Agronomic evaluation and genetic studies of *Lagenaria siceraria* populations derived from landraces collected from KwaZulu-Natal, South Africa

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Declaration

I, Lungani Siyabonga Nkosi, declare that this dissertation is my original work, except where otherwise acknowledged or indicated. This dissertation has not been submitted for any examination or degree at any other university. This dissertation does not contain any other persons' data, graphs, pictures or writing and tables, unless precisely acknowledged as being sourced from other researchers. Where their exact words have been used, their writing has been placed in quotation marks and referenced accordingly.

Signed

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As the candidate's supervisors, we agree to the submission of this dissertation:

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Abstract

Lagenaria siceraria is one of the most important cucurbitaceous vegetables due to its prolific bearing habit, utility as a cooked vegetable and its low cost of cultivation. The fruit is a vital source of minerals, vitamins, cucurbitacins, campesterol, polyphenols and 1.6% choline in sitosterol on dry weight basis. The edible fleshy parts of the vegetable are leaves, shoots, fruit and seeds. The crop is grown under subsistence farming with no genetically improved cultivars. No previous studies have reported the crossing of *L. siceraria* landraces collected from KwaZulu-Natal, South Africa to generate new populations. Therefore, there was a need to develop F_1 populations from *L. siceraria* landraces with desirable traits and evaluate their agronomic and nutritional attributes for commercial and subsistence farming. The first objective of the study was to evaluate the morpho-agronomic traits among the selected parental landraces and created F_1 progenies, and the second one was to analyse the nutritional variability of the selected parental landraces and created *L. siceraria* F_1 progenies.

In the first study, the diversity present among 19 genotypes of (seven parental landraces and twelve F_1 offspring) was investigated using morpho-agronomic qualitative and quantitative traits. Significant differences ($P < 0.05$) were observed among quantitative traits suggesting considerable genetic variability. The genotypes displayed significant variation in most qualitative traits of fruits (colour, texture and shape) and seeds (colour, size and shape). The genotypes evaluated in the present study exhibited a wide range of diversity in quantitative traits including days to emergence, emergence percentage, seedling height, leaf area, main vine length, days to flowering, days to edible harvest maturity, days to drying harvest maturity, fruit neck length, seed width and leaf growth percentage. The first five principal components on quantitative traits among the evaluated 19 genotypes contributed 74.84% of the variability. For quantitative traits, emergence percentage, seedling height, cotyledons size, leaf area, vine length, number of leaves, number of branches, number of seeds per fruit, fruit mass, fruit rind thickness, hundred seed mass, total seed mass, fruit length, fruit width, seed width, fruit yield, yield per plant, leaf chlorophyll content, total fruit mass per plot, fruit neck length and seed length were correlated positively with the first principal component (PC1), which accounted for 34.46% of the total variation. Days to emergence, days to flowering, days to edible harvest maturity, days to drying harvest maturity, shoot growth percentage, cotyledon leaf area, leaf area, vine length, number of leaves, number of branches, fruit mass, fruit rind thickness, hundred seed mass, fruit width, seed length and seed width correlated positively with PC2, which accounted 14.48% of total variability. The biplot and dendrogram clustered genotypes into five clusters according to their vegetative, fruit and seed traits. F_1 progenies displayed more heterosis or hybrid vigour than parental landraces in 15 quantitative traits (53.57%) out of the 28 evaluated traits. Hence, the F_1 progenies can be used for further *Lagenaria siceraria* genetic improvement.

In the second study, the diversity present among 19 genotypes was investigated using taste (very sweet, sweet, neutral, bitter and very bitter) and mineral nutrients. With a participant's test, we observed a major difference in taste between the 19 genotypes. A very sweet genotype (RRPxNSC) and sweet genotype (NqSCxNSC) would typically be utilized to make jams and sweets. There were insignificant differences ($P > 0.05$) among mineral contents of the genotypes, suggesting no considerable mineral composition variability. The first five components among the 19 genotypes evaluated contributed 89.97% of the variability. The following minerals/nutrients, namely, crude protein, calcium, magnesium, zinc, copper, manganese, iron, and phosphorus positively correlated with the first principal component (PC1), which accounted for 42.42% of the total variation. Calcium, magnesium, potassium, sodium, zinc, manganese, iron, and aluminium had a positive correlation with the second principal component (PC2), which accounted for 24.78% of the total variation. The biplot and dendrogram clustered genotypes into five clusters according to the macronutrients and micronutrients. The NSRC genotype was unique because of its characteristics of having high contents of crude protein, phosphorus, magnesium, zinc, manganese and iron but low potassium content, and thus making it suitable to supply minerals for human consumption. The differences in the *Lagenaria siceraria* genotypes also occur in the taste and in the composition of the various nutrients present in them and not only in the morphological traits. Since we have obtained the variation in mineral content among the genotypes, the analysis of vitamins and phytonutrients is recommended for future studies.

Keywords: F_1 progenies, qualitative traits and quantitative traits, mineral nutrients, taste and genotypes.

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Dedication

This dissertation is dedicated to my mother Dingeni Sibiya and my late father Mzikayifani Nkosi.

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Chapter 1

1. Introduction

Lagenaria siceraria (Molina) Standly, commonly known as bottle gourd or calabash gourd (English), iSelwa (isiZulu and isiXhosa), moraka (seSotho) and segwana (seTswana), belongs to the family Cucurbitaceae (Sithole 2014*)*. It is one of the earliest crops known to originate from Africa (Sithole et al. 2016). In South Africa, *L. siceraria* is mostly produced in the Limpopo, Gauteng, Eastern Cape, KwaZulu-Natal and North-West provinces (Department of Agriculture Forestry and Fisheries 2016). It is a nutrient rich leafy vegetable (Kumar et al. 2014), which possesses some medicinal properties (Malviya et al. 2017b). The leaves are usually consumed as a relish with maize staples (Chimonyo and Modi 2013). The mature dried fruit can be used as a container to store water and milk, and as an ornament or musical instrument (Milind and Satbir 2011).

For the prevention of root-borne disease-causing organisms such as *Fusarium oxysporum*, *Lagenaria siceraria* is used as a rootstock in winter production of watermelons and squashes (Mashilo et al. 2016). It contains high levels of choline, which is a compound that is used to cure mental disorders (Sithole 2014). It also contains sodium, potassium, essential elements and trace minerals (Chimonyo and Modi 2013). The tender edible fruit are also prepared into jams, curries and sweets (Ghuge et al. 2016). In addition, the oil from the seeds is nutritious and diuretic, and can be used to relieve headaches (Malviya et al. 2017a)

This crop exhibits significant genetic variation in fruit shape and size (Yetisir et al. 2008), which includes variation in fruit width, fruit length and fruit rind thickness (Koffi et al. 2009). Landraces are still used as the germplasm resource for the cultivation of this crop with no improved varieties available in the market (Sithole et al. 2016). These landraces have undergone many years of selection by subsistence farmers and may form a vital germplasm resource for upcoming crop improvement programs (Mabhaudhi and Modi 2010). The average yield of *Lagenaria siceraria* landraces is very low (less than 25 t/ha) as compared to the Asian hybrid varieties (more than 40 t/ha) (Chimonyo and Modi 2013).

The development of high yielding varieties through hybridisation is important for maximizing the yield of bottle gourd (Kumar et al. 2014). The selection of parents based on combining ability is a significant breeding method in crop improvement (Malviya et al. 2017a). There is great opportunity to exploit hybrid vigour in the crop (Milind and Satbir 2011). Therefore, there is huge potential to increase *L. siceraria* production in South Africa. The advantage is that the fruits are larger in size with greater number of seeds per fruit and the seed rate requirement per unit area is low (Balat et al. 2020). However, information on genetically improved cultivars of *Lagenaria siceraria* is largely missing from the literature. Part of the gap in literature includes information on the agronomy of the crop, which is an important basic aspect of crop production.

1.1 Problem Statement

No genetically improved cultivars of *Lagenaria siceraria* have been developed in South Africa yet. The crop is grown under subsistence farming where landraces are the available cultivars. No studies have associated the mating of *Lagenaria siceraria* landraces collected from KwaZulu-Natal with the agronomic and nutritional composition in South Africa. It was reported that the average yield of *Lagenaria siceraria* landraces is very low as compared to the hybrid varieties in Asia. Therefore, there was a need to develop F_1 populations from *Lagenaria siceraria* landraces with desirable traits and evaluate their morpho-agronomic and nutritional attributes for commercial and subsistence farming systems.

1.2 Justification for the study

In South Africa *Lagenaria siceraria* is one of the traditional under-utilized crops, which is very much under researched. This crop possesses many nutritional benefits and has industrial and medicinal uses. The cultivation of this crop is largely dependent on indigenous unimproved landraces, which are not scientifically selected and bred due to the absence of improved cultivars. The hybridisation study conducted in *L. siceraria* at Bangladesh with half diallel mating design generated high yielding F_1 progenies (Quamruzzaman et al. 2020). Like the present study, North Carolina design ii was also used for cucumber (*Cucumis sativus* L.) in China by Liu et al. (2021). It was necessary to select and cross the landraces that show the desirable traits was and evaluate the morpho-agronomic characteristics. In addition, it was crucial to assess the taste and nutritional composition of their offspring to develop new cultivars.

1.3 Aim of the study

The aim of the study was to develop *Lagenaria siceraria* F_1 populations from landraces with desired traits in South Africa and evaluate their morpho-agronomic characteristics, assess the taste and nutritional composition to develop improved cultivars.

1.4 Research questions

The research questions of the study were as follows:

- What are the differences in the morpho-agronomic traits between the parents and F_1 offspring of *Lagenaria siceraria* landraces from South Africa?
- How does the taste and nutritional composition vary among *L. siceraria* genotypes?

1.5 Objectives

The objectives of the study were:

- To evaluate the morpho-agronomic traits among the selected parental landraces and created *Lagenaria siceraria* F_1 progenies.
- To assess the taste and analyse the nutritional variability of the selected parental landraces and created *L. siceraria* F_1 progenies.

1.6 Research hypotheses

H_0 = *Lagenaria siceraria* landraces from South Africa and their F_1 progenies do not vary in the fruit and seed morpho-agronomic traits.

H_1 = Wide variation exists in the fruit and seed morpho-agronomic traits of *L. siceraria* landraces from South Africa and their F_1 progenies.

H_0 = Fruit taste and mineral content do not differ between *Lagenaria siceraria* parental landraces and their F_1 progenies.

H_1 = *L. siceraria* F_1 progenies has a general sweeter taste and has higher fruit nutritional and mineral content than parental landraces.

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Chapter 2

2. Literature Review

2.1 Taxonomy and origin of *Lagenaria siceraria*

Lagenaria siceraria belongs to the Cucurbitaceae family, which comprise of 118 genera and 825 species (Milind and Satbir 2011). It is the most cultivated species in the genus *Lagenaria* along the warmer regions of the world. The genus *Lagenaria* contains five other wild species, namely, *L. brevifilora* (Benth) Roberty, *L. rufa* (Gilg) C Jeffrey, *L sphaerica* E Mey, *L. abyssinia* (Hook. F.) C Jeffrey and *L. guineensis* (G Den) C Jeffrey (Chimonyo and Modi 2013). Archaeological evidence shows this crop as a first species domesticated by humans for at least 12 000 years (Buthelezi et al. 2019). It is believed that *Lagenaria siceraria* originated in Africa (Sithole 2016).

This crop has a branching growth habit, and it is an annual herbaceous plant with variable and alternate leaves (Chimonyo and Modi 2013). The plants have well developed branched root system. The landraces differ widely in size, texture and shape of both fruits and seeds from one location to another (Yetisir et al. 2008). The fruit shape can be cylindrical, long, curved, necked, oblong, round, flat-round, conical, pear-shaped, and club-shaped (Singh 2008). There are many seeds in a fruit (Iqbal et al. 2019). Furthermore, it has a wide environmental adaptation because of its large genetic variability as a desired characteristic (Uddin et al. 2014).

Lagenaria siceraria exhibits rich variability in economic traits and is highly cross-pollinated (Malviya et al. 2017a). The flowers are pollinated mainly by hawk moths at night, but during the day cucumber beetles, bumble bees and other insects are active (Sithole 2014). Like the situation with other members of the Cucurbitaceae family, the ratio of male: female flower is high. This is a white flowered monoecious plant that produces staminate flowers five days before pistillate flowers on average (Uddin et al. 2014). First staminate and pistillate flowers open at approximately 45 and 50 days after seedling emergence, respectively (Buthelezi et al. 2019). It was reported that the male: female sex ratio for *L. siceraria* is 26:1 (Morimoto et al. 2004).

2.2 Uses of *Lagenaria siceraria*

The crop is grown for its tender fruits and is mainly used as a cooked vegetable (Malviya et al. 2017a). In addition, the leaves, flowers and young shoots of this crop are used for food as a vegetable in the same way as a pumpkin and can be consumed as relish to accompany maize staples such as pap (South Africa), phutu (South Africa), sadza (Zimbabwe), ugali (Kenya) and nsima (Malawi) (Mavengahama et al. 2013). The seed is rich in oils and proteins, as it contains about 50% oil and up to 35% protein (Hassan et al. 2007). Thus, the seed is used by the livestock feed industry for feed production (Buthelezi et al. 2019). The seed of *L. siceraria* can be roasted and consumed as snacks or grounded to powder to thicken stew as a condiment (Sithole et al. 2016). This crop contains potassium, sodium, trace amounts of iron and is rich in vitamins B and C and essential minerals as well as other trace elements (Milind and Satbir 2011). Therefore, *L. siceraria* has the potential to contribute to both food security and dietary diversity (Chimonyo and Modi 2013). Landraces have been used for food in KwaZulu-Natal, so it is also important to know the concentrations of minerals and phytonutrients that would be obtained in F_1 progenies.

Medicinal properties of the crop include the use of fruit juice to lower blood cholesterol and it also helps in weight loss due to its high dietary fibre (Milind and Satbir 2011). It is claimed that the fruit juice is helpful in curing conditions such as flatulence, urinary disorders, diabetes mellitus, insomnia, hypertension, premature greying of hair, liver diseases and as a diuretic (Mashilo et al. 2016). *Lagenaria siceraria* contains choline, which is an important compound to retain and enhance memory because it serves as a precursor of the neurotransmitter acetylcholine (Sithole 2014). The dried fruit is used to store sour milk and water in rural communities and also to make musical instruments (Buthelezi et al. 2019).

2.3 Ecology and Production

Lagenaria siceraria grows well in tropical, temperate and sub-tropical climates and in arid to semi-arid climates of low-lying areas (Grubben and Denton 2004). It is now distributed along the tropical regions of the African continent. It grows well over a wide range of soils with good drainage, but sandy loams to loamy are ideal for cultivation and requires a near neutral pH such as 6.5 (Milind and Satbir 2011). It requires areas with annual rainfall ranging from 400 to 600 mm and planting is carried out from

September to early December (Department of Agriculture, Forestry and Fisheries 2016). However, the crop is intolerant to water logging. Warm temperatures between 25 °C and 35 °C are conducive for the growth of the crop (Singh et al. 2015). The optimum germination temperature is between 20 °C and 25 °C (Chimonyo and Modi 2013). Germination rate is reduced by temperatures above 35 °C and below 15 °C (DAFF 2016).

Photoperiodism affects the flowering, where long days with short nights (summer) stimulate pistillate flower formation with a greater fruit yield, but short days with long nights (winter) promote staminate flower establishment with a less fruit yield (Buthelezi et al. 2019). In South Africa, there is an information gap on the nutrients obtainable in *L. siceraria* and its consumption health benefits. Hence, it is under produced (subsistence produced with traditional farming practices), where women are the main custodians of its husbandry. It responds well in both organic and inorganic fertilizer to obtain macronutrients (nitrogen, potassium and phosphorus) which are needed in larger quantities. Hybrid varieties in Asia have recorded yields of more than 40 t/ha under optimum conditions, while local landraces produced less than 25 t/ha (Chimonyo and Modi 2013).

2.4 Importance of landraces in cultivar development and crop genetic improvement

A landrace is a cultivated, genetically diverse plant variety that has grown in a certain eco-geographical area and is thus adapted to the edaphic and climatic conditions and to its traditional management and uses (Casañas et al. 2017). The main contribution of landraces to plant breeding is based on their desirable characters for more effective nutrient uptake and utilization, as well as useful genes for adaptation to stressful environments such as water stress, salinity and high temperatures (Azeez et al. 2018).

Commercial cultivars are generally more productive than landraces, although in recent years, landraces have become important as sources of genetic variability in the search of genes for tolerance or resistance to biotic and abiotic factors of interest in agriculture (Chimonyo and Modi 2013). Landraces are divided into two groups, namely primary and secondary, depending on their maintainers. In primary landrace, the informal maintainers propagate the crop repeatedly over several generations to develop desired traits (Cardoso and Maxted 2014). Secondary landrace is a crop that has been developed for seed saving and commercial purposes, which is genetically dissimilar from the original bred material (Buthelezi et al. 2019).

Genetic improvement in plants is a procedure based on theoretical principles and methods for obtaining varieties of crop plants with high and stable yields of the products grown with the required quality (Council for Agricultural Science and Technology 2017). Plant breeding is a process adopted by society to create new strains of plants that have more desirable traits (Acquaah 2012). The crop improvement process, with respect to a given species, typically involves several steps. Defining the traits to be improved is the first step. Second is the identification of the germplasm with desired traits and favourable genes that can contribute to an improved cultivar (i.e., identifying parents). Crossing chosen parents is the third step, followed by evaluating and selecting the outstanding progeny of intermated plants (which may be repeated for several generations) and lastly is the field testing in the potential market region to determine if the performance of any selected offspring is sufficient to warrant release of an improved cultivar (CAST 2017). To characterize the genetic parameters in improvement of cross-pollinated crops, North Carolina designs are usually used (Kalola and Pandya 2016).

2.5 Mating designs

Breeders use these mating designs to understand the genetic control or behaviour of the trait of interest and they also generate a base population to initiate a breeding program (Acquaah 2012). There are different mating designs as follow: Biparental mating; Polycross; North Carolina design I; North Carolina design ii; North Carolina design III; Triple test cross; Complete diallel mating; Partial diallel mating; Line x Tester mating design and Topcross (Muthoni and Shimelis 2020). In any breeding program, the key is the choice of the correct parents (Agarwal et al. 2017). For the selection of suitable parents for hybridization, the knowledge about combining ability is vital. Combining ability is the capacity of the parent to combine during hybridisation process among each other such that desirable characters or genes are transmitted to their offspring (Fasahat et al. 2016).

2.5.1 Biparental mating (or pair crosses)

In biparental mating design, the breeder chooses many plants (n) at random and crosses them in pairs to generate 1/2n full-sib families (Acquaah 2012). The restraint of this otherwise simple to implement design is its inability to offer the needed information to estimate all the parameters required by the model (Klein et al. 1973). The offspring from the design comprises full-sibs or unrelated individuals (Nduwumuremyi et al. 2013). There is no further relatedness among individuals in the offspring (Mather 1982). To estimate the genetic and environmental variance, the breeder must make unjustifiable assumptions (Hill et al. 1998). It is not ideal for selection, and it is not good for estimating genetic gains from additive variance (Muthoni and Shimelis 2020).

2.5.2 Polycross

This is a mating arrangement for intermating a group of cultivars or clones utilising natural hybridization in an isolated crossing block (Klein et al., 1973). It works well in species that are obligate cross-pollinators, but especially to those that can be vegetatively propagated crops such as cassava, sugarcane and sweet potato (Acquaah 2012). The design provides equal opportunity for each clone or parent to naturally cross with each other in the block such that self-pollination is prevented (Nduwumuremyi et al. 2013). Because of several problems, placing the system in jeopardy of deviating from random mating, the ideal requirements are hard to meet in practice. Mating will not be random if all the entries do not flower together, (Acquaah 2012).

2.5.3 North Carolina Design I

It is used for evaluation of full- and half-sib recurrent selection as well as in estimation of additive and dominance variances, (Acquaah 2012). It is not of practical application in breeding species that are not capable of producing large amounts of seed because it requires sufficient seed for replicated evaluation trials (Muthoni and Shimelis 2020). It is applied in both self- and cross-pollinated species that meet this principle (Nduwumuremyi et al. 2013). As a nested design, each member of a group of parents used as males is mated to a different group of parents. It is a hierarchical design with non-common parents nested in common parents. This design is most widely used in animal studies, and it has been extensively used in maize breeding for estimating genetic variances (Acquaah 2012).

2.5.4 North Carolina Design II

North Carolina Design II is a factorial mating design in which each member of a group of parents used as males is mated to each member of another group of parents used as females in evaluation of inbred lines for combining ability (Nduwumuremyi et al. 2013). This is a modification of NC design I that has greater precision (Muthoni and Shimelis 2020). In North Carolina Design II, two independent estimates of general combining ability allow determination of maternal effect and calculation of heritability based on male variance, which is free from maternal effect (Fasahat et al. 2016). NCD II used in the present study also allows the breeder to measure not only GCA but also SCA. The design provides good information on parents and full-sib families, provides estimates of both additive and dominance effects, and provides estimates of genetic gains from both additive variance and dominance variance. This mating design generates adequate data for estimation of all parameters because of the relatedness among the offspring families (Muthoni and Shimelis 2020). This design is used in the present study since it can be applied in cross-pollinated crops like *Lagenaria siceraria*. North Carolina design ii was also used for yam bean (*Pachyrhizus* spp.) in Uganda by Agaba et al. (2021).

2.5.5 North Carolina Design III

In this design, a random sample of F2 plants is backcrossed to the two inbred lines from which the F2 was descended (Acquaah 2012). Comstock and Robinson (1948) developed this mating design. The estimation of dominance is not subjected to any assumption regarding allele frequencies in this mating design, that is an advantage of this design over designs I and II. In this design, estimates of genetic components tend to be more biased from epistasis than in design I and design II (Muthoni and Shimelis 2020).

2.5.6 Triple Test cross

Kearsey and Jinks (1968) extended North Carolina Design III to include a third tester (L3). Third tester is the F1 from original parents L1 x L2 (Muthoni and Shimelis 2020). The extended form is called triple test cross (TTC) (Le Clerg 1966). It is capable of testing for epistatic interactions and estimate dominance and additive variances (Acquaah 2012).

2.2.7 Diallel mating designs

Diallel crossing is a vital procedure for conducting animal and plant breeding experiments, particularly for estimating combining ability effects of lines (Ghosh and Biswas 2003). In Complete **diallel mating design** parents are crossed in all possible combinations, including reciprocals and selfs (Acquaah 2012). Hardy-Weinberg equilibrium in a population is achieved with complete diallel mating design (Muthoni and Shimelis 2020). The recent use of this method started with the concept of specific and general combining abilities by Sprague and Tatum (1942). Selfing does not donate to recombination of genes between parents. Moreover, recombination is attained by crossing in one direction making reciprocals needless (Nduwumuremyi et al. 2013). A **partial diallel mating design** is utilised when there is a large number of entries , which allows all parents to be mated to some but not all other parents in the set (Acquaah 2012).

2.2.8 Line x Tester mating design

Line x Tester mating design utilises inbred lines as the base population, and it is an amendment of North Carolina Design II (Muthoni and Shimelis 2020). Randomly selected f inbreds used as females (designated as testers) are mated to each of the randomly chosen m inbreds used as males (referred to as lines), to generate mf offspring families which are normally F1 hybrids (Sharma 2006). This mating design is not limited to the multiflowered crop species because it allows mating all the m inbred lines to all the f testers (Comstock and Robinson 1952). Kempthorne (1957) proposed this mating design, it provides specific combining ability of each cross, and it is not providing general combining ability of lines only but of the testers also, as liner and tester both are different sets of genotypes (Nduwumuremyi et al. 2013). Hence, it is most appropriate for animal experiment (Sharma 2006). Furthermore, it is useful in estimating several kinds of gene actions significant in the expression of quantitative traits (Rashid et al. 2007).

2.2.9 Topcross mating design

This design involves the crossing of several selections, lines, or clones to a common parent (tester) which may be a cultivar, an inbred line, a single cross, where the tester is the same for each mating (Muthoni and Shimelis 2020). For preliminary evaluation of combining ability of new inbred lines, this design has been widely utilised (Mosa 2010). The offspring from individual plants are tested with this design and these progenies are half-sib families (Nduwumuremyi et al. 2013). It is mainly used in cross-pollinated crops like maize, where it is commonly an inbred-cultivar cross (Muthoni and Shimelis 2020).

2.6 Heritability, genetic and phenotypic variance

Heritable genetic variation is transmitted from parent to offspring. Some genetic variation is not strictly heritable because of the dominance or epistatic interactions that are not directly passed from parent to progeny (Harriman and Nwammadu 2016). Heritability is a measure of the proportion of the genetic variance out of the total phenotypic variance existing in a population and it is characterized into broad sense heritability (H^2) and narrow sense heritability (h^2) (Rukundo et al. 2017).

For a specific characteristic, heritability displays the degree to which progeny can be expected to resemble their parents (Rukundo et al. 2017). When breeding sexually propagated crops, heritability in the narrow sense is important because alleles responsible for non-additive genetic variations are not fully recovered due to reshuffling of genes (Sleper and Poehlman 2006). Broad sense heritability is useful in the breeding of clonally propagated species such as *Ipomoea batatas* in which both additive and non-additive gene actions are stable and transmitted from parent to progeny (Acquaah 2012).

Phenotypic variance is the overall variance among individuals within a population when grown in a variety of environments of interest to the plant breeder (Harriman and Nwammadu 2016). The total genetic variance is the portion of the phenotypic variance that can be attributed to genetic differences among phenotypes (Suzuki et al. 1981). Narrow sense heritability can only be low if additive variance is low and dominance, epistasis and environmental variances are large. Broad sense heritability is associated with genetic variance which includes additive, dominance, and epistasis variance. It can be high when these components are high, and the environmental variance is low. A high heritability (especially narrow sense – when additive variance is high) means the correlation between the genotype and phenotype is high and simple phenotypic selection can be done (Acquaah 2012).

A main factor restraining the rate of progress in plant breeding is the low heritability of quantifiable traits such as yield. The main role of heritability in the genetic study of quantitative characters is its predictive part to specify the reliability of the phenotypic value as a guide to the breeding value (Harriman and Nwammadu 2016). Heritability is expressed as a value between zero and one, thus we can find heritability numbers that indicate 0.40 or 0.60 or some such proportion to indicate the effect of the genes in the variability of a certain attribute (Acquaah 2012). Consequently, genetics at about 30% and the environment at 70% influence the variation of a trait that has a heritability of 0.30. Zero heritability means there is no variation in the genes (Harriman and Nwammadu 2016).

Broad sense heritability (H^2) is the proportion of trait variance that is due to all genetic aspects including dominance and gene-gene interactions, whereas narrow-sense heritability (h^2) is the proportion of trait variance that is due to additive genetic factors (Harriman and Nwammadu 2016). Narrow-sense heritability is expressed as (h^2) = V_A/V_P where V_A is additive variance and V_P is phenotypic variance. Broad sense heritability is expressed as (H^2) = V_G/V_P where V_G is the genetic variance [$V_G = V_A + V_D + V_I$; where V_D is the dominance variance and V_I is the epistatic variance; $V_D + V_I$ = non-additive variance] (Falconer and Mackay 1996). Total phenotypic variance, $V_P = V_G + V_E$. These considerations lead to the following equations: $V_P = V_A + V_I + V_D + V_E$, where V_P and V_E are total phenotypic variance and environmental variance respectively (Acquaah 2012).

2.7 Potential for genetic improvement in *Lagenaria siceraria*

Lagenaria siceraria landraces are morphologically different from one another based on vegetative and reproductive traits. These landraces show broad variation in their seed and fruit attributes, so specifically that they can be simply recognized by these traits (Buthelezi et al. 2019). These characteristics of each landrace are transmitted from parents to progeny during crossing. The heterosis can be attained by crossing parents with complementary traits. Heterosis refers to the super performance of a hybrid exhibiting increased biomass, yield, size, growth rate, or fertility relative to its parents (Feng et al. 2015).

Lagenaria siceraria is a diploid (2n = 2x = 22) vine crop that is mainly grown for its fruits (Mashilo 2016). *L. siceraria* being monoecious in sex expression can be beneficially utilized to produce hybrid seeds at inexpensive rate, by eliminating staminate flowers in seed parents and allowing hand pollination method only (Milind and Satbir 2011). The high number of hybrid seeds per cross makes it more economical (Ghuge et al. 2016). Additionally, the seed requirement per hectare for large-scale cultivation would be low and cost effective because the crop is cultivated at wider spacing. Hence, *L. siceraria* offers greater scope for exploitation of heterosis on commercial scale to increase the production and productivity (Ghuge et al. 2016).

In cross-pollinated crops, controlled pollination is made on individual plants by bagging flowers before they open, requiring resources for each population and family to be advanced (Kumar et al. 2013). Cucurbit populations can be improved by recurrent selection by using natural inter-crossing of selected families in isolation blocks if the rate of natural outcrossing can be increased (Kumar and Wehner 2011). Inter-crossing can play a vital role in genetic gain (Wehner and Cramer 1996). The pollination of *L. siceraria* plants can be manipulated by adjusting plant spacing. Moreover, close plant spacing may be used to enhance cross-pollination, therefore facilitating inter-crossing among families in a recurrent selection program (Kumar et al. 2013). Quantitative traits are improved by using recurrent selection (Hallauer and Miranda 1988). Increasing the plant spacing might increase the amount of self-pollination for individual plant selection where required in methods such as pedigree breeding (Kumar et al. 2013). The pollen flow in cucurbit crops is affected by the environment. which in turn, affects the rate of cross or self-pollination (Stanghellini and Schultheis 2005).

2.8 Bitterness and sweetness traits

Flavour is an influential basis of human consumption behaviour (Forestell 2017). Many factors from different sensory modalities influence multisensory flavour perception and liking (Wang et al. 2019). Peroxide and polyphenol oxidase are enzymes that cause browning in *Lagenaria siceraria* fruits, thus the colour of fruit juice is brown with unpleasant taste (Gajera and Joshi 2015). For protection against animal and insect predators, *Lagenaria siceraria* plants produce cucurbitacins (tetracyclic triterpenoid structured bitter compounds) which produce a distinctive aroma (Chimonyo and Modi 2013). Hence, there is abnormal high level of cucurbitacins in bitter gourds than in less bitter types. Plant based cucurbitacins, flavonoids, phenols, terpenes, isoflavones, glucosinolates, and other compounds that are present in the everyday diet are almost always bitter, acrid, or astringent, but they lower rates of cancer and coronary heart disease (Drewnowski and Gomez-Carneros 2000).

Growing confirmation exists on the protective role of vegetable intake to lower risk for chronic diseases (Sophie et al. 2012). Children are born with a biological tendency to prefer sweet and to avoid bitter foods such as green leafy vegetables for discouraging the consumption of toxins (Forestell 2017). Bitterness in foodstuffs is often linked with toxic compounds and a 59-year-old male died in South Delhi due to alleged consumption of bitter *L. siceraria* juice (Sharma et al. 2012). Excessive bitter taste is the main problem in fruit and vegetable industry worldwide because it reduces the quality and commercial value of the product, and thus the research on genetic improvement conducted to eliminate bitterness genes in these fruits and vegetables is necessary (Kore and Chakraborty 2015).

2.9 Conclusion

This chapter reviews the taxonomy, origin, uses, ecology, and production of *Lagenaria siceraria*. It also reviews the importance of landraces, mating designs, heritability, variances, bitterness and sweetness, genetic components and potential of *L. siceraria* landraces for crop improvement. The cultivation of this crop is largely dependent on indigenous unimproved landraces, which are not scientifically selected and bred due to the absence of improved cultivars. As a breeding program, the present study crossed landraces that show the desirable traits and evaluated the morpho-agronomic attributes, taste and nutritional composition of their offspring, for efficient utilization of this crop.

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Chapter 3

Morpho-agronomic evaluation of parental landraces and F_1 offspring of *Lagenaria siceraria*

Abstract

Lagenaria siceraria is one of the most important cucurbitaceous vegetables due to its prolific bearing habit, edibility as a cooked vegetable and its low cost of cultivation. The objective of this study was to evaluate variation in the morpho-agronomic traits among selected parental landraces and the *Lagenaria siceraria* F_1 populations (progenies). The selected landraces were crossed based on the North Carolina II genetic design to develop early F_1 populations. The twelve F_1 populations along with seven parental landraces were grown in a randomized complete block design with three replications. Significant differences ($P < 0.05$) were observed among quantitative traits suggesting considerable genetic variability. The genotypes displayed significant variation in most qualitative traits of fruits (colour, texture and shape) and seeds (colour, size and shape). The seven parental landraces and twelve F_1 progenies evaluated in the present study exhibited a wide range of diversity in quantitative traits including days to emergence, emergence percentage, seedling height, leaf area, main vine length, days to flowering, days to edible harvest maturity, days to drying harvest maturity, fruit neck length, seed width and leaf growth percentage. The first five principal components on quantitative traits among the evaluated 19 genotypes contributed 74.84% of the variability. For quantitative traits, emergence percentage, seedling height, cotyledons leaf area, leaf area, vine length, number of leaves, number of branches, number of seeds per fruit, fruit mass, fruit rind thickness, hundred seed mass, total seed mass, fruit length, fruit width, seed width, fruit yield, yield per plant, leaf chlorophyll content, total fruit mass per plot, fruit neck length and seed length were correlated positively with the first principal component (PC1), which accounted for 34.46% of the total variation. The days to emergence, days to flowering, days to edible harvest maturity, days to drying harvest maturity, shoot growth percentage, cotyledon leaf area, leaf area, vine length, number of leaves, number of branches, fruit mass, fruit rind thickness, hundred seed mass, fruit width, seed length and seed width correlated positively with PC2, which accounted for 14.48% of total variability. The biplot and dendrogram clustered the genotypes into five clusters according to their vegetative, fruit and seed traits. The highest value for broad sense estimate was recorded for days to drying harvest maturity (early maturing) trait. F_1 progenies displayed more heterosis or hybrid vigour than parental landraces in 15 quantitative traits (53.57%) out of the 28 evaluated traits. Hence, the F_1 progenies can be used for further *Lagenaria siceraria* genetic improvement.

Keywords: F_1 progenies, qualitative traits and quantitative traits.

3.1 Introduction

Lagenaria siceraria is an important cucurbitaceous vegetable due to its prolific bearing habit, its edibility as a cooked vegetable and its low cost of cultivation (Doloi et al. 2018). The primary gene centre of the *L. siceraria* is tropical Africa (Mladenovic et al. 2012). Its white flowers and characteristic seed, fruit and leaf shapes differentiate this crop from other pumpkin varieties (Mladenovic et al. 2012). The flower size and monoecious condition makes hybridization easy and appropriate (Doloi et al. 2018). The quantity of cross-pollination ranges between 60 and 80% for this crop (Doloi et al. 2018). Its landraces in South Africa display great diversity for morphological traits, mostly in fruit shape and size (Yetisir et al. 2008). The valuable tools for preliminary assessment of genetic variability are morpho-agronomic descriptors because they allow fast insight into the range of diversity that exists (Jesus et al. 2013).

Knowledge of genetic diversity in a germplasm collection is important for parental selection in hybridization (Keneni et al. 2005). Significant genetic diversity occurs in this crop, which can be applied for the exploitation of hybrid vigour (heterosis) (Doloi et al. 2018). Heterosis breeding provides the way to overcome the yield barriers (Kumar et al. 2011). The heterosis can be attained by crossing parents with complementary traits. Heterosis refers to the super performance of a hybrid exhibiting increased biomass, yield, size, growth rate, or fertility relative to its parents (Feng et al. 2015).

Quantitative variables are vital descriptors, particularly when used to evaluate the agronomic potential of a new cultivar or germplasm accession (Jesus et al. 2013). The cultivation of *L. siceraria* is largely dependent on indigenous unimproved landraces, which are not scientifically selected and bred, due to the absence of improved cultivars. Hybrid varieties of *L. siceraria* in Asia have recorded yields of more than 40 t/ha under optimum conditions, while local landraces produced less than 25 t/ha (Chimonyo and Modi 2013). Yield is a generic term used by crop producers to describe the quantity of the part of a crop plant of interest that is harvested from a given area at the termination of the cropping season or within a given period (Acquaah 2012). It was necessary to select and cross the landraces from KwaZulu-Natal that show desirable traits and evaluate the morpho-agronomic characteristics of their offspring for future development of new cultivars. Therefore, the current study was undertaken to evaluate the performance of *Lagenaria siceraria* genotypes (seven parental landraces and twelve F_1 offspring) using morpho-agronomic traits.

3.2 Materials and Methods

3.2.1 Planting material

The planting materials for this study consisted of seven *Lagenaria siceraria* landraces as the parents and the twelve F_1 progenies. The seven landraces (Table 3.1) were collected from various geographic locations of KwaZulu-Natal, South Africa. The selection was based on their early growth, high yield and high number of flower traits, following a study conducted by Buthelezi et al. (2019). These landraces were collected from Dundee (28.1650° S, 30.2343° E), Khangelani (29.0106° S, 31.2211° E), Nquthu (28.2195° S, 30.6746° E) and Rorke's Drift (28.3492° S, 30.5351° E) (Buthelezi et al. 2019). The parental landraces were named according to their origin and fruit morphology (Buthelezi et al. 2019). Four landraces that were used as male parents were KSR from Khangelani, NSRC and NqSC from Nquthu, as well as RRP from Rorke's Drift. Three that were used as female parents were DSI from Dundee, KSP from Khangelani, and NSC from Nquthu. The female parents were selected based on their greater average number of pistillate flowers than the ones for male parents, whereas the male parents were selected based on their high number of staminate flowers than the ones for female parents for ease of hand pollination (Buthelezi et al. 2019). Furthermore, the female parents were selected based on their superior performance in several traits including germination percentage and fruit yield, whereas the male parents were selected since they were superior in most vegetative traits including number of leaves, number of branches and leaf chlorophyll content (Buthelezi et al. 2019). Moreover, fruit shape and fruit texture were considered in selecting the female parents and the male parents for proper mixing of traits in the generated F_1 offspring.

Table 3.1: Description of parental landraces according to their origin as well as fruit and seed morphology (Buthelezi et al. 2019)

PLR	Area	Fruit colour	Fruit texture	Fruit shape	Seed type	Seed colour	Seed texture	Seed size	Seed shape
KSP	Khangelani	Pale green	Smooth	Pear	Asiatica	Brown	Leathery	Large	Slightly oblong to rectangular
KSR	Khangelani	Green	Rough	Isodiametric	Siceraria	Dark brown	Leathery	Large	Slightly oblong to rectangular
DSI	Dundee	Dark green	Smooth	Isodiametric	Siceraria	Dark brown	Smooth	Large	Oblong
RRP	Rorke's Drift	Pale green	Rough	Pear	Asiatica	Light brown	Leathery	Large	Rectangular
NSC	Nquthu	Pale green	Smooth	Curvilinear	Asiatica	Light brown	Leathery	Medium	Slightly oblong
NSRC	Nquthu	Green	Semi-rough	Curvilinear	Intermediate	Brown	Leathery	Medium	Slightly oblong
NqSC	Nquthu	Pale green	Smooth	Semi-curvilinear	Asiatica	Light brown	Leathery	Medium	Slightly oblong

Note: PLR-Parental Landraces-from Khangelani area (KSP) with smooth pear-shaped and (KSR) rough isodiametric shape fruits; from Rorke's Drift area (RRP) with rough pear shaped; from Nquthu area (NqSC) and (NSC) with smooth curvilinear shaped and (NSRC) semi-rough curvilinear shaped and from Dundee area (DSI) with smooth isodiametric shape

Crossing

The crossing study was carried out in pots under semi-controlled environmental conditions (net house) at North-West University (25º49´34´´S, 25º36´34´´E) from November 2019 to April 2020. The soil to fill the ten litre pots of 30 cm diameter was collected from the Crop Science research field. Soil was sieved using a 5 mm sieve before filling the pots. Three seeds were planted per pot. Fertilizer NPK 13: 7: 10 (30) + 0.5% Zn + 5% S + 3% Ca at a rate of 3.6 g/pot was mixed with the soil thoroughly in each pot as basal dose before sowing. Limestone Ammonium Nitrate (LAN 28) was applied with irrigation water where 3.6 g was dissolved in one litre of water one month after planting. At flowering the same rate of LAN fertigation was applied to avoid cutting of roots. Pots were hand weeded and the plants were sprayed with Malasol (Mercaptothion) to control pests and irrigation water was supplied where necessary

At planting, the temperature was 28 ˚C in the net house, whereas outside it was 31 ˚C with 0% precipitation. The laboratory thermometers were used to measure the temperature in the net house. The average minimum day temperature was 16 ˚C and the average maximum day temperature was 31 ˚C in the net house during growing season. The pots were labelled for identification and after planting they were irrigated adequately.

The selected landraces were crossed based on the North Carolina II genetic design to develop F1 progenies. Blocking was performed in this study to allow all mating involving a single group of males to a single group of females to be kept intact as a unit (Table 3.2) (Acquaah 2012). The pistillate flowers of female parent and staminate flowers of male parent were bagged separately a day before opening and hand pollination was done the next morning immediately followed by bagging for two to three days. The cross pollination was performed early in the morning (before 06:00 am) using ear buds to transfer pollen grains from staminate flower to pistillate flower. An ear bud was used once for each crossing and discarded afterwards. The pollinated flowers were tagged for identification.

Table 3.2: Arrangement of North Carolina Design II for crosses

Female parents **Male parents**	**KSP**	**NSC**	**DSI**
KSR	KSRxKSP	KSRxNSC	KSRxDSI
NSRC	NSRCxKSP	NSRCxNSC	NSRCxDSI
NqSC	NqSCxKSP	NqSCxNSC	NqSCxDSI
RRP	RRPxKSP	RRPxNSC	RRPxDSI

Note: Female Parents- smooth pear shaped KSP from Khangelani area, smooth curvilinear shaped NSC from Nquthu area and smooth isodiametric shaped DSI from Dundee area. Male parents- rough pear shaped KSR from Khangelani, smooth curvilinear shaped NSRC and smooth semi-curvilinear shaped NqSC from Nquthu and rough isodiametric shaped RRP from Rorke's Drift area.

3.2.2 Experimental site

A field experiment for the first season was conducted at North-West University (NWU) Crop Science field (25º49´34´´S, 25º36´34´´E) from October 2020 to January 2021. The second season field experiment was conducted at Molelwane North-West University research farm, (25°48′ S, 25°38′ E) from January to May 2021. Both sites are located at Mafikeng, South Africa. The area usually receives mean annual rainfall of 571 mm during summer seasons (Kasirivu et al. 2011). The minimum temperatures vary from 7 °C to 11.4 °C while the maximum varies from 26.9 °C in June to 37 °C in January. Soil samples were collected (0-30 cm) before planting and sent to analytical laboratory of KwaZulu-Natal Department of Agriculture and Rural Development for analysis (Table 3.3), using the rapid procedures described by Hunter (1975). The soils of the site belong to the Hutton series according to the South African Soil Classification, with a loamy sand to sandy clay loam texture (Kasirivu et al. 2011).

Table 3.3: Physico-chemical properties of experimental sites during planting seasons

Soil Physico-chemical properties	**NWU crop science field (Season 0ne)**	**Molelwane farm (Season two)**
Sample density (g/mL)	1.14	1.34
Phosphorus (mg/L)	1	14
Potassium (mg/L)	244	275
Calcium (mg/L)	2961	643
Magnesium (mg/L)	2961	286
Exchangeable acidity (cmol/L)	0.07	0.07
Total cations (cmol/L)	21.07	6.34
Acid saturation (%)	0	1
pH (KCL)	6.24	6.49
Zinc (mg/L)	0.1	1.3
Manganese (mg/L)	14	22
Copper (mg/L)	2.2	1.1
Organic carbon (%)	1.5	< 0.5
Nitrogen (%)	0.08	0.06
Clay (%)	20	17

3.2.3 Experimental design and field establishment

Field evaluation

The twelve F_1 progenies along with seven parental landraces were grown in a randomized complete block design with three replications. The field was divided into 19 equal sized gross plots with three replicates per genotype, which made a sum of 57 gross plots in total. Each gross plot was 9 m^2 (3 × 3) with 1 m inter-row spacing and 1 m intra-row spacing, resulting in 16 plants per plot. There were four rows per gross plot. The spacing between plots was 1.5 m. The seeds were sown directly into the soil at 5 cm depth.

Soil analysis was conducted before sowing. Fertilizer NPK 13: 7: 10 (30) + 0.5% Zn + 5% S + 3% Ca at a rate of 40 g/m^2 was mixed with the soil thoroughly in each plot in equal amount as basal dose before sowing. Nitrogen fertilizer Limestone Ammonium Nitrate (LAN 28% N) of the same rate was broadcasted around each plant, one month after planting, and the second one was given at flowering. The field was irrigated to field capacity after fertilizer application and the supplementary irrigation was applied three times a week until maturity. Weed control was performed manually using hand-hoes, and Malasol (Mercaptothion) was used to control aphid infestations.

3.2.4. Data collection

a. Seedling traits

Days to emergence were counted from the date of seeding until 50% of the plants had emerged and the mean was calculated. Emergence percentage was calculated by counting the total number of plants emerged in each plot and emergence percentage was calculated with the following formula (Iqbal et al. 2019).

$$Emergence\ percentage = \frac{Number\ of\ seedlings\ emerged}{Number\ of\ seeds\ planted} \times 100$$

The emergence percentage was calculated from 16 seeds sown per plot in the field. The seedling height (cm) and cotyledon size (mm^2) were measured at the first true leaf stage using a 300-millimetre ruler. Height was measured from the ground surface to the apical growth tip of the uniform seedlings for each genotype**.** Cotyledon size was measured using the following formula: Cotyledon leaf area (cm^2) = length (cm) × width (cm) × 0.88 (Blanco and Folegatti 2005).

b. Shoot and leaf growth

The apical point of the desired vines was marked with the artline90 permanent marker just below the first true leaf. At 43 days after planting (DAP), the initial shoot length (from the base of the marker towards the apical tip of the leaf) and initial leaf area (length × width × 0.88) of the first true leaf was measured. At 50 days after planting, the final shoot length was measured from the base of the initial point towards the apical end of the shoot. At 50 DAP, the final leaf area (length × width × 0.88) of the first true leaf was measured. Shoot and leaf growth was calculated using the following formula: Growth (%) = ((final -initial) ÷ initial) × 100 (Buthelezi et al. 2019).

c. Vine traits

Length of the main vine (cm) was measured from net plants in each plot with a measuring tape and a string (which traced the vine length due to its flexibility properties) and the mean was calculated. A measuring tape was used to measure the vine length from the stem base to the leaf apex. On the same vines measured for length, the number of branches and number of leaves were counted. The leaf area (cm^2) was measured from net plants per plot using the formula: LA = 0.88LW (Blanco and Folegatti 2005). The following qualitative traits were recorded namely early plant vigour, plant growth habit, stem shape, presence or absence of tendrils, tendril type, tendril branching, stem colour, colour of leaves, leaf shape and leaf margin.

d. Chlorophyll content index

The leaf chlorophyll content was taken from net plot using the chlorophyll meter (model CCM-200 plus) in chlorophyll content index (cci) units. CCM-200 plus output, expressed in chlorophyll content index, is the ratio of radiation transmission from a light emitting diode centred at 931 nm to radiation transmission from light emitting diode cantered at 653 nm (Parry et al. 2014). The data was collected from the third true leaf, where one reading was recorded per leaf.

e. Flower traits

The number of days to male and female flowering were counted from the date of emergence to appearance of 50% flowers and the mean was calculated. At flowering stage, the number of staminate and pistillate flowers were recorded from net plots for each of the three replicates. Following Buthelezi et al. (2019), the flower sex ratio was calculated using the following formula: Flower sex ratio (FSR) = (number of staminate flowers ÷ number of pistillate flowers). The following flower traits were recorded: place of flowers, flower colour, flower size and number of petals.

f. Days to fruit maturity

The number of days were counted from the date of sowing to fruit maturity and the mean was calculated. Fruits were considered mature when they were still green in colour, but with a hard rind. At fruit maturity, the plants lost most of their leaves.

g. Fruit yield and agronomic traits at harvest

The number of fruits per plot were recorded at maturity. The number of fruits per plant was recorded from each net plot at every picking. The total number of fruits picked were counted and then averaged to number of fruits per plant. Fruit mass was measured using the analytical weighing scale in grams (KERN KB 10000-1N) and fruit size was measured using a Vernier calliper to measure the length (cm) and width (cm) of the fruits. Harvested fruits of each plot were weighed with an analytical weighing scale and their mean was determined to find the fruit mass.

Total mass of all the harvested fruits per plot from each picking was weighed and fruit yield per hectare was calculated. Following Oloyede et al. (2013), fruit yield per hectare was calculated using the following formula:

$$Fruit\ yield\left(\frac{\mathrm{kg}}{\mathrm{ha}}\right) = \frac{fruit\,\frac{mass}{plot}\,(\mathrm{kg}) \times 10\ 000\ \mathrm{m}^2}{Area\ of\ the\ plot(\mathrm{m}^2)\ \times 1\ kg}$$

The cutting of uniform fruits longitudinally was performed, and the rind thickness (mm) was measured using a Vernier calliper. Fruit neck length was measured in cm with a Vernier calliper. The following qualitative traits were recorded: fruit colour, fruit shape, fruit texture and fruit pulp colour.

h. Seed size and mass

At harvest, the seeds were removed from the fruit pulp and were air dried at room temperature (25 °C) for one week. A Vernier calliper was used to measure the seed length (mm) and width (mm). One hundred seed mass and total seed mass from the fruits of each plot were measured using the analytical weighing scale in grams (KERN KB 2000-2N) for each of 19 genotypes (12 F_1 progenies and seven parental landraces). The following traits were recorded, namely, number of seed per fruit, seed size, seed colour, seed shape, seed texture and presence or absence of seed lines.

3.2.5 Statistical analysis

The data collected were subjected to analysis of variance (ANOVA) using the general linear model (GLM) of Statistical Analysis System program (SAS software version 9.4) (SAS Institute Inc. 2016). The differences between male and female parents and their interactions were measured based on the Tukey's HSD test at 5% significance level. Correlations and principal component analysis (PCA) were performed to determine multi-character variation. Cluster analysis through biplot and dendrogram were performed to study the differences and similarities of landraces and F_1 progenies. Data for both growing seasons was combined and analysed. Following Beavis and Lamkey (2016), linear model used for data analysis was: $Y_{ijk} = + m_i + f_{ij} + mf_{ij} + r_k + e_{ijk}$

Where, μ = mean; m_i = the effect of male *i*; f_j =the effect of female *j*; mf_{ij} = the interaction effect of female *j* when crossed to male *i*; r_k = replication effect and e_{ijk} = the residual.

3.2.5.1 Variance components estimation

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

- Environmental variance (δ^2e) = MSE
- Genotypic variance (δ^2g) = $\frac{(MSG-MSE)}{r}$
- Phenotypic variance (δ^2p) = $\delta^2g + \delta^2e$

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

- Phenotypic coefficient of variation (PCV) = $\frac{\sqrt{\delta^2 p}}{X} \times 100$
- Genotypic coefficient of variation (GCV) = $\frac{\sqrt{\delta^2 g}}{X} \times 100$

Where: δ^2p = Phenotypic variation, δ^2g = Genotypic variation, X = Grand mean of the character to be 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:

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

Genetic advance (GA) was estimated as per formula given by Allard (1960) and cited by Meena et al. (2017).

$\boldsymbol{G_A} = (\mathbf{k})(\sqrt{(\delta^2 p)})\ (H^2)$

Where: GA = expected genetic advance, H^2 = heritability and k = The standard selection differential at 5% selection intensity (k = 2.063).

3.3 Results

3.3.1 Qualitative traits evaluation

Spreading was the dominant growth habit exhibited by 95% of the genotypes (18 genotypes), while one genotype (5%) had a bushy growth habit (Table 3.4). There were differences in leaf margins amongst studied genotypes, with the following types per genotype: crenate (15.8%), spiny (15.8%), sinuate (5.3%), ciliate (10.5%), ciliate-crenate (5.3%), spiny-crenate (10.5%), spiny-sinuate (10.5%), spiny-ciliate (5.3%), spiny-undulate (10.5%), undulate (5.3%), and denticulate (5.3%) margins (Table 3.4 and Figure 3.1). The dominant stem shape was angular, displayed by 89% (17 genotypes), while two genotypes (11%) had round stem shape. Green was the dominant colour of leaves, exhibited by 84% (16 genotypes), while three genotypes (16%) exhibited dark green leaf colour (Table 3.4 and Figure 3.1).

Table 3.4: Variation in vegetative and reproductive qualitative traits among *Lagenaria siceraria* genotypes

Genotype	**Growth Habit**	**Stem Shape**	**Leaves Colour**	**Leaf Margin**
KSP	spreading	Angular	Dark Green	Crenate
DSI	Spreading	Angular	Dark Green	Spiny
NSC	Bushy	Angular	Green	Sinuate
NqSC	Spreading	Angular	Green	Spiny
KSR	Spreading	Angular	Green	Ciliate
NSRC	Spreading	Angular	Green	Crenate
RRP	Spreading	Angular	Green	Ciliate-Crenate
NqSCxKSP	Spreading	Angular	Green	Spiny
NqSCxDSI	Spreading	Angular	Green	Spiny-Crenate
NqSCxNSC	Spreading	Angular	Dark Green	Spiny-Sinuate
KSRxKSP	Spreading	Angular	Green	Spiny-ciliate
KSRxDSI	Spreading	Round	Green	Spiny-undulate
KSRxNSC	Spreading	Angular	Green	Spiny-sinuate
NSRCxKSP	Spreading	Round	Green	Ciliate
NSRCxDSI	Spreading	Angular	Green	Undulate
NSRCxNSC	Spreading	Angular	Green	Spiny-crenate
RRPxKSP	Spreading	Angular	Green	Denticulate
RRPxDSI	Spreading	Angular	Green	Spiny-undulate
RRPxNSC	Spreading	Angular	Green	Crenate

KSP
DSI
NSC
NqSC
KSR
NSRC
RRP
NqSCxKSP
NqSCxDSI
NqSCxNSC
KSRxKSP
KSRxDSI
KSRxNSC
NSRCxKSP
NSRCxDSI
NSRCxNSC
RRPxKSP
RRPxDSI
RRPxNSC

Figure 3.1: Diversity in leaf colour and leaf margin among *Lagenaria siceraria* genotypes (bold-parents and normal-F_1 progenies)

In the current study, the genotypes displayed significant variation in most fruit and seed traits. The dominant fruit colour amongst genotypes was dark green exhibited by 47.4% of entries (9 genotypes), while seven genotypes (36.8%) displayed green colour and three genotypes (15.8%) had light green fruit colour (Table 3.5 and Figure 3.2). Smooth was the dominant fruit texture amongst genotypes exhibited by 57.9% of entries (11 genotypes), while five genotypes (26.3%) displayed both rough and smooth texture and three genotypes (15.8%) had rough fruit texture (Table 3.5 and Figure 3.2). The following differences in fruit shape were recorded amongst studied genotypes, as represented by the number and percentage: curvilinear (4, 21.1%); isodiametric (4, 21.1%); pear (3, 15.8%); pear and isodiametric (2, 10.5%); isodiametric and curvilinear (2, 10.5%); pear and semi-curvilinear (2, 10.5%); semi-curvilinear (1, 5.3%) and curvilinear and pear (1, 5.3%) shapes (Table 3.5 and Figure 3.2).

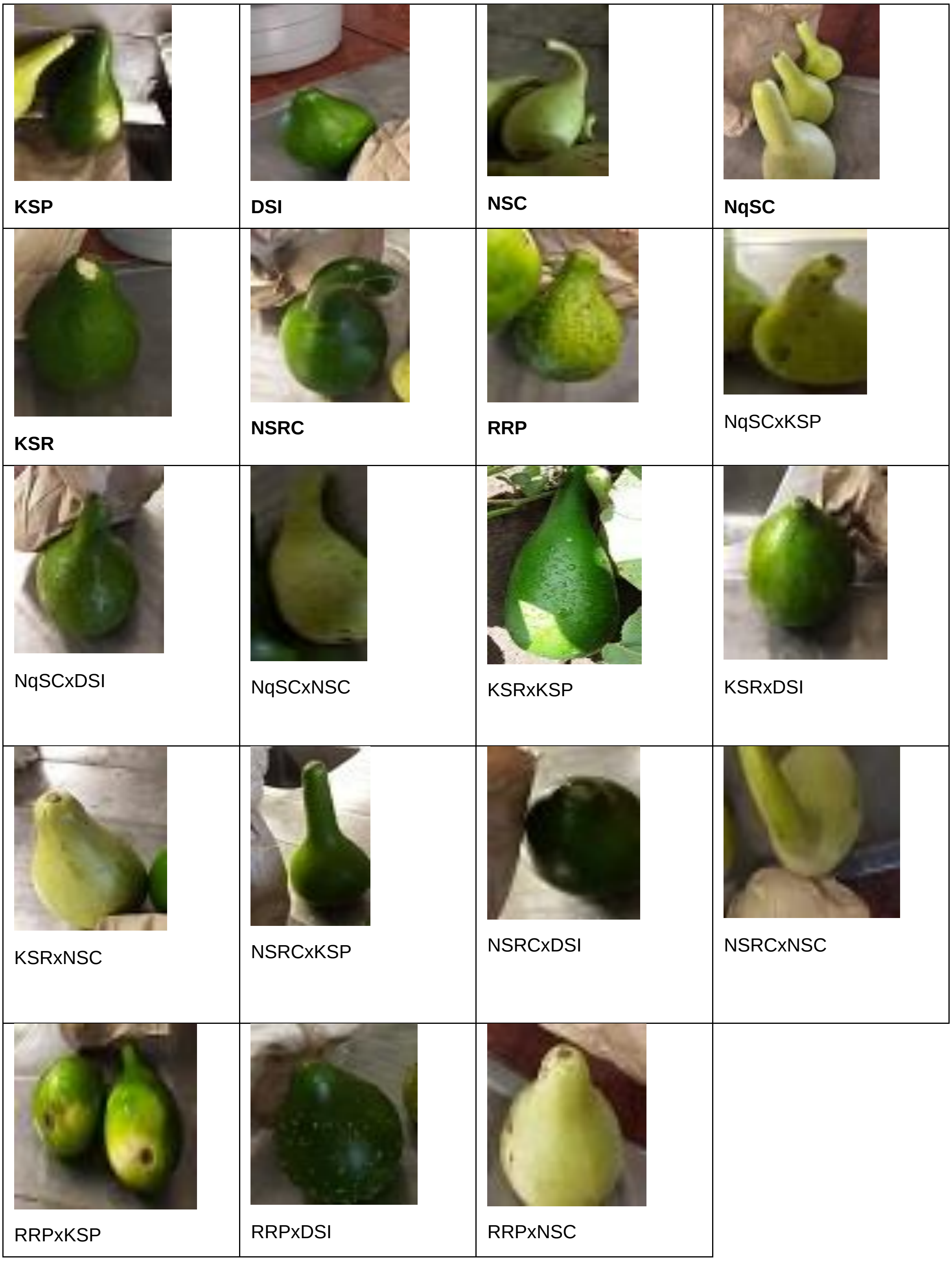
Table 3.5: Description of genotypes according to the fruit and seed morphology

Genotype	**Fruit colour**	**Fruit texture**	**Fruit Shape**	**Seed colour**	**Seed shape**	**Seed size**
KSP	Dark green	Smooth	Pear	Brown	Oblong	Small
DSI	Dark green	Smooth & rough	Isodiametric	Brown	Oblong	Small
NSC	Light green	Smooth	Curvilinear	Brown	Ovate	Medium
NqSC	Green	Smooth	Semi-Curvilinear	Brown	Ovate	Large
KSR	Dark green	Rough	Pear	Brown	Oblong	Medium
NSRC	Dark green	Smooth	Curvilinear	Light brown	Obovate	Large
RRP	Green	Rough	Isodiametric	Brown	Ovate	Large
NqSCxKSP	Green	Smooth	Curvilinear & Pear	Brown	Ovate	Medium

Table 3.5 continued.

Genotype	**Fruit colour**	**Fruit texture**	**Fruit Shape**	**Seed colour**	**Seed shape**	**Seed size**
NqSCxDSI	Dark green	Smooth & rough	Isodiametric & curvilinear	Brown	Ovate	Medium
NqSCxNSC	Light green	Smooth	Curvilinear	Brown	Ovate	Large
KSRxKSP	Dark green	Smooth	Pear	Brown	Oblong	Medium
KSRxDSI	Dark green	Smooth & rough	Isodiametric	Brown	Oblong	Small
KSRxNSC	Green	Smooth	Pear & semi-curvilinear	Brown	Ovate	Large
NSRCxKSP	Dark green	Smooth	Pear & semi-curvilinear	Light brown	Oblong	Large
NSRCxDSI	Dark green	Smooth & rough	Pear & isodiametric	Brown	Oblong	Medium
NSRCxNSC	Green	Smooth	Curvilinear	Light brown	Ovate	Large
RRPxKSP	Green	Smooth & rough	Pear & isodiametric	Dark brown	Ovate	Medium
RRPxDSI	Green	Rough	Isodiametric	Brown	Oblong	Small
RRPxNSC	Light green	Smooth	Isodiametric & curvilinear	Brown	Ovate	Small

Seed size (Length x Width): Small- 140-159 (mm^2); Medium- 160-179 (mm^2) and Large- 180-210 (mm^2)

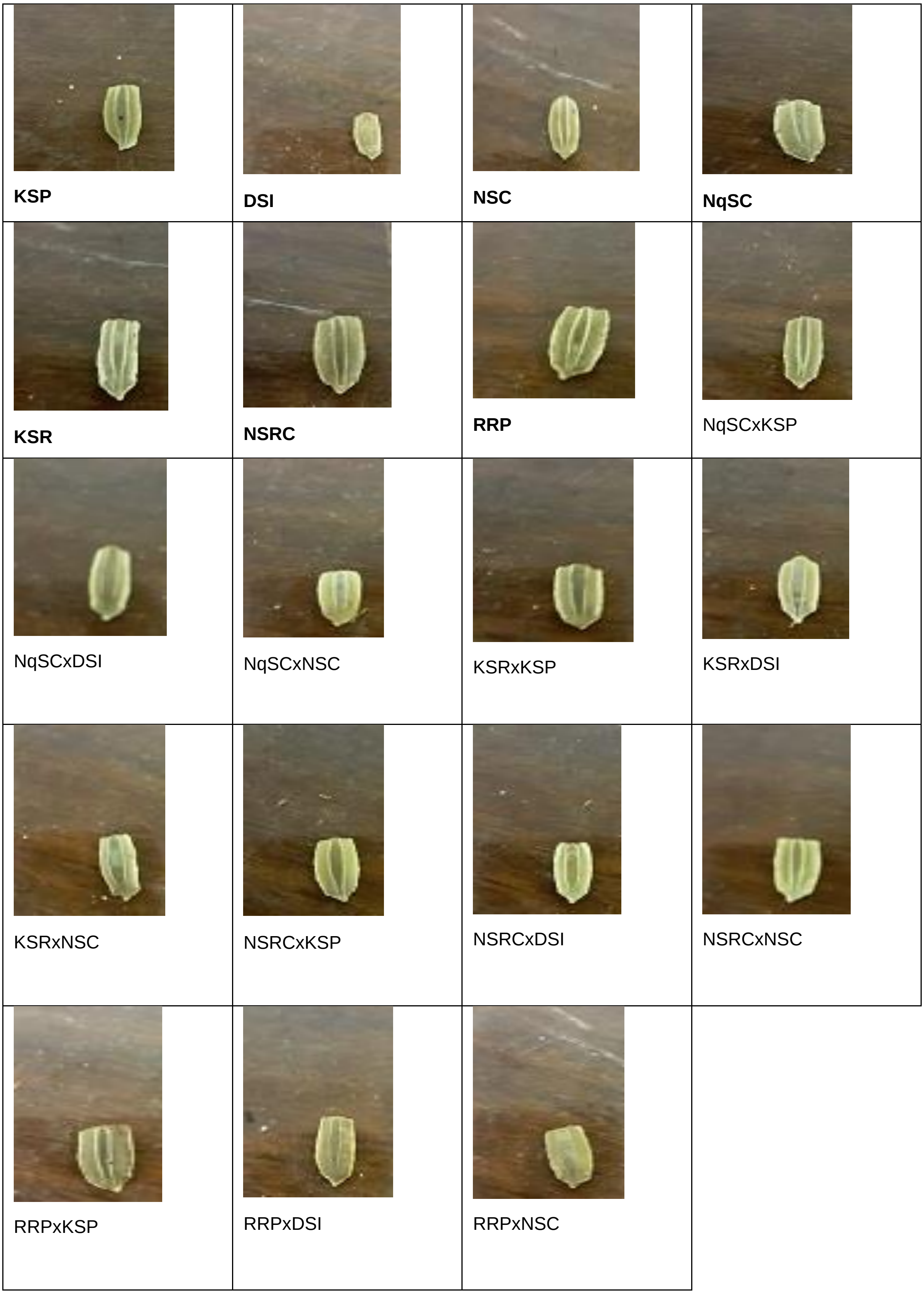


KSP
DSI
NSC
NqSC
KSR
NSRC
RRP
NqSCxKSP
NqSCxDSI
NqSCxNSC
KSRxKSP
KSRxDSI
KSRxNSC
NSRCxKSP
NSRCxDSI
NSRCxNSC
RRPxKSP
RRPxDSI
RRPxNSC

Figure 3.2: Diversity in fruit colour, fruit shape and fruit texture among *Lagenaria siceraria* genotypes (bold-parents and normal-F_1 progenies)

The dominant seed colour amongst genotypes was brown exhibited by 15 genotypes (78.9%), while three genotypes (15.8%) displayed light brown colour and one genotype (5.3%) had dark brown seed colour (Table 3.5 and Figure 3.3). The recorded differences in the seed shape among genotypes were in the following descending order (number and percentage): ovate (10, 52.6%); oblong (8, 42.1%) and obovate (1, 5.3%) shapes (Table 3.5 and Figure 3.3). The detected diversity amongst studied genotypes with regards to seed size based on seed width and seed length were as follows: large (7, 36.8%); medium (7, 36.8%), and small (5, 26.3%) sizes (Table 3.5 and Figure 3.3).

All evaluated genotypes exhibited vigorous plants with a rapid ground cover habit, and the tendrils were produced by all genotypes. White flower colour, large flower size, green stem colour and white fruit pulp colour were observed in all genotypes. Seeds of all genotypes had lines and had rough texture. All genotypes showed a heart shaped leaf. The tendril type was coiled and branched for all genotypes. There were five petals observed in all genotypes and staminate flowers were exhibited in the main vine, whereas the pistillate flowers were borne in the lateral branches.



KSP **DSI** **NSC** **NqSC**

KSR **NSRC** **RRP** NqSCxKSP

NqSCxDSI NqSCxNSC KSRxKSP KSRxDSI

KSRxNSC NSRCxKSP NSRCxDSI NSRCxNSC

RRPxKSP RRPxDSI RRPxNSC

Figure 3.3: Diversity in seed colour, seed shape and seed size among *Lagenaria siceraria* genotypes (bold-parents and normal-F_1 progenies)

3.3.2 Quantitative traits evaluation

Analysis of variance for fruit yield based on North Carolina Design II

Analysis of variance revealed significant ($P < 0.05$) difference among male parents for fruit yield (Table 3.6), indicating genetic variability in the trait. Replications also showed significant ($P < 0.05$) difference for fruit yield. Sites, genotypes, and female parents showed non-significant ($P > 0.05$) differences for fruit yield. Non-significant ($P > 0.05$) males-by-females, site-by-genotype, site-by-males, site-by-females, site-by-males-by-females interactions effect was observed for fruit yield.

Table 3.6: Analysis of variance for fruit yield among *L. siceraria* genotypes in different sites based on North Carolina design ii (NCD-II)

Source of variation	df	Sum of squares	Mean squares
Site (S)	1	100837.9ns	100837.9ns
Replications (site)	4	2422644773***	605661193.3***
Genotypes (G)	11	1965999740ns	178727249.1ns
Males (M)	3	1052008806*	350669601.9*
Females (F)	2	239939781.3ns	119969890.7ns
M x F	6	674051153.1ns	112341858.9ns
S x G	11	665878432.4ns	60534402.9ns
S x M	3	91095282.1ns	30365094ns
S x F	2	85906527.2ns	42953263.6ns
S x M x F	6	488876623.1ns	81479437.2ns
Residuals	44	4153006670	94386515.2

Note: DF-Degrees of Freedom. *Significant at $P < 0.05$ and ns- non-significant.

Components of variance for fruit yield based on NCD-II across site

Additive variance (28636835) was higher than dominance variance (11970229.1) for fruit yield, suggesting role of additive variance in the inheritance of this trait (Table 3.7). No negative estimate of variance components was observed in NCD-II for this trait. Male variance (13240430.2) was higher than female variance (317834.7), whereas genotypic variance (14056788.9) was lower than environmental variance (15731085). The male x female interaction variance was 2992557.3, general combing ability variance was 7159208.3 and heritability was 0.7 for fruit yield.

Table 3.7: Estimates of genetic components of variance from 12 *Lagenaria siceraria* crosses for fruit yield based on NCD-II

Variance components across site	Fruit yield (kg/ha)
Genotype Variance	14056788.9
Male Variance	13240430.2
Female Variance	317834.7
Male x Female Variance	2992557.3
GCA Variance	7159208.3
Additive Variance	28636835
Dominance Variance	11970229.1
Environmental Variance	15731085
Broad-sense Heritability	0.7

Note: GCA -general combining ability

a. Seedling traits

The results displayed significant differences ($P < 0.05$) in some traits. Seedlings of RRP and NSRC genotypes emerged earlier (9 days), while genotype NqSC took long to emerge (19 days), with a mean of 12 days (Table 3.8). The KSRxKSP hybrid had significantly high (81.95%) emergence percentage, whereas genotype NqSC had the least (32.92%) with a mean of 61.94% (Table 3.8). Significantly taller seedlings (a) were obtained with RRP, NqSCxKSP, NqSCxDSI and NSRCxDSI genotypes, but KSP had the shortest (b) and the other genotypes exhibited significant letters of ab (Table 3.8). Differences were not significant ($P > 0.05$) among all genotypes in terms of cotyledons leaf area (Table 3.8).

Table 3.8: Variation in seedling traits at 25 days after planting; vegetative traits (50 DAP) and growth traits among *Lagenaria siceraria* genotypes

Genotype	**DG**	**EP**	**SH**	**CLA**	**LA**	**VL**	**NOL**	**NOB**	**LCC**	**DF**	**DEM**	**DHM**
KSP	16^{c}	44.58^{ef}	3.58^{b}	12.25	122.9^{c}	23.70^{d}	9.17	0.83	20.72	59^{a}	78^{d}	119^{a}
DSI	14^{d}	66.13$^{a\text{-}d}$	4.47^{ab}	12.93	183.4^{bc}	60.40^{cd}	12.33	1.33	25.06	57^{c}	80^{a}	120^{a}
NSC	17^{bc}	45.83^{def}	4.47^{ab}	12.81	152.9^{bc}	75.47^{bcd}	15.17	2.33	25.62	58^{ab}	78^{d}	119^{a}
NqSC	19^{a}	32.92^{f}	5.18^{ab}	14.63	167.8^{bc}	111.37^{abc}	23.83	3.33	24.25	58^{b}	79^{b}	119^{a}
KSR	18^{b}	49.78$^{c\text{-}f}$	5.17^{ab}	15.08	171.6^{bc}	84.40$^{a\text{-}d}$	19.17	3.33	23.25	57^{c}	79^{c}	119^{a}
NSRC	9^{f}	53.47$^{b\text{-}f}$	4.53^{ab}	14.14	228.0^{ab}	108.73^{abc}	19.17	3.17	22.59	58^{ab}	79^{c}	118^{b}
RRP	9^{f}	70.62^{abc}	5.77^{a}	16.62	310.6^{a}	143.92^{a}	24.17	3.67	21.28	56^{d}	78^{d}	117^{c}
NqSCxKSP	12^{e}	72.33^{ab}	5.50^{a}	13.98	175.5^{bc}	129.35^{ab}	19.83	3.17	24.64	55^{e}	78^{d}	116^{d}
NqSCxDSI	12^{e}	67.45^{abc}	5.40^{a}	15.33	169.5^{bc}	86.18$^{a\text{-}d}$	17.33	2.67	23.84	56^{e}	79^{b}	117^{d}
NqSCxNSC	12^{e}	67.47^{abc}	4.53^{ab}	13.49	169.1^{bc}	94.43^{abc}	18.50	3.33	24.21	56^{e}	78^{d}	117^{d}
KSRxKSP	10^{f}	81.95^{a}	5.22^{ab}	15.07	196.3^{bc}	98.68^{abc}	17.67	3.17	22.22	55^{f}	80^{a}	116^{e}
KSRxDSI	12^{e}	*61*.42$^{a\text{-}e}$	5.20^{ab}	15.13	179.2^{bc}	107.07^{abc}	17.33	3.33	23.57	56^{e}	79^{b}	117^{d}
KSRxNSC	12^{e}	67.08$^{a\text{-}d}$	5.32^{ab}	15.81	177.3^{bc}	106.17^{abc}	17.0	2.17	26.73	55^{e}	75^{e}	117^{d}
NSRCxKSP	10^{f}	70.50^{abc}	5.30^{ab}	15.72	206.5^{bc}	132.90^{ab}	18.50	3.0	24.49	54^{f}	74^{f}	115^{f}
NSRCxDSI	12^{e}	68.75^{abc}	5.63^{a}	12.93	213.2^{abc}	117.63^{abc}	17.67	2.83	26.23	55^{f}	79^{b}	117^{d}
NSRCxNSC	10^{f}	66.53$^{a\text{-}d}$	4.47^{ab}	12.72	151.3^{bc}	103.05^{abc}	17.83	2.17	54.51	54^{f}	78^{d}	116^{e}
RRPxKSP	10^{f}	70.50^{abc}	5.25^{ab}	13.63	188.5^{bc}	119.83^{abc}	17.67	2.50	25.90	55^{f}	74^{f}	116^{e}
RRPxDSI	13^{e}	53.50$^{b\text{-}f}$	5.18^{ab}	14.45	211.9^{abc}	110.80^{abc}	16.83	2.0	25.0	55^{f}	78^{d}	117^{d}
RRPxNSC	12^{e}	65.97$^{a\text{-}e}$	4.83^{ab}	15.65	198.8^{bc}	109.70^{abc}	17.0	2.33	22.62	57^{cd}	78^{d}	117^{d}
Significance	***	***	*	ns	***	*	ns	ns	ns	***	***	***
P_Value	<.0001	<.0001	0.0120	0.4028	0.0002	0.0142	0.7328	0.3606	0.4446	<.0001	<.0001	<.0001
HSD	1.13	21.41	1.80	549.23	11674	65.20	14.77	3.45	37.0	0.51	0.34	0.49
CV	4.35	16.42	17.14	16.01	25.94	30.59	39.67	61.52	68.62	0.43	0.20	0.19
MSG	54.55	871.0	1.72	122575.19	112888931	4506.41	66.54	3.33	309.59	13.69	16.89	13.08
MSE	0.29	103.45	0.74	68080.1	30758151	959.46	49.26	2.69	309.02	0.06	0.03	0.05
Grand Mean	12.35	61.94	5.0	14.39	188.1	101.25	17.69	2.67	25.62	55.95	77.89	117.11

Variables: DG-Days to emergence, EP-Emergence Percentage (%), SH-Seedling Height (cm), CLA-Cotyledon leaf area (cm²), LA-Leaf area (cm²), VL-Vine length (cm), NOL-Number of leaves, NOB-Number of branches, LCC-Leaf Chlorophyll content (cci), DF-Days to 50 % flowering, DEM-Days to edible harvest maturity and DHM-Days to drying harvest maturity. Means with the same letter within the column are not significantly different ($P > 0.05$). The explanation of genotypes is in Table 3.1. HSD-Honestly significant difference, CV-Coefficient of variation, MSG-Mean square due to genotype and MSE-Mean square of error. * and *** indicate significant at $p < 0.05$ and $p < 0.001$ respectively, whereas ns indicate non-significance ($p > 0.05$).

b. Vegetative traits

There were no significant differences ($P > 0.05$) on the number of branches, number of leaves and leaf chlorophyll content among all genotypes (Table 3.8). The tallest vines (143.92 cm) were obtained with RRP, but KSP had the shortest (23.70 cm), with the mean vine length of 101.25 cm (Table 3.8). RRP exhibited significantly large leaf area (310.6 cm^2), while KSP had the smallest (122.9 cm^2) with the mean of 188.1 cm^2 (Table 3.8). Differences were not significant among all genotypes in terms of shoot growth percentage (Table 3.9). Significantly higher leaf growth percentage (a) were obtained with KSP, DSI, NSC, NqSC, KSR, NSRC, NqSCxNSC, NqSCxDSI, KSRxKSP, KSRxDSI, KSRxNSC, NSRCxKSP, NSRCxDSI, NSRCxNSC and RRPxNSC genotypes, but NqSCxKSP had the lowest (b) and the other genotypes (RRP, RRPxKSP and RRPxDSI) exhibited significant letters of ab (Table 3.9).

c. Reproductive traits

NSRCxKSP, NSRCxDSI, NSRCxNSC, KSRxKSP, RRPxKSP and RRPxDSI crosses exhibited significantly ($P < 0.05$) fewer days to flowering, while genotype KSP took long to flower (Table 3.8). Number of male flowers ranged from 7.3 to 11 and pistillate flowers had a mean of one. There was no significant difference in flower sex ratio among the studied *Lagenaria siceraria* genotypes.

d. Fruit yield and agronomic traits at harvest

The crosses NSRCxKSP and RRPxKSP exhibited significantly fewer days to edible harvest maturity (74 days), while genotype DSI and KSRxKSP took long to reach edible harvest maturity (80 days) with a mean of 77.89 days. NSRCxKSP cross had significantly less days to drying harvest maturity, while genotypes DSI, KSP, NSC, NqSC and KSR took long to reach drying harvest maturity. Differences were not significant ($P > 0.05$) among all genotypes in terms of fruit yield per plot, fruit mass, fruit width, fruit length, total fruit mass per plot, fruit rind thickness, yield per plant and number of fruits per plant (Table 3.9). Fruit neck length ranged significantly from 2.07 cm (KSRxDSI) to 11.60 cm (NSC) with an average of 7.75 cm (Table 3.9).

Table 3.9: Variation in fruit traits, seed traits and shoot and leaf growth (43-50 DAP) and yield traits among *Lagenaria siceraria* different genotypes

Genotype	TFM	SF	FM	FR	FP	HSM	TSM	FL	FW	FNL	SL	SW	LG	SG	FY	YP
KSP	7.5	144.3	673.9	3.8	1.5	38.8	118.5	16.6	9.3	7.1^{c-f}	17.7	8.8^{e}	154.0^{a}	273.2	6357	0.5
DSI	11.8	141.5	1474.6	4.6	1.7	41.0	216.4	15.8	11.9	3.9^{fg}	18.8	7.5^{f}	135.7^{a}	410.4	15492	1.4
NSC	8.9	158.8	863.3	4.2	2.2	46.6	204.5	23.1	11.3	11.6^{a}	18.2	9.8^{a-e}	75.6^{a}	411.6	9685	0.9
NqSC	7.1	172.5	1095.8	4.2	2.5	54.4	253.7	21.5	12.7	7.8^{a-f}	18.0	10.5^{ab}	43.7^{a}	329.4	13462	1.3
KSR	7.1	163.0	1331.1	4.8	1.5	55.4	278.3	23.4	12.5	7.3^{b-f}	18.3	9.2^{cde}	49.4^{a}	290.1	13527	1.3
NSRC	6.8	146.3	1917.5	5.0	1.2	57.6	178.3	25.1	13.9	7.1^{c-f}	20.3	10.3^{ab}	67.9^{a}	526.5	9252	0.8
RRP	4.9	223.8	1402.0	5.5	1.0	59.2	242.3	18.9	14.3	5.5^{d-g}	19.0	10.7^{a}	26.4^{ab}	177.7	9554	0.9
NqSCxKSP	9.7	219.3	1465.0	4.8	1.3	61.9	274.8	26.7	12.2	11.4^{ab}	19.0	9.4^{b-e}	23.9^{b}	242.6	15123	1.4
NqSCxDSI	9.9	164.2	1349.4	4.9	2.0	53.7	303.3	20.4	11.4	6.7^{c-f}	17.7	9.4^{b-e}	41.1^{a}	276.1	15492	1.4
NqSCxNSC	8.9	161.5	958.9	4.5	1.5	53.9	239.1	22.9	10.9	9.3^{a-d}	19.0	10.0^{a-d}	88.5^{a}	402.3	11016	0.9
KSRxKSP	9.7	302.0	2045.3	4.6	1.5	62.2	316.7	27.3	12.9	8.9^{a-d}	18.3	9.0^{de}	50.6^{a}	315.7	13801	1.3
KSRxDSI	4.5	180.7	1414.5	5.3	1.2	56.6	243.1	15.6	13.2	2.1^{g}	17.5	9.0^{de}	71.8^{a}	840.3	9811	0.9
KSRxNSC	9.7	152.7	1859.6	4.7	1.3	53.0	206.5	25.5	12.2	8.4^{a-e}	18.5	9.8^{ae}	39.6^{a}	232.5	13193	1.2
NSRCxKSP	13.6	179.5	1152.1	4.0	1.8	50.9	252.7	24.9	12.3	11.5^{a}	18.8	9.8^{ae}	51.9^{a}	470.5	19472	1.8
NSRCxDSI	15.9	203.0	1771.1	5.2	1.2	52.5	191.6	23.7	12.9	7.2^{b-f}	17.8	9.9^{a-e}	33.7^{a}	247.6	23039	2.1
NSRCxNSC	8.9	236.9	1186.1	4.6	1.5	53.8	223.8	23.2	11.7	9.6^{a-d}	18.3	10.2^{abc}	126.5^{a}	292.8	15682	1.5
RRPxKSP	6.2	257.0	1620.6	4.7	1.7	51.9	319.5	25.9	13.1	10.1^{abc}	18.7	9.5^{b-e}	27.7^{ab}	460.0	11185	1.1
RRPxDSI	10.3	214.8	2342.2	5.3	1.2	61.9	236.4	20.9	15.3	4.2^{efg}	15.3	9.8^{a-e}	26.2^{ab}	249.1	17022	1.6
RRPxNSC	6.3	159.3	1037.3	4.4	1.3	43.2	244.1	18.3	11.8	7.6^{a-f}	17.0	9.0^{de}	60.6^{a}	246.1	6357	0.9
Significance	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	***	*	ns	ns	ns
P-Value	0.144	0.778	0.087	0.082	0.863	0.099	0.668	0.119	0.256	0.0027	0.135	<.0001	0.043	0.332	0.456	0.498
HSD	11.1	228.3	1468.2	1.7	1.4	28.2	261.4	8.7	3.7	4.2	4.1	1.1	131.4	690.2	17866	1.7
CV	59.6	57.6	49.2	17.5	44.3	25.3	51.9	18.6	13.9	25.6	10.6	5.4	99.3	93.1	64.1	67.6
MSG	49.13	11445.2	1107054	1.21	0.88	271.32	14465.9	79.98	10.30	40.87	6.02	3.18	8900.2	138187.5	97718210	0.87
MSE	27.7	11765.7	486467.3	0.7	0.46	179.8	15424.9	16.9	3.0	3.9	3.7	0.3	3896.6	107504.5	72038336	0.67
Grandmean	8.8	188.5	1418.9	4.7	1.5	53.1	239.1	22.1	12.4	7.8	18.2	9.6	62.9	352.3	13235.6	1.2

Variables: TFM-Total fruit mass per plot (kg), SF-Number of seeds per fruit, FM-Fruit mass (g), FR-Fruit rind (mm), FP-Number of fruits per plant, HSM-100 seed mass (g), TSM-Total seed mass (g), FL-Fruit length (cm), FW-Fruit width (cm), FNL-Fruit neck length (cm), SL-Seed length (mm), SW-Seed width (mm), LG-Leaf growth%, SG-Shoot growth%, FY-Fruit yield (kg/ha) and YP-Yield per plant (kg). Means with the same letter within the column are not significantly different ($P > 0.05$). The explanation of genotypes is in Table 3.1. HSD-Honestly significant difference, CV-Coefficient of variation, MSG-Mean square due to genotype and MSE-Mean square of error. *, ** and *** indicate significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively, whereas ns indicate non-significance ($p > 0.05$).

e. Seed traits

Differences were not significant (*P* > 0.05) among all genotypes in terms of one hundred seed mass, total seed mass, seed length and number of seeds per fruit (Table 3.9). Seed width varied significantly from 7.50 mm for DSI to 10.67 mm for RRP with a mean of 9.56 mm (Table 3.9).

3.3.3 Significant correlation among traits

Emergence percentage correlated positively with number of seeds per fruit (r = 0.54) and total seed mass (r = 0.45) (Table 3.10). Number of fruits per plant was also negatively correlated with emergence percentage (r = -0.44). Positive correlations were observed between number of branches with hundred seed mass (r = 0.70), total seed mass (r = 0.51), fruit width (r = 0.46), seedling height (r = 0.65), cotyledons leaf area (r = 0.55), leaf area (r = 0.48), vine length (r = 0.69), number of leaves (r = 0.87) and seed width (r =0.53). Leaf growth percentage was negatively correlated with the number of branches (r = -0.63).

Total seed mass positively associated with seedling height (r = 0.62), hundred seed mass (r = 0.49), vine length (r = 0.46), number of leaves (r = 0.47), number of seeds per fruit (r = 0.57) and cotyledon leaf area (r = 0.46). Also, leaf growth percentage was negatively correlated with total seed mass (r = -0.58) (Table 3.10). Total fruit mass per plot correlated positively with the fruit yield in kg/ha (r =0.84). Fruit yield correlated positively with the seedling height (r =0.49). Fruit rind thickness correlated positively with seedling height (r =0.59), hundred seed mass (r = 0.62), vine length (r = 0.48), fruit width (r = 0.73) and leaf area (r = 0.62). Fruit rind thickness also correlated negatively with number of fruits per plant (r = -0.63), fruit neck length (r = -0.57) and leaf growth percentage (r = -0.49). Cotyledon leaf area correlated positively with the seedling height (r = 0.66), number of leaves (r = 0.58), vine length (r = 0.57), fruit width (r = 0.45) and leaf area (r =0.57). A negative correlation coefficient was observed between cotyledons leaf area with leaf growth percentage (r = -0.62).

Table 3.10: Correlation among morpho-agronomic traits of *Lagenaria siceraria* genotypes

Variables	EP	SH	CLA	LA	VL	NOL	NOB	LCC	TFM	SF	FR	FP	HSM	TSM	FL	FW	FNL	SL	SW	LG	SG	FY
SH	0.43^{ns}																					
CLA	0.26^{ns}	**0.66****																				
LA	0.35^{ns}	0.58**	0.57*																			
VL	0.41^{ns}	**0.79*****	0.57*	**0.68****																		
NOL	0.06^{ns}	**0.69****	0.58**	0.57*	**0.81*****																	
NOB	0.18^{ns}	**0.65****	0.55*	0.48*	**0.69*****	**0.87****																
LCC	0.11^{ns}	-0.17^{ns}	-0.35^{ns}	-0.27^{ns}	0.07^{ns}	0.01^{ns}	-0.18^{ns}															
TFM	0.28^{ns}	0.14^{ns}	-0.28^{ns}	-0.08^{ns}	0.001^{ns}	-0.23^{ns}	-0.21^{ns}	0.13^{ns}														
SF	0.54*	0.43^{ns}	0.09^{ns}	0.27^{ns}	0.45*	0.31^{ns}	0.28^{ns}	0.26^{ns}	0.01^{ns}													
FR	0.31^{ns}	0.59**	0.34^{ns}	**0.62****	0.48*	0.40^{ns}	0.42^{ns}	-0.05^{ns}	-0.14^{ns}	0.26^{ns}												
FP	-0.44*	-0.16^{ns}	-0.17^{ns}	-0.50*	-0.26^{ns}	0.01^{ns}	-0.07^{ns}	0.03^{ns}	0.09^{ns}	-0.19^{ns}	**-0.63****											
HSM	0.26^{ns}	**0.64****	0.42^{ns}	0.43^{ns}	**0.65****	**0.68****	**0.70*****	0.03^{ns}	-0.08^{ns}	0.58**	**0.62****	-0.30^{ns}										
TSM	0.45*	**0.62****	0.46*	0.15^{ns}	0.46*	0.47*	0.51*	-0.04^{ns}	-0.11^{ns}	0.57*	0.23^{ns}	0.21^{ns}	0.49*									
FL	0.29^{ns}	0.33^{ns}	0.04^{ns}	0.04^{ns}	0.43^{ns}	0.36^{ns}	0.3^{ns}	0.15^{ns}	0.29^{ns}	0.46*	-0.06^{ns}	0.06^{ns}	0.51*	0.37^{ns}								
FW	0.11^{ns}	**0.62****	0.45*	**0.73*****	**0.68****	0.57*	0.46*	-0.11^{ns}	-0.09^{ns}	0.40^{ns}	**0.73*****	-0.40^{ns}	**0.69****	0.29^{ns}	0.18^{ns}							
FNL	0.14^{ns}	-0.04^{ns}	-0.18^{ns}	-0.26^{ns}	0.16^{ns}	0.11^{ns}	0.11^{ns}	0.22^{ns}	0.24^{ns}	0.21^{ns}	-0.57*	0.39^{ns}	-0.01^{ns}	0.18^{ns}	**0.74*****	-0.36^{ns}						
SL	0.22^{ns}	-0.05^{ns}	-0.04^{ns}	0.17^{ns}	0.13^{ns}	0.25^{ns}	0.34^{ns}	0.01^{ns}	-0.06^{ns}	-0.06^{ns}	-0.09^{ns}	0.02^{ns}	0.04^{ns}	-0.02^{ns}	0.38^{ns}	-0.13^{ns}	0.37^{ns}					
SW	-0.17^{ns}	0.32^{ns}	0.22^{ns}	0.37^{ns}	0.58**	**0.70*****	0.53*	0.20^{ns}	-0.11^{ns}	0.17^{ns}	0.19^{ns}	0.003^{ns}	0.49*	-0.03^{ns}	0.43^{ns}	0.35^{ns}	0.31^{ns}	0.15^{ns}				
LG	-0.22^{ns}	**-0.89*****	**-0.62****	-0.53*	**-0.77*****	**-0.68****	**-0.63****	0.32^{ns}	-0.02^{ns}	-0.37^{ns}	-0.49*	0.10^{ns}	**-0.67****	-0.58**	-0.49*	**-0.65****	-0.12^{ns}	0.10^{ns}	-0.45*			
SG	-0.07^{ns}	-0.14^{ns}	-0.06^{ns}	-0.13^{ns}	-0.01^{ns}	-0.09^{ns}	0.19^{ns}	-0.08^{ns}	-0.26^{ns}	-0.14^{ns}	0.08^{ns}	0.04^{ns}	-0.01^{ns}	0.02^{ns}	-0.19^{ns}	0.09^{ns}	-0.24^{ns}	0.17^{ns}	-0.18^{ns}	0.18^{ns}		
FY	0.29^{ns}	0.49*	-0.06^{ns}	0.08^{ns}	0.31^{ns}	0.15^{ns}	0.09^{ns}	0.27^{ns}	**0.84*****	0.25^{ns}	0.18^{ns}	0.06^{ns}	0.29^{ns}	0.20^{ns}	0.35^{ns}	0.25^{ns}	0.09^{ns}	-0.11^{ns}	0.08^{ns}	-0.29^{ns}	-0.18^{ns}	

Note: *, ** and *** significant Correlation at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively and ns—no significant correlation. High correlations have value >0.6. Variables: EP-Emergence Percentage (%), SH-Seedling Height (cm), CLA-Cotyledon leaf area (cm²), LA-Leaf area (cm²), VL-Vine length (cm), NOL-Number of leaves, NOB-Number of branches, LCC-Leaf Chlorophyll content (cci), TFM-Total fruit mass per plot (kg), SF-Number of seeds per fruit, FR-Fruit rind (mm), FP-Number of fruits per plant, HSM-100 seed mass (g), TSM-Total seed mass (g), FL-Fruit length (cm), FW-Fruit width (cm), FNL-Fruit neck length (cm), SL-Seed length (mm), SW-Seed width (mm), LG-Leaf growth%, SG-Shoot growth% and FY-Fruit yield (kg/ha)

Seed width positively interrelated with number of leaves (r = 0.70), vine length (r = 0.58) and hundred seed mass (r = 0.49). A negative correlation was observed between seed width with leaf growth percentage (r = -0.45). Number of seeds per fruit associated positively with the vine length (r = 0.45), hundred seed mass (r = 0.58), and total seed mass (r = 0.57) and fruit length (r = 0.46). Positive correlations were observed between seedling height with leaf area (r = 0.58), vine length (r = 0.79), number of leaves (r = 0.69), hundred seed mass (r = 0.64) and fruit width (r = 0.62). Seedling height correlated negatively with leaf growth percentage (r = -0.89). Leaf area positively associated with vine length (r = 0.68), number of leaves (r = 0.57) and fruit width (r = 0.73). Negative correlations were observed between leaf area with number of fruits per plant (r = -0.50) and leaf growth percentage (r = -0.53). Vine length positively associated with number of leaves (r = 0.81), number of seeds per fruit (r = 0.45), fruit width (r = 0.68), hundred seed mass (r = 0.65) and seed width (r = 0.58). Vine length was also positively associated with leaf growth percentage (r = -0.77).

Positive correlations were observed between number of leaves with hundred seed mass (r = 0.68), seed width (r = 0.70) and fruit width (r = 0.57). Number of leaves correlated negatively with leaf growth percentage (r = -0.68). Hundred seed mass correlated positively with number of seeds per fruit (r = 0.58), fruit length (r = 0.51), fruit width (r = 0.69) and seed width (r = 0.49). A negative correlation was observed between hundred seed mass with leaf growth percentage (r = -0.67). Fruit length correlated positively with fruit neck length (r = 0.74) and again fruit length significantly correlated negatively with leaf growth percentage (r = -0.49). Fruit width correlated negatively with leaf growth percentage (r = -0.65).

3.3.4 Principal component analysis

There was a diversity among genotypes identified by principal component analysis (Table 3.11). The first five components contributed 74.84% of the variability (Table 3.11). Emergence percentage, seedling height, cotyledons leaf area, leaf area, vine length, number of leaves, number of branches, number of seeds per fruit, fruit mass, fruit rind thickness, hundred seed mass, total seed mass, fruit length, fruit width, seed width, fruit yield, yield per plant, leaf chlorophyll content, total fruit mass per plot, fruit neck length and seed length were correlated positively with the first principal component (PC1), which accounted for 34.46% of the total variation. Days to emergence, days to flowering, days to edible harvest maturity, days to drying harvest maturity, number of fruits per plant, leaf growth percentage and shoot growth percentage correlated negatively with PC1.

The days to emergence, days to flowering, days to edible harvest maturity, days to drying harvest maturity, shoot growth percentage, cotyledon leaf area, leaf area, vine length, number of leaves, number of branches, fruit mass, fruit rind thickness, hundred seed mass, fruit width, seed length and seed width associated positively with PC2, which accounted 14.48% of total variability. Emergence percentage, leaf chlorophyll content, total fruit mass per plot, number of seeds per fruit, number of fruits per plant, total seed mass, fruit length, fruit neck length, leaf growth percentage, fruit yield and yield per plant correlated negatively with PC2. There was no strong correlation identified by PC1 and PC2 in all evaluated morphological traits.

Table 3.11: Loadings of the variables for the first five principal components

Variables	PC1	PC2	PC3	PC4	PC5
DG	-0.20	0.07	0.10	0.43	-0.12
EP	0.19	-0.18	-0.15	-0.32	-0.16
SH	0.28	0.05	0.01	0.18	-0.17
CLA	0.18	0.22	0.07	-0.01	-0.29
LA	0.21	0.22	-0.07	-0.06	0.17
VL	0.29	0.04	0.12	-0.02	0.07
NOL	0.23	0.17	0.26	0.13	0.09
NOB	0.22	0.18	0.24	0.05	-0.01
LCC	0.01	-0.26	-0.01	-0.09	0.29
DF	-0.23	0.28	0.13	0.09	0.12
DEM	-0.07	0.16	-0.21	0.20	0.06
DHM	-0.23	0.21	0.04	0.25	0.15
TFM	0.04	-0.36	-0.16	0.25	0.13
SF	0.20	-0.13	-0.03	-0.13	-0.09
FM	0.21	0.02	-0.27	0.06	0.09
FR	0.20	0.22	-0.27	0.003	0.12
FP	-0.11	-0.12	0.31	0.29	-0.24
HSM	0.26	0.09	0.03	0.06	0.09
TSM	0.19	-0.03	0.11	0.02	-0.49
FL	0.17	-0.22	0.27	-0.004	0.14
FW	0.24	0.20	-0.14	0.10	0.13
FNL	0.02	-0.29	0.41	-0.07	0.04
SL	0.02	0.003	0.27	-0.23	0.27
SW	0.15	0.06	0.29	0.07	0.41
LG	-0.26	-0.09	-0.11	-0.19	0.14
SG	-0.03	0.11	-0.02	-0.19	-0.12
FY	0.15	-0.29	-0.15	0.33	0.09
YP	0.16	-0.28	-0.15	0.33	0.05
Eigenvalue	9.65	4.05	3.13	2.45	1.67
Variability%	34.46	14.48	11.19	8.74	5.97
Cumulative%	34.46	48.94	60.13	68.87	74.85

Note: PC1-5: Principal components 1-5. Variables: DG- Days to emergence, EP-Emergence Percentage (%), SH-Seedling Height (cm), CLA-Cotyledon leaf area (mm²), LA-Leaf area (cm²), VL-Vine length (cm), NOL-Number of leaves, NOB-Number of branches, LCC-Leaf Chlorophyll content (cci), DF-Days to 50 % flowering, DEM-Days to edible harvest maturity and DHM-Days to drying harvest maturity, TFM-Total fruit mass per plot (kg), SF-Number of seeds per fruit, FM-Fruit mass (g), FR-Fruit rind (mm), FP-Number of fruits per plant, HSM-100 seed mass (g), TSM-Total seed mass (g), FL-Fruit length (cm), FW-Fruit width (cm), FNL-Fruit neck length (cm), SL-Seed length (mm), SW-Seed width (mm), LG-Leaf growth %, SG-Shoot growth %, FY-Fruit yield (kg/ha) and YP-Yield per plant (kg)

3.3.5 Cluster analysis

Evaluated genotypes clustered according to their vegetative, fruit and seed traits as shown in the biplot (Figure 3.4) and dendrogram (Figure 3.5). A first Cluster (I) in the biplot and Group I of the dendrogram consisted of RRP genotype with tall seedlings, large cotyledon size, least shoot growth percentage, high number of branches, high number of leaves, large leaf area, long vine length, large rind thickness and large seed width (Table 3.8 and 3.9). RRP fruits were green in colour, rough in texture, isodiametric shaped and had white pulp, whereas the seeds were brown, rough, ovate, large and had lines (Table 3.5). Second Cluster (II) in the biplot and Group II of the dendrogram consisted of RRPxNSC and NSRC genotypes with similar vine length, leaf chlorophyll content, total fruit mass per plot and leaf growth percentage. Fruits of these genotypes were smooth with white fruit pulp, while the seeds were rough with lines. Third Cluster (III) in the biplot and Group III of the dendrogram consisted of RRPxDSI, NSRCxDSI and NSRCxKSP genotypes with similar leaf area and seed width. Fruits of these genotypes exhibited white pulp and the seeds were rough, oblong and had lines.

Fourth Cluster (IV) in the biplot and Group IV of the dendrogram consisted of RRPxKSP, NSRCxNSC, KSRxDSI, NqSCxNSC, NSC, KSRxKSP, KSRxNSC, NqSC, NqSCxDSI, NqSCxKSP, DSI and KSR genotypes with similar fruit width, low number of leaves, leaf area and seed length. The fruits of these genotypes were smooth with white pulp, whereas the seeds were brown, rough and had lines. Fifth Cluster (V) in the biplot and Group V of the dendrogram consisted of KSP genotype with short seedlings, small cotyledon size, high leaf growth percentage, low number of branches, low number of leaves, small leaf area, short vine length, small rind thickness and low fruit yield. KSP fruits were dark green, smooth, pear shaped with white pulp and the seeds were brown, rough, oblong and small in size with lines.

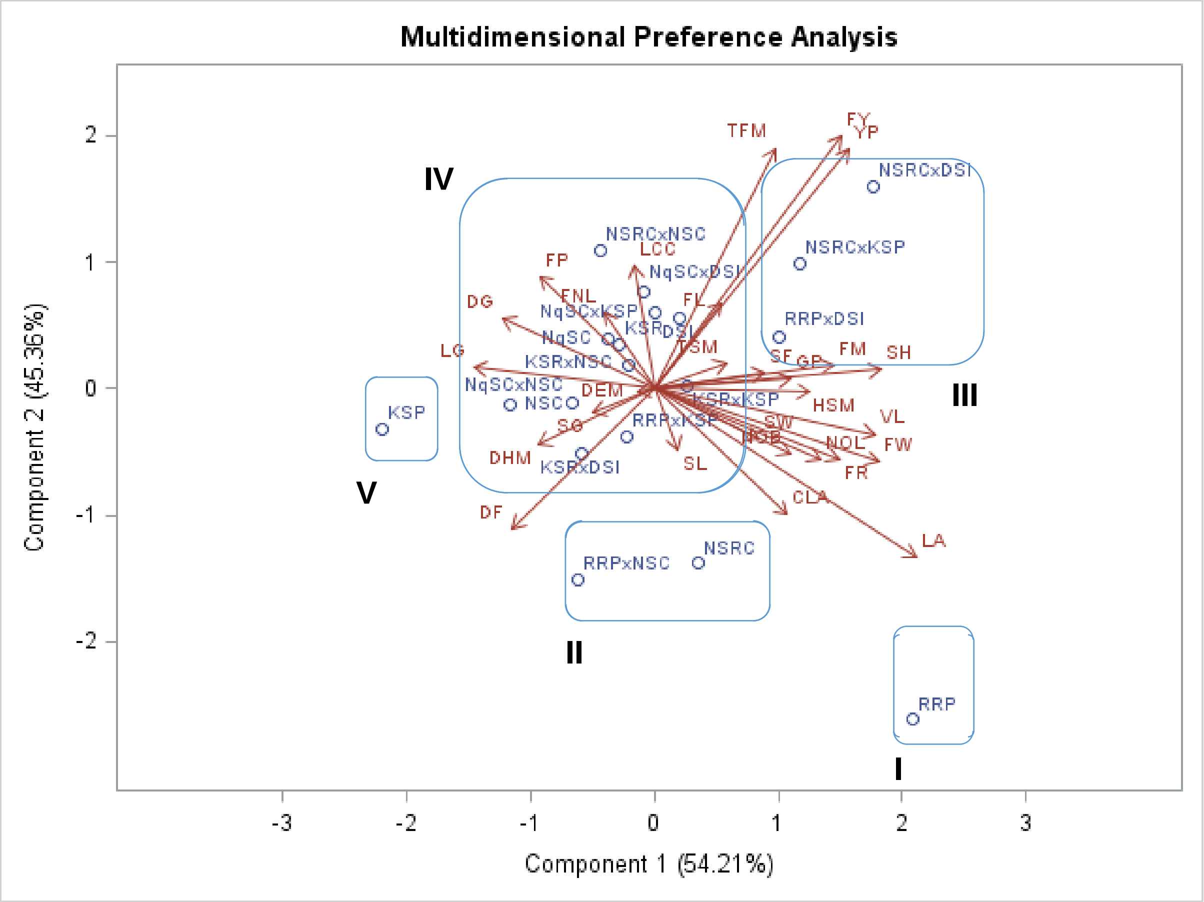


Figure 3.4: Biplot of *Lagenaria siceraria* genotypes and agronomic traits. Genotypes and agronomic traits (variables) are described in Table 3.5 and Tables 3.8 and 3.9, respectively

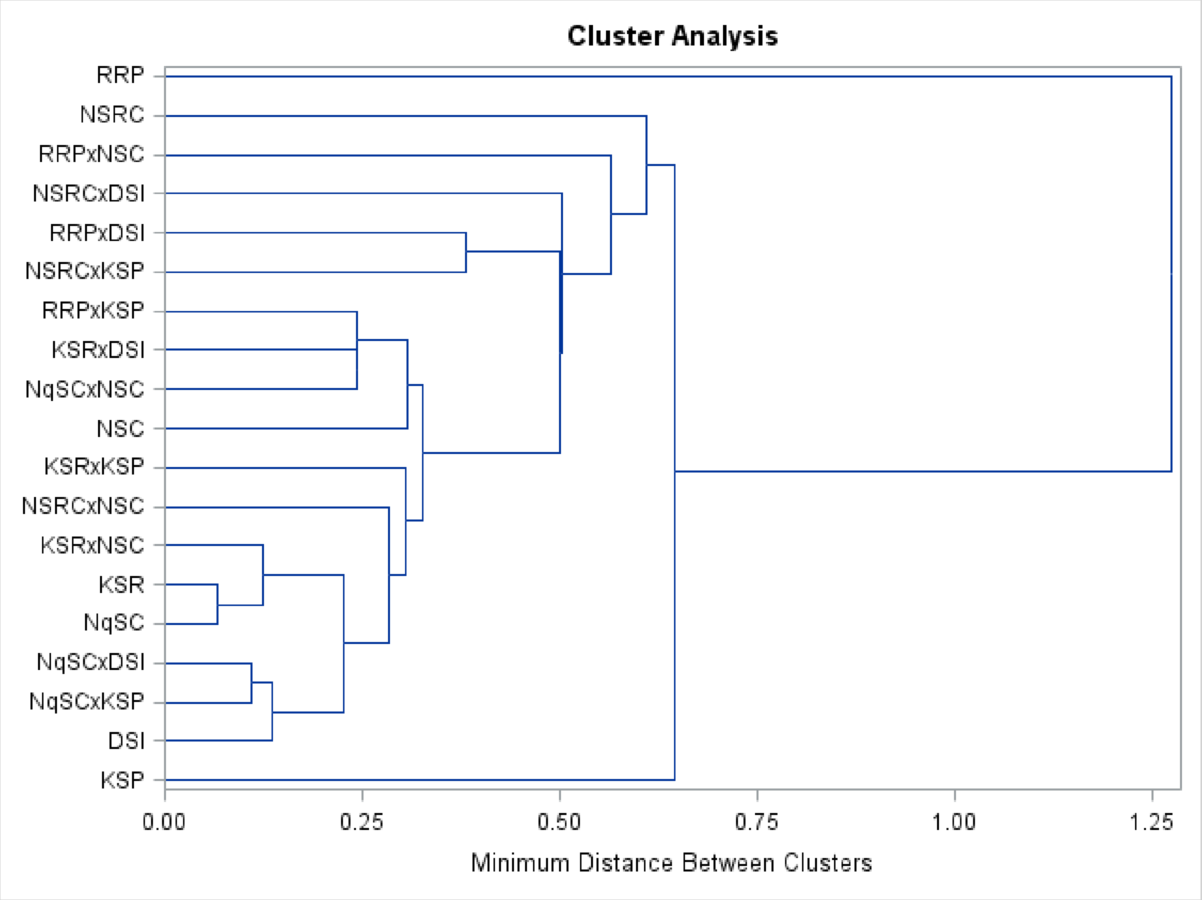


Figure 3.5: Hierarchical cluster showing similarities amongst *Lagenaria siceraria* genotypes using the complete linkage method. Description of genotypes is in Table 3.5

3.3.6 Genetic parameters

In the current study the high genotypic variance was recorded for leaf area (14188463.3), whereas a low genotypic variance was recorded for yield per plant (0.03) (Table 3.12). A high environmental variance was recorded for fruit yield (72038336), whereas the lowest environmental variance was recorded for days to edible harvest maturity (0.03). High value of phenotypic variance was recorded for fruit yield (169756546), whereas a low value of phenotypic variance was recorded for number of fruits per plant (1.3). We recorded a high phenotypic coefficient of variation for leaf growth percentage (179.9%), whereas the low phenotypic coefficient of variation was recorded for days to drying harvest maturity (3.1%). We obtained highest genotypic coefficient of variation for leaf growth percentage (45.93%), whereas the low genotypic coefficient of variation was recorded for leaf chlorophyll content (1.20). A high value for broad sense estimate was recorded for days to edible harvest maturity

(16.6%), while the lowest value was recorded for leaf chlorophyll content (0.02%). Days to emergence, days to 50 % flowering, days to edible harvest maturity and days to drying harvest maturity exhibited the largest value of genetic advancement (0.84%), while the lowest value was recorded for leaf chlorophyll content (0.03%).

Table 3.12: Genetic parameters for agronomic traits of *Lagenaria siceraria* genotypes

Variable	**δ^2g**	**δ^2e**	**δ^2p**	**PCV**	**GCV**	**H^2**	**GA**
DG	9.04	0.29	54.8	59.9	24.35	16.5	0.84
EP	127.93	103.45	974.5	50.4	18.26	13.1	0.75
SH	0.16	0.73	2.5	31.3	8.0	6.7	0.54
CLA	9082.52	68080.1	190655.3	26.8	5.85	4.8	0.45
LA	14188463.3	30758151	146647082	56.6	17.31	9.7	0.64
VL	591.16	959.46	5465.9	73.0	24.01	10.8	0.68
NOL	2.88	49.26	115.8	60.8	9.59	2.5	0.33
NOB	0.11	2.69	6.0	91.9	12.42	1.8	0.28
LCC	0.095	309.02	618.6	97.1	1.20	0.02	0.03
DF	2.27	0.06	13.8	6.6	2.69	16.5	0.84
DEM	2.81	0.03	16.9	5.3	2.15	16.6	0.84
DHM	2.17	0.05	13.1	3.1	1.26	16.5	0.84
TFM	3.57	27.73	76.9	99.2	21.37	4.6	0.44
FM	103431.1	486467.3	1593521.3	88.9	22.66	6.5	0.53
FR	0.085	0.68	1.9	29.3	6.22	4.8	0.45
FP	0.07	0.46	1.3	75.5	17.29	5.2	0.47
HSM	15.25	179.82	451.1	40.0	7.36	3.4	0.38
FL	10.51	16.97	96.9	44.6	14.68	10.8	0.68
FW	1.22	3.01	13.3	29.4	8.91	9.1	0.62
FNL	6.16	3.95	44.8	86.4	32.02	13.7	0.76
SL	0.39	3.71	9.7	17.1	3.43	3.9	0.41
SW	0.48	0.27	3.5	19.4	7.25	14.1	0.77
LG	833.93	3896.61	12796.8	179.9	45.93	6.5	0.53
SG	5113.83	107504.5	245691.9	140.7	20.29	2.1	0.29
FY	4279979	72038336	169756546	98.4	15.63	2.5	0.33
YP	0.03	0.69	1.6	102.3	14.19	1.9	0.28

Note: δ^2g-genotypic variance, δ^2e-environmental variance, δ^2p-phenotypic variance, GCV-genotypic coefficient of variation, PCV-phenotypic coefficient of variation, H^2-broad sense heritability, GA-genetic advancement. Variables: DG-Days to emergence, EP-Emergence Percentage (%), SH-Seedling Height (cm), CLA-Cotyledon leaf area (mm^2), LA-Leaf area (cm^2), VL-Vine length (cm), NOL-Number of leaves, NOB-Number of branches, LCC-Leaf Chlorophyll content (cci), DF-Days to 50 % flowering, DEM-Days to edible harvest maturity and DHM-Days to drying harvest maturity, TFM-Total fruit mass per plot (kg), FM-Fruit mass (g), FR-Fruit rind (mm), FP-Number of fruits per plant, HSM-100 seed mass (g), FL-Fruit length (cm), FW-Fruit width (cm), FNL-Fruit neck length (cm), SL-Seed length (mm), SW- Seed width (mm), LG-Leaf growth%, SG-Shoot growth%, FY-Fruit yield (kg/ha) and YP-Yield per plant (kg)

3.4 Discussion

3.4.1 Qualitative traits evaluation

The 19 genotypes (seven parental landraces and 12 F_1 progenies) evaluated in the present study showed a wide range of diversity in qualitative traits including growth habit, stem shape, leaves colour, leaf margin, fruit colour, fruit texture, fruit shape, seed colour, seed shape and seed size (Table 3.4 and 3.5). Related findings were reported on *L. siceraria* landraces characterisation, where the greatest variation was in fruit shape, fruit texture, fruit colour, seed size, seed shape and seed colour (Buthelezi et al. 2019). The present results revealed that different landraces display different morphology. However, plant vigour, tendrils, stem colour, leaf shape, flower colour, flower size, tendrils branching, tendril type, fruit pulp colour and seed texture were the same for all evaluated genotypes. Corresponding findings were reported by Kalyanrao et al. (2016) who observed that *L. siceraria* genotypes were similar in stem colour, tendrils, tendril branching, leaf shape and flower colour, but differ in fruit and most seed traits.

Observed diversity in fruit and seed qualitative traits among the genotypes in the present study could be due to the different sites of origin of the seven parental landraces crossed and the mixing of genes in controlled cross pollination performed for generating 12 first filial generations (F_1 progenies). It was observed that the F_1 offspring inherited either the male or female fruit morphological traits (fruit colour, fruit texture and fruit shape) in the current findings. For example, the cross between DSI (maternal parent) with dark green smooth isodiametric fruits and RRP (paternal parent) with green rough isodiametric fruits generated green rough isodiametric fruits of RRPxDSI (F_1 progeny) (Table 3.5).

3.4.2 Quantitative traits evaluation

Similarly, on the previous study on cowpea genotypes indicated non-significant site x genotype (S x G) on grain yield, seed number per plant, seed number per pod, hundred seed weight, pod weight per plant and days to flowering (Mofokeng et al. 2020). In the present study, the observed non-significant S x G for fruit yield suggests that the genotypes studied were equitably stable across test environments. Across different sites, a stability analysis is vital for identification of sources of germplasm with desirable traits for commendation.

Seven parental landraces and twelve F_1 progenies evaluated in the present study showed a wide range of diversity in quantitative traits including days to emergence, emergence percentage, seedling height, leaf area, main vine length, days to flowering, days to edible harvest maturity, days to drying harvest maturity, fruit neck length, seed width and leaf growth percentage (Table 3.8 and 3.9). There was no significant difference for days to 50% emergence in a study by Mashilo (2016) in *Lagenaria siceraria* landraces collected from Limpopo, whereas in the present study there was a significant difference in days to emergence among 19 evaluated genotypes. Variation observed in the days to emergence was mainly due to differences in sites of origin for the parental landraces selected and mixing of genes in the obtained F_1 progenies. In the current study two parental landraces (RRP and NSRC) reached 50% emergence before all F_1 offspring and one parental landrace (NqSC) emerged later than all F_1 progenies. Similar findings were reported on significance of emergence percentage among *L. siceraria* genotypes that ranged from 65.8% to 85.3% (Iqbal et al. 2019).

In the present study the highest emergence percentage was obtained from F_1 progeny (KSRxKSP), while the lowest was observed from parental landrace (NqSC). Thus, F_1 progenies had general higher emergence percentage than parental landraces. This means F_1 progenies displayed heterosis/ hybrid vigour, where the progeny of a cross performs better than either parent (Wallace et al. 2014). Accessions of *L. siceraria* collected from Turkey displayed the same range in seedling height (22-56 mm), but their mean (39 mm) was lower than the one obtained in the present study (Yetisir et al. 2008). This observation confirms that different growing environments affect the performance of *Lagenaria siceraria* populations. Buthelezi et al (2019) obtained lower cotyledons leaf area that ranged from 880-1726 mm^2 and a mean of 1289 mm^2 amongst the landraces of *L. siceraria*. The diversity in seedling traits obtained in the current study may be due to the genetics of different *L. siceraria* genotypes (Uddin et al. 2014).

A shorter main vine length was obtained in the present study than the one reported amongst *Lagenaria siceraria* lines (4.8 to 6.8 m) by Uddin et al. (2014). In the present study we observed a larger leaf area (2581 to 7000 mm^2) than the results reported on *L. siceraria* landraces by Mashilo (2016). The findings obtained in the current study agree with the observations on days to flowering in *L. siceraria* that ranged from 44.0 to 65.8 days from a study by Iqbal et al. (2019). The current findings on days to drying harvest maturity correspond with published findings, that *L. siceraria* reach maturity from 60-120 days after planting (DAFF 2016). Mashilo (2016) reported a longer maximum fruit neck length (24.1 cm) and shorter minimum fruit neck length (0.53 cm) than for the present study amongst *L. siceraria* landraces. Thus, different genotypes differ in the fruit neck length that make their use to be different when the fruits are dry. For example, isodiametric shaped fruits have short neck, therefore the matured dried fruits are used for storing of water and milk. The dried curvilinear shaped fruits with long necks are used as cups for traditional beer in rural communities.

Results on seed width from the present study agreed with reported study on African *L. siceraria* accessions, where the seed width ranged from 5.30 to 12.34 mm (Mladenović et al. 2012). A greater *L. siceraria* leaf growth percentage with a mean of 5281% was recorded on the previous study by Buthelezi et al. (2019). However, cotyledon leaf area, number of leaves, number of branches, leaf chlorophyll content, total fruit mass per plot, number of seeds per fruit, fruit mass, fruit rind thickness, hundred seed mass, total seed mass, fruit length, fruit width, seed length, shoot growth percentage, fruit yield and yield per plant were not significantly different ($P > 0.05$) for the evaluated genotypes in the current study (Tables 3.8 and 3.9). Results of this present study disagree with reported results, where significant differences ($P < 0.05$) were obtained in most quantitative traits of *Lagenaria siceraria* (Mashilo 2016).

Correlation study provided a vibrant picture of traits association which is usually due to linkage, pleiotropy, physiological association in developmental and biochemical pathway (Chitralekha et al. 2018). Positive correlation between desirable traits is supposed to be favourable and help to breeder in selection, whereas negative selection hinders the recovery of combinations in both traits. The positive significant correlation between emergence percentage with fruit yield, number of seeds per fruit and vine length indicates that the selection of genotypes with high emergence percentage would result in genotypes with higher fruit yield, many seeds per fruit and longer vines. Positive correlation was observed in the current study between number of seeds per fruit with vine length, number of branches and total fruit mass, which is in accordance with reported findings (Koffi et al. 2009). Similar correlations were reported between number of branches with vine length h (r = 0.614) and number of leaves (r = 0.678) (Buthelezi et al. 2019).

A comparable positive correlation was obtained by Mashilo (2016) between hundred seed mass with vine length (r = 0.45) and number of branches (r = 0.42). Seed length was also reported to be positively correlated with fruit length and fruit rind thickness also correlated positively with fruit circumference in the comparable study (Mlandenovic et al. 2012). The present results are like those of Buthelezi et al. (2019) who also reported that seed mass positively correlated with hundred seed mass (r = 0.966) and fruit width (r = 0.732); fruit yield was also reported to correlate positively with fruit width (r = 0.702) and seed width was also reported to be positively correlated to tendril traits. Hence, the current study suggested that selection of *Lagenaria siceraria* genotypes with greater fruit width will result in fruit yield improvement. Total fruit mass positively correlated with all other variables studied on *L. siceraria* accessions in a study by Mlandenovic et al. (2012).

The first principal component (PC1) composed of several traits that contributed for the highest variation than PC2, as shown by principal component analysis on quantitative traits studied. Fruit width and fruit mass were negatively correlated with first principal component. In the first and second principal components, the traits with high coefficients were considered more important for explanation of total variability. Seedling height, cotyledon size, vine length, leaf area, number of leaves, number of branches, fruit mass, fruit rind thickness, fruit width, hundred seed mass and number of seeds per fruit established the greatest variability among the genotypes. Mashilo (2016) reported comparable findings, where principal component analysis indicated that most of the variation in *L. siceraria* is contributed by fruit and seed traits.

Clustering of genotypes according to fruit texture agreed with a previous study conducted on *Lagenaria siceraria* landraces by Buthelezi et al. (2019). It was reported that genotypes with analogous morphology belong to one cluster (Chimonyo and Modi 2013) and that genotypes with identical fruit shape, fruit length, fruit mass and fruit circumference grouped together (Mlandenovic et al. 2012). Cluster analysis conducted on quantitative traits grouped the evaluated genotypes into five clusters showing an adequate heritable variation that could permit rational selection. Genotypes found in cluster III could be recommended for selection and for further breeding program because they had high fruit yield, total fruit mass per plot, individual fruit mass and large fruit width.

Genotypic coefficient was generally lower than the phenotypic coefficient of variability signifying a robust impact of the environment concerning the expressiveness of genes in the phenotypic display (Table 3.12). Supportive results obtained in a study conducted on the genotypes *of Pisum sativum*, where the genotypic coefficient was generally lower than the phenotypic coefficient of variability (Meena et al. 2017). Higher coefficient of variation was obtained with leaf growth percentage in the current study, but pod bearing length exhibited higher value on the study conducted on field pea by Meena et al. (2017). Heritability is classified as low (<30%), medium (30-60%) and high (≥60%), according to Johnson et al. (1955). Traits under study that recorded low heritability values suggested that genetic variance (which includes additive, dominance, and epistasis variance) was low, and the environmental variance was high. A low heritability means the correlation between the genotype and phenotype is low and phenotypic selection cannot be done easily (Acquaah 2012). Higher heritability estimates were recorded for fruit length, vine length, seed width, fruit weight, rind thickness, leaf area, thickness of seed, number of seeds per plant and fruit circumference on another study (Mlandenovic et al. 2012). In most of the traits evaluated in the current study, the genetic advance was lower than the one obtained a study conducted on *Corchorus* accessions by Dube et al. (2019). Mofokeng et al. (2020) also reported high genetic advance values for days to flowering (538.29%), number of branches (613.72%) and hundred seed mass (1235.31%) in cowpea.

3.5 Conclusion

Wide variation exists in morpho-agronomic traits of the studied *Lagenaria siceraria* landraces and their F_1 progenies. In the current study, it was observed that the F_1 offspring inherited either the male or female fruit morphological traits. For example, the cross between DSI with dark green smooth isodiametric fruits and RRP with green rough isodiametric fruits generated green rough isodiametric fruits of RRPxDSI. F_1 progenies had superior performance than parental landraces in 15 quantitative traits

(53.57%) out of the 28 evaluated traits. Cluster III genotypes (NSRCxDSI, NSRCxKSP and RRPxDSI) outperformed all genotypes with respect to high fruit yield, total fruit mass per plot, individual fruit mass and large fruit width. Hence, these F_1 progenies can be used for further *Lagenaria siceraria* genetic improvement. A molecular evaluation of these genotypes by simple sequence repeat markers as an example is recommended for future studies as it is more precise in differentiating the variation.

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Chapter 4

Sensory evaluation and nutritional variability among parental landraces and F_1 progenies of *Lagenaria siceraria* from South Africa

Abstract

Lagenaria siceraria fruit is a vital source of minerals, vitamins, cucurbitacin, campesterol, polyphenols and 1.6% choline in sitosterol on dry weight basis. Edible fleshy parts of *L. siceraria* which are leaves, shoots and fruit as well the seeds contain different nutritional minerals from one landrace to another. The objective of this study was to assess the taste and analyse the nutritional variability of the selected parental landraces and created *L. siceraria* F_1 progenies. Selected landraces were crossed based on the North Carolina II genetic design. Twelve F_1 progenies along with seven parental landraces were grown in a randomized complete block design with three replications. With a participant's test, we observed a major difference in taste between 19 *Lagenaria siceraria* genotypes. A very sweet genotype (RRPxNSC) and sweet genotype (NqSCxNSC) would typically be utilized for making jams and sweets. There were no significant differences ($P > 0.05$) observed among mineral contents for *L. siceraria* genotypes, suggesting no considerable mineral composition variability. The first five components among the 19 genotypes evaluated contributed 89.97% of the variability. In the present study the following minerals/nutrients namely, crude proteins, calcium, magnesium, zinc, copper, manganese, iron, and phosphorus were correlated positively with the first principal component (PC1), which accounted for 42.42% of the total variation. Calcium, magnesium, potassium, sodium, zinc, manganese, iron, and aluminium were correlated positively with the second principal component (PC2), which accounted for 24.78% of the total variation. Biplot and dendrogram clustered genotypes into five clusters according to the macronutrients and micronutrients. Highest value for broad sense estimate was recorded for copper (18.67). NSRC genotype was unique because of its characteristics of having high contents of crude proteins, phosphorus, magnesium, zinc, manganese and iron, but poor in potassium making it suitable to be used for human consumption to supply all these minerals. Differences in the *Lagenaria siceraria* genotypes also occur in the taste and in the composition of the various nutrients present in them and not only in the morphological traits.

Keywords: Mineral nutrients, taste and genotypes

4.1 Introduction

The edible fleshy parts of *Lagenaria siceraria* which are leaves, shoots and fruit as well the seeds contain different nutritional minerals from one landrace to another (Buthelezi et al. 2019). Its fruit is a vital source of minerals, vitamins, cucurbitacin, campesterol, polyphenols and 1.6% choline in sitosterol on dry weight basis (Gajera and Joshi 2015). Seeds are sources of protein and oil (Hassan et al. 2007). Nutritional composition of the seeds is about 50% oil and up to 35% protein (Hassan et al. 2007). This crop had high values of ash content (4.25%) and high fat content value of 48.75% than the other six Cucurbitaceae seeds studied by Ogunbanjo et al. (2016). One hundred grams edible portion of the raw *L. siceraria* contained 3.4 g carbohydrates, 95.5 g water, 0.6 g proteins, 0.5 g total dietary fibre, 0.02 g fats, 150 mg potassium, 2 mg sodium, 26 mg calcium, 10.1 mg ascorbic acid, 13 mg phosphorus and 0.320 mg niacin (Gajera and Joshi 2015).

This crop is regarded as a main source of nutrient and mineral elements for resource poor rural communities (Buthelezi et al. 2019). The improved availability of knowledge on the nutritional status of the *Lagenaria siceraria* will increase its cultivation to solve malnutrition problems and achievement of household food security in the subsistence farming regions of South Africa (Sithole 2014). Although the morphological variation of *L. siceraria* landraces is receiving some attention from researchers in South Africa, the nutritional and mineral element content of the hybrids of these landraces has never been documented. It is hypothesised that the *L. siceraria* F_1 offspring may have a higher nutritional value than the parental landraces because of the expected heterosis or hybrid vigour. Therefore, this study aimed to determine taste preference and variability in nutritional and mineral content among *Lagenaria siceraria* genotypes (seven parental lines and their twelve F1 progenies).

4.2 Materials and Methods

4.2.1 Assessment of sweetness and bitterness

Thirteen Taletso TVET college undergraduate students (seven females and six males) aged 21–30 years were the study participants for evaluation of *Lagenaria siceraria* sweetness and bitterness. Each student was invited to participate if in a self-reported good state of health, and if not limited by any food intolerance(s) and/or allergies.

Participants were first briefed about the study and were then requested to sign a written consent form as a way of indicating their willingness to conduct sensory evaluation of different *L. siceraria* genotypes. Participants were requested not to eat, drink (except for water) or smoke for at least one hour prior to the session. Students (n = 13) were evaluated on liking of the flavour of the 19 most diverse *L. siceraria* cooked fruits sliced into 5 cm x 5 cm sizes. The 19 samples were evaluated in one session to maintain similar participants for the study.

Each student was given three pieces of each genotype to taste with a plastic cup. They held the sample on their tongue for 5 seconds before estimating the bitterness or sweetness intensity on five sensory levels (very sweet, sweet, neutral, bitter and very bitter). Students were asked to rinse their mouth with water and to wait between samples until they felt they had recovered a neutral taste sensation on their tongue. Data were captured using micro soft excel and the mean bitterness and sweetness from sampled fruits was used for the analysis. Ethical approval for this study was obtained from the Faculty of Natural and Agricultural Sciences Ethics Committee at the North-West University (ethics clearance number NWU - 01147 - 20 - A9).

4.2.2 Determination of nutrient and mineral content

At fruit maturity, the fruits of *Lagenaria siceraria* genotypes were collected from the same trial as specified in chapter three. Fruit samples from all three replicates in both two growing seasons were collected per plot representing 19 genotypes. Distilled water was used to rinse the fruits and they were cut using a stainless-steel knife into about five cm × five cm pieces or slices. Fruit pulp was removed from the fruit rind, separated from the seeds; sun dried for 24 hours and then it was oven dried at 60 °C for 24 hours (Labcon incubator, model 5016LC). Samples were ground into powder through a 0.84 mm sieve, using a laboratory grinder (Hammer mill SMC). Milled fruit pulp samples of the genotypes were sent to an analytical laboratory of KwaZulu-Natal Department of Agriculture and Rural Development (Cedara) for nutrient analysis.

These samples were analysed for macronutrients (nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg) and calcium (Ca)) and micronutrients (zinc (Zn), sodium (Na), copper (Cu), iron (Fe), manganese (Mn) and aluminium (Al)). The sub samples of the pulp of the fruit material were dried and ashed at 450 °C overnight. The ash was dissolved in one Moles (M) hydrochloric acid (HCl). The supernatant was analysed for Ca, Al, Cu, Mg, K, Mn, Zn and Na by atomic absorption spectroscopy (AAS) (Manson and Roberts 2000). The determination of P concentration was performed colorimetrically on a two millilitres (mL) aliquot of filtrate using a modification of the Murphy and Riley (1962) molybdenum blue system (Hunter 1974). Nitrogen content was converted into crude proteins by multiplying it with a constant value of 6.25 (Mariotti et al. 2008).

4.2.3 Data Analysis

Data was subjected to ANOVA using the SAS software (version 9.4) (SAS Institute Inc. 2016). Means were separated using Tukey’s HSD at the 5% significance level. Correlations and principal component analysis (PCA) were performed to determine multi-character variation. Cluster analysis through biplot and dendrogram was performed to study the differences and similarities of F_1 progenies. Variance components were calculated the same way as indicated in chapter three. Data for both sites was combined and analysed.

4.3 Results

4.3.1 Taste evaluation

The results of observations on taste among 19 genotypes from 13 participants are displayed in Table 4.1. In the present study RRPxNSC genotype was considered very sweet among all other genotypes as suggested by 38.46% of entries (5 participants). NqSCxNSC cross was considered a sweet genotype amongst all other evaluated genotypes as suggested by 61.54% of entries (8 participants). KSP was a neutral genotype among all evaluated genotypes as suggested by 92.31% of entries (12 participants). NSRCxKSP was considered a bitter genotype as suggested by 61.54% of entries (8 participants). There were no very bitter genotypes in the present study. Furthermore, we evaluated the differences in minerals present in these genotypes, to find which minerals are responsible for its taste.

Table 4.1: Assessment of sweetness and bitterness among nineteen different *Lagenaria siceraria* fruits

Genotypes	**Very sweet**	**Sweet**	**Neutral**	**Bitter**	**Very bitter**
KSP	0	1	12	0	0
DSI	0	2	9	2	0
NSC	0	3	9	1	0
NqSC	0	3	8	2	0
KSR	0	2	9	2	0
NSRC	0	5	8	0	0
RRP	1	4	7	1	0
NqSCxKSP	0	5	3	5	0
NqSCxDSI	1	4	7	1	0
NqSCxNSC	0	8	3	2	0
KSRxKSP	0	1	7	5	0
KSRxDSI	0	7	3	1	2
KSRxNSC	3	4	2	2	2
NSRCxKSP	1	1	3	8	0
NSRCxDSI	0	0	9	4	0
NSRCxNSC	1	6	6	0	0
RRPxKSP	0	5	6	2	0
RRPxDSI	1	6	2	3	1
RRPxNSC	5	4	3	0	1

4.3.2 Nutrients composition

In the current study there were no significant differences (*P* > 0.05) in the analysed minerals among the genotypes (Table 4.2). NSRC had high crude protein content (13.19%), whereas genotype NqSCxNSC had the least (8.31%) with a mean of 1.61% (Table 4.2). NqSC and NSC had the greatest calcium content (0.39%), whereas genotype KSRxDSI had the least (0.19%) with mean of 0.27%. High magnesium was obtained for NSRC (0.28%), whereas genotypes DSI, KSRxDSI and NSRCxDSI had the least (0.21%) with mean of 0.24%. KSR exhibited the highest potassium (12.29%), but NSRC had the least (7.87%) with a mean of 9.81%. NSRC had high phosphorus (0.42%), whereas genotype KSP had the least (0.19%) with a mean of 0.24%.

KSR had a high sodium content (894.9 mg/kg), whereas KSRxKSP had the least (347.8 mg/kg) with a mean of 519.30 mg/kg (Table 4.2). Higher zinc was obtained with NSRC (111.17 mg/kg), but RRPxKSP had the least (20.33 mg/kg) with a mean of 28.77 mg/kg. KSRxDSI displayed high copper (6.68 mg/kg), whereas RRPxKSP had the least (1.97 mg/kg) with a mean of 3.86 mg/kg. A high concentration of manganese was recorded for NSRC (42.50 mg/kg), whereas the least was recorded for NSRCxDSI (20.83 mg/kg) with a mean of 30.62 mg/kg. NSRC had a higher iron (1567.3mg/kg), whereas KSRxKSP had the least (110.80 mg/kg) with a mean of 434.44 mg/kg. KSR had a high value of aluminium content (332.17 mg/kg), while KSRxDSI had the least (160.50 mg/kg) with a mean of 234.59 mg/kg.

Table 4.2: Variation in fruit nutrients composition among *Lagenaria siceraria* different genotypes

Genotype	N	Ca	Mg	K	Na	Zn	Cu	Mn	Fe	P	Al
KSP	10.31	0.37	0.25	11.12	557.0	23.17	3.48	40.67	213.5	0.19	279.0
DSI	11	0.22	0.21	8.66	512.6	34.0	4.62	26.0	226.3	0.29	173.17
NSC	9.31	0.39	0.27	8.76	492.8	29.67	4.07	31.17	450.7	0.24	247.67
NqSC	8.69	0.39	0.24	9.81	594.8	21.33	4.10	33.67	460.0	0.21	248.67
KSR	8.5	0.25	0.23	12.29	894.9	20.83	3.70	26.0	171.8	0.21	332.17
NSRC	13.19	0.29	0.28	7.87	399.9	111.17	6.67	42.50	1567.3	0.42	217.67
RRP	9.94	0.30	0.25	10.95	617.5	24.17	3.90	35.17	419.8	0.23	236.83
NqSCxKSP	11	0.31	0.24	11.09	622.7	22.17	4.42	29.0	191.8	0.25	207.50
NqSCxDSI	10.75	0.22	0.22	9.50	405.9	21.17	2.63	24.50	163.8	0.23	234.83
NqSCxNSC	8.31	0.23	0.22	10.75	468.5	25.0	4.45	35.50	738.5	0.22	251.50
KSRxKSP	9.31	0.26	0.23	9.71	347.8	22.67	3.60	25.83	110.8	0.25	178.83
KSRxDSI	11.5	0.19	0.21	9.25	406.2	23.83	6.68	23.33	174.0	0.27	160.50
KSRxNSC	9.81	0.23	0.23	10.32	541.7	26.0	4.58	36.0	804.5	0.25	237.17
NSRCxKSP	10.38	0.30	0.25	8.83	380.6	22.67	3.90	27.83	213.5	0.27	235.67
NSRCxDSI	9.5	0.22	0.21	9.85	402.7	23.33	3.05	20.83	225.5	0.22	170.0
NSRCxNSC	10.44	0.28	0.27	9.52	506.5	23.50	4.25	28.83	546.3	0.23	229.17
RRPxKSP	9.56	0.28	0.22	10.14	518.5	20.33	1.97	29.83	358.0	0.20	251.17
RRPxDSI	10.25	0.25	0.23	9.43	546.2	22.67	2.90	26.67	275.2	0.23	318.17
RRPxNSC	9.5	0.23	0.24	8.51	650.1	29.0	3.78	38.50	942.8	0.23	247.50
Significance	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
P_Value	0.3331	0.7914	0.2092	0.2460	0.7672	0.4997	0.3075	0.7575	0.7995	0.1833	0.6034
HSD	0.69	0.23	0.11	5.20	510.18	87.3	5.12	26.69	1917.9	0.16	212.64
CV	20.59	39.95	22.03	25.19	46.67	144.15	62.98	41.41	209.73	32.0	43.06
MSG	0.204	0.021	0.003	7.189	97486.9	2458.75	5.92	222.98	830161.3	0.014	12393.5
MSE	0.11	0.012	0.0026	6.10	58742.1	1720.03	5.911	160.839	791453.6	0.006	10204.7
Grand Mean	10.06	0.27	0.24	9.81	519.30	28.77	3.86	30.62	434.44	0.24	234.59

Variables: N- crude proteins (%), Ca- Calcium(%), Mg- Magnesium(%), K- Potassium(%), Na- Sodium (mg/kg), Zn- Zinc (mg/kg), Cu- Copper (mg/kg), Mn- Manganese (mg/kg), Fe- Iron (mg/kg), P- Phosphorus(%) and Al- Aluminium (mg/kg). The explanation of genotypes is in chapter 3 (Table 3.1). HSD- Honestly significant difference, CV- Coefficient of variation, MSG- Mean square due to genotype and MSE- Mean square of error.

4.3.3 Correlation analysis

Crude protein content was positively and significantly associated with zinc (r = 0.66), copper (r = 0.55) and phosphorus (r = 0.78) (Table 4.3). A negative significant correlation was observed between crude proteins with potassium content (r = -0.51). Calcium content was positively correlated with magnesium (r = 0.66). Positive correlations were observed between magnesium content with manganese (r = 0.58), iron (r = 0.51) and zinc (r = 0.49). Potassium content was positively correlated with sodium (r = 0.61). Negative correlations were observed between potassium content with zinc (r = -0.50) and phosphorus (r = -0.61). Sodium content was positively correlated with aluminium (r = 0.65). Zinc content was positively associated with phosphorus (r = 0.89), iron (r = 0.78), copper (r = 0.59) and manganese (r = 0.49). Copper content was positively correlated with phosphorus (r =0.72) and iron (r =0.48). Manganese content was positively associated with iron (r = 0.74). Iron content was positively correlated with phosphorus (r =0.60).

Table 4.3: Correlation among fruit nutrients content of *Lagenaria siceraria* genotypes

Variables	**N**	**Ca**	**Mg**	**K**	**Na**	**Zn**	**Cu**	**Mn**	**Fe**	**P**	**Al**
Ca	-0.13^{ns}										
Mg	0.28^{ns}	__0.66**__									
K	-0.51*	0.08^{ns}	-0.24^{ns}								
Na	-0.39^{ns}	0.15^{ns}	0.04^{ns}	__0.61**__							
Zn	__0.66**__	0.04^{ns}	0.49*	-0.50*	-0.23^{ns}						
Cu	0.55*	-0.09^{ns}	0.26^{ns}	-0.32^{ns}	-0.17^{ns}	0.59**					
Mn	0.11^{ns}	0.43^{ns}	0.58**	-0.05^{ns}	0.17^{ns}	0.49*	0.26^{ns}				
Fe	0.31^{ns}	0.02^{ns}	0.51*	-0.41^{ns}	-0.05^{ns}	__0.78***__	0.48*	__0.74***__			
P	__0.78***__	-0.13^{ns}	0.36^{ns}	__-0.61**__	-0.38^{ns}	__0.89***__	__0.72***__	0.23^{ns}	__0.60**__		
Al	-0.39^{ns}	0.29^{ns}	0.24^{ns}	0.42^{ns}	__0.65**__	-0.14^{ns}	-0.40^{ns}	0.29^{ns}	0.04^{ns}	-0.38^{ns}	

Note: *, ** and *** significant Correlation at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively and ns—no significant correlation. High correlations have value >0.6. Variables: N- crude proteins(%), Ca- Calcium(%), Mg- Magnesium(%), K- Potassium(%), Na- Sodium (mg/kg), Zn- Zinc (mg/kg), Cu- Copper (mg/kg), Mn- Manganese (mg/kg), Fe- Iron (mg/kg), P- Phosphorus(%) and Al- Aluminium (mg/kg).

4.3.4 Principal component analysis

There was a considerable diversity among genotypes as determined by principal component analysis (Table 4.4). The first five components among the 19 genotypes evaluated contributed 89.97% of the variability (Table 4.4). In the present study, the following minerals namely, crude proteins, calcium, magnesium, zinc, copper, manganese, iron, and phosphorus were correlated positively with the first principal component (PC1), which accounted for 42.42% of the total variation. Potassium, sodium, and aluminium correlated negatively with PC1. Calcium, magnesium, potassium, sodium, zinc, manganese, iron, and aluminium were associated positively with the second principal component (PC2), which accounted for 24.78% of the total variation. Crude proteins, copper, and phosphorus correlated negatively with PC2. However, there was a weak correlation (< 0.6) of all evaluated nutrient contents with PC1 and PC2.

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

Variables	PC1	PC2	PC3	PC4	PC5
N	0.3559	-0.1538	0.0060	0.3493	0.4268
Ca	0.0093	0.4075	-0.5991	0.3052	-0.1370
Mg	0.2412	0.3995	-0.3532	0.1716	0.1291
K	-0.3188	0.1889	0.3117	0.4135	-0.2004
Na	-0.1994	0.3645	0.4625	0.2833	0.1599
Zn	0.4229	0.0996	0.1410	-0.0419	0.1862
Cu	0.3391	-0.0541	0.2733	0.4637	-0.4126
Mn	0.2101	0.4465	0.0784	-0.2260	-0.3852
Fe	0.3479	0.2433	0.2506	-0.4416	-0.1849
P	0.4311	-0.0889	0.1211	0.1260	0.1953
Al	-0.1696	0.4504	0.1647	-0.1595	0.5396
Eigenvalue	4.666	2.726	1.142	0.761	0.603
Variability%	42.42	24.78	10.38	6.910	5.480
Cumulative%	42.42	67.20	77.59	84.50	89.98

Note: PC1-5: Principal components 1-5. Variables are described in Tables 4.2

4.3.5 Cluster analysis

Evaluated genotypes clustered according to the macronutrients and micronutrients as shown in a biplot (Figure 4.1) and dendrogram (Figure 4.2). A first Cluster (I) in the biplot and Group I of the dendrogram consisted of NSRC genotype, which was rich in crude proteins, phosphorus, magnesium, zinc, manganese, and iron, but lower potassium than other genotypes (Table 4.2). Second Cluster (II) in the biplot and Group II of the dendrogram consisted of KSR genotype with higher potassium, sodium, and aluminium contents than other genotypes. Third Cluster (III) in the biplot and Group III of the dendrogram consisted of RRPxNSC genotype with second high sodium and iron content among evaluated genotypes. Fourth Cluster (IV) in the biplot consisted of KSRxDSI, NqSCxDSI, NSRCxDSI, NSRCxKSP and KSRxKSP genotypes without significant difference in contents of crude protein, calcium, magnesium, potassium, zinc, copper, manganese, iron, and aluminium. Fifth Cluster (V) in the biplot consisted of RRPxKSP, RRPxDSI, NSRCxNSC, NqSCxNSC, NSC, KSRxNSC, NqSC, NqSCxKSP, DSI, KSP and RRP genotypes with insignificant difference in calcium, magnesium, potassium, sodium, copper, manganese, iron, and aluminium content.

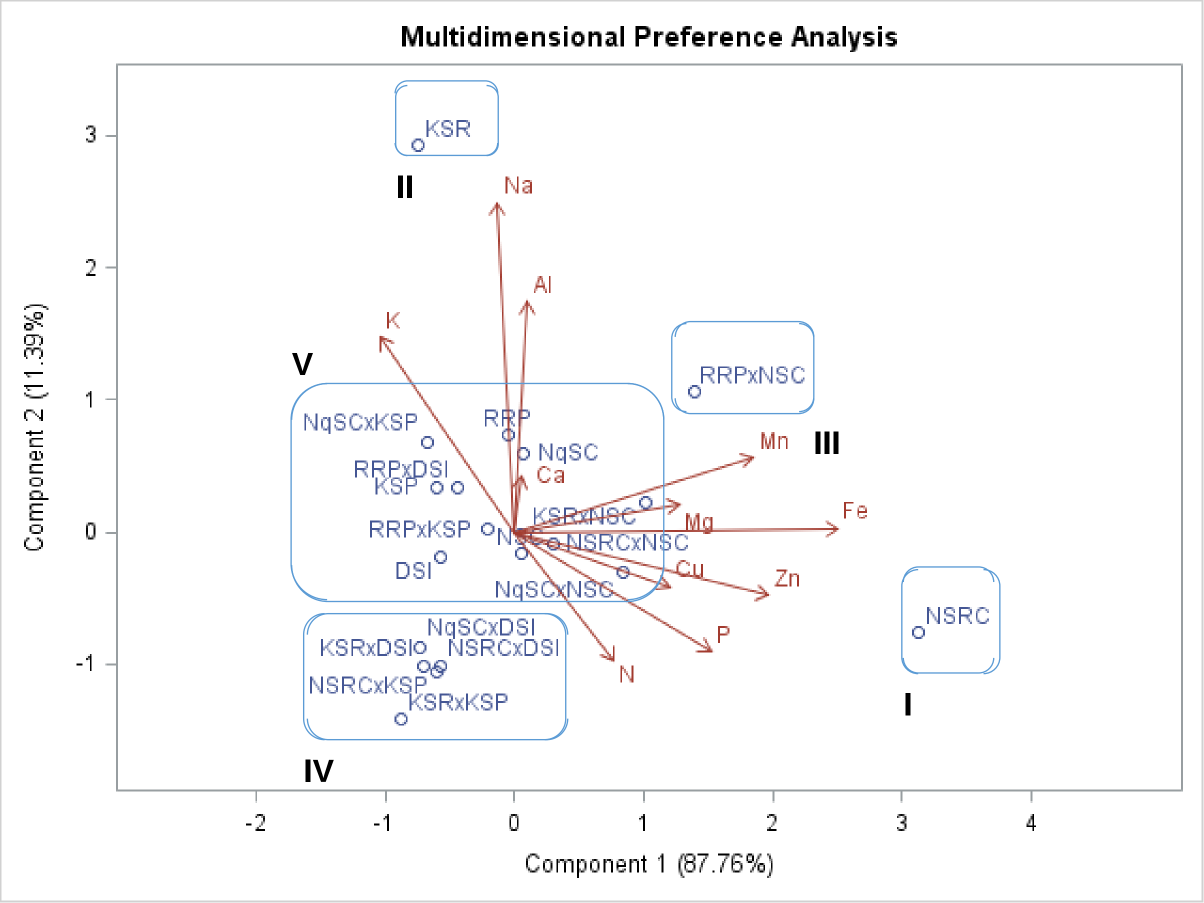

Figure 4.1: Biplot of *Lagenaria siceraria* genotypes and fruit nutrients composition. Genotypes and fruit nutrients composition (variables) are presented in Table 4.2

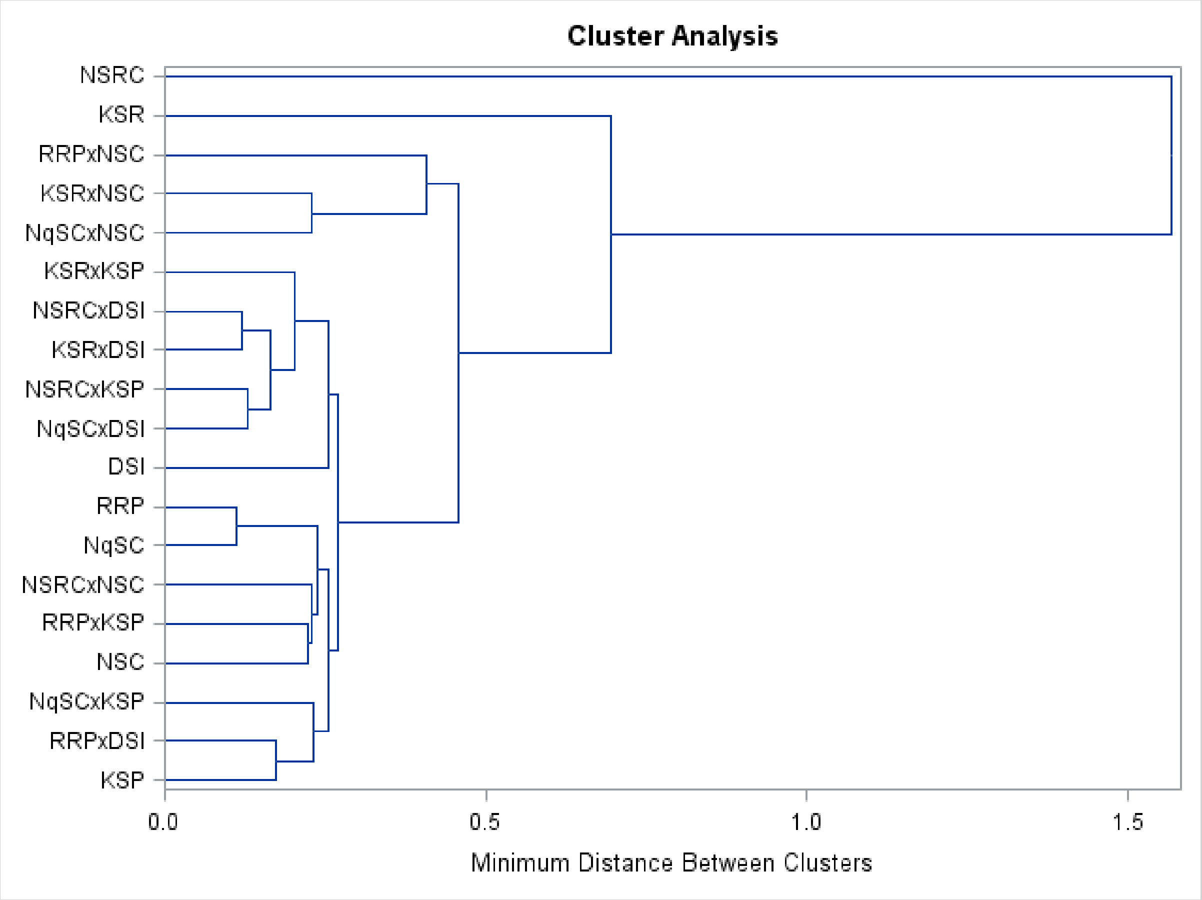

Figure 4.2: Hierarchical cluster showing similarities amongst *Lagenaria siceraria* genotypes using the complete linkage method. Description of genotypes is in Table 4.2

4.3.5 Genetic parameters

In the current study a high genotypic variance was recorded for sodium (6457.5), whereas a low genotypic variance was recorded for magnesium (0.00007) (Table 4.5). A greater environmental variance was recorded for iron (791453.6), whereas the lowest environmental variance was recorded for magnesium (0.0026). The uppermost value of phenotypic variance was recorded for iron (797904.9), while the lowest value of phenotypic variance was recorded for magnesium (0.0033). Higher value of phenotypic coefficient of variation was recorded for iron (205.6%), but the low phenotypic coefficient of variation was recorded for crude protein (3.53%). We obtained the greatest genotypic coefficient of variation for zinc (38.55%), whereas the lowest genotypic coefficient of variation was recorded for copper (1.16%). Phosphorus and calcium showed a large value for broad sense estimate (14.29%), while the smallest value was recorded for copper (0.03%). The highest value for genetic advancement was recorded for sodium (52.23%), while the least value was recorded for copper (0.002%).

Table 4.5: Genetic parameters for fruit nutrients composition of *Lagenaria siceraria* genotypes

Variables	δ^2g	δ^2e	δ^2p	PCV	GCV	H^2	GA
N	0.016	0.11	0.126	3.53	1.26	12.69	0.09
Ca	0.002	0.012	0.014	43.82	16.56	14.29	0.03
Mg	0.00007	0.0026	0.0033	23.94	3.49	2.12	0.003
K	0.181	6.10	6.28	25.55	4.34	2.88	0.15
Na	6457.5	58742.1	65199.6	49.17	15.47	9.90	52.23
Zn	123.12	1720.03	1843.15	149.17	38.55	6.68	5.92
Cu	0.002	5.91	5.912	62.99	1.16	0.03	0.002
Mn	10.36	160.84	171.2	42.73	10.51	6.05	1.63
Fe	6451.28	791453.6	797904.9	205.61	18.49	0.81	14.93
P	0.001	0.006	0.007	34.86	13.18	14.29	0.02
Al	364.79	10204.7	10569.5	43.82	8.14	3.45	7.32

Note: δ^2g-genotypic variance, δ^2e-environmental variance, δ^2p-phenotypic variance, GCV-genotypic coefficient of variation, PCV-phenotypic coefficient of variation, H^2-broad sense heritability, GA-genetic advancement. Variables: N- crude proteins (%), Ca- Calcium(%), Mg- Magnesium(%), K- Potassium(%), Na- Sodium (mg/kg), Zn- Zinc (mg/kg), Cu- Copper (mg/kg), Mn- Manganese (mg/kg), Fe- Iron (mg/kg), P- Phosphorus(%) and Al- Aluminium (mg/kg).

4.4 Discussion

With a participant’s test, a major difference in taste between the 19 genotypes was observed. A very sweet genotype (RRPxNSC) and sweet genotype (NqSCxNSC) would typically be utilized to make jams and sweets, because the tender edible fruit are prepared into jams, curries and sweets (Ghuge et al. 2016). RRPxNSC was rich in potassium, zinc, manganese, iron, sodium and aluminium, but its contents of crude protein, calcium and copper were low and finally its phosphorus and magnesium contents were medium (equal to mean). NqSCxNSC was poor in zinc but rich in copper that made it less sweet than RRPxNSC. It was observed in the current study that the lower the contents of crude protein, calcium and copper, the sweeter the fruit.

A neutral genotype (KSP) can be boiled and eaten like pumpkin. KSP had a plenty of crude protein, calcium, magnesium, potassium, sodium, manganese and aluminium, but was poor in zinc, copper, iron and phosphorus than average. Bitter cross (NSRCxKSP) could be utilized for medicine. It was reported that the fruit juice is helpful in curing conditions like flatulence, urinary disorders, diabetes mellitus, insomnia, hypertension, premature greying hair, liver diseases and as a diuretic (Mashilo 2016). The bitter genotype had a generally low potassium and zinc content. It may contain high cucurbitacin content (Chimonyo and Modi 2013).

The mineral analysis of the genotypes exhibited high concentrations of sodium, aluminium, and iron. Sodium and potassium contents in these genotypes agree with those determined for *Lagenaria siceraria* seeds: 1400 and 3001 mg/100g respectively, in a study by Hassan et al. (2007). High potassium prevents excessive sodium intake, and it has been reported that the high blood pressure would be reduced if a ratio of sodium ion to potassium ion less than one is maintained (Ossamulu et al. 2014). Hence, all 19 genotypes would be appropriate for this function. In a comparable study on two *Lagenaria siceraria* landraces (M01 and M03), the iron contents from leaf analysis were 12.17 mg/100g and 13.92 mg/100g respectively (Sithole 2014). For production of haemoglobin, iron is required in transportation of oxygen with blood from the lungs to the tissues (Ossamulu et al. 2014). High iron content found in *L. siceraria* could be beneficial in anaemia prevention (Dube et al. 2019).

Concentrations of aluminium in this study could be compared with those found in the work of Sithole (2014) who analysed leaves of two landraces (9.5 and 13.6 mg/100g). In a similar study on *L. siceraria* seeds, the zinc content varied from 0.15 to 0.30 mg/100g and manganese ranged from 16.2 to 26.3 mg/100g (Hassan et al. 2007). Zinc is important in cellular differentiation and protein synthesis, whereas manganese is playing a role in pyruvate metabolism and urea formation (Achikanu et al. 2013). In a similar study on *L. siceraria* seeds, the calcium content ranged from 3.1 to 3.7 mg/100g and magnesium ranged from 146 to 568 mg/100g (Hassan et al. 2007). Calcium and magnesium contents obtained in the present study were higher than these values. Hence, *L. siceraria* could be used to supplement calcium and magnesium in the diet.

It was reported that on *L. siceraria* leaves, crude protein ranges from 6419 to 6794 mg/100g and phosphorus ranges from 306 to 322 mg/100g (Sithole 2014). High crude protein content that fluctuated from 16.84% to 35.0% was reported on *L. siceraria* seeds by Hassan et al. (2007). Nitrogen is required for protein and nucleic acid synthesis (Adebayo et al. 2019). In a comparable study on *L. siceraria* seeds, the copper content ranged from 13 to 40.9 mg/100g (Hassan et al. 2007). Copper is vital in erythrocyte function, regulating the survival of red blood cell and in the erythropoiesis process (Ossamulu et al. 2014). The findings of the present study agree with the findings reported on *L. siceraria*, that it contains sodium, potassium, essential elements and trace minerals (Chimonyo and Modi 2013). Hence, *L. siceraria* would be used to supply dietary diversity and contribute to food security. Diversity in mineral concentration among genotypes in the current study may be due genetics of parental lines used in crossing.

The positive correlation between magnesium with manganese, zinc and iron indicates that the selection of genotypes with high magnesium result in genotypes with higher manganese, zinc and iron. In the first and second principal components, the minerals with high coefficients were considered more important for explanation of total variability. Divergent results were reported on *Corchorus olitorius*, where copper was positively associated with the second principal component (Mncwango et al. 2019). Biplot and dendrogram based on mineral contents classified the genotypes into five clusters revealing nutrients variation. Genotypes were not grouped based on place of origin for the parental lines signifying sharing mineral concentration. It was reported that *L. siceraria* selection must have been determined by specific socio-cultural use and preferences of farmers (Mashilo 2016). Cluster I genotype (NSRC) was unique by its characteristics of being rich in crude proteins, phosphorus, magnesium, zinc, manganese and iron, but lower in potassium making it suitable for human consumption to supply all these minerals. This genotype had a neutral taste.

The present study displayed that the assessed minerals had low heritability estimates of < 20%, indicating less significant genetic variation for selection. Genotypic coefficient was generally lower than the phenotypic coefficient of variability (Table 4.5). Corresponding results recorded on the accessions of *Corchorus* study, where the genotypic coefficient was generally lower than the phenotypic coefficient of variability (Dube et al. 2019).

4.5 Conclusion

Differences of *Lagenaria siceraria* also occur in the taste and in the composition of various nutrients present in them, do not end at morphological traits only. This crop has significant contents of phytochemicals and nutrients which make them therapeutically beneficial and nutritionally. F_1 progenies had higher level of sweetness (RRPxNSC) and higher level of bitterness (NSRCxKSP) than parental landraces, where all parental landraces were neutral in taste. The current study revealed that the lower the contents of crude protein, calcium and copper in the abundance of potassium and zinc mineral, the sweeter the *L. siceraria* fruit. All parental landraces were neutral in taste. Parental landraces outperformed F_1 progenies for ten minerals (90.9%) out of eleven evaluated minerals. Cluster I genotype (NSRC) outperformed all genotypes through higher contents of crude protein, phosphorus, magnesium, zinc, manganese and iron. Since there was a variation in mineral contents obtained in the present study among *Lagenaria siceraria* genotypes, the evaluation of vitamins and phytonutrients is recommended for future studies.

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Chapter 5

Final summary, Conclusion and Recommendations

5.1 Research overview

Lagenaria siceraria is one of the most essential cucurbitaceous vegetables due to its prolific bearing habit, value as a cooked vegetable and low cost of cultivation. Its fruit is a vital source of minerals, vitamins, cucurbitacin, campesterol, polyphenols and 1.6% choline in sitosterol on dry weight basis. The edible fleshy parts of *L. siceraria* which are leaves, shoots and fruit as well as the seeds contain different nutritional minerals from one landrace to another. This crop is grown under subsistence farming with no improved cultivars. No studies associated crossing of *L. siceraria* landraces collected from KwaZulu-Natal with the agronomic and nutritional composition in South Africa. Therefore, there was a need to develop F_1 progenies from *Lagenaria siceraria* landraces with desirable traits and evaluate their morpho-agronomic and nutritional attributes for commercial and subsistence farming.

It was hypothesized that wide variation existed in fruit and seed morpho-agronomic traits of *Lagenaria siceraria* landraces from South Africa and their F_1 progenies. It was also hypothesized that *L. siceraria* F_1 progenies had a general sweeter taste and had higher fruit nutritional and mineral content than the parental landraces because of heterosis. This study aimed to develop *L. siceraria* F_1 progenies from landraces with desired traits in South Africa and evaluate their morpho-agronomic characteristics, assess taste and nutritional composition to develop improved cultivars. The research comprised of two experiments focusing on the following: Morpho-agronomic evaluation of parental landraces and F_1 offspring of *Lagenaria siceraria*; as well as the assessment of taste and nutritional variability among parental landraces and F_1 progenies of *Lagenaria siceraria* from South Africa.

5.2 Research organisation

The crossing study was carried out in pots under semi-controlled environmental conditions (net house) at North-West University from November 2019 to April 2020. A field experiment for the first season was conducted at North-West University (NWU) crop science field (25º49´34´´S, 25º36´34´´E) from October 2020 to January 2021. The second season field experiment was conducted at Molelwane North-West University research farm, (25°48′ S, 25°38′ E) from January to May 2021. Twelve F_1 progenies along with seven parental landraces were grown in a randomized complete block design with three replications to evaluate the qualitative traits, quantitative traits, taste and mineral contents of these genotypes.

5.3 Main findings from the study

A wide range of variation was observed for quantitative and qualitative traits among the evaluated *Lagenaria siceraria* genotypes. The current study established that the F_1 offspring inherited either the male or female fruit morphological traits (fruit colour, fruit texture and fruit shape). For example, the cross between DSI (maternal parent) with dark green smooth isodiametric fruits and RRP (paternal parent) with green rough isodiametric fruits generated green rough isodiametric fruits of RRPxDSI (F_1 progeny). Genotypes such as NSRCxDSI, NSRCxKSP and RRPxDSI exhibited suitable quantitative traits such as high fruit yield, total fruit mass per plot, individual fruit mass and large fruit width. The differences of *L. siceraria* also occur in the taste and in the composition of the various minerals present in them, do not end at the morphological traits only. All parental landraces were more neutral in taste, whereas most F_1 progenies were either bitter or sweet in different levels. Parental landraces were dominant than F_1 progenies in ten minerals (90.9%) out of eleven evaluated minerals.

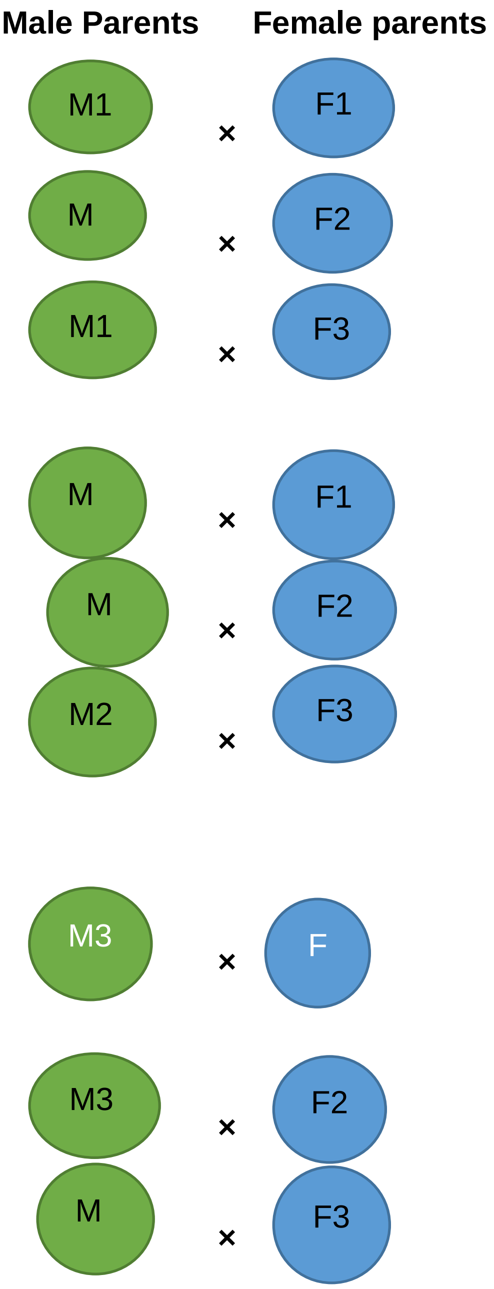
5.4 Conclusion and recommendations

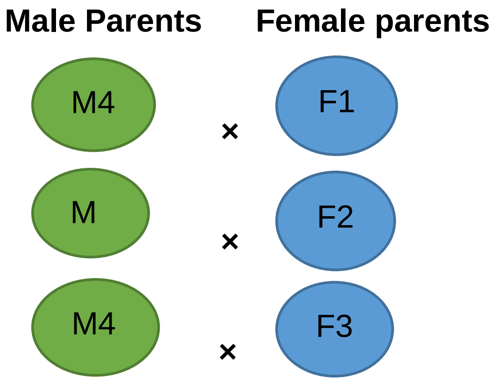
Wide variation exists in morpho-agronomic traits of *Lagenaria siceraria* landraces from South Africa and their F_1 progenies. F_1 progenies had superior performance than parental lines in 15 quantitative traits (53.57%) out of the 28 evaluated traits. In the first study, genotypes in cluster III (NSRCxDSI, NSRCxKSP and RRPxDSI) outperformed all genotypes through high fruit yield, total fruit mass per plot, individual fruit mass and large fruit width. F_1 progenies had higher level of sweetness (RRPxNSC) and higher level of bitterness (NSRCxKSP) than parental landraces, where all parental landraces were neutral in taste. The present study revealed that the lower the crude protein, calcium and copper in the abundance of potassium and zinc mineral, the sweeter the *L. siceraria* fruit. In the second study, cluster I genotype (NSRC) outperformed all genotypes with higher contents of crude proteins, phosphorus, magnesium, zinc, manganese and iron. The F_1 progenies that exhibited desirable morpho-agronomic traits can be used for further *L. siceraria* genetic improvement, molecular evaluation of these genotypes by simple sequence repeat markers as an example is recommended for future studies. The evaluation of vitamins, phytonutrients and phytochemicals among these *Lagenaria siceraria* genotypes is recommended for future studies. One of the recommendations can be to look for genotypes with exceptionally high nutritional contents and use them as parents for generation of additional populations. This could be an important strategy in efforts to develop highly nutritious *L. siceraria* genotypes.

Appendices

Appendix 1: Experimental design for the study

a. Crossing





b. Field evaluation of 19 genotypes (seven parents and twelve F_1 progenies)

Rep1	Rep2	Rep3
KSP	NqSCxDSI	RRPxNSC
DSI	KSRxNSC	RRPxDSI
NSC	KSP	RRPxKSP
NqSC	NqSCxNSC	NSRCxNSC
KSR	RRPxNSC	NSRCxDSI
NSRC	RRPxKSP	NSRCxKSP
RRP	KSRxDSI	KSRxNSC
NqSCxKSP	KSRxKSP	KSRxDSI
NqSCxDSI	RRPxDSI	KSRxKSP
NqSCxNSC	NqSC	NqSCxNSC
KSRxKSP	NqSCxKSP	NqSCxDSI
KSRxDSI	RRP	NqSCxKSP
KSRxNSC	DSI	RRP
NSRCxKSP	KSR	NSRC
NSRCxDSI	NSC	KSR
NSRCxNSC	NSRCxDSI	NqSC
RRPxKSP	NSRC	NSC
RRPxDSI	NSRCxKSP	DSI
RRPxNSC	NSRCxNSC	KSP

Appendix 2: Taste assessment instrument

a. Study information and Consent form

Investigator: Lungani Nkosi, North-West University

Introduction

You are invited to take part in a research study (taste assessment). This research will study sweetness and bitterness of *Lagenaria siceraria* (bottle gourd) fruits. It is your choice if you want to be part of this study or not. We have 19 cooked different genotypes of *L. siceraria* fruits. You will be given three pieces of each genotype to taste with a plastic cup. You will hold a sample on your tongue for 5 seconds before estimating the bitterness or sweetness intensity on five sensory levels (very sweet, sweet, neutral, bitter, and very bitter). Please rinse their mouth with water and to wait between samples until they felt they had recovered a neutral taste sensation on your tongue. You will be given the sheet to record the taste intensity of each genotype. Research studies are ways of finding out new information that might add value in science and human life. This form explains why we are doing the study and it also tells you about any risk with this study. This information will help you decide whether you wish to be part of the study.

What is the purpose of the study?

The main reason for doing this study is to help answer the following question:

- Which *Lagenaria siceraria* genotype is sweeter and which one is bitter among these ones?

Who can take part in the study?

You can participate in this study if: you are in a good state of health; you are not limited by any food intolerance(s) and/or allergies and finally you have not eaten, drank (except for water) or smoked for at least one hour prior to this session.

I ………………………………………agree to be part of the study……………………….

Name and Surname Signature

b. Participants recording sheet

Genotypes	Very sweet	Sweet	Neutral	Bitter	Very bitter
KSP					
DSI					
NSC					
NqSC					
KSR					
NSRC					
RRP					
NqSCxKSP					
NqSCxDSI					
NqSCxNSC					
KSRxKSP					
KSRxDSI					
KSRxNSC					
NSRCxKSP					
NSRCxDSI					
NSRCxNSC					
RRPxKSP					
RRPxDSI					
RRPxNSC					

Please tick on one column per sample corresponding to your answer after tasting.