the body and is essential for energy metabolism (Gharibzahedi and Jafari 2017).

The Zn content of the evaluated *L. siceraria* landraces was between $0 - 120.33$ mg kg^{-1} with a mean of 39.9 mg kg^{-1}. These amounts were higher compared to those reported for some commonly consumed cucurbits, for example, *Cucumis sativus* ($0.28 - 0.45$ mg/100g with an average of 0.37 mg/100g) from Nigeria by Abbey *et al.* (2017) and *Cucurbita pepo* accessions ($0.67 - 2.15$ mg kg^{-1} with an average content of 1.1 mg kg^{-1}) from Kolova (Aliu *et al.* 2012). A study on *L. siceraria* from India also recorded a lower Zn content, averaging at 0.70 mg/100g (Rahman 2003). Zinc is an integral part of many enzymes; it is vital for genetic material and protein synthesis and for sexual maturation and healthy fetal development, and improves digestion (Gharibzahedi and Jafari 2017).

The copper (Cu) content of *L. siceraria* landraces from northern KwaZulu-Natal ranged from $0.33 - 10.67$ mg kg^{-1} and averaged 4.60 mg kg^{-1}. Comparable studies on *Cucurbita pepo* recorded a higher Cu content range of ($0.1 - 1.3$ mg kg^{-1} with a lower mean of 3.1 mg kg^{-1}) from Spain by Martinez- Valdivieso *et al.* (2014) and ($0.56 - 3.50$ mg kg^{-1} with a mean of 1.17 mg kg^{-1}) from Kolova (Aliu *et al.* 2012). Copper is essential for the production of red blood cells and protein (Stern *et al.* 2007). It is also vital for iron breakdown and digestion, and is important as an antioxidant defence and for healthy immune system function (Bosta *et al.* 2016).

Aluminium (Al) content of *L. siceraria* landraces from northern KwaZulu-Natal ranged from $11.01 - 525.53$ mg kg^{-1} with an average of 69.35 mg kg^{-1}. The Al content of the current study was noticeably higher than that of ryegrass with a mean of 0.2 g kg^{-1} (Pontigo *et al.* 2017). Aluminium has no beneficial biological function in humans, but it is detrimental to human health as the most common contaminants contain aluminium (Strunecka *et al.* 2016).

Boron content from the current study on the *L. siceraria* landraces from northern KwaZulu-Natal ranged from $18.80 - 38.32$ mg kg^{-1} with an average of 0.0025 g/100g.

Boron is essential for regulating oestrogen in post-menopausal women for optimum mental performance and healthy bones (Gharibzahedi and Jafari 2017).

4.4.4 Correlation

Correlation measures the similarities and dissimilarities between variables. Thus, the correlation of ($r \geq 0.60$ and $r \geq -0.60$) in this study was a strong correlation. This study simulated the studies conducted by, Nagar *et al.* (2018) on *Cucurbita moschata* genotypes. The findings of the current study on *L. siceraria* landraces from northern KwaZulu-Natal agrees with the findings on *Luffa acutangula* and *Momordica charantia* from Pakistan (Hussain *et al.* 2009). According to Hussain *et al.* (2009), protein has a very low correlation with fat ($r= 0.3$) and ash ($r= 0.4$), hence no correlations were recorded for the current study.

Magnesium correlated with fat ($r= 0.689$), potassium ($r= 0.656$), sodium ($r= 0.637$), copper ($r= 0.774$) and boron ($r= 0.738$). This suggests that when selecting for genotypes with high magnesium content, those genotypes will subsequently be rich in fat and essential salts as well as being genotypes that facilitate faster digestion as a result of high copper content (Bosta *et al.* 2016). In the current study phosphorus correlated positively with nitrogen ($r= 0.640$) and copper ($r= 0.610$). This assures that upon selecting genotypes with high phosphorus, the selected genotypes will also contain high nitrogen and copper content.

4.4.5 Principal component analysis

The principal component analysis identified diversity among landraces in terms of their nutritional composition. It measured the contribution of each nutritional trait towards the total cumulative variability (Nayik and Nanda 2015). The cumulative variance of 80.270% of the first three components with an eigenvalue of >1.0 is indicative of the amount of influence nutritional traits have within each principal component on the nutritional composition of different *L. siceraria* landraces. A factor loading with a total

value of ≥ 0.60 was deemed a significant contributor towards the total variability of nutritional composition, positively or negatively (Colonna *et al.* 2016) (Figure 4.1).

Nutritional variability in PC1 (42.076%), PC2 (29.266%) and PC3 (8.928%) in the current study was comparable to variability in PC1 (48.34%), PC2 (23.04%) and PC3 (19.07%) among several leafy vegetables (Kumar *et al.* 2018). The nutritional traits of the current study; ash, namely, fat, acid detergent fibre, neutral detergent fibre, calcium, magnesium, potassium, sodium, copper and boron correlated positively with the first principal component (PC1). These traits contributed 42.076% of the total variability. A study on *Cantharellus cibarius* obtained similar findings as K, Mg, Na and Cu correlated positively with PC1, accounting for 66% of the total variability (Drewnowska and Falandysz 2015).

The nutritional traits of protein, phosphorus, nitrogen, manganese, iron and aluminium correlated positively with second principal component (PC2). These traits contributed 29.266% to the total variability. A study on *Cyperus esculentus* documented similar results, with protein, phosphorus and aluminium correlating positively with PC2 and contributing 21.78% to the total variability (Bado *et al.* 2015).

A study on different leafy vegetables also documented positive correlation of potassium (r= 0.7407) with PC1 (Kumar *et al.* 2018) as in the current study. Positive correlation of calcium in PC1 and phosphorus in PC2 among *L. siceraria* landraces were in PC2 and PC3, respectively (Kumar *et al.* 2018). However, a study on *Abelmoschus esculentus* contradicts the findings of the current study as Ca, Mg, K, Na and Cu were all negatively correlated with PC1 (dos Santos *et al.* 2013).

4.4.6 Cluster analysis

Multivariate cluster analysis based on nutritional traits resulted in the dendrogram shown in Figure 4.2. The cluster analysis displayed the presence of diversity among the 14 landraces in terms of nutritional traits studied. The dendrogram showed four distinct

clusters. Each cluster grouped landraces with similar proximate and mineral traits. The genetic variances and heritability estimates revealed positive results in terms of high variability amongst the germplasm with nutritional traits that are highly heritable - which is ideal for genetic manipulation and future breeding programmes in the quest for revealing the most nutritional *L. siceraria* landraces.

A total variance of 71.35% for two principal components of a biplot among *L. siceraria* landraces was comparable to the 66.16% in *Cucurbita maxima* and *Cucurbita moschata* morphotypes (Mbogne *et al.* 2015) and 79.70% amongst *Abelmoschus esculentus* cultivars (dos Santos *et al.* 2013). Grouping of *L. siceraria* landraces in the dendrogram according to their fruit morphology and area of origin was similar to the grouping of *Cucurbita maxima* germplasm (Kazminska *et al.* 2018).

4.4.7 Genetic parameters

The analysis of variance revealed significant differences among the genotypes regarding their nutritional traits. The genotypic coefficient of variation and the heritability estimate are indicative of how much of the variation id heritable (Jha *et al.* 2017). Thus, a high heritability estimate value and genetic advancement value are accurate parameters for an effective artificial selection based on the phenotypic display (Dalamu 2011).

In the current study on *L. siceraria* landraces from northern KwaZulu-Natal the phenotypic coefficient of variance (PCV) was slightly higher than the genotypic coefficient of variation (GCV), suggesting that the environment played a vital role in the expression of these nutritional traits (Jain *et al.* 2017). A high to moderate (>15%) phenotypic coefficient of variation was noted in the current study for aluminium (160.9%), iron (72.4%), zinc (69.6%), ash (19.5%), and boron (15.9%). Similar findings were documented on *Momordica charantia* genotypes from India, where iron recorded a lower PCV at 25.30% (Dalamu 2011).

The highest value for broad sense heritability estimate was noted for aluminium, boron, ADF, protein, nitrogen, fat, NDF, calcium, phosphorus, potassium, magnesium, zinc, sodium, copper, manganese and ash. A comparable study yielded similar results and the following highest heritability estimates were noted: calcium (99.90%), iron (99.70%), sodium (99.20%), potassium (97.50%), phosphorus (95.50%), zinc (93.30%) and manganese (90.00%) (Dalamu 2011). The current study recorded higher genetic advances for potassium (1.8) and phosphorus (1.8) compared to the findings recorded by Dalamu (2011).

4.5 Conclusion

In proximate composition traits, landraces KSP, NRC and DSI recorded the highest ash, fat, ADF, NDF and protein content. In mineral composition traits, landrace KSP outperformed all landraces for most mineral traits. Nutritional traits only correlated positively with each other. In principal component analysis, the majority of the nutritional composition traits were positively associated with three informative principal components. The biplot and dendrogram clustered landraces according to their fruit morphology and area of origin. Among genetic parameters, the phenotypic coefficient of variation was higher than the genotypic coefficient variation for most traits. The heritability and genetic advance was significantly high for nutritional traits. The next chapter (Chapter 5) focuses on the outstanding landraces in terms of their morphological and nutritional traits, and makes recommendations based on the findings of the current study.

Chapter 5
Conclusions and recommendations

Significant variations (P<0.001) in morphological traits and nutritional composition were recorded among the *Lagenaria siceraria* landraces. In seedling traits, landrace DSI outperformed all landraces, with the highest germination percentage. Landrace KSC recorded the tallest seedlings, while landraces NSRC and DSI had the broadest cotyledonous leaves. Landraces DSI, KSC and NSRC can be recommended for selection for maximum germination and vigorously growing seedlings with broad cotyledonous leaves. Landrace KSC had the most vigorously growing shoots and leaves while NSRP, NSRC and NRC had the longest vines. Landraces KRI and NRC had the greatest number of branches, and KRI, KSC, NSRP and NSRC had the highest number of leaves. Landraces with vigorous vegetative growth are a potential source of adequate leafy vegetables for consumption purposes. These landraces can also be selected for superior leaf canopy, which minimizes weed growth around plants thus allowing optimum utilization of resources (water and nutrients) by the investigated plants.

All landraces had high chlorophyll content, except landraces NqRC and NSRP. Landraces RSP, RRP, NqRC and NqSC had the longest tendrils. These landraces can be recommended for the robustness of their vegetative structures due to the anchoring by the longer tendrils onto firm climbing support, thus permitting trellising. Landraces KSC and RSP recorded high fresh and dry shoot mass and low moisture content. High shoot mass and low moisture content result in a longer shelf life of leafy vegetables, which is an ideal agronomic trait to be enhanced and inherited. Landraces KSP, MSC and NSRP recorded the highest shoot moisture content. These landraces are recommended for succulent shoots due to the highwater content they have.

Landraces KRI, NSC, KSP, NqRC and NSRC had the highest number of flowers per plot. Landraces MSC, RRP and NqRC had the maximum flower sex ratio. These landraces can be selected as potentially high yielding landraces due to the high number

of flowers and flower sex ratio, which increases the probability of pollination and fruit formation. All landraces yielded comparable amounts of fruits. However, landraces KRI and KSP outperformed the rest, and can therefore be recommended for numerous fruits and high fruit yield per plot. Landraces RSP, NSRP and KSP had the heaviest, longest and widest fruits. These landraces are potential suppliers of large fruits essential for consumption purposes.

Landrace NSRC had the longest pedicels, which increases the probability of pollination and fruit formation as pistillate flowers will be held up above the dense leaf canopy of *L. siceraria*. Landrace KRI had the thickest fruit rind, which is essential for cucurbits in the defense against pests and diseases such as fruit flies and *Fusarium* fruit rot. Landraces NqRC, KSP and RSP had the largest and heaviest seeds compared to other landraces. Landraces with larger seeds normally produce vigorously-growing plants from the seedling stage and tend to produce more numerous and larger fruits.

Landrace KSP outperformed all other landraces in terms of high ash, fat, ADF, NDF and protein content. However, landraces KSC, KRI, NSRC and DSI also had high ash, ADF and NDF content. Landrace NRC also recorded highest protein content. These landraces can be selected due to the abundance of proximate nutritional traits indicative of their potential as alternate food sources. Landraces KSP and NSRC had high calcium content, outperforming all other landraces, thus their consumption can be recommended for proper bone and teeth development as well as assisting with the digestion of food. Landraces KSP, KRI, NRC and DSI recorded high magnesium content and they are potential landraces for protein synthesis, a healthy immune system, and constipation relief.

Landraces KSP, KSC, NSRC and DSI had high potassium and sodium content. These landraces can be recommended for selection as potassium is vital for regulating blood pressures and waste elimination, whereas sodium maintains electrolyte balance, suggesting that upon selection these landraces are good alternative source of energy. Landraces, KSP, NRB and NRC had high phosphorus, nitrogen, manganese and iron

content. KSC and NSC also had a high manganese content. They can be selected due to their essential properties in ensuring proper functioning of the body's vital organs such as the brain, lungs and heart.

Landraces KSC, NSRC and DSI documented high zinc content and these landraces can be recommended as zinc is essential for making protein and genetic material. Landrace KSP had high copper content and is recommended for selection as copper is vital for stimulating the immune system to fight infections. Landrace NRC recorded the highest aluminium content. NRC is not recommended, due to high aluminium content since Al is detrimental to human health. Landraces KSP, KSC, NSRC and DSI outperformed all other landraces in terms of boron content. Landrace KSP outperformed all other landraces in terms of nutritional composition and is recommended as a primary germplasm for nutritional composition enhancement, followed by NSRC, DSI, NRB and NRC, respectively.

Correlation analysis revealed significant positive correlations among vegetative traits (vine length, number of branches, number of leaves, shoot dry mass, tendril basal length and tendril lobes), reproductive traits (number of staminate flowers, fruit yield per plot, fruit mass, fruit width, fruit rind thickness, seed length, seed width, 100seed mass and total seed mass) and also among the majority of nutrients (ADF, NDF, ash, fat, protein, calcium, magnesium, potassium, phosphorus, nitrogen, manganese, sodium, iron, copper, boron and aluminium). Therefore these traits could be used as indices for selection of genotypes with vigorously growing vegetative structures, high yielding potential, and abundant nutritional composition.

The first principal component (PC1) for morphological traits, with 26.635% of the total variation, was positively associated with shoot fresh mass, shoot dry mass, tendril basal length, tendril longer lobe length, tendril shorter lobe length, fruit mass, fruit width, seed width, 100 seed mass and total seed mass. The first principal component (PC1) for nutritional traits, with 42.076% of the total variation, was positively associated with ash, fat, ADF, NDF, calcium, magnesium, potassium, sodium, copper and boron. These

traits are recommended as they are responsible for majority of variations among the landraces.

In morphological traits and nutrient analyses, biplot and dendrogram grouped landraces mainly according to fruit and seed morphology and then their origin. This concludes that the phenotypic traits are good indicators of variations among *L. siceraria* landraces as well as the area of origin. The genotypic variations are not expressed in a vacuum but in the environment, hence there is great variation among landraces of different origins. Therefore these cluster analyses are recommended for identifying closely and distantly related landraces of different origins regarding morphological and nutritional traits.

High heritability estimate values of morphological traits were recorded for: fruit yield; seed width; fruit rind thickness; fruit width; total seed mass per fruit; fruit mass; fruit length; seed length; number of branches; 100 seed mass; number of leaves; seedling height; tendril basal length; pedicel length; vine length and tendril longer lobe length; nutritional traits (aluminium; boron; ADF; protein; nitrogen; fat; NDF; calcium; phosphorus; potassium; magnesium; zinc; sodium; copper; manganese and ash). Selection based on these traits has a high transmissibility potential, therefore their use as indices for favourable traits is highly recommended, especially when breeding for high yield and nutritional content.

High genetic advance values were documented for: fruit mass; fruit width; fruit rind thickness; seed width; total seed mass per fruit; fruit length; number of branches; 100 seed mass per fruit; number of leaves; seedling height; tendril basal length; tendril longer lobe length; cotyledonous leaf area; vine length; pedicel length; leaf growth; shoot fresh mass, tendril shorter lobe length and all nutritional traits. These traits are recommended, as traits with high heritability and genetic advance are considered to be under the additive gene action. Additive gene action results from the combination of more than one gene, where each gene has a positive and equal contribution towards the phenotype, hence landraces possessing these traits stand a better chance of betterment upon selection.

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