Agronomic, genetic, and nutritional characterization of Nightshade (*Solanum* spp.) accessions

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DECLARATION

This dissertation is my original work, and it has not been presented for a degree at any other institution.

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DECLARATION

1. I understand what Plagiarism is and I am aware of the University's policy in this regard
2. I declare that this dissertation is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other peoples' work or my own work has been used, this has properly been cited and referenced in accordance with the North-West University requirements.
3. I have not sought or used the services of any professional agencies to produce this work.
4. I have not allowed, and shall not allow anyone to copy my work with the intention of passing it off as his/her own work.

Signed: Date: July 2022

DEDICATION

I dedicate this study to my mother Juliah Anna Shongwe (1966-2016) who supported me with her love, care and inspiration throughout my life and was my source of strength, May her soul rest in peace.

To my son, Innocent Tsepo Prince Mahlalela who loves, cares, supports and inspires me.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest: No potential conflicts of interest reported by the author for the study.

LIST OF ABBREVIATIONS AND ACRONYMS

Access:	Accession
ALVs:	African leafy vegetables
ANOVA:	Analysis of variance
BLarea:	Biggest leaf area
BT:	Branching type
CA:	Cluster analysis
CC:	Cotyledon colour
ChloroC:	Leaf chlorophyll content
CoN:	Colour of nodes
CV %:	Coefficient variation as a per cent
DAFF:	Department of agriculture, forestry, and fisheries
DF:	Degrees of freedom
FFM:	Fruit fresh mass
FLC:	Flower colour
FLT:	Flowering type
FRA:	Fruit attachment
FRCS:	Mature fruit colour and surface
FRP:	Fruit type
FRS:	Mature fruit shape
Fruit_Y:	Fruit yield kg ha^{-1}
FY_gp:	Fruit yield per plant
GA:	Genetic advance
GAM:	Genetic advance as mean percentage
GCV:	Genetic coefficient of variation
GM/X:	Grand mean
GML:	General Linear Model
GV:	Genetic variance
H:	Shannon-Weaver diversity index
H^2:	Broad sense heritability
HrvstInd:	Harvest index
HSD:	Tukey's honest difference
LC:	Leaf colour

LDM:	Leaf dry mass
Leaf_Y:	Leaf yield kg ha^{-1}
LFM:	Leaf fresh mass
LS:	Leaf surface
LSP:	Leaf shape
LTP:	Leaf type
LY_gp:	Leaf yield per plant
ND_F:	number of days to 50% flowering
ND_FF:	number of days to fruit forming
ND_FR:	number of days to fruit ripening
Nfruit:	Number of fruits
NLS:	Number of leaves
NPBr:	Number of primary branches
PBR:	Plant branching habit
PCA:	Principal component analysis
PCV:	Phenotypic coefficient of variation
PH:	Plant height
Psprd:	Plant spread
PV:	Phenotypic variance
Rep:	Replication
SAS:	Statistical Analysis Software
SBC:	Colour of stem and branches
SC:	Seed colour
SD:	Stem diameter
SDM:	Stem dry mass
SFM:	Stem fresh mass
SLarea:	Small leaf area
StD:	Standard deviation
Stem_Y:	Stem yield kg ha^{-1}
SY:	Seed type
SY_gp:	Stem yield per plant
TBimass:	Total biomass

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General Abstract

Nightshades (commonly referred to as African and/or Black nightshade) comprise several species within a section of the genus *Solanum*. There are 30 species reported in the section in the world, and about 12 species are found in Africa. Nightshade is an indigenous leafy vegetable in Sub-Saharan Africa (including South Africa) valued for its high nutrient content and therefore nutritional security and a potential to generate income especially in subsistence farming. Its use as a food crop needs to be promoted and for this to be achieved there is a need to understand the genetic background and generate knowledge for cultivation through breeding and precision agronomic studies. Therefore, the limited information available on this neglected and utilized family hinders their development as well as sustainable conservation. Existing knowledge on the agronomic and nutrient potential of these plants is limited and the information regarding the morphological and nutritional assessment of Solanum species found in South Africa is scarce. Therefore, the objectives of this study were: (i) to assess and evaluate the genetic variability in 15 nightshade accessions for agronomic, yield and yield components characteristics, (ii) to assess the variability in nightshade accessions for nutrient traits, (iii) to identify potential parents for future use in breeding programs in South Africa. For two consecutive years (2020 and 2021) trials were carried out on the fields of the North-West University research garden on the Mafikeng campus by executing a randomized complete block design (RCBD) with three replications. The raw data were subjected to analysis of variance using SAS software (version 9.4) at a 5% probability level. Shannon-weaver results exhibited the least diversity as evidenced by low diversity indices for all qualitative descriptors ranging from 0.077 to 0.227 with the lowest average diversity index (0.067), thus suggesting low levels of polymorphism. Multivariate analyses were performed to determine patterns of diversity among accessions. Morpho-agronomic characters for the first two PCs accounted for 62.10% of the total variation and identified stem diameter (mm), fruit fresh mass, leaf fresh mass, leaf dry mass, stem fresh mass, stem dry mass, leaf area, days to 50% flowering, leaf yield, stem yield, and total biomass as the major contributors to variability, while the first two components accounted for 74.61% of the total variation in nutritional traits, Ca, Mg, Zn, Fe, Al, N, K, and P contributed more to the variability. The principal component biplots and cluster analysis among accessions revealed a significant level of variability in both morphological and nutrient traits studied. Cluster analysis resulted in 14 clusters based on accession dissimilarity and similarity. Genetic parameters were estimated for agronomic and nutrient characteristics

and showed a considerable amount of variability. The high mean value, high range of variability and genotypic variance was observed for all traits. The phenotypic variance and phenotypic coefficient of variation were more than the genotypic variance and genotypic coefficient of variation under study, thus indicating the role of environmental influence on tested traits. High genotypic coefficient of variation (GCV %), broad-sense heritability (H^2), and genetic advance expressed as a percentage of the mean (GAM %) were calculated (82.28%, 70.59%, and 142.40%, respectively) for the smallest leaf area (cm^2). On the other hand, the biggest leaf area (cm^2) (172.92%, 50.94%, and 254.25%) had high GCV, moderate H^2, and high GAM%, while Fe and Al had moderate (32.35%, 34.78%) heritability coupled with high (277.12%, 435.72%) genetic gain at 5% selection intensity, thus suggesting the effects of additive gene action in the inheritance of these characters and selection may be effective. The study revealed sufficient genetic variability for traits among the 15 nightshade accessions studied, which could be exploited for the improvement of the nightshade breeding programme in the country. Furthermore, the study identified accessions Scabrum, Ncampus, Nshad40, N5547, SRetrflx, NigSN18, ManTown, and Nshad9 as superior accessions for high yield and nutrient-dense of the 15 nightshade accessions.

Keywords: Agronomic, Genetic advance, Heritability, Morphological, Nightshade, Nutrient, Correlation, Traits

Chapter 1:

General Introduction

1.1 Background

South Africa is a country rich in cultural diversity and biodiversity, with many people still relying on plants for food, water, shelter, fuel, medicine and other necessities (Van Wyk & Gericke, 2000). Leafy vegetables, also known as leafy greens or vegetable greens, are plant leaves, tender petioles, and shoots that are consumed as a vegetable, relish or sauce. Traditional leafy vegetables (also known as African leafy vegetables or ALVs) are indigenous or traditional vegetables that are consumed for their leaves, young shoots, and flowers (Chweya & Eyzaguire, 1999; Maundu, 1999). Some of the ALVs available in South Africa include *Amaranthus* spp., *Corchorus olitorios*, *Cucurbita maxima*, *Solanum retroflexum*, *Moringa oleifera*, and *Cleome gynandra* (Smith & Eyzaguirre, 2007; van Rensburg et al., 2007).

South Africa is endowed with a diverse range of biodiversity (Reinten & Coetzee, 2002), including wild vegetable species-rich with macro-nutrients, micro-nutrients, and vitamins (van den Heever, 1995). These species play an important role in the food security of many resource-poor farming families (Nandhini et al., 2014) and have potential value as a genetic resource for the global community. As a result, it is regrettable that African researchers, policymakers, and farmers are said to have overlooked the potential of these crops to reduce food insecurity and poverty. Leafy vegetables, including several African indigenous vegetables (AIVs), are highly valued as accompaniments to carbohydrate-based staples in the typical West and East African diet, but not so much in Southern Africa, where the main vegetables are exotic. Because of its high nutritional value and ability to grow under drought conditions and resistance to pests and diseases, *Solanum retroflexum*, also known as the broad-leafed nightshade (family Solanaceae), has been identified as a significant food crop that can be included in sustaining nutrition-intervention programs aimed at combating hidden hunger and malnutrition among Sub-Saharan rural communities (van Averbeke et al., 2004).

Nightshades are herbaceous plants that grow in an erect, branched form and bloom once or twice a year. They are well-known for their small, shiny, black to purple-black fruit, which is used to make jam. The nightshade complex is made up of many different species, and its taxonomy is complicated. The most common species in South Africa are *S. americanum*, *S.*

nigrum, and S. retroflexum. When used as a leafy vegetable, the leaves and tender shoots of nightshade are harvested and cooked but reports in the literature indicate that the leaves can also be eaten raw. Ripe fruit is also popular, whether fresh or preserved, but green fruit is poisonous. According to Werner and Petra (2010), fruits are used in preserves, jams, and pies. Fruits have a pleasant musky taste (Hammington, 1974), which improves lightly after a frost.

Nightshade (English) is known as nastergal, galbessie and nagskade in Afrikaans; ixabaxaba in isiNdebele; umsobo, sheshoabohloko and umsobo-sobo in isiXhosa; umsobo, isihlalakuhe, udoye, umagqa, umgwaba, umsobo-sobo (fruit) and umqunbane in isiZulu; lethotho in SePedi; seshoa-bohloko, sehloabohloko and momoli in Sesotho; msobo and umsobo in siSwati; muxe in Tshivenda; kophe in Xitsonga and musaka in Shona (Fox & Young, 1982; Bromilow, 1995; Van Wyk & Gericke, 2000; Vorster et al., 2002; van Rensburg et al., 2007).

Afuape et al. (2011) reported that proper management and actual utilization of plant genetic resources depends on a detailed understanding of their genetic variability. The failure to identify appropriate and potential parents would influence the outcomes of breeding programs, which is dependent on the degree of genetic variability present in the source population (Tseng et al., 2002). There are various methods available to measure genetic variation among crop genetic resources. These methods rely on the availability of data based on pedigree, plant morphology, agronomic performance, biochemical and molecular analyses (Mohammadi & Prasanna, 2003). Field phenotyping is a common technique for genetic variation between and within genotypes (Yoshida, 2004). The knowledge concerning the extent of genetic variation and genetic associations among genotypes are important considerations for designing effective breeding and conservation programs (Singh et al., 2013).

1.2 Problem statement

Edible wild leafy vegetables play an important role in African agricultural and nutritional systems. Chweya (1997) listed several leafy vegetables used in Kenya. Some of these vegetables are treated as weeds in different parts of the country, and as indigenous vegetables in others (Rwig, 2016). Such is the case of Solanum species, related to the nightshades, the subject of this study. Nevertheless, not much is known about agronomic variation, genetic and nutritional characterization using morphological descriptors among different nightshade accessions and the heritability of traits in South Africa. Nightshade germplasm shows varying

amounts of phenotypic variation mainly in their vegetative features (Rwig, 2016). Nonetheless, it is not clear if the phenotype reflects genetic diversity, which is critical for future breeding programs of these accessions. There is little research work done in South Africa regarding the genetic improvement of this species, and there is no current commercial production in RSA.

1.3 Justification for the study

Nightshade is a highly nutritious leafy vegetable with the capacity to improve food and nutritional security in South Africa and elsewhere. The leaves, young tender shoots, and flowers are consumed, while the fruits or berries, when ripe, are harvested to manufacture jams. This plant is not only eaten but is also used for medicinal purposes. The study was undertaken as there is little research done in South Africa regarding the morphological, genetic, and nutritional variation in Nightshade accessions. Nightshade is an important crop in some parts of South Africa, but it is not cultivated, because even though people eat the crop it is still considered a weed. Because it is not cultivated or commercialized there is not enough to meet the demand of the consumers. Only conventional vegetables such as these listed below are found on the market; Swiss chard (*Beta vulgaris* L. var. cicla), Mustard green (*Brassica juncea*. subsp. integrifolia var. crispifolia), Kale (*Brassica oleracea* var. virids) and Cabbage (*Brassica oleracea* var. capitata). One of the reasons cited for the lack of cultivation of nightshade and other traditional leafy vegetables is the non-existence of improved genotypes. Currently, most nightshade consumers in South Africa harvest nightshade from the wild or buy from the street vendors, who purchase from farmworkers. A lot of variation has been observed to exist among the wild biotypes and landraces because of natural hybridization which could be exploited for crop improvement. There is thus a need and an opportunity for the genetic improvement of nightshade in South Africa. According to Adebola and Morakimyo (2006), the use of morphological descriptors is the first stage in any crop genetic improvement program.

A concerted effort is being made to collect and preserve *Solanum* section. Solanum germplasm is important for facilitating systematic evaluation and breeding or selection programs for genetic enhancement of desirable characteristics. The morphological and genetic characterization will yield new insights into its genetic structure, enhancing identification and the evaluation of discrete accessions. Understanding phenotypic characteristics will allow for the introgression of important yield and nutritional genes into adapted nightshade accessions to combat the 'hidden hunger.' Malnutrition and other diseases caused by micronutrient

deficiencies should be avoided by eating leafy vegetables, especially nightshade plants, due to their nutritional composition (van Rensburg et al., 2004). Having this information, it is a significant stage in genetically and nutritionally improving and developing this species to contribute to food and nutritional security in the South Africa. Information on morpho-genetic variation and nutritional content is important for plant breeders when they want to improve any given crop.

1.4 Research aim

The primary aim of this study was to characterize the available nightshade accessions morphologically and nutritionally, as well as the genetic parameters to identify and select superior accessions for future breeding programs.

1.5 Research objectives

The specific objectives of the study were:

i. To characterize the germplasm collection of *Solanum* species at the NWU-Mafikeng campus agronomically and morphologically.

ii. To analyse variability in yield and yield contributing traits of accessions of nightshade, and to determine the contribution of the component traits towards yield.

iii. To evaluate the nutritional composition and heritability of nutritional traits of nightshade accessions.

1.6 Research hypotheses

i. There are no agronomic, morphological, and genetic variations of different accessions of the same species grown under similar environmental conditions.

ii. There are no variations in yield and its contributing traits of different accessions of the same species.

iii. There is no nutritional existence in different accessions of the same species grown under similar environmental conditions.

1.7 Structure of the Dissertation

The dissertation outline is shown in Table 1.1. The dissertation is in the form of discrete research chapters, each with its format, like that of a stand-alone research paper (whether the chapter has already been published or not). This is the most common dissertation format used by the University. As a result, some references and introductory information are inevitably repeated among chapters.

Table 1.1: Dissertation outline

Chapter	Objectives
Chapter 1	General Introduction
Chapter 2	Literature review
Chapter 3	Morphological characterization of nightshade accessions using phenotypic (qualitative) traits.
Chapter 4	To assess and evaluate genetic variability of nightshade accessions based on their agronomic-morphological traits.
Chapter 5	To evaluate the performance of nightshade accessions and a significant amount of variation and correlation for yield and yield contributing traits.
Chapter 6	To assess and evaluate nutritional variability among nightshade accessions
Chapter 7	General discussion, conclusion and recommendations

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Chapter 2:

Literature Review

2.1 Introduction

The review of literature in this chapter aims to provide information associated with the agronomic, morphological, and nutritional characterization of nightshades (*Solanum* section, *Solanum*) using morphological descriptors.

2.2 Indigenous leafy vegetables and their consumption

Indigenous leafy vegetables (ILVs) are plant species that are native to a particular region or those species that were intentionally or accidentally introduced to that region for an extended period and have become part of the region's dietary culture (van Rensburg et al., 2007). Traditional leafy vegetables (TLVs), African leafy vegetables (ALVs), and wild vegetables (WV) are all names for indigenous leafy vegetables (van Rensburg et al., 2007; Odhav et al., 2007; Schönfeldt & Pretorius 2011). They are known by various vernacular languages in South Africa (Table 2.1), with the Nguni tribe referring to them as imifino and the Sothos collectively referring to them as morogo (Van Rensburget al., 2007; Van Der Hoeven et al., 2013; Njeme et al., 2014; van Jaarsveld et al., 2014). Sub-Saharan Africa (SSA) has over a thousand plant species consumed as leafy vegetables (Odhav et al., 2007), with South Africa (SA) alone having over a hundred (Dweba & Mearns, 2011). Indigenous leafy vegetables are well known for their high nutrient content and other important health benefits for humans (Njeme et al., 2014).

ILVs' soft, tender leaves, fresh shoots, succulent stems, and flowers are harvested in the wild and cooked as a relish to accompany stiff porridge (phuthu or pap) (Abukutsa-Onyango, 2014; Chweya & Eyzaguire, 1999; Maundu, 1997; van Rensburg et al., 2004; Lewu & Mavengahama, 2010). Other ingredients, such as cooking oil, onions, tomatoes, peanuts, and even spices, are added to enhance the flavour and aroma (Darkwa & Darkwa, 2013; van Rensburg et al., 2014). ILV preparation methods vary depending on the individual's preferences. The boiling method requires water, with the first boiling water being replaced with fresh water to reduce the bitter taste of the vegetable, depending on the species. The steaming process utilizes very little water and takes very little time, especially when using very

young and fresh plants. However, van Averbeke et al. (2007) and Funke (2011) suggested that the optimal cooking methods still need to be tested because certain preparation methods, particularly the boiling method, have been reported to deplete the nutritional content of the ILV.

Table 2.1: Indigenous/African leafy vegetables consumed in South Africa

		South African names		
Scientific name	**Common English name**	**Zulu/Swati**	**Sotho/Pedi**	**Family**
Solanum nigrum/retroflexum	Nightshade	Umsobo	Momoli/lethotho	Solanaceae
Amaranthus hybridus	Pigweed	Imbuya	Thepe	Asteraceae
Corchorus olitorius	Jute mallow	Igushe/ligusha	Thelele	Malvaceae
Vigna unguiculata	Cowpea	Imbumba	Nawa	Fabaceae
Bidens pilosa	Blackjack	Umgadolo	Monyane	Asteraceae
Momordica balsamina	Balsam pear	Nkaka	Mohodu	Cucurbitaceae
Cleome gynandra	Spider plant	Ulude/bangala	Morotho/lerotho	Capparaceae
Cucumis melon	Muskmelon	Ikhabe	Phara	Cucucrbitaceae
Chenopodium album	Fat hen	Imbikicane	Umbicana	Chenopodaceae
Abelmoschus esculentus L.	Okra			Malvaceae

Adapted from: Schippers, (2000); van Rensburget al. (2004); Odhav et al. (2007); Uusiku et al. (2010); Mavengahama et al. (2013).

Indigenous leafy vegetables are high in nutrients and vitamins essential for effective human health maintenance (Table 2.2), especially for children and the elderly, who are prone to malnutrition and illness (Kwenin et al., 2011; Acho et al., 2014).

There is empirical evidence that African leafy vegetables have several advantages and values, including high micronutrient content, medicinal properties, and a variety of agronomic advantages that contribute to food and nutrition security as well as income generation (Schippers, 2002; Mnzava, 1997; Abukutsa-Onyango, 2007). They are high in minerals like zinc, iron, copper, manganese, magnesium, potassium, phosphorus, sodium, and beta-carotenes (Odhav et al., 2007; Ndlovu & Afolayan, 2008; Van Jaarsveld et al., 2014). African leafy vegetables comprise high levels of vitamin A, vitamin C, iron, calcium, and amino acids are a valuable source of nutrition in rural areas where they contribute substantially to protein, mineral and vitamin intake (Abukutsa-Onyango, 2003).

They are compatible with starchy staples and provide affordable nutrition to the population's resource-poor sector. Most ALVs, such as vegetable amaranths (*Amaranthus hybridus*), slender leaf (*Crotalaria brevidens* Benth), spider plant (*Cleome gynandra*), vegetable cowpeas (*Vigna unguicalata*), pumpkin (*Cucurbita maxima*) leaves, and jute mallow (*Corchorus olitorius*), have more than 100 per cent of the recommended daily allowances for vitamins and minerals at 40 per cent (Chweya, 1985; Abukutsu-Onyango, 2007). ILVs have been reported to be highly nutritious or comparable to common vegetables such as spinach, cabbage, lettuce, and kale (Flyman & Afolayan, 2006; Afolayan & Jimoh, 2009; Legwaila et al., 2011).

Table 2.2: Mineral composition in 100g of African leafy vegetables consumed in South Africa

African leafy vegetables (AFLs)											
Genotype/ Accession		**Ca**	**K**	**P**	**Mg**	**Mn**	**Zn**	**Cu**	**Fe**	**Na**	**Reference**
Amaranthus		410 159 260.0 2.57	4.91	103 557 43.0	248 2.92	3.333 11.66	10.81 2.87	1.49	8.9 7.61-2.87 7.8	-	FAO, 1968, 1966; Kumar-Maurya and Dr Pratitha Arya, 2018; Sarker et al. 2020
Solanum spp (African Nightshade, Black nightshade, Nightshade)		90-442 99-442	30.84 3.084	75 239 0.239	667 0.667	83	4.2 42	16	49.6 1.0-4.2	61 3.2 0.061	Edmonds and Chweywa, 1997; van Rensburg et al.2004; Potawale et al.2008; Afolayan and Jimoh, 2009; Jimoh et al. 2010; Padmashree et al. 2014)
Corchorus olitorous		266.0 34.7 12120 13874	1669 45493 42897	122.0 6690 7800	0.560 3338 2941	115 65 120	0.6	14 11	27.4 7.7 135 118	290 211 163	Schippers, 2000; van Resburg et al. 2004; Frison et al. 2006; Ndlovu and Afolayan, 2008; Acho et al. 2014; Sarnker et al.2014; Nemba et al. 2012
Cleome gynandra		288 232	374	111 138	76	-	1.0	-	6.0 2.1	15	van Rensburget al. 2004; van Jaarsveld et al.2014
Vigna unguiculata		176	1280	303	4.8	-	5.1	-	2.6	102	Iqbal et al.2006
Bidens pilosa		340 111 1358	-	67 39 508	658	-	22	-	2.3 17	290	Nesamuuni et al. 2001; Barolome et al. 2013; Odhav et al. 2014
Momordica balsamina		271	389	78.2	159	-	1.6	-	16.3	15.2	Steyn et al. 2001
Abelmoschus esculentus L.	Leaves Fruit	70 90	267 -	56 -	45.3 43	- -	- 0.233	- 0.102	- -	- 18.3	Grubben, 1977; Hamon and Charrier, 1997
Commercial/ exotic vegetables											
Swiss chard greens		51	379	46	-	81	0.36	0.366	1.8	-	Yuan et al. 2018
Lettuce		18	141	20	7	-	0.15	0.025	0.41	-	Yuan et al. 2018
Mustard green		118	-	-	-	-	-	-	1.69	-	Xiao et al. 2016; Yuan et al 2018
Kale		187	246	68	45	-	32	0.053	1.6	3.2	Xiao et al. 2016; Yuan et al. 2018

	254	348	55	33		0.39				
Cabbage	92 47	192	40 0.122	0.279	-	0.0314	-	0.6 0.7 0.056	57	FAO; Xiao et al. 2016; Ndlovu and Afolayan,2008
Spinach	99 1400 0.6007	558 3840	49 400 0.437	79 0.2 0.426	-	0.53 0.37 0.106	0.13	2.71 3.10 0.411	4.0	Ndlovu and Afolayan, 2008; Meilmann et al.2017; Yuan et al.2018
Recommended daily allowance	800	1400	800	120	-	10	-	10	300	National Research Counsil, 1989

Ca- calcium; K- potassium; P- phosphorus; N- nitrogen; Mg- magnesium; Mn- manganese; Zn- zinc; Cu-copper; Fe- iron; B- boron; Na- sodium; Al- aluminum; - not determined

2.3 Origin and distribution of Nightshades

According to DAFF (2013), black nightshade originated from South Africa. It was sparingly adapted on the Eyre Peninsula. It is widespread in the tropics and can be found throughout East Africa (van Rensburget al., 2014). Nightshade is a widespread weed that can be found on all inhabited continents. It has a long and well-documented history as a food source for many different societies. It is one of the most widely used and well-documented wild foods on the planet, rivaled only by weed species like Lamb's quarters, amaranth, and stinging nettle (Schmidt et al., 2004).

The diploid section *Solanum* species have their origins in South and North America, whereas the majority of polyploid species (tetraploids and hexaploids) have their origins in Europe or Africa. The most widely cultivated vegetable nightshades, *Solanum scabrum* (hexaploid) and *S. villosum* (tetraploid) have the greatest diversity in Africa. Nightshades are among the most popular and common leafy vegetables, especially in Africa's warm and humid regions, and are only second to amaranths in importance among indigenous leafy vegetables. They are reportedly the most important leafy vegetable crop in some areas of West Africa (Berinyuy et al., 2002).

2.4 *Solanum* species biology and morphology

Nightshades are annual herbs that can reach a height of 0.6 m. Black nightshades bloom from July to September in temperate regions, and the seeds ripen from August to October (Taab, 2009). Insects pollinate the flowers, which are hermaphrodites. The fruits are approximately 9 mm in diameter. The colour of the fruits varies, with 66 per cent of nightshades producing black fruits and 34 per cent producing pomes that never turn black (Zachary, 2010). The cotyledons of seedlings are covered with short hair along the margins, and the roots are fibrous and shallow. Nightshades have stems that are weak, unarmed, smooth, and usually hairless, while they branch extensively and freely (Jennifer & James, 1977).

The leaves are alternate, dark green, soft, and thin, often riddled with large holes, like those of amaranth, which they often resemble. The underside of the leaf surface may have a coppery or purplish sheen. The shape and size of the leaves vary greatly, ranging from ovate to lanceolate to diamond-shaped (Schmidt et al., 2004). Petioles range in length from 1.2 to 2.5 inches and

have a faint wing on each side (Poczai & Hyvonen, 2011). *Solanum retroflexum* has a distinctive rhomboid leaf shape and *S. scabrum* leaves are usually broadly ovoid with long petioles. As with other vegetative characters in this group, leaf morphology can be extremely variable within a species or even in a single plant. Many of the intraspecific names in *S. nigrum* are based on variations in leaf morphology, particularly concerning the margins whether they are lobed or not (Särkinen et al., 2018).

The fruits are roughly the size of a blueberry and are green at first before turning purplish-black when ripe (Rwig, 2016). Berry colour can range from green (*S. nitidibaccatum*, *S. opacum*, and some populations of *S. tarderemotum*) to greenish-yellow or yellow (some populations *of S. nigrum* and *S. villosum*, *S. palitans*), bright orange (*S. villosum*) or varying shades of purple (many species). Colour polymorphisms are common in this group of species; for example, *S. nigrum* and *S. tarderemotum* have individuals and populations with green or purple berries, while *S. villosum* has either yellow or orange berries. Schilling and Andersen (1990) suggested that berry colour in *S. villosum* may be affected by environmental factors; accessions labeled as having yellow or orange berries in the field in India all had orange berries in cultivation. Manoko (2007) demonstrated that the mature berry colour (green or black) did not distinguish groups within *S. nigrum*. Regardless of this variation, berry colour is an important identification aid in this group, but it is frequently not recorded on herbarium labels, particularly for older specimens (Särkinen et al., 2018).

Natural hybridization has also been observed to occur. Although black nightshade species are naturally self-pollinating, out and cross-pollinating can occur, and natural inter and genetic diversity has been reported at higher polyploidy levels, particularly among the smallest-flowered diploids (Kantety & Rota, 2002).

2.5 *Solanum* taxonomy

The Solanaceae family represents around 85 genera of flowering plants consisting of over 2800 species globally (D'Arcy, 1986; Albuquerque et al., 2006). Its cosmopolitan distribution is best characterized in tropical and temperate regions, with Australia and Latin America as major dispersal centres (Barroso et al., 1991). Hunziker (1979) indicated that the greatest species diversity is reported in tropical South America and is the origin of Solanaceae.

According to Lim (2013) and Rwig (2016), the Solanaceae family, to which *Solanum nigrum* belongs, is a cosmopolitan family containing numerous crucial vegetables and fruits along with potatoes, tomatoes, green and red peppers. According to Chweya (1997), the Solanaceae family has a global distribution, with the widest diversity hotspots in Central, South America, and Australia. It comprises angiosperms such as *Solanum* (black nightshade), *Capsicum* (pepper), *Physalis* (Chinese lantern), *Lycium* (kraal honey thorn), and *Nicotiana* (tobacco), amongst many others (Rwig, 2016). According to Terri and Lynn (2007), Solanum is the major species, with 1700-1750 species, including plants such as *S. nigrum, S. tuberosum, S. lycopersicon,* and *S. dulcamara*.

Table 2.3: Nightshade taxonomy

Rank	**Scientific name & common English name**
Kingdom	Plantae – plantes, vegetal
Subkingdom	Viridi plantae – green plants
Superdivision	Embryophyta
Division	Tracheophyta – vascular plants, tracheaphyte spermatophytes
Subdivision	Spermatophytina – seed plants
Class	Magnoiosida
Order	Solanales
Family	Solanaceae
Genus	Solanum L. nightshade
Species	Solanum section – direct families • *S. nigrum* • *S. scabrum* • *S. Americanum* Miller • *S. retroflexum* • *S.luteum* • *S. triflorum* • *S. villosum*

Source: ITTIS –Report: Solanum – Integrated Taxonomic Information

Complexity has long plagued nightshade taxonomy, resulting in significant similarity and uncertainty. Historical factors, morphological plasticity, genetic diversity, polyploidy, natural hybridization, and indistinct variation all contribute to such complexity. Most of this complexity has already been resolved for Europe, Asia, the Americas, and Australia, leaving Africa and South-East Asia as the two major geographic regions in which a complete and accurate taxonomic framework is sorely missing (Edmonds & Chweya, 1997). The taxonomic complexity of species associated with the section Solanum has long been accepted (Stebbins

& Paddock, 1949; Heiser, 1963; Symon, 1970; Venkateswarlu & Rao, 1972; Darcy, 1973; Darcy, 1974). Various classical, experimental, and numerical studies have demonstrated that the complexity is attributable to several causes (Edmonds, 1972; 1977; 1979). Among these causes, there is phenotypic plasticity (Baylis, 1958; Henderson, 1974), genetic variation, polyploidy, natural hybridization, and discordant variation (Jardine, 1971). *S. nigrum* (Solanaceae) is one of 1500 species within the genus Solanum. Taxonomic resolution is required not just for effective biodiversity conservation, but for evaluating the crucial prospects that these genetic resources might have. Due to various inter and intraspecific hybridization, phenotypic plasticity, and polyploidy, the taxonomy of black nightshades are unresolved (Lana & Krystic, 2002). The five-petaled flowers are whitish and about a half-inch across (Poczai & Hyvonen, 2011). They are protected by a five-parted persistent calyx that is slightly smaller in diameter than the fruit (Rudy, 1998). The skins are slightly tough, like tomato skin, and encase a smooth, juicy interior with several seeds.

2.6 Optimal ecological requirements

The black nightshade is found around the world in a variety of habitats ranging from tropical to temperate, and from sea level to altitudes exceeding 3500 m (Edmonds & Chweya, 1997; Albouchi et al., 2018). Seeds are used to propagate African nightshade. It appears to be growing well in a variety of climatic conditions but grows best in cool, moist environments at medium and high altitudes (Ondieki et al., 2011a). Total plant weight and leaf yield are both reduced in shady conditions. Though African nightshade can tolerate shade, it grows best when it is exposed to direct sunlight (Ojiewo et al., 2013a). Annual rainfall of 500–1200 mm is required for adequate growth of African nightshades (Masinde et al., 2006).

Nightshade species prefer warm, humid conditions and perform well in areas with an annual rainfall of 500–1200 mm, with optimum altitudes ranging from 0–2400 m above sea level depending on the species (MOALF/SHEP PLUS). The ideal temperature for growth, according to van Resburg et al. (2007), is between 20 °C and 30 °C, but most species can tolerate temperatures ranging from 15 °C to 35 °C. Maximum growth and biomass production are obtained when the plants are exposed to full sunlight during the winter, whereas 60 per cent shading can be beneficial during the summer (Edmonds & Chweya, 1997). Nightshades prefer rich, nitrogen- and phosphorus-rich soils (Van Averbeke & Juma, 2006b). It can grow in a

variety of soil conditions. It can also grow in soils that are acidic, neutral, or basic. In temperate areas, most species are frost-sensitive and die with the first frost (Nzioka, 1994).

2.7 Cultivation and utilization

In South Africa, nightshade species are usually harvested from the wild and not cultivated, but there are some regions where they are semi-domesticated (van Rensburg et al., 2007). When cultivated, nightshade species are generally propagated by seeds (Emongor et al., 2004; DAFF, 2013), however, a study by Ademuyiwa (2018) indicated that propagation can also be done using branch and stem cuttings. Seeds are sown directly in the field, either by broadcasting or by line sowing. However, due to seed dormancy, the germination of the untreated seed is poor (Adebooye et al., 2005). Solanum species are thought to be high in nutrient properties (Schippers, 2000). According to Abukutsa-Onyango (2003), the nutrient composition of this vegetable has been revealed and can provide 100 per cent of an adult's recommended dietary intake of calcium, iron, *b*-carotene, and ascorbic acid, as well as 40 per cent of protein, when 100 g of the fresh vegetable is consumed. *Solanum retroflexum* is native to the Horn of Africa and Southern Africa. It is the primary species grown by small-scale farmers in South Africa's Limpopo province (van Rensburg et al., 2007). The statistical production of nightshades, on the other hand, is unknown. *Solanum scabrum* is the largest species, reaching 1.5 m in height and with large fruits and leaves. The species comes from humid West Africa and was introduced to East and Southern Africa (Rwigi, 2016). Nightshade is mostly gathered from commercial farms by farmworkers in Mpumalanga, where many farms grow Solanaceae species such as chilies, green peppers and tomatoes.

2.8 Constraints to Nightshade production

A significant hindrance to crop improvement is insufficient knowledge about existing genetic diversity, notably for indigenous or wild species. Negligence and negative stereotypes have resulted in the plant being regarded as a weed consumed by the deprived in society (Mnzawa, 1997). According to Blackman and Eastop (2006), many other barriers hinder the production of nightshade, including pest species such as locusts (*Zonocerus variegatus*), beetles (*Podagrica species*), black aphids (*Aphis fabae*), nematodes (*Meloidogyne* species), and flea beetles (*Epilachna hirta*). In perhaps the most successful prevention, active control of these pests is attained using insecticides or insect repellents. Aphid infestation tends to occur beneath

the leaves, causing curving or curling, and the bottoms of young, infested plants can stimulate growth (Epenhuijsen, 1974).

Ralstonia solanacearum and *Cladosporium oxysporum* cause bacterial wilt and seedling blight in black nightshades, respectively. According to some other reports, the high cost of certified seeds for some indigenous vegetables has negatively impacted the large-scale production of such vegetables (Masayi & Netondo, 2012). Geographical location and racial differences continue to be obstacles to adopting these species (Kimiywe et al., 2007; Uusiku et al., 2010). Consumer preferences, according to Masayi and Netondo (2012), are a barrier to the production of these vegetables, especially among the younger generation, who are uninterested in eating them because they are considered unconventional and outmoded. Farmers also lack certified seeds and they, therefore, use seeds from old crops (Maundu et al., 2004). There has been very little utilization of germplasm derived from species belonging to the Solanum group in research, crop improvement, and breeding studies. No collection expeditions have been organized yet, largely because the plants used as vegetables are regarded as weeds or volunteers crops and have not been fully domesticated (Chweya, 1997).

2.9 Germplasm collection and utilization in crop improvement

Germplasm is a collection of genetic resources that can be used to preserve a population's species (Volis & Blecher, 2010). Germplasm collection and preservation of natural resources are critical for preventing the extinction of landraces and wild relatives of any cultivated crop (Rosenow and Dahlberg, 2000). In other regions of South Africa, nightshade is grown as a vegetable, but it is also considered a weed or a volunteer crop. Some local collections are being made in South Africa, but there are not enough records of Nightshade accessions collected from provinces like Mpumalanga and Kwa-Zulu Natal. Germplasm collectors and farmers could perhaps work together to conserve all nightshade genetic resources in South Africa. The Asian Vegetable Research and Development Center (AVRDC), DAFF, as well as the Agricultural Research Council (ARC), have made significant contributions to the germplasm collection, conservation, and distribution of indigenous species. To enhance and sustain the diversity of nightshade species in South Africa, breeding programs should be used to conserve germplasm.

2.10 Genetic resources and breeding in African nightshades

Seeds are used to propagate African nightshades. They are mostly autogamous, though there is some outcrossing. The lack of a self-incompatibility system aids in stabilizing any crossings, resulting in new populations that are sufficiently uniform after only 2-3 generations (Ronoh et al, 2019). Cultivars can thus be created in a short time. Farmers prefer cultivars that are high yielding, have a long season of picking, are uniform, and are resistant to biotic and abiotic stresses (Afari-Sefa et al., 2012). Quality and nutritional value are significant to consumers. Improved cultivars with high leaf and seed yield are sought after by seed companies (Dinssa et al., 2015). Unfortunately, there are still gaps in this section's taxonomy knowledge (Ronoh et al., 2017). Furthermore, there is little information available about breeding potential.

2.11 Economic importance

Indigenous leafy vegetables play a significant role in the diets of many households in Sub-Saharan Africa, both urban and rural. Indigenous vegetables are preferred because they are readily available in rural areas and have a pleasant flavour (Nzioka, 1994). Some indigenous vegetables are harvested from the wild or as weeds in farms. Black nightshades are grown as a food crop for both their leaves and fruits. Fruits are used in preserves, jams, and pies, according to Werner and Petra (2010). Fruits have a pleasant musky flavour that improves slightly after a frost (Hammington, 1974).

Solanine is a toxin found in unripe fruit (Timothy & Kokwaro, 1991). Fruit contains 2.5 per cent protein, 0.6 per cent fat, 5.6 per cent carbohydrates, 1.2 per cent ash, minerals (iron and phosphorus), and vitamins B. *Solanum nigrum* is a nutrient-rich plant high in beta-carotene (Dhellot et al., 2006). Seeds are high in minerals such as iron and calcium, as well as proteins, particularly methionine, an essential amino acid (Onyango, 1993). It also contains a lot of vitamin A and C. Young leaves and new shoots are added to soups raw or cooked as a potherb (Reid, 1997). The amount of ascorbic acid in food is determined by the method of cooking. The ascorbic acid content decreases as the cooking time and volume of water used increase.

Both leaves and berries are used to make dyes that are used to colour sisal baskets (Nzioka, 1994) Purple berries from both *Scabrum scabrum* and *Solanum americanum* are used to make ink. Saponins and glycoalkaloids have larvicidal properties and can thus be used as pesticides (Silva et al., 2005; Chowdhury et al., 2008). Molluscs have been controlled using extracts from

black nightshades (Shoeb et al., 1990; Chowdhury et al., 2008). The species has been found effective at removing and detoxifying polychlorinated biphenyls from soil (Duke & Ayensu, 1985), particularly when infected with the bacterial parasite *Agrobacterium tumefaciens* (Anderson, 2006).

Solanum species are sold as vegetables in both rural and urban markets, providing a source of income for rural farmers, the majority of whom are women (Nzioka, 1994). They can be purchased in Cameroon, Ghana, Kenya, Madagascar, and Nigeria. In South Africa, Zulu women are known to take baskets full of berries to nearby villages and towns to sell, generating income for family use (Sergel & Irina 2009, Jagatheeswari et al., 2013).

In most parts of the world, species related to black nightshades are notorious agricultural weeds. They primarily affect barley, wheat, melon, onion, pea, bean, potato, sorghum, tea, tomato, sugarcane, and a variety of vegetables (Nzioka, 1994). They compete for moisture, light, and nutrients with these crops, lowering yields and, in the long run, income (Rwigi, 2016).

2.12 Importance of morphological characterization in plants

Morphological characterization of plant species is primarily used to conduct a thorough investigation of genetic diversity in germplasm collection, and it provides valuable information for breeding programs and conservation strategies (Idris & Saad, 2001; Borokini et al., 2010; Benor et al., 2012). Characterization, according to Perry and Bettencourt (1995), is an assessment of traits that are highly heritable, visually accessible, and expressed similarly in all environments. Morphological characterization is a necessary precondition for selecting valuable attributes in crop improvement programs. Knowing a species' morphological variability is the first step toward genetic improvement (Adebola & Morakinyo, 2006), so the information obtained through characterization should be documented and made available to both germplasm collectors and breeders (Idris & Saad, 2001; Ghosh et al., 2013).

The description of plant material, accessions, genotypes, or species in a germplasm collection is referred to as characterization. The authenticity, homonyms, and duplicates in germplasm collections are validated through characterization and evaluation. Characterization aids in the identification of potential lines that can be evaluated further to produce cultivars (Idris & Saad, 2001; Galovi et al., 2006). Morphological characterization can provide information on genetic

diversity, species relationships, and heritability estimates for specific traits (Gerrano et al., 2015). Plant height, leaf shape, flower colour, and other characteristics may be included in the characterization data (Bar-Hen et al., 1995). Characterization also aids in the removal of redundant or unnecessary material from gene banks (Engels & Visser, 2003). Morphological characterization may include a list of morphological descriptors used to label the plant's phenotype (Osawaru et al., 2012). The International Plant Genetic Resources (IPGR) and World Vegetable Center (WVC) descriptor lists provide an efficient method of data scoring in morphological characterization. Herbarium specimens are excellent sources of variation documentation and are used as a reference after the material has been removed from the field. Hard-pressed mounted plants, seeds, fluid-preserved flowers and fruits, microscope slides as well as frozen DNA extractions are examples of herbarium specimens (Shenoy et al., 2007). Pictures are taken during the characterization process to taxonomically classify the species and record features that show variation (Osawaru et al., 2012).

Agronomic characterization is a subset of morphological characterization that focuses on performance characteristics and aids in germplasm utilization. The traits considered during agronomic assessment differ by species, most notably between perennial and annual plants (Idris & Saad, 2001). Agronomic characteristics in most crops and indigenous leafy vegetables, however, may include days to emergence, days to 50% flowering, yield, and yield components, along with uniformity of characteristics (K'opondo, 2011).

2.12.1 Morphological characterization of African nightshade accessions

In research carried out by Mwai et al. (2007) to identify, characterize and delimit the indigenous nightshade species, fifty African nightshade accessions from Eastern, Western, and Southern Africa were obtained and raised in pots filled with vermiculite and peat soil under standard greenhouse cultivation at Radboud University Nijmegen's Botanical and Experimental Garden. The strategy employed was to use morphological features of living materials to differentiate accessions from distinct species. The taxonomic complexity of *Solanum nigrum* has long been recognized (Mwai et al., 2007). Plants with purple corollas, brown anthers, and purple leaves and stems on the one hand, and those with white corollas, yellow anthers, and green leaves and stems on the other, displayed distinct morphological diversity. Berinyuy et al. (2002) and Fontem and Schippers (2004) discovered 12 recognizable variants of *S. scabrum* in Cameroon, where it is widely grown as a leafy vegetable, while Olet (2004) discovered two *S. scabrum*

subspecies in Uganda. Other authors' observations also suggested the existence of two subspecies known as *S. villosum* in Africa (Schippers, 2002; Manoko & van der Weerden, 2004b; Olet et al., 2005). The diversity of the other species as offered by Mwai (2007) was quite low, unlike the previous investigations which revealed high genetic diversity.

2.12.1.1 Use of Descriptor list to score for morphological characteristics

An original descriptor list was created, which included all the morphological characteristics mentioned by Edmonds and Chweya (1997). The resulting descriptor list was amended several times to account for the observed variation in the plants growing in the greenhouse at the time of the study, resulting in a final edition with 48 morphological characters. To cover the entire range of variation reported in the growing plants, character states were ascertained for each descriptor.

2.12.1.2 Scoring for morphological characters

Mwai et al. (2007) scored 47 morphological characters on flowering and fruiting plants using the final version of the descriptor list. Hair-type features were rated using a magnifier, and the presence or absence of stone cells in mature berries was checked in all 50 accessions. Characters were scored given most plants (qualitative) or the mean of ten random observations (quantitative). Statistical analysis was performed on a computer as a matrix of character states, and cluster analysis was performed using NT-SYS software, SIMQUAL (similarities for qualitative data) with UPGMA and simple matching, and SAHN (sequential agglomerative hierarchical clustering).

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Chapter 3:

Qualitative morphological variation of *Solanum* section *Solanum* (nightshade) accessions

3.1 Abstract

The family *Solanaceae* is composed of a broad list of species, which includes both commercial and wild accessions with vast medicinal importance. The diversity in the morphological traits of 15 accessions of nightshade germplasm sourced from ARC, DAFF gene banks, and some germplasms collected from two different provinces in South Africa was investigated. Morphological characterization was conducted using standard descriptors published by Berinyuy et al. (2002). However, new descriptors were created also for this study. The objective of the study was to evaluate nightshade accessions using phenotypic traits in the field experiments to analyze the variation in morphological characteristics relevant to agronomic performance. Fifteen accessions of nightshade were evaluated in the field using a randomized complete block design with 3 replications. The frequencies for each qualitative character were tabulated. The Shannon Weaver diversity index was calculated and there was low diversity among the accessions which varied from 0.077 to 0.227, indicating the existence of little diversity among the species tested. The information obtained from this study could be used for genetic improvement of nightshade species in South Africa for the development of accessions, however, the study suggests using more accessions or entries per species for morphological characterization to attain more diversity for the species.

Keywords: Characterization, Nightshade, Morphological, Qualitative, Shannon Weaver, Trait

3.2 Introduction

Solanum retroflexum Dun. (Nightshade) is a South African species that is part of the *S. nigrum* complex (Grabandt, 1985; Schippers, 2002). This complex contains a variety of leafy vegetable species harvested from the wild or cultivated by rural Africans (Edmonds & Chweya, 1997). *S. retroflexum* cultivation in South Africa is a recent development that is currently limited to the Vhembe District of the Limpopo Province according to Van Averbeke and Juma (2006 cited in Averbeke, 2007).

Black nightshade is a common herb or short-lived perennial shrub that can be found in a variety of wooded and disturbed habitats. *Solanum nigrum* is sometimes confused with the much more toxic and deadly nightshade, *Atropa belladonna*, which belongs to a different Solanaceae genus entirely. A comparison of the fruits reveals that the black nightshade berries grow in clusters, whereas the deadly nightshade berries grow singly. Solanum is made up of several species, including the *Solanum nigrum* complex, also known as African nightshades or Black nightshade. African nightshade grows to a height of 75 cm on average. The leaves are ovate or lanceolate in shape, with a serrated or entire margin, and are green, with a glabrous or pubescent surface. Venkateswarlu et al. (1998), reported leaves that were 4-7.5 cm long and 2-5 cm wide, ovate to heart-shaped with wavy or large-toothed edges, both surfaces were hairy or hairless and petioles were 1-3cm long. African nightshade flowers have prominent yellow anthers that are surrounded by white petals. The berries can be dull black, purple-black, red, or orange (Matasyoh et al., 2015). Another strain found in India has berries that turn red when ripe (Venkateswarlu et al., 1998).

However, while Nightshades have long been thought of as unpalatable poisonous plants and tiresome agronomic wild plants in other areas (Schilling & Andersen, 1989; Onyango, 1993; Edmonds & Chweya, 1997; Schippers, 2000), their story is quite different in Western, Eastern, and Southern Africa, as well as India, Indonesia, and China, where they have long been used as leafy herbs and vegetables, as a source of fruits and dye (Onyango, 1993; Edmonds & Chweya, 1997; Schippers, 2000).

Morphological traits have long been used by plant breeders to study variability, genetic variation patterns, and correlation in plant populations and accessions. This technique entails a lengthy survey of plant growth, which is expensive, labour intensive, and susceptible to

environmental conditions (Vega, 1993). Morphological data are affected by environmental interaction, so descriptions must be made with sufficient replication, and valid comparisons can only be made between descriptions taken at the same location and time of year (Smith & Smith, 1988). These phenotypic expressions are also strongly influenced by management practices and human interpretation. As a result, it is significant to characterize the available nightshade accessions for their qualitative morphological traits in the field. The goal of this study was to evaluate nightshade accessions in field experiments using phenotypic traits to analyse variation in morphological traits relevant to agronomic performance.

3.3 Materials and Methods

3.3.1 Plant materials, experimental design, and treatments

The study used 15 Nightshade accessions. The accessions were sourced from various origins within South Africa. Detailed information about each accession is given in Table 3.1. The study was laid out in a randomized complete block design with three (3) replicates, each accession represented by a 5 row plot of 2.5 m length with inter-row and intra-row spacings of 50 cm, respectively. The trial was carried out for 2 growing seasons.

Table 3.1: Fifteen nightshade accessions that were evaluated during the study

Entry No.	Given codes	Accessions	Source of origin in South Africa
1	ManTown	ManTown	ARC
2	NigSN18	NigSN18	ARC
3	*SRetrflx*	*Solanum retroflexum*	ARC
4	Nshad9	Nshad9	ARC
5	Nshad5	Nshad5	ARC
6	Nshad40	Nshad40	ARC
7	Scabrum	*Solanum scabrium* (Unizulu)	University of Zululand
8	SNig2495	*S. nigrum* 2495	DAFF
9	Ncapmus	Nshad NWU	NWU Mafikeng-campus
10	Nfarm	Nshad NWU	NWU Farm
11	Timbali	Nshad Timbali	Komatiport- Mpumalanga
12	N0096	Nshad 0096	Naas- Mpumalanga
13	N5547	N5547	ARC
14	SABGA	SABGA	ARC
15	Sharon	Sharon	ARC

NWU- North-West University, ARC- Agricultural Research Counsil, DAFF- Department of Agriculture, Forestry and Fisheries

3.3.2 Description of the research site, trial establishment, and maintenance

Experimental accessions were planted in seedling trays in the Net-house facility of the controlled environment research unit at the North-West University (NWU) Mafikeng. Five weeks after planting the plants were transplanted directly to the field to the NWU research garden (25. 810S and 25. 630 E, with an altitude of 1276 m). The trial was conducted during the 2020 summer seasons (January to April 2020 and December 2020 to February 2021) for both seasons. The precipitation and average temperature for both cropping seasons in the NWU Mafikeng campus are presented in Table 3.2. The total supplementary irrigation in the first season was 720 mm, while it was 660 mm in the second season.

No fertilizer was applied during the study period. Malasol® 2.5 E.C was sprayed regularly to control black aphids, Efekto Redspidercide was also used to control red spider mites, using a knapsack sprayer based on manufacturer's recommendation for use on tomatoes. The signs and symptoms of aphid infestation were shown by the colonies of aphids clustered on young leaves, resulting in leaf curling, (Figure 3.1). Manual weeding was done four times for both seasons regularly by hand hoeing.

Figure 3.1: Black aphids (*Aphis solanella*) on nightshade leaves noted under study.

Figure 3.2: South African map showing the three North-West University campuses (Mafikeng campus, where the trails were conducted).

Table 3.2: Weather conditions at NWU Mafikeng garden

Month	Year	Minimum Temperature (°C)	Maximum Temperature (°C)	Rainfall (mm)	Relative humidity (%) average
Season 1					
January	2020	20	30	179.3	51
February	2020	20	30	95.7	49
March	2020	18	29	97.2	45
April	2020	15	26	59.2	45
November	2020	20	30	229.5	46
Season 2					
December	2020	20	29	168.2	52
January	2021	17.4	30.8	335.8	58
February	2021	16.8	29.5	259.3	63

Source: South African Weather Service (SAWS) records

3.4 Data collection

3.4.1 Phenotypic data

Six plants from each accession were randomly selected in the field and recorded on different days before the recording of qualitative morphological traits for the study. The seeds

(germplasm) used in the experiment were a collection of accessions obtained from gene banks and other regions of Mpumalanga and the North West province (Table 3.1). Seventeen qualitative traits were characterized based on Berinyuy et al. (2002) and Schippers (2000)'s list of modified African nightshade descriptors: cotyledonous leaf colour, leaf colour; surface; shape; type, the colour of stem and branches, the colour of nodes, mature fruit colour and surface, mature fruit shape, fruit type, fruit attachment, flowering type, flower colour, branching type, plant branching habit, seed colour and seed size (Table 3.3). All observations for each trait were made on the same day for all accessions after 50% flowering to avoid differences in the developmental stages of growth.

Table 3.3: Characters, codes, and description used for characterization of qualitative traits in nightshade accessions under study

Qualitative traits	**Code**	**Description**
Cotyledon colour	CC	Green (1)
Leaf colour	LC	Green (4), light green (3), dark green (7), Greyish green (1)
Leaf surface	LS	Smooth (3), hairy (12)
Leaf shape	LSP	Ovate (7), lanceolate (1), ovate-lanceolate (3), rhomboid or diamond (3), Mix of ovate and ovate-lanceolate (1)
Leaf type	LTP	Small (6), medium (7), large (2)
Colour of stem and branches	SBC	Green (3), purplish-green (4), a mixture of green & purplish (1), greenish-purple (3), purplish-black (4)
Colour of nodes	CoN	Green (2), purplish-green (9), purplish-black (4)
Mature fruit colour and surface	FRCS	Shiny purple (2), shiny black (9), dull purple (1), dull black (3)
Mature fruit shape	FRS	Slightly flattened (6), round (9)
Fruit type	FRP	Small (3), medium (3), large (9)
Fruit attachment	FRA	Fruit remaining on plant when ripe (a), falling from calyxes when ripe (b), falling with pedicel still attached (c)
Flowering type	FLT	Early (5), medium (6), late (4)
Flower colour	FLC	White (8), purplish (6), both with yellow anthers.
Seed colour	SC	Brown (12), greenish (3)
Seed type	SY	Small (3), medium (3), large (9)
Plant branching habit	PBR	Primary (0), secondary (0), both primary and secondary (15)
Branching type	BT	Erect (5), semi-erect (8), spreading (2)

Berinyuy et al. (2002) and Schippers (2000); Numbers in brackets on the right-hand side are the corresponding descriptor codes listed in the FAO publication with modifications during the development of the list

3.5 Data analysis

The Shannon-Weaver diversity index (H'), as described by Perry and Mclntosh (1991), was used to analyze and interpret phenotypic diversity for each trait based on phenotypic frequencies (calculate phenotypic frequency distribution of all characters for all accessions). A hand touch and feel method was also used to determine the hairiness of the leaves and stems.

$$H' = -\Sigma\, pi \ln (pi)$$

Where: H' – is the Shannon –Weaver Diversity Index

Pi – is the relative abundance of each group of organisms

3.6 Results and Discussion

Morphological characterization

3.6.1 Qualitative Traits of nightshade accessions

Morphological characteristics were visually assessed using criteria described by Jennifer and James (1997) along with Berinyuy et al. (2002) as well as Nandhini et al. (2014). The accessions displayed qualitative morphological variation in traits studied. The most distinctive variations were observed for leaf, stem, and fruit-related traits for nightshade evaluated.

3.6.1.1 Plant/Growth habit, branching and stem related traits

Eight (53 per cent), five (34 per cent), and two (13 per cent), accessions respectively, had semi-erect, erect, and spreading growth habits. ManTown, Nshad5, Nshad40, Ncampus, Nfarm, N0096, Timbali and SNig2495 were semi-erect (intermediate) plant habit accessions (Table 3.4). There are three main plant habits in African nightshade plants, according to descriptors by Berinyuy et al. (2002). These are erect, medium, and prostrate; however, three plant habits were observed in this study, as reported by Mwai et al. (2007). This could be attributed to the fact that the studies of Ronoh et al. (2019) and Berinyuy et al. (2002) were based on an assessment of a large number of nightshade plant accessions collected from many parts of the world, as appeared differently concerning the study that was based on an assessment of only a small number of nightshade accessions from South Africa. In their study, Ronoh et al. (2019) found only two growth habits (prostrate and upright).

All the accessions studied had primary and secondary branching only. The stem and branches of four (27 per cent) of the plants had a purplish-black colour, three (20 per cent) had a green colour, three (20 per cent) had a greenish-purple colour, and one (7 per cent) had a mixture of green and purplish stemmed plants (Figure 3.4). Plant genotypes can be classified into various categories including growth habit, stem colour and plant alignment. The proportion of accessions in each category may vary due to either inherent characteristics or environmental or human selection of genotypes (Teman et al., 2021). Growth habit is very important in the cropping system of nightshade plants and an influential character in the harvesting of the plant (Mnzava, 1997). Six of the 15 accessions had hard and rough young shoots at harvest making harvesting of nightshade ineffective. Accessions with a green stem and branches were observed

to be characterized by nightshade plants with a semi-erect plant habit, except for accession Sharon which was recorded to be spreading.

3.6.1.2 Leaf (attributes) - Related traits of nightshades

The findings revealed that some nightshade accessions (Scabrum and Nshad40) had broad/large leaves and fewer branches than other evaluated solanum species. *S. scabrum* in the present study was noted to have large leaves and only primary and secondary branches, resulting in a high yield. Other solanum species, on the other hand, had smaller leaves but a bushy growth habit. Some *S. villosum* accessions and *S. tarderemotum* formed compact plants with only primary and secondary branches in the third growth habit. Based on Mwai's (2007) three main growth habits, the study observed and grouped accessions based on leaf size: Scabrum and Nshad40 had large leaves, but Scabrum only had primary and secondary branches, whereas Nshad40 had tertiary branching. As a result, only the primary and secondary branches were recorded in this study. Timbali, N0096, Ncampus, Nfarm, SNig2495, and Sharon had smaller leaves but more primary branches at various stages of growth, resulting in either semi-erect or spreading plants. This category is consistent with the findings of Mwai (2007) and Berinyuy et al. (2002). The current study classified leaf size into three categories: small, medium, and large, resulting in a group of plants with medium (intermediate) leaves and few to more branches, some with only primary and secondary branches, and some with tertiary branches (accessions includes SABGA, Nshad5, ManTown, and Nshad9). In the case of N5547, the accession had a mixture of primary and secondary branches, as well as some plants with tertiary branches. The current study's findings are consistent with previous reports on nightshade (Abukutsa-Onyango et al., 2005; Manoka, 2007; Mwai, 2007; Mwai et al., 2007; Monako et al., 2008, 2012; Stoilova et al., 2015; Ronoh et al. (2019). DAFF (2013), Ojiewo et al. (2013), Rwigi (2016) and Berinyuy et al. (2002) reported additional leaf shapes such as oval, ovate-rhomboid, mature berry/fruit colour such as red, yellow/orange, pinkish-purple berries with dull or shiny/glossy fruit surface.

3.6.1.3 Flower, fruit, and seed-related traits of nightshades

The observed flower colours of the accessions evaluated in this study are presented in Table 3.4. Seven of the accessions produced white flowers, six produced purplish flowers, and two produced plants with a mixture of both white and purplish flowers (Table 3.4), and all

accessions had yellow anthers. Scabrum and N5547 had flowers that were a mixture of white and purplish/light purple (Table 3.4). Mwai et al. (2007) and Abukatsa-Onyango et al. (2005) made similar observations on 50 accessions studied, which were divided into nine *Solanum* section *solanum* groups. Eight of the accessions studied had white flowers (white corolla and yellow anthers), five had purplish (striped) flowers and two had a mixture of light purple (striped) flowers and yellow anthers. The reports by Mwai et al. (2007) and Abukutsa-Onyango et al. (2005) support the findings of the current study; however, Berinyuy et al (2002) reported additional flower colours for *S. scabrum*, such as violet, purple-striped flowers. DAFF (2013) and van Rensburget al. (2007) both reported white flowers on *S. nigrum* and *S. retroflexum* Dunal. This could be attributed to differences in environmental factors such as temperature, nutrients and stress in the locations where the evaluations were conducted. The findings of this study revealed the two main flower colours identified by Mwai et al., 2007. Six of the accessions had medium flowering, five had early flowering, and four had late flowering (Table 3.5). Early flowering and extreme fruiting limit leaf expansion, resulting in lower leaf yields.

There were two mature fruit shapes among the accessions: nine accessions were slightly flattened mature fruit shapes and six were round mature fruit shapes. Mature fruit colour showed the most variation among the nightshade accessions studied, with 60%, 20%, 13%, and 7%, being shiny black, dull black, shiny purple and dull purple respectively, based on mature fruit score (supplementary Figure 3.5). Large, medium and small fruit types differed slightly in size (Figure 3.5). Six of the fruit attachment accessions assessed fell from the calyxes, while nine remained on the plant when fully ripe. Ojiewo et al. (2013) and Mwai et al. (2007) characterized nightshade accessions with various fruit attachment characteristics, including fruit remaining on the plant when fully ripe, fruit falling from calyxes and fruit falling with pedicels still attached when ripe. The current study, however, only recorded two of the three main characteristics: accessions with fruit that remained on the plant when fully ripe and fruit that fell from calyxes when ripe (Table 3.5). Previous reports on fruit colour have reported dark purplish-black fruits that remain on the plant when ripe, light green fruits that fall with pedicels still attached, orange fruits that fall from calyxes when ripe, and purple fruits that fall from calyxes when ripe. However, the current study did not include accessions with fruits that fell with pedicels still attached, nor did it include mature yellowish-green, yellow/orange, and red fruits. According to Matasyoh et al. (2015), mature berries are either dull black, purple-black, red, or orange. Ronoh et al. (2019), found purple berries in *S. scabrum* and yellow/orange berries in *S.villosum*, while Rwigi (2016) found purple and red mature fruits in *S. scabrum*.

The 15 accessions showed two groups of seed colour and three seed sizes: had brown seeds, and three had greenish seeds in colour, while seed size varied from large, medium, and small seeds (Figure 3.6.). Abukutsa-Onyango (2015) reported on broad-leaved African nightshade indicated a great diversity in seeds colour. The seeds are brown and numerous (Akubugwo et al., 2007). According to DAFF (2013), the seeds were reported to be light brownish yellow or purple for *Solanum retroflexum* Dunal.

3.6.2 Genetic Diversity Analysis

Table 3.6 displays the Shannon-Weaver diversity index for the qualitative morphological traits that were evaluated. The estimates for morphological diversity indices (H') for traits ranged from 0.077 for cotyledon colour to 0.277 for leaf colour and stem and branch colour, with an average diversity index of 0.067. The standardized Shannon and Weaver (1949) diversity index was divided into three categories: low (0 – 0.33), intermediate (0.34 – 0.66), and high (0.67 – 1). All traits had low levels of polymorphism (H' < 0.277). A low (H') indicates an extremely unbalanced frequency of classes for an individual trait and a lack of diversity.

Table 3.4: Morphological descriptors for 15 nightshade accessions

Access	LC	LS	LSP	LTP	STC	FRCS	FRS	FRP	FLT	FLC	PBR	BT
ManTown	Darkgreen	Hairy	Ovate	Medium	Purplish green	Dull black	Slight-flattened	Large	Medium	Purplish	Primary & secondary	Intermediate/semi-erect
NigSN18	Green	Smooth	Ovate	Medium	Purplish	Dull black	Slightly flattened	Large	Medium	Purplish	Primary & secondary	Spreading
SRetrfx	Green	Hairy	Rhomboid /Diamond	Medium	Darkgreen	Shiny purple	Slightly flattened	Large	Medium	White	Primary	Upright/Erect
Nshad9	Darkgreen	Hairy	Rhomboid /Diamond	Medium	Purple black	Shiny black	Slightly flattened	Large	Medium	Purplish	Primary & secondary	Upright/Erect
Nshad5	Green	Smooth	Rhomboid /Diamond	Medium	Green	Shiny black	Slightly flattened	Large	Medium	White	Primary & secondary	Intermediate/semi-erect
Nshad40	Green	Hairy	Ovate	Large	Darkgreen	Shiny black	Round	Large	Late	Purplish	Primary & secondary	Intermediate/semi-erect
Ncampus	Darkgreen	Hairy	Ovate-lanceolate	Small	Purlish-black	Dull black	Round	Medium	Early	White	Primary & secondary	Intermediate/semi-erect
Nfarm	Darkgreen	Hairy	Ovate-lanceolate	Small	Purplish black	Dull black	Round	Medium	Early	White	Primary & secondary	Intermediate/semi-erect
N0096	Lightgreen	Smooth	Ovate	Small	Green	Shiny purple	Round	Small	Early	White	Primary & secondary	Intermediate/semi-erect
N5547	Green	Mixture of smooth and hairy	Ovate-lanceolate & lanceolate	Medium	Green & purplish	Shiny-black	Slightly flattened	Large	Late	Mixture of white & purplish	Primary & secondary	Upright/Erect
Scabrum	Green	Hairy	Ovate	Large	Greenish-purple	Shiny-black	Slightly flattened	Large	Late	Mixture of white & purplish	Primary	Upright/Erect
SNig2495	Darkgreen	Hairy	Ovate-lanceolate	Small	Purplish black	Dull purple	Round	Medium	Early	White	Primary & secondary	Intermediate/semi-erect

Sharon	Green	Hairy	Lanceolate	Small	Green	Dull purple	Round	Small	Late	White	Primary & secondary	Spreading
SABGA	Darkgreen	Smooth	Ovate	Medium	Green	Shiny-black	Slightly flattened	Large	Medium	Purplish	Primary & secondary	Upright/Erect
Timbali	Lightgreen	Smooth	Ovate	Small	Green	Shiny purple	Round	Small	Early	White	Primary & secondary	Intermediate/semi-erect

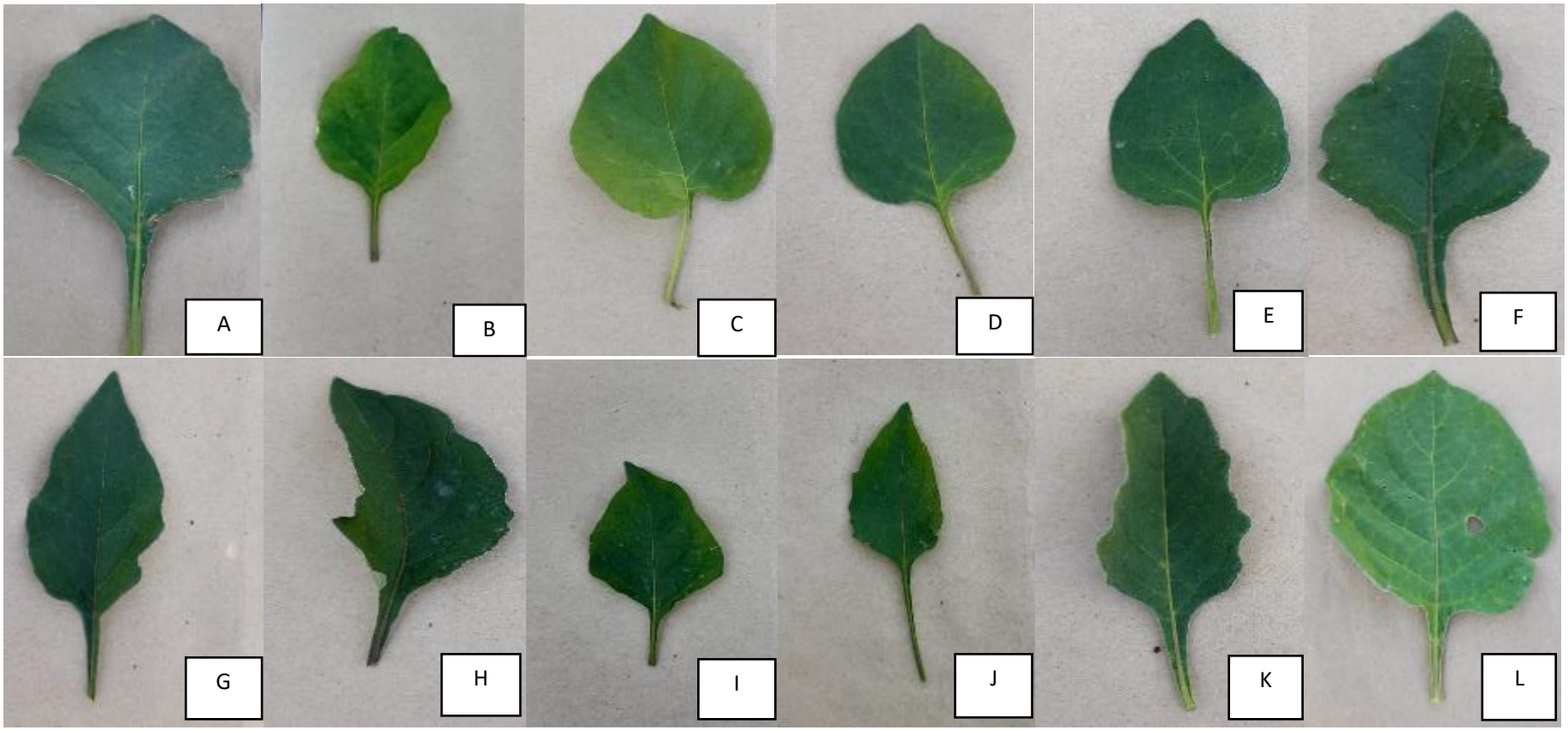
Table 3.5: Frequency distribution (%) of nightshade accessions based on qualitative morphological traits

Qualitative traits	Descriptor	Number of samples	Total (%)
Cotyledon colour	Green	15	100
	Total	15	100
Leaf colour	Green	4	26.7
	Light green	3	20.0
	Dark green	7	46.7
	Greyish green	1	6.7
	Total	15	100
Leaf surface	Smooth	3	20.0
	Hairy	12	80.0
	Total	15	100
Leaf shape	Ovate	7	46.6
	Lanceolate	1	6.7
	Ovate-lanceolate	3	20.0
	Rhomboid/ Diamond	3	20.0
	Mix of ovate and ovate-lanceolate	1	6.7
	Total	15	100
Leaf type	Small	6	40
	Medium	7	46.7
	Large	2	13.3
	Total	15	100
Colour of stem and branches	Green	3	20.0
	Greenish purple	3	20.0
	Purplish green	4	26.7
	Purplish black	4	26.7
	Mixture green and purplish.	1	6.7
	Total	15	100
Colour of nodes	Green	2	13.3
	Purplish green	9	60.0
	Purplish black	4	26.7
	Total	15	100
Fruit colour and surface	Shiny purple	2	13.3
	Shiny black	9	60.0
	Dull purple	1	6.7
	Dull black	3	20.0
	Total	15	100
Mature fruit shape	Slightly flattened	9	60.0
	Round	6	40.0
	Total	15	100
Fruit type	Small	3	30.0
	Medium	3	30.0
	Large	9	60.0
	Total	15	100
Fruit attachment	Fruit remains on plant when fully ripe	9	60.0

	Falling from calyxes when ripe	6	40.0
	Falling with pedicels still attached		
	Total	0	0.0
		15	100
Flowering type	Early	5	33.3
	Medium	6	40.0
	Late	4	26.7
	Total	15	100
Flower colour	White	8	53.3
	Purplish	5	33.3
	Mixture of both white and purplish	2	13.3
	Total	15	100
Branching habit	Primary	0	0.0
	Secondary	0	0.0
	Primary and secondary branches only	15	100
	Total	15	100
Plant type	Erect	5	33.3
	Semi-erect	8	53.3
	Spreading	2	13.3
	Total	15	100
Seed colour	Brown	12	80.0
	Greenish	3	20.0
	Total	15	100
Seed size	Small	3	20.0
	Medium	3	20.0
	Large	9	60.0
	Total	15	100

Table 3.6: Shannon-Weaver diversity index for 17 qualitative morphological descriptors for 15 nightshade accessions evaluated

Descriptor	**Diversity-index (H')**
Cotyledon colour	0.077
Leaf colour	0.199
Leaf surface	0.127
Leaf shape	0.227
Leaf type	0.166
Colour of stem and branches	0.227
Colour of nodes	0.166
Fruit colour and surface	0.199
Mature fruit shape	0.127
Fruit type	0.166
Fruit attachment	0.166
Flowering type	0.127
Flower colour	0.166
Branching habit	0.166
Plant type	0.166
Seed colour	0.127
Seed size	0.166
Average Diversity index	0.067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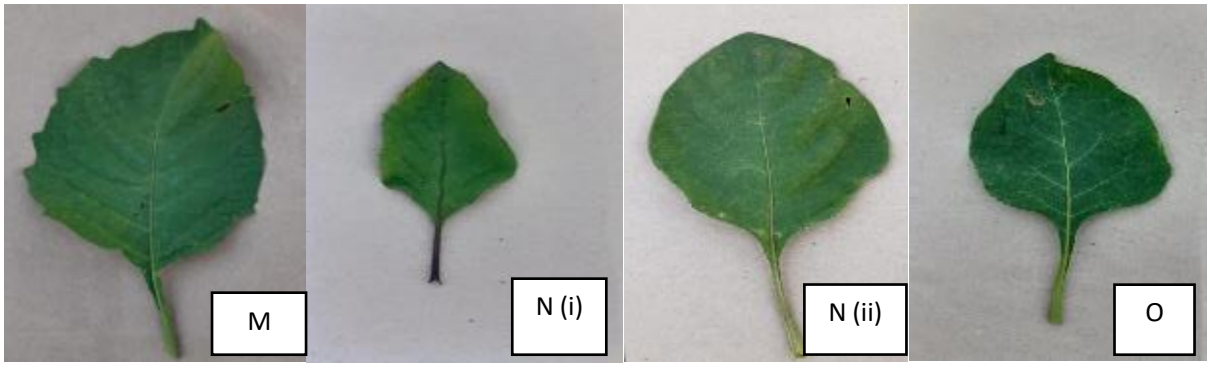

Figure 3.3: Different types of leaves of the nightshade species. A-Mantown, B- Nigsn18, C- Sretrflx, D- Nshad9, E- Nshad5, F- Ncampus, G- Nfarm, H- SNig2495, I- N0096, J- Timbali, K-Sharon, L- Scabrum, M- Nshad40, N(i) & (ii) N5547, O- SABGA.

D

E

F
G
H
I
J

Figure 3.4: Variation in plant/growth habit and stem–related traits of nightshade accessions evaluated

Figure 3.5: Variation in matured and ripened fruits in fruit colour, shape and size of Nightshade accessions used in the study

Figure 3.6: Variation in seed colour and size of some Nightshade accessions used for the study

3.7 Conclusion

According to the findings from this study, accessions with large fruits had medium to larger leaf size and stems, with shiny black and/or dull purple fruits that remained on the plant when fully ripe, producing large brown and medium brownish seeds. The accessions Scabrum and Nshad40 outperformed the others in this category. N0096 and Timbali had small light green leaves with many branches, shiny/glossy small round purple fruits that fell from calyxes when ripe. Primary, secondary, and tertiary branches were observed for these accessions. Ncampus, Nfarm, and SNig2495 were distinguished by their dull purple-black medium round fruits, medium brownish seeds, purple-black stems and branches with short hairs, dark green lanceolate hairy leaves and early flowering plants with white flowers. Futhermore, this present study observed a very low level of diversity among accessions that were assessed using qualitative traits.

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Chapter 4:

Agronomic, genetic and quantitative traits characterization of Nightshade accessions

4.1 Abstract

Nightshades are among many underutilized and neglected African indigenous leafy vegetable (AILVs) species, and if adequately exploited, nightshade could improve food, nutrition, and income among the rural population. Morphological characterization of the available accessions is key for the breeder to identify and select superior parentals for utilization in future development. Fifteen accessions of nightshade were assessed in the field for morpho-agronomic traits implemented in a randomized complete block design with three replicates across the two growing seasons. Nightshades exhibited significant ($p<.0001$) differences in all quantitative traits evaluated. Evaluation of 15 accessions of nightshade (*Solanum* section *Solanum*) revealed the highest fresh leaf yield in Scabrum (805.30 g/plant), followed by Ncampus (718.60 g/plant). While for fruit fresh yield the accession NigSN18 (1782.00 g/plant) recorded the highest followed by ManTown (1507.90 g/plant). The accession N5547 had the tallest plants (66.83 cm) followed by accession Timbali (62.31 cm). The first four principal components (PCs) accounted for 86.82% of the total variation, which had an eigenvalue greater than 1. Cluster analysis grouped the accessions into 14 clusters based on their genetic similarity. Results of genetic studies revealed that phenotypic coefficient of variation was higher than genotypic coefficient of variation for all parameters studied, indicating the environmental influence on the expression of these traits. Both GCV and PCV were higher for the largest leaf area, moderate to high for the remaining characters, and low for leaf fresh yield per plant. High heritability coupled with genetic advance as a mean percentage (H^2-70.59%, GAM-142. 4%), indicates the presence of additive gene effects. Hence, selection can be employed for the improvement of this trait in nightshades. The study revealed sufficient genetic variability in nightshade accessions which can be exploited for crop improvement.

Keywords: Correlation, Genetic, Heritability, Morphological, Nightshade, Variability

4. 2 Introduction

Black nightshades (cultivated species from the genus *Solanum* section *Solanum*) are indigenous leafy vegetables that have the potential to improve food and nutritional security in Sub-Saharan Africa (Ronoh et al., 2019). Indigenous leafy vegetables are a diverse group of unrelated species whose leaves are consumed by African communities (Rwigi, 2016). According to Rwigi, these vegetables play a major role in household food security (Nandhin et al., 2014). (2016). According to Jagatheeswari et al. (2013), human consumption of their leaves and fruits as food is widespread, especially in Africa and South-East Asia. Nightshade is mostly gathered from the fields by agricultural labourers in South Africa's Mpumalanga province, who work in farms that grow and produce Solanaceae species such as chilies, green peppers and tomatoes.

Genetic variations occur within cultivars within each species as well as between species, and it is these variations that cause genetic diversity. Some of these species may be phenotypically similar, posing a taxonomic challenge. Though they are morphologically similar, they differ genetically, which is why African nightshade has been reported to be genetically diverse (Ojiewo et al., 2013 a). Plant growth habits, stem colour and ridging, leaf shape and pubescence, and other phenotypic differences between species can be observed. Their flower and berry sizes significantly decrease during senescence, becoming fewer and smaller than usual. Broad leafed African nightshade cultivar (*Solanum scabrum*) is one of the most common and promising African nightshade species in Kenya, distinguished by its vigorously growing broad leaves and large purple berries. It varies in leaf size and plant height, but its leaf production remains higher than that of narrow-leafed species such as *Solanum villosum* and *Solanum eldoretianum*. One of the most widely distributed and consumed African nightshade species in the country is *Solanum scabrum* (Abukutsa et al., 2005). Although nightshades have traditionally been regarded as inedible poisonous plants or troublesome agronomic weeds in Europe and the Americas (Schilling & Andersen, 1990; Onyango 1993; Edmonds & Chweya, 1997), their status is completely different in western, eastern and southern Africa as well as India, Indonesia and China, where they have long been used as leafy herbs and vegetables, a source of fruits and dye, and for various medicinal uses according to the presentation of Onyango (1993 cited in Mwai et al., 2007). The study's goal was to conduct a quantitative morphological characterization of available nightshade accessions with the specific goal of

computing genetic variance components and using them to identify superior lines exhibiting desirable traits that can be used as breeding lines or recommended as cultivars.

4.3 Materials and Methods

4.3.1 Plant materials, experimental design, and treatments

Fifteen Nightshade accessions were used in the study. The accessions came from a variety of sources within South Africa. Table 3.1 in Chapter 3 provides detailed information on each accession. The study was set up in three blocks with a randomized complete block design. Each accession representing a treatment was planted in a 2.5-m long, 5-row plot with inter-row and intra-row spacing of 50 cm. The experiment was conducted over two summer growing seasons (January–April 2020 and December 2020–February 2021).

4.3.2 Description of the research site, trial establishment, and maintenance

Accessions were planted in seedling trays at the North-West University (NWU) Mafikeng's Net-house facility of the controlled environment research unit. Plants were transplanted directly onto the field of the NWU research garden in South Africa five weeks after planting (25. 810 S and 25. 630 E, with an altitude of 1276 m). The precipitation and average temperature for both cropping seasons in the NWU Mafikeng campus are presented in Table 3.2. The total supplementary irrigation in the first season was 720 mm, while it was 660 mm in the second season. During the study period, no fertilizer was used. Malasol® 2.5 E.C was sprayed regularly to control black aphids, and Efekto Redspidercide was sprayed regularly to control red spider mites, using a knapsack sprayer at a rate of 12.5 ml/10 litres of water based on the manufacturer's recommendation for tomatoes. Hand-hoeing was used for manual weeding twice per growing season. Soil samples were collected from the study site and sent to CEDARA, which is part of the KZNDARD and has soil analytical facilities, for soil analytical service and analyses. Table 4.1 presents the results of the soil physical and chemical characteristics within the top 30 cm. According to the South African soil taxonomic system, the soil is classified as Coega form.

Table 4.1: Physical and chemical characteristics of the soil in the field trial site

Soil properties	2019/20	2020/21
Sample density g/mL	1.14	1.17
P mg/L	1	1
K mg/L	244	279
Ca mg/L	2961	1857
Mg mg/L	680	478
Zn mg/L	0.1	0.5
Mn mg/L	14	16
Cu mg/L	2.2	2.5
pH- (KCI)	6.24	6.07
Exchangeable acidity cmol/L	0.07	0.07
Total cations cmol/L	21.07	13.98
Acid saturation (%)	0	1
Soil Organic Carbon (%)	1.5	1.3
N (%)	0.08	0.09
Clay (%)	20	19

P-phosphorus; K-potassium; Ca-calcium; Mg-magnesium; Zn-zinc; Cu-copper; pH-potential hydrogen; N-nitrogen.

4.4 Data collection

4.4.1 Quantitative traits

In each plot, data for morphological traits were collected from six randomly selected plants per accession. For this study, the following quantitative traits were assessed: plant height was measured in cm using a metre from the base of the tip of the main stem, the number of leaves was recorded by physically counting mature leaves per plant, the number of primary branches (branches attached to the main stem of the plant) was counted, using a digital veneer calliper the stem diameter in millimetres was measured at the base of the first internode from all data plants, and the leaf chlorophyll content index nm was measured using the CCM-200 PLUS GPS CHLOROPHYLL CONTENT. The plant spread in millimetres was measured using a tape measure graduated in millimetres, centimetres, and metres, leaf fresh and dry mass in grams, stem fresh and dry mass in grams were also measured, Leaf length and Leaf width were taken from a total of 12 leaves per net plot for both small (the 3rd leaf from the top of the main stem) and the biggest leaf and were recorded in centimetres using a metre ruler. The leaf area (cm^2) was calculated using the recorded leaf length and width as follows:

$$\text{Leaf area } (cm^2) = LL * LW * 0.75,$$

Where 0.75 is a constant, as determined by the non-destructive length * width method described by Saxena and Singh (1965 cited in Bvenura & Afolayan, 2013). The number of days from transplanting to when approximately half of the plants in each plot flowered, the number of days from transplanting to fruit-forming, and the number of days from transplanting to fruit ripeness were all recorded. After seedling transplantation, the field was monitored daily to determine phenological attributes such as the number of days to 50% flowering, days to fruit forming, and matured fruit ripeness.

The yield was determined as fresh yield (total biomass) in grams per plant and also per plot. It was portioned into leaf, stem, and fruit yield. Harvesting of leaves and young shoots was done once at trial termination. Harvesting was done by pinching off tender and new shoot leaves early in the morning to get fresh weight and avoid weight loss during the day. All plants in the net plots were harvested, however, the trial was harvested only once for research purposes. At the harvesting stage, the fresh weight, which included leaves and tender new shoots, as well as the number of fruit and fruit weight, were recorded. Nightshade fruits were counted using two techniques: manually and/or with a seed counting machine. Small to medium fruits were counted using a Numigral CHOPIN machine (the Numigral Analyzer accurately counts 1000 grains using optical sorting), while large fruits were manually counted. Total biomass was computed as the sum of total above-ground mass in grams per plant, and harvest index was calculated as the ratio of leaf fresh mass to total above-ground mass in percentage.

4.5 Data analyses

Statistical analysis was done using the GLM procedures of SAS (Version 9.4). Analysis of Variance (ANOVA) was performed with a significance set at $P< 0.05$. Tukey's honestly significant difference (HSD) test (Gomez and Gomez, 1984) was used to separate means at $P< 0.05$. The similarities and dissimilarities were determined using hierarchical clustering analysis of unweighted pair group method of arithmetic (UPGMA) mean using SAS software. SAS was used to identify influential traits for selection using principal component analysis (PCA) based on the correlation matrix. SAS was used to construct a PCA multidimensional preference analysis biplot to demonstrate the associations between accessions based on observed traits. To describe the relationship between the morphological traits, SAS was used to perform Pearson's correlation analysis.

Heritability in the broad-sense was calculated using a formula by Falconer (1989 cited in Al-Tabba & Al-Fraihat, 2012) as follows;

$$H^2 = \frac{\sigma^2 g}{\sigma^2 p},$$

Where H^2 = broad-sense heritability; $\sigma^2 g$ is the genotypic variance; $\sigma^2 p$ is the phenotypic variance were obtained from the analysis of variance table. The (phenotype) is the mean of genotype across *m* trials (environments) and *r* replicates per trial. This has variance

$$\sigma^2 p = \sigma^2 g + \frac{\sigma^2 ge}{m} + \frac{\sigma^2}{rm}$$

Where $\sigma^2 ge$ is the genotype-environment interaction variance and σ^2 is the residual error variance according to a formula of Falconer and Mackay (1996 cited in Piepho & Mohring, 2007). Heritability was categorized as low, moderate, and high following Robinson et al. (1949 cited in Mangena, 2018) as follows; as low (< 10%), moderate (10–20%) and high (> 20%). Estimation of variance components**;** the genotypic and phenotypic variance was calculated according to Comstock and Robinson's equations (1952 cited in Al-Tabba & Al-Fraihat, 2012) using;

Genotypic variance $\sigma^2 g = \frac{MS1-MS2}{rs}$ and phenotypic variance: $\sigma^2 p = \frac{MS1}{rs}$

Where MS1 is the mean square due to genotype, MS2 is the mean square for species by season interaction, S= season and *r* is the number of replications. The mean values were used for genetic analyses to determine the Genetic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV) were computed according to Singh and Chaudhary of 1985 (cited in Al-Tabba & Al-Fraihat, 2012) and expressed as a percentage as follows; genotypic covariance (GCV%) $= \frac{\sqrt{\sigma^2 g}}{X} \times 100$ and phenotypic covariance (PCV%) $= \frac{\sqrt{\sigma^2 p}}{X} \times 100$; Where $\sigma^2 p$ = phenotypic variation and $\sigma^2 g$ = genotypic variation; X= Grand mean of the characters studied. Genetic advance (GA) was calculated by selecting 5% of the superior accessions and calculated using the following formula from Allard's work of 1960 (cited in Al-Tabba and Al-Fraihat, 2012) as:

GA = $k.\ \sigma^2 p\ .\ h^2$

Where GA= expressed genetic advance, $\sigma^2 p$ = square root of phenotypic variance, and k: constant = selection differential (2.06) at 5% selection intensity. GA as % of mean (GAM) = $\frac{GA}{X} \times 100$

4.6 Results and Discussion

4.6.1 Analysis of Variance

All measured traits showed highly significant ($p < 0.001$) differences between accessions (Table 4.2). The significant differences revealed a high degree of variability among the accessions that could be used to improve nightshade.

Table 4.2: Analysis of variance and significance tests for nightshade accessions for combined data of two seasons

Mean of Squares						
Source of Variance	**SEASON**	**Rep**	**Access**	**S*Access**	**Residual Error**	**GM**
DF	**1**	**2**	**14**	**14**	**508**	
PH cm	37500.00	2272.20	1541.61	972.97	170.12	55.54
NLS	386082.81	126599.01	28148.89	6127.77	3822.37	122.77
NBPr	18.51	31.57	56.33	13.14	5.70	7.72
SD mm	941.84	13.59	152.50	105.78	57.79	10.83
ChloroC nm	171.92	682.19	857.30	375.56	120.26	27.63
Psprd mm	180950.41	332508.37	52391.55	38209.14	8513.04	436.26
Nfruit	1803360.06	16647.53	145177.30	75054.82	17846.65	163.87
FFM g	471865.09	24732.61	73300.68	31490.02	4823.89	79.18
LFM g	41018.36	3167.15	4806.58	2650.23	1434.49	54.73
LDM g	4411.95	269.51	337.36	109.12	91.66	13.42
SFM g	209762.49	54980.50	15658.43	16752.00	3511.77	85.82
SDM g	16668.55	2718.57	456.95	617.37	179.64	19.34
SLarea cm^2	22.97	38.43	154.27	17.61	57.79	5.80
BLarea cm^2	998.11	2009.37	23268.50	5545.11	433.21	31.43
ND_F	4611.26	270.06	481.20	148.12	12.29	30.32
ND_FF	10454.40	131.40	357.28	91.40	11.75	45.00

ND_FR	1092.26	168.80	314.17	112.83	6.63	51.80
Source of variance	**SEASON**	**Rep**	**Access**	**S*Access**	**Residual Error**	**GM**
DF	**1**	**2**	**14**	**14**	**58**	
LY_gp	1224365.41	64213.01	90383.08	83521.08	43006.78	593.19
SY_gp	5775868.26	1220950.45	419336.89	338286.70	136080.38	972.99
FY_gp	14048539.10	545708.29	1944116.32	1003041.28	147601.51	871.17
TBiomass_gp	52677869.68	4210043.66	3895404.18	2601992.42	693867.4	2437.36
HrvstInd %	785.40	289.58	224.65	101.48	31.46	27.85

DF- degree of freedom, Rep-replication, Access-accession, S*Access- season by accession interaction, GM-grand mean. PH- plant height, NLS- number of leaves, NPBr- number of primary branches, SD- stem diameter, ChloroC- leaf chlorophyll content, Psprd- plant spread, Nfruit- number of fruits, FFM- fruit fresh mass, LFM-leaf fresh mass, LDM- lead dry mass, SFM-stem fresh mass, SDM- stem dry mass, SLarea- smallest leaf area, BLarea- biggest leaf area, ND_F- number of days to 50% flowering, ND_FF- days to fruit forming, ND_FR- days to fruit repines. LY_gp-leaf yield in grams per plant, SY_gp-stem yield in grams per plant, FY_gp-fruit yield in grams per plant, TBiomass_gp - total biomass in grams per plant, HrvstInd- harvest Index percentage

4.6.2 Variation among accessions

The quantitative morphological traits showed considerable mean variation as presented in Table 4.3. There were higher variations among the accessions in plant height, the number of (leaves, fruits), fresh mass (fruit, leaf fresh, and stem fresh mass), and the biggest leaf area. Plant height varied from 43.50 to 66.83 cm with a mean of 55.54 cm. The tallest accessions were N5547, Timbali, N0096, ManTown, and Nshad9. While accessions with the most numerous leaves were Timbali, Sharon, SNig2495, Nfarm, N5547, N0096, Ncampus, and SABGA. The number of primary branches varied significantly (Table 4.3). Accessions with bigger stems were Nshad40, Scabrum, ManTown, SABGA, NigSN18, and SRetrflx, as stem diameter (mm) range between 8.03 mm (N0096) and 16.42 mm (Nshad40) with a mean of 10.82 mm. The number of fruits ranged from 82.67 (N5547) to 300.06 (Sharon) with a mean of 163.86, however, accessions with a high number of fruits were Sharon, Timbali, N0096, Nshad40, Ncampus, NigSN18, Nshad9, and SNig2495.

Fruit fresh mass ranged from 18.74 g/plant (Nfarm) to 140.32 g/plant (SABGA) with a mean of 79.18 g/plant. The mean of 54.72 g was observed in leaf fresh mass (g) as it varied from 37.85 to 74.87 g/plant, with Ncampus, Scabrum, Nshad40, and SABGA having high fresh mass. The mean stem fresh mass ranged from 52.20 g (NigSN18) to 122.97 g/plant (Scabrum) with a mean of 85.82 g/plant. The mean leaf area cm^2 (small leaf) ranged from 3.15 cm^2 (Sharon) to 9.76 cm^2 (Scabrum) with a mean of 5.80 cm^2, while the mean leaf area (biggest leaf) ranged from 9.15 cm^2 (Sharon) to 101.84 cm^2 (Scabrum) with a mean of 31.43cm^2. The number of days to flowering ranged between 26.33 (Nfarm and SNig2495) and 36.83 (Sharon) with a mean of 30.32. On average, the Nfarm and SNig2495 flowered earlier (approximately 26 days from transplanting) than Sharon flowering late (approximately 37 days from transplanting). The mean number of days to fruit forming varied between 41.83 and 51.50 with a mean of 45.00 (approximately 45 days from seedling transplanting). The number of days to fruit ripening ranged from 47.66 to 58.66, with a mean of approximately 52 days from transplanting.

The analysis of variance also showed variation in leaf yield per plant ranging from 301.60 g (Sharon) to 805.30 g (Scabrum), with a mean of 539.19 g/plant. The mean stem yield per plant was between 466.50 g (Sharon) to 1376.90 g (N5547) with a mean of 972.19 g/plant, while the

fruit yield ranged from 173.40 g (Nfarm) to 1782.00 g (NigSN18), with a mean of 871.16 g/plant. The total biomass varied between 1044.00 g (Sharon) and 3347.20 g (ManTown), with a mean of 2437.37 g/plant. Finally, the harvest index in percentage varied between 19.60% (SRetrflx) and 38.87% (Nfarm), with a mean of 27.85% (Table 3.2). This study showed great variation among accessions in terms of the traits of importance and agronomic yield, however, a slightly significant variation was observed in leaf yield. Among the morphological characteristics that were measured for this present study, the biomass of leaf fresh mass (measured as an average leaf fresh mass g/plant), leaf area and the number of leaves, and days to 50% flowering contribute to the amount of harvested biomass that can be used for consumption.

According to the findings of Ronoh et al. (2019), the traits of agronomic importance, such as leaf yield expressed as leaf fresh mass, leaf area, plant height, fruit mass, and stem diameter varied significantly between and within accessions used in their study, while in this present study the findings are in agreement as it showed a significant variation in plant height, number of leaves, number of primary branches, number of fruits, and fruit mass, while leaf fresh mass showed a slightly significant variation among accessions. The study revealed that accessions with the biggest leaf area (size) resulted in high leaf fresh mass, and these accessions are Scabrum, Nshad40, and ManTown. This agrees with the recommendations by Masinde et al. (2010) as well as Ronoh et al. (2019) who stated that leaf yield in African nightshades should be considered in terms of leaf fresh weight as well as the number of leaves. The number of days to flowering influences the harvest in that of the onset of flowering, so late flowering allows a prolonged period of harvesting. Flower removal as soon as they appear has been suggested to increase leaf yield and prolong harvesting periods as stated by Ojiewo et al. (2013 cited in Omondi et al., 2017; Ronoh et al., 2019). From this present study, five of fifteen accessions (Nfarm, Ncampus, SNig2495, N0096, and Timbali) flowered early at the seedling stage (6 weeks from seed sowing in seedling trays), however, flowers were removed a day before seedling transplanting.

Table 4.3: Variation for 22 traits in 15 nightshade accessions and means under combined analysis

Access	PH (cm)	NLS	NBPr	SD (mm)	ChloroC nm	Psprd mm	Nfruit	FFM (g)	LFM (g)	LDM (g)	SFM (g)	SDM (g)
ManTown	61.33ab	94.67df	6.41de	12.16ab	23.59c	418.89bd	136.47ce	123.41a	48.82ac	10.38bc	83.70ad	16.67bc
NigSN18	43.50d	104.14de	7.78ad	11.58ab	24.95bc	443.61ac	161.42be	111.17ab	43.01bc	10.68bc	52.20d	11.34c
SRetrflx	56.11bc	101.28de	6.97be	11.57ab	26.50bc	453.33ac	116.42de	120.97ab	49.21ac	10.89bc	91.15ad	19.09ac
Nshad9	59.83ac	101.19de	8.31ac	10.82ab	25.71bc	457.22ac	155.39be	130.68a	52.85ac	11.12bc	94.61ad	19.79ac
Nshad5	49.61cd	113.31be	5.97de	10.18b	28.25bc	359.17d	98.39e	108.15ab	50.55ac	12.01bc	81.72ad	17.53ac
N5547	66.83a	138.22ae	7.61ad	10.94ab	25.29bc	405.28bd	82.67e	67.20ac	59.83ac	13.34ac	109.87ab	22.72ab
Nshad40	57.89ac	90.39ef	7.08cd	16.42a	22.20c	460.00ac	210.67ad	100.65ac	71.33ab	17.82ab	114.10ab	21.22ac
Ncampus	51.83bd	127.08be	8.86ab	9.83b	39.34a	469.72ab	188.47be	24.03d	74.88a	15.97ac	82.07ad	21.32ac
Nfarm	53.67bd	150.08ac	9.31a	9.84b	33.69ab	459.44ac	119.36de	18.74d	54.67ac	14.20ac	81.70ad	19.16ac
N0096	61.63ab	135.06be	8.33ac	8.03b	25.62bc	390.56cd	241.94ac	30.88d	39.24c	9.62c	57.77cd	16.66bc
Scabrum	56.81ac	85.47f	5.06e	12.54ab	22.92c	433.06bd	122.03de	107.43ab	73.49a	19.91a	122.97a	27.75a
SNig2495	50.39cd	140.33ad	8.81ab	9.39b	32.68ab	446.25ac	150.97be	26.36d	55.55ac	12.59ac	73.32bd	18.16ac
Sharon	45.28d	153.92ab	8.39ab	9.33b	23.25c	516.94a	300.06a	45.14cd	49.31ac	17.11ac	76.85ad	19.09ac
SABGA	56.14bc	121.14be	7.53ad	11.63ab	32.60ab	432.22bd	115.81de	140.32a	60.31ac	13.98ac	105.00ad	21.28ac
Timbali	62.31ab	185.36a	9.42a	8.18b	27.86bc	398.33bd	257.94ab	32.58d	37.85c	11.65bc	60.31cd	18.33ac
HSD	**	***	***	**	**	**	***	***	**	*	**	**
Fpr	<.0001	<.0001	<.0001	0.0010	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0016
StD	6.54	27.29	1.25	2.06	4.88	38.15	63.50	45.12	11.56	3.06	20.85	3.56
GM	55.54	122.77	7.72	10.83	27.63	436.27	163.87	79.18	54.73	13.42	85.82	19.34

Access- accession, PH- plant height, NLS- number of leaves, NPBr- number of primary branches, SD- stem diameter, ChloroC- leaf chlorophyll content, Psprd- plant spread, Nfruit- number of fruits, FFM- fruit fresh mass, LFM-leaf fresh mass, LDM- lead dry mass, SFM-stem fresh mass, SDM- stem dry mass, SLarea- smallest leaf area, BLarea- biggest leaf area, ND_F- number of days to 50% flowering, ND_FF- days to fruit forming, ND_FR- days to fruit repines. LY_gp-leaf yield in grams per plant, SY_gp-stem yield in grams per plant, FY_gp-fruit yield in grams per plant, TBiomass_gp - total biomass in grams per plant, HrvstInd- harvest Index percentage, and Fpr- F probability for accessions, HSD- Honest Significant difference at 5% probability level, StD- standard deviation, and GM- grand mean. * -p<0.05, ** - p<0.01, *** - p<0.001. Tukey pairwise comparisons analysed at $p < 0.05$ significant level. Means within columns that do no share a letter are significantly different.

Table 4.3. Cont'.

Access	SLarea (cm^2)	BLarea (cm^2)	ND_F	ND_FF	ND_FR	LY_gp	SY_gp	FY_gp	TBiomass_gp	HrvstInd (%)
ManTown	6.10b	51.61c	28.33bc	43.83cf	52.17cf	651.90ab	1187.30ac	1507.90ab	3347.20a	20.77ed
NigSN18	6.50b	22.36de	28.50bc	44.67be	53.33be	532.60ab	765.10ac	1782.00a	3079.70ab	21.86ce
SRetrflx	6.68b	33.26d	30.16b	45.83bd	52.33cf	611.70ab	1206.60ac	1354.20ac	3172.60ab	19.60e
Nshad9	6.24b	24.66de	27.83bc	43.83cf	51.67df	612.50ab	1086.90ac	1363.70ac	3063.10ab	21.29ed
Nshad5	6.14b	23.19de	27.83bc	42.83ef	49.17gh	580.20ab	956.10ac	1433.70ac	2970.00ac	26.30be
N5547	6.64b	23.40de	35.33a	51.50a	55.33b	685.50ab	1376.90a	657.10ce	2719.50ad	26.47be
Nshad40	9.72a	70.31b	36.50a	47.00b	53.50bd	679.00ab	1192.80ac	879.80be	2751.60ac	29.42ae
Ncampus	4.14c	20.49de	28.17bc	43.67df	47.67h	718.60ab	911.60ac	228.20e	1858.40ad	37.68ab
Nfarm	4.00c	14.30e	26.33c	41.83f	48.17h	612.00ab	856.80ac	173.40e	1642.20bd	38.87a
N0096	3.38c	10.17e	29.83b	41.83f	51.17fg	425.80ab	615.30bc	304.80e	1346.00cd	30.41ae
Scabrum	9.77a	101.84a	34.67a	46.00bd	53.83bc	805.30a	1252.00ab	1132.70ad	3190.10ab	30.19ae
SNig2495	4.41c	20.63de	26.33c	41.83f	48.17h	573.40ab	880.30ac	289.80e	1743.50ad	33.22ac
Sharon	3.16c	9.16e	36.83a	51.50a	58.67a	301.60b	466.50c	275.90e	1044.00d	29.39ae
SABGA	6.53b	35.13cd	27.83bc	42.33ef	51.33ef	622.30ab	1161.50ac	1331.70ac	3115.50ab	21.00ed
Timbali	3.52c	10.99e	30.33b	46.50bc	50.50fg	485.50ab	679.10ac	352.50de	1517.20bd	31.33ad
HSD	**	***	*	***	***	*	**	***	***	***
Fpr	<.0001	<.0001	<.0001	<.0001	<.0001	0.0250	0.0013	<.0001	<.0001	<.0001
StD	2.07	25.42	3.66	3.15	2.95	122.72	264.37	569.22	805.75	6.12
GM	5.80	31.43	30.32	45.00	51.80	593.19	972.99	871.16	2437.37	27.85

Access- accession, SLarea- smallest leaf area, BLarea- biggest leaf area, ND_F- number of days to 50% flowering, ND_FF- days to fruit forming, ND_FR- days to fruit repines. LY_gp-leaf yield in gram per plant, SY_gp-stem yield in gram per plant, FY_gp-fruit yield in gram per plant, TBiomass_gp - total biomass in grams per plant, HrvstInd- harvest Index percentage, and Fpr- F probability for accessions, HSD- Honest Significant difference at 5% probability level, StD- standard deviation, and GM- grand mean. * -$p<0.05$, ** - $p<0.01$, *** - $p<0.001$. Means within columns that do not share a letter are significantly different.

4.6.3 Phenotypic correlation for quantitative traits

There was a strong and positive correlation among some traits in the accessions under study (Table 4.4). The significance, strength, and nature of correlation among quantitative traits were analyzed. Correlation analysis varied significantly at 0.05 probability level, with positive and negative correlations ranging from strong to moderate, and to weak. Positive and significant relationships were found between stem diameter, fruit fresh mass, leaf fresh mass, leaf dry mass, stem fresh mass, stem dry mass, small and biggest leaf area, and days to 50% flowering (Table 4.4). Another strong positive correlation was observed between the number of leaves and the number of primary branches, while leaf yield was also strongly correlated with stem yield per plant (r= 0.85). Negative and significant associations were observed for the number of leaves with stem diameter (r= -0.76), fruit fresh mass (r= -0.77), and biggest leaf area (r= -0.72), fruit fresh yield (r= - 0.74), as well as total biomass (r= -0.82).

Positive and moderate associations were found for the number of leaves and harvest index, the number of primary branches and the leaf chlorophyll content, the stem diameter and fruit yield, the fresh fruit mass and the largest leaf area (r= 0.51), and the stem dry mass and stem yield (r= 0.53) (Table 4.4). Negative moderate interactions were observed between the number of leaves and stem diameter (r= -0.50), the number of primary branches and leaf yield (r= -0.48), and the leaf chlorophyll content and days to 50% flowering (r= -0.52). Plant height, the number of primary branches, stem diameter, plant spread, dry leaf mass, stem fresh mass, smallest and largest leaf area, and stem yield all had positive weak associations. Negative weak associations were observed for plant height with leaf chlorophyll content (r= -0.21), plant spread (r= -0.43), number of fruits per plant(r= -0.16), leaf dry mass (r= -0.18), and fruit yield(r= -0.08) per plant. When selecting desirable parental lines for yield improvement, correlation analysis is useful for determining traits that influence variation as well as interrelatedness (Mohammed et al., 2012). The analysis findings revealed several correlations between the traits under consideration. Fruit yield and total biomass were positively and significantly correlated, whereas stem diameter, leaf fresh mass, stem fresh mass, leaf area, and the number of leaves were positively and significantly correlated with stem fresh mass, biggest leaf area, leaf yield, and total biomass. The primary branches, on the other hand, exhibited a negative and significant correlation with fruit fresh mass, small and biggest leaf area, and total biomass per plant. These findings are consistent with those reported by Ronoh et al. (2019) and Ravi et al. (2013). Plant

height was also found to have a weak positive relationship with stem fresh mass, stem dry mass, leaf yield and stem yield per plant. These positive correlations among plant height, number of leaves, plant spread, leaf fresh mass, fruit fresh mass, leaf area and number of days to 50% flowering imply that tall plants that flower later have larger stems, more time to accumulate photosynthesis, and more time to spread and produce leaves with high fresh weight. According to Ronoh et al. (2019), a positive correlation between fresh mass and yield descriptors such as leaf area, plant height, and stem indicates that such traits should be prioritized when selecting for high yield.

As reported in eggplant, positively correlated traits may have a common genetic and physiological basis (Kaushik et al., 2018). The strong positive correlation suggests that indirect selection is possible; thus, selecting for one trait would result in the selection of another trait that is strongly correlated with the selected trait. As a result, a negative correlation between agronomic and yield-related traits indicates that plants with a high number of leaves may not be achieved simultaneously with stem diameter and leaf yield. This suggests that an increase in one would result in a decrease in the other (Ganguly & Bhat 2012). When applying selection to a trait of interest, knowledge of correlation is essential to obtain the expected response of the other trait. Correlation estimates allow for the evaluation of one or more traits' behaviour using the behaviour of the other as a reference. There is a thorough understanding of indirect selection. This is especially true for traits that are difficult to quantify and are associated with low heritability (Lambardi et al., 2015). Positively correlated traits imply gene linkage and pleiotropic effects according to Kearsey and Pooni as well as Kisau et al. (1996, 2015, cited in Nundwe, 2018, respectively). According to Kisau et al. (2015), strong positive correlations among genotypes indicate that such traits are heritable and genetically controlled, implying that they can be transmitted to the desired genotypes.

Table 4.4: Estimation of phenotypic correlation coefficient among nightshade accessions and its yield contributing traits

Characters	PH (cm)	NLS	NBPr	SD (mm)	ChloroC nm	Psprd (mm)	Nfruit	FFM (g)	LFM (g)	LDM (g)	SFM (g)	SDM (g)	SLarea (cm^2)	BLarea (cm^2)	ND_F	ND_FF	ND_FR	LY_gp	S
PH (cm)	1																		
NLS	0.03	1																	
NBPr	-.06	**0.77****	1																
SD (mm)	0.07	-.76**	-.58	1															
ChloroC nm	-.21	0.36*	**0.51****	-.40	1														
Psprd (mm)	-.43	-.02	0.30*	0.18	0.07	1													
Nfruit	-.16	0.45*	0.47	-.28	-.17	0.35	1												
FFM (g)	0.06	-.77**	-.70	**0.62****	-.44*	-.12	-.46	1											
LFM (g)	0.01	-.42	-.31	**0.56****	0.24	0.32	-.29	0.09	1										
LDM (g)	-.18	-.13	-.24	0.42*	-.02	0.47*	0.12	-.09	**0.79****	1									
SFM (g)	0.36	-.50	-.56	**0.67****	-.18	0.12	-.46	0.45*	**0.77****	**0.63****	1								
SDM (g)	0.39	-.15	-.33	0.28	0.02	0.12	-.21	0.04	**0.75****	**0.74****	**0.85****	1							
SLarea (cm^2)	0.17	-.80**	-.77	**0.89****	-.45	-.07	-.47	**0.71****	**0.56****	0.40*	**0.76****	0.45*	1						
BLarea (cm^2)	0.18	-.72**	-.77	**0.76****	-.41	0.01	-.29	**0.51****	**0.62****	**0.57****	**0.73****	**0.59****	**0.87****	1					
ND_F	0.17	-.07	-.29	0.39	-.64	0.25	0.34	0.03	0.29	**0.61****	0.46	0.46	0.38	0.39	1				
ND_FF	0.12	0.15	-.09	0.18	-.52	0.29	0.26	-.01	0.09	0.38	0.29	0.29	0.15	0.08	**0.86****	1			
ND_FR	0.01	-.13	-.29	0.29	-.77	0.33	0.28	0.28	-.05	0.29	0.26	0.14	0.27	0.22	**0.82****	**0.83****	1		
LY_gp	0.32	-.58	-.48	0.57	0.17	-.13	-.69	0.34	**0.75****	0.34*	**0.69****	**0.58****	**0.70****	**0.69****	-.04	-.15	-.28	1	
SY_gp	0.49	-.63**	-.59	**0.66****	-.13	-.18	-.76	**0.60****	**0.55****	0.16	**0.81****	**0.53****	**0.77****	**0.64****	0.12	0.08	0.03	**0.85****	
FY_gp	-.08	-.74*8	-.67	**0.51****	-.40	-.23	-.49	**0.94****	-.07	-.26	0.20	-.20	**0.62****	0.40	-.13	-.11	0.18	0.29	
TBiomass_gp	0.15	-.82	-.74	**0.67****	-.30	-.24	-.71	**0.91****	0.25	-.08	**0.51****	0.12	**0.79****	**0.60****	-.05	-.07	0.09	**0.64****	**0**
HrvstInd (%)	-.12	0.51	0.48	-.33	**0.51****	0.16	0.29	-.87	0.30	0.43	-.13	0.24	-.39	-.16	-.00	-.11	-.40	0.01	

PH- plant height, NLS- number of leaves, NPBr- number of primary branches, SD- stem diameter, ChloroC- leaf chlorophyll content, Psprd- plant spread, Nfruit- number of fruits, FFM- fruit fresh mass, LFM-leaf fresh mass, LDM- lead dry mass, SFM-stem fresh mass, SDM- stem dry mass, SLarea- smallest leaf area, BLarea- biggest leaf area, ND_F- number of days to 50% flowering,

ND_FF-number of days to fruit forming, ND_FR- number of days to fruit repines. LY_gp-leaf yield in grams per plant, SY_gp-stem yield in grams per plant, FY_gp-fruit yield in grams per plant, TBiomass_gp - total biomass in grams per plant, HrvstInd- harvest Index percentage. **= Significant at 1% level; * = Significant at 5% level.

4.6.4 Principal component analysis (PCA)

The first four principal components (PC1, PC2, PC3, and PC4) with eigenvalues greater than 1.00 explained 86.82 per cent of the total variation for all traits studied. These were extracted and are shown in Table 4.5. The first principal component (PC1) accounted for 42.03 per cent of total variation and had an eigenvalue of 9.25. Traits that contributed to this variation included both the largest and the smallest leaf area, stem diameter, stem yield and total biomass. The eigenvalue of the second PC was 4.41, and it accounted for 20.07 per cent of the total variation. Days to 50% flowering, days fruit forming, leaf dry mass, harvest index, leaf fresh mass, and plant spread were all components in this. The third PC accounted for 16.41% of total variation and had an eigenvalue of 3.61. The traits that contributed to this variation were leaf chlorophyll content, leaf yield, harvest index and leaf fresh mass. Finally, the fourth PC accounted for 8.31 per cent of total variation and had an eigenvalue of 1.83, with plant height being the major contributor.

Components with eigenvalues less than 1.00 were estimated in the principal component analysis, as explained by Chatfield and Collins (1980, cited in Nundwe, 2018). Component loadings of ±0.03 are considered meaningful by Hair et al. (1998), so they were selected. According to the current study's findings, the first four principal component axes explained 86.82 per cent of the variation, indicating significant diversity among the traits studied, which agrees with Berinyuy et al. (2002) and, Fontem and Schippers (2004) findings of similar variation. From the first component (9.25) to the fourth component (1.83), the eigenvalues decreased significantly. Though there are no clear guidelines for determining the significance of a character coefficient, one rule of thumb is to consider coefficients greater than 0.6 to have a large enough effect to be considered significant according to Jeffers (1967) as well as Balkaya (2009 cited in Cakir et al., 2017). Small leaf area, largest leaf area, stem diameter, stem yield and total biomass were the traits that contributed the most to the first component, accounting for 42.03 per cent of the total variation. This implies that selection in the first component should be centred on these characteristics. The first and second components showed the most variation among the accessions, with a cumulative variation of 62.10 per cent, indicating a high degree of association among the traits studied (Kisua et al., 2015).

Table 4.5: Principal component analysis for 22 quantitative traits indicating Eigenvectors, Eigenvalues and proportion of variation explained with the four PCs axes.

	Eigenvectors			
Characters	**PC1**	**PC2**	**PC3**	**PC4**
PH (cm)	0.08	0.01	0.05	**0.65**
NLS	-.27	0.12	0.01	**0.24**
NBPr	-.27	0.06	0.08	0.00
SD (mm)	**0.28**	0.06	-.04	-.18
ChloroC	-.14	-.02	**0.42**	-.14
Psprd (mm)	-.02	0.25	-.07	-.46
Nfruit	-.19	0.20	-.24	-.10
FFM g/plant	**0.26**	-.22	-.14	-.09
LFM g/plant	0.19	0.26	**0.27**	-.16
LDM g/plant	0.12	**0.40**	0.07	-.20
SFM g/plant	0.27	0.19	0.11	0.11
SDM g/plant	0.17	**0.31**	0.18	**0.20**
SLarea cm^2	**0.31**	0.02	-.01	-.05
BLarea cm^2	0.28	0.11	0.04	-.06
ND_F	0.11	0.34	-.29	0.13
ND_FF	0.05	0.28	-.32	0.17
ND_FR	0.10	0.18	**-.44**	0.03
LY_gp	0.24	0.02	**0.32**	0.06
SY_gp	**0.28**	-.04	0.15	**0.21**
FY_gp	0.22	-.31	-.14	-.15
TBiomass_gp	0.28	-.22	-.00	-.03
HrvstInd (%)	-.16	**0.28**	**0.28**	-.03
Eigenvalue (explained variance)	9.25	4.41	3.61	1.83
Proportion of total variance (%)	42.03	20.07	16.41	8.31
Cumulative variance (%)	42.03	62.10	78.51	86.82

PC- principal component, PH- plant height, NLS- number of leaves, NPBr- number of primary branches, SD- stem diameter, ChloroC- leaf chlorophyll content, Psprd- plant spread, Nfruit- number of fruits, FFM- fruit fresh mass, LFM-leaf fresh mass, LDM- lead dry mass, SFM-stem fresh mass, SDM- stem dry mass, SLarea- smallest leaf area, BLarea- biggest leaf area, ND_F- number of days to 50% flowering, ND_FF- to fruit forming, ND_FR days to fruit repines. LY_gp-leaf yield in grams per plant, SY_gp-stem yield in grams per plant, FY_gp-fruit yield in grams per plant, TBiomass_gp - total biomass in grams per plant, HrvstInd- harvest Index percentage.

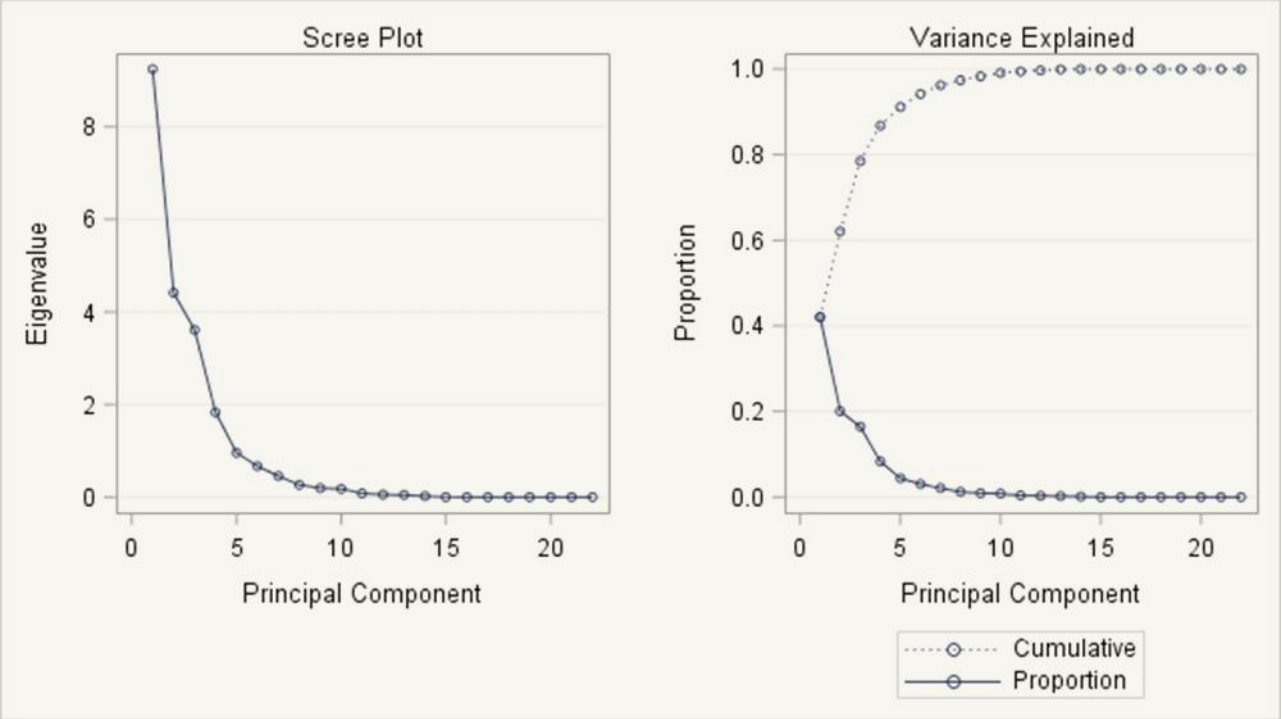

Figure 4.1: A presentation of both the scree plot and variance explained

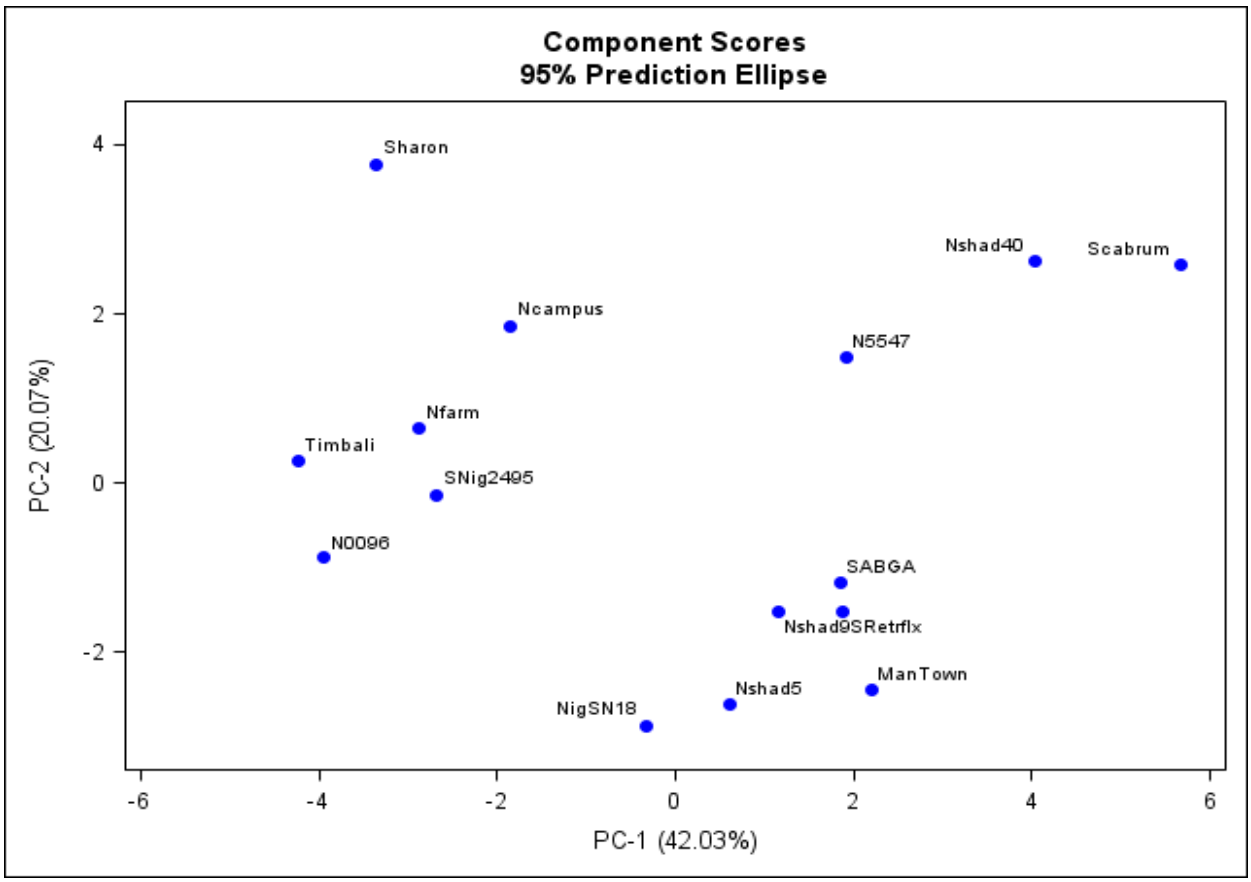

Figure 4.2: A representation biplot of the cumulative variance from principal components 1 and 2. • – indicate the position of accession within the quadrant.

4.6.5 Principal Component biplot

A multidimensional biplot in Figure 4.3 illustrates the observed phenotypic diversity among the nightshade accessions under study. The biplot represents the relationship among phenotypic traits and accessions with the principal components. According to Mwadzingeni et al. (2016), traits with small angles between dimension vectors in the same direction have a high correlation in discriminating accessions. Such relationships were found to be positively and significantly correlated in this study between leaf fresh mass and plant height, stem diameter, and small leaf area. Accessions that excelled in a specific trait were plotted closer to the vector line and further in the direction of that vector. For example, stem diameter and small leaf area, leaf fresh mass and plant height were all positively and significantly correlated. This implies that selecting for small leaf area can be accomplished through selection for thicker and succulent stems while selecting for leaf fresh mass can be accomplished through selection for tall plants. According to the biplot, accessions were grouped based on their associations with phenotypic traits. According to Figure 4.3, Scabrum was associated with plant height and leaf fresh mass per plant, whereas Nshad40 was associated with stem fresh mass, stem yield and leaf yield per plant.

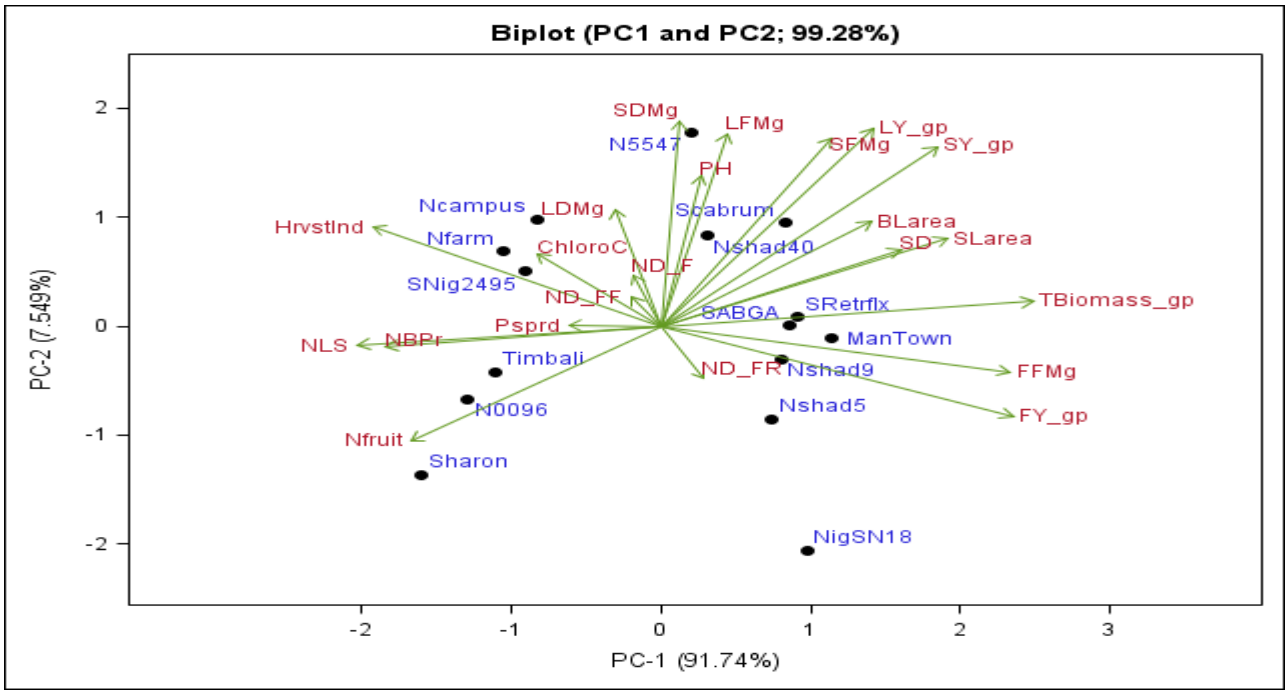

Figure 4.3: Principal component analysis biplot describing the overall variation among nightshade accessions estimated using 22 traits. ●– indicate the position of accession within the quadrant. ➚ – is the vector line indicating the direction of the dimension vector (trait) associated accession within quadrants.

4.6.6 Cluster analysis

A cluster history of accessions (Table 4.7), which is not presented in this subsection illustrated the grouping of accessions. Using the SAS procedure single method, the dissimilarity and similarity indices were used to group similar accessions. To evaluate the phenotypic relationships among accessions, the minimum distances between all pairs of individual accessions were constructed by plotting on a dendrogram (Figure 4.4). To gain a better understanding of the overall diversity of nightshade accessions, the data were analyzed using cluster analysis, which revealed the distribution of phenotypic diversity and is shown in Figure 4.4. SRetrflx, SABGA, and Nshad9 had the lowest dissimilarity index (0.00) (Table 4.7). Cluster 1 accessions had the highest dissimilarity index of 0.8652 and the mean distance between observations was 1245.994. Cluster analysis was used in this study and grouped the accessions into 14 clusters. Cluster 1 was a component of all accessions attributed to the following clusters: 4, 5, 10, 11, and 12. These clusters contained accessions that are not high yielding and are early flowering. Cluster 2 was a component of clusters 3, 6, 7, 8, 9, 13, and 14, and all accessions in these clusters performed well in terms of agronomic and yield-related traits, with three accessions (N5547, Nshad40, and Scabrum) showing late flowering. Cluster 6 included two of the five late-flowering accessions (Nshad40 and N5547) as well as four of the seven tallest accessions (N5547, ManTown, Nshad40 and Scabrum).

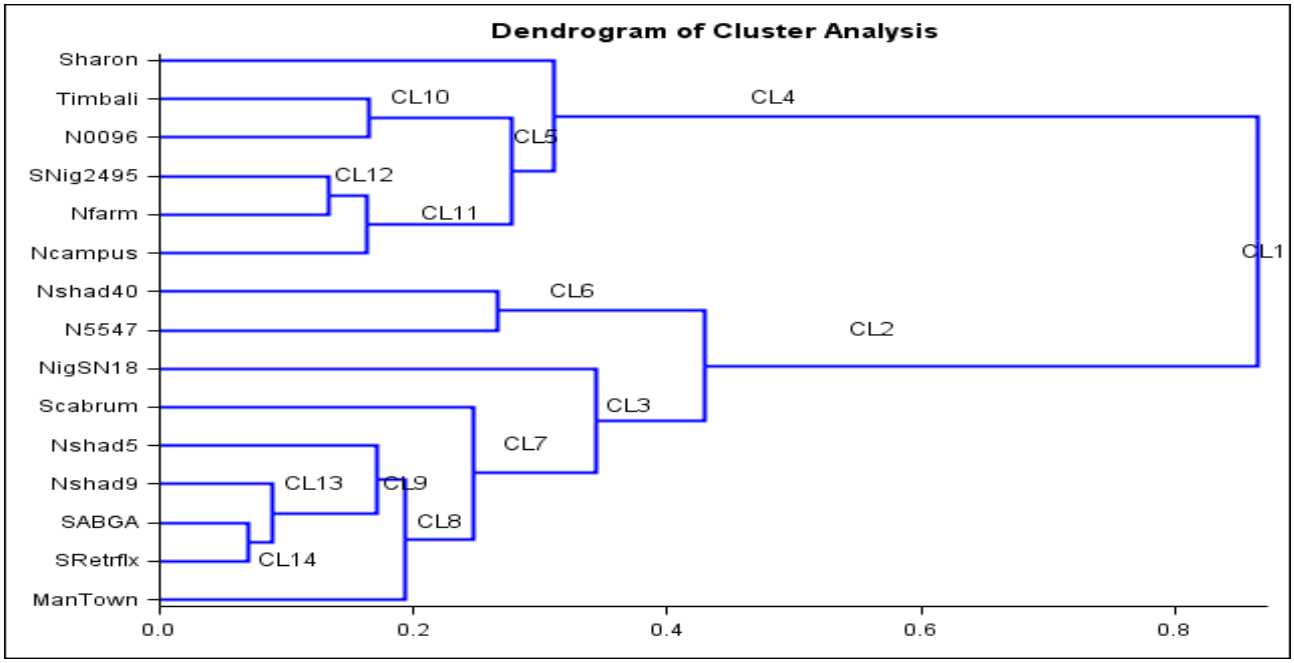

Figure 4.4: Cluster analysis showing phenotypic association among nightshade accessions. CL-cluster

4.7 Genetic parameters

For all traits studied, the results revealed significant genotypic and phenotypic variances among the accessions (Table 4.6). Table 4.6 displays estimates of mean, genotypic variance, genotypic coefficient of variability (GCV), phenotypic coefficient variability (PCV), broad-sense heritability (H^2), genetic advance (GA), and genetic advance expressed as a percentage of the mean (GAM %) for various traits. The genetic variance ranged from 7.20 for the number of primary branches to 215568.63 for the total biomass per plant, while the phenotypic variance ranged from 14.72 for the number of primary branches to 1632209.40 for the total biomass per plant. The estimates for the phenotypic coefficient of variation (PCV) were higher than those for the genetic coefficient of variation for all traits, indicating that environment influences trait expression. GCV values ranged from 5.70 per cent for leaf yield per plant to 172.92 per cent for the largest leaf area, while PCV values ranged from the biggest leaf area to fruit fresh mass. The results of broad-sense heritability revealed that the studied traits had high significant estimates. The estimates for leaf yield per plant ranged from 2.28 per cent to 70.59 per cent for the smallest leaf area. The expected genetic advance as a percentage of the mean (GAM) ranged from 1.77 per cent for leaf yield to 254.25 per cent for the largest leaf area, followed by 142.40 per cent for the smallest leaf area.

The analysis of trait variability and association of a trait with other traits contributing to crop yield would be important in planning a successful breeding program (Mary & Gopalan, 2006). The observed variability is a composite of genetic and environmental effects, of which only the former is inherited. Estimates of heritability, on the other hand, do not provide an idea of the expected gain in the next generation and must be considered in conjunction with estimates of genetic advance, the change in mean value among successive generations (Al-Tabbal & Al-Fraihat, 2012; Ravi et al., 2013; Shukla et al., 2016).

The magnitude of GCV and PCV values indicate the degree of variability shown by different parameters. GCV revealed that the extent of genetic variability in the studied characters ranged from moderate to high. This suggests that there was a greater environmental influence on the expression of these traits, and selection may be effective in improving nightshade. Furthermore, for all traits studied, GCV values were low in magnitude when compared to PCV. This suggests that direct selection would be ineffective in these traits. These findings are consistent with Singh et al. (2000) on poppy and Singh and Singh (1999) on lemongrass. Although the

genotypic coefficient of variation revealed the extent of genetic variability present in accessions for various traits, it does not provide a complete view of heritable variation. Heritable variation is useful for permanent genetic improvement according to Singh (2000 cited in Al-Tabbal & Al-Fraihat, 2012); however, the extent of heritable variation cannot be ascertained using GCV alone. Thus, heritability estimates indicate the efficacy with which selection could be expected to exploit existing genetic variability as stated by Bilgin et al. (2010 cited in Nyadanu & Dikera, 2014).

Phenotypic and genotypic variances in a crop population are important for successful plant breeding. Broad-sense heritability (H^2) is expressed as the percentage of the ratio between the genotypic variance and phenotypic variance. According to Johnson et al. (1955) heritability is classified as low (below 30%), medium (30 – 60%) and high (above 60%); and genetic advance (as a percentage of mean) is classified as low (<10%), moderate (10 – 20%) and high (> 20%). In the current study, the smallest leaf area had a high heritability in the broadest sense. The highest heritability estimates were for the smallest leaf area (70.59%), followed by a relatively high moderate heritability for the biggest leaf area (50.94%) (Table 4.6). The heritability estimates for the number of leaves, number of primary branches, leaf dry mass, biggest leaf area, days to 50% flowering, days to fruit forming, and days to fruit ripening were 49.79, 48.88, 35.26, 50.94, 42.18, 48.18, and 36.84%, respectively, whereas all other traits had low estimates.

Though heritability estimates may not provide clear predictability of breeding values, they are usually more useful than simple heritability values in predicting the resultant effect of selecting the best individual. According to Johnson et al. (1955), heritability coupled with genetic advance is a significant factor in predicting the resultant effect for selecting the best individuals. Harvest index was low in GA with moderate GAM, revealing that the trait was governed by additive gene action and low heritability, revealing that this trait was influenced by environmental influence. For the number of leaves and largest leaf area, there was moderate broad sense heritability combined with high genetic advance, indicating that the traits are primarily controlled by the additive type of genes. However, for many primary branches, leaf dry mass, days to 50% flowering, days to fruit forming, and days to fruit ripening, respectively, moderate heritability was observed, along with low GA and high GAM (Table 4.6), respectively. As a result of these findings, it was determined that the action of additive genes involved in the expression of these traits, as well as the potential for improvement through

selection must be rewarding. According to Osman and Khirdir (1974 cited in Méndez-Natera et al., 2012), low heritability combined with low genetic advance indicates non-additive gene effects, and thus a low genetic gain from a selection is expected. Plant height, stem diameter, leaf chlorophyll content, leaf fresh mass per plant, and harvest index all had low heritability and low genetic advance, indicating the involvement of non-additive genes in the expression of these traits and the limited scope for improvement through direct selection. The current study's findings are consistent with previous reports on amaranths by Sharma et al. (2010) and Adeniji (2018). Akter (2010) discovered a low heritability (16.09%) with a low genetic advance (0.16) for seed weight in Mustard, while Saifullah (2010) discovered a high heritability (65.03%) with a low genetic advance (0.31%).

The heritability of the number of fruits, fruit fresh mass, stem yield, fruit yield and total biomass was low, despite high genetic advance (Table 4.6). Such traits are more influenced by the environment, making selection ineffective for them as indicated by Sharma et al. (2017 cited in Suganthi et al., 2018). Furthermore, traits that exhibit high heritability with moderate or low genetic advance can be improved by inter-mating the superior accessions of the segregating population developed from multiple crosses and the desirable genes can be accumulated in the lines as stated by Liang and Walter (1968 cited in Suganthi et al., 2018). On the other hand, an association of high heritability with high genetic advance is an indication of additive gene effects and consequently, a high genetic gain from selection would be anticipated according to Panse (1957 cited in Ravi et al., 2013).

According to the results of this study, if the top performing 5% were selected as parents, the mean biggest leaf area, smallest leaf area, fruit fresh mass, number of leaves, number of primary branches and days to 50% flowering for progenies would improve by 79.91 cm^2, 8.26 cm^2, 93.61 g/plant, 88.06, 16.88 g/plant, and 9.97 days, respectively. When combined with heritability estimates, the estimate of genetic advance is more useful as a selection tool according to Johnson et al. (1955 cited in Al-Tabbal & Al-Fraihat, 2012). Estimating genetic progress aids in understanding the type of gene action involved in the expression of various polygenic traits (Al-Tabbal & Al-Fraihat, 2012; Nundwe, 2018).

High GA values indicate additive gene action, whilst low values indicate non-additive gene action (Singh & Narayanan, 1993; Al-Tabbal & AL-Fraihat, 2012; Nundwe, 2018). As a result, the heritability estimates would be useful if they are accompanied by a high genetic advance.

According to the findings of this study, the highest expected GAM was observed for the largest leaf area, smallest leaf area, fruit fresh mass, number of leaves, number of fruits per plant, leaf dry mass, number of primary branches, fruit yield, leaf chlorophyll content, days to 50% flowering, and leaf fresh mass, (Table 4.6), respectively. Ravi et al. (2013), Yadav et al. (2008), and Suganthi et al. (2018) reported similar findings of high genetic advance over a mean number of leaves and other related traits. According to Al-Tabbal and Al-Fraihat (2012), high values indicate that these traits are simply inherited, and the heritability is most likely due to additive gene effects, and selection may be effective in early generations for these traits. As a result, selecting traits with high GAM will improve the performance of nightshade accessions for these traits.

Table 4.6: Estimation of genetic parameters for agronomic and yield contributing traits of 15 nightshade accessions selected at 5% selection intensity

Variables	Mean Range	X	$\sigma^2 g$	$\sigma^2 p$	H^2 (%)	GCV (%)	PCV (%)	GA at 5%	GAM (%)
PH cm	43.50 - 66.83	55.54	94.77	609.62	15.55	17.53	44.45	7.91	14.24
NLS	85.47 - 185.36	122.77	3670.19	7371.14	49.79	49.35	69.93	88.06	71.73
NBPr	5.06 - 9.41	7.72	7.20	14.72	48.88	34.75	49.70	3.86	50.05
SD mm	8.03 - 16.42	10.83	7.79	70.31	11.08	25.79	77.50	1.91	17.68
ChloroC	22.20 - 39.34	27.63	80.29	288.12	27.87	32.43	61.43	9.74	35.27
Psprd mm	359.17 - 515.94	436.27	2363.73	22888.89	10.33	11.14	34.68	32.19	7.38
Nfruit	82.67 - 300.06	163.87	11687.08	52188.94	22.39	65.97	139.41	105.39	64.31
FFM g	18.74 - 140.32	79.18	6968.44	23517.40	29.63	105.43	193.68	93.61	118.22
LFM g	37.85 - 74.88	54.73	359.39	1923.59	18.68	34.64	80.14	16.88	30.84
LDM g	9.62 - 19.91	13.42	38.04	107.88	35.26	45.96	77.39	7.54	56.22
SLarea cm^2	3.16 - 9.77	5.80	22.78	32.27	70.59	82.28	97.95	8.26	142.40
BLarea cm^2	9.16 - 101.84	31.43	2953.90	5798.66	50.94	172.92	242.28	79.91	254.25
ND_F	26.33 - 36.83	30.32	55.52	131.62	42.18	24.57	37.84	9.97	32.88
ND_FF	41.43 - 51.50	45.00	44.32	91.97	48.18	14.79	21.31	9.52	21.15
ND_FR	47. 67 - 58.67	51.80	33.56	91.08	36.84	11.18	18.42	7.25	13.98
LY_gp	301.60 - 805.30	593.19	1143.67	50072.00	2.28	5.70	37.72	10.53	1.77
SY_gp	466.50 - 1376.90	972.99	13508.37	205331.78	6.58	11.95	46.57	61.41	6.31
FY_gp	173.40 - 1782.00	871.16	156845.84	682966.73	22.97	45.46	94.86	390.97	44.88

TBiomass_gp	1044.00 - 3347.60	2437.37	215568.63	1632209.40	13.07	19.05	52.42	347.59	14.26
HrvstInd (%)	19.60 - 38.88	27.85	20.53	76.51	26.83	16.27	31.41	4.83	17.36

MR- mean range, X-grand mean, $\sigma^2 g$ -genotypic variance, $\sigma^2 p$ – phenotypic variance, H^2- Broad sense heritability, PCV% -phenotypic coefficient of variation, GCV% - genotypic of variation, GA- genetic advance, GAM% -genetic advance as mean percentage. PH- plant height, NLS- number of leaves, NPBr- number of primary branches, SD- stem diameter, ChloroC- leaf chlorophyll content, Psprd- plant spread, Nfruit- number of fruits, FFM- fruit fresh mass, LFM-leaf fresh mass, LDM- lead dry mass, SFM-stem fresh mass, SDM- stem dry mass, SLarea- smallest leaf area, BLarea- biggest leaf area, ND_F- number of days to 50% flowering, ND_FF- days to fruit forming, ND_FR- days to fruit repines. LY_gp-leaf yield in grams per plant, SY_gp-stem yield in grams per plant, FY_gp-fruit yield in grams per plant, TBiomass_gp - total biomass in grams per plant, HrvstInd- harvest Index percentage.

4.8 Conclusion

In the current analyses of phenotypic diversity of nightshade accessions using both agronomic and quantitative morphological traits, a variety of observations were made. For all traits, all 15 accessions varied significantly from others, indicating the presence of significant variation among the accessions. Correlation studies are important in breeding programs since they help breeders understand the interrelatedness among various agronomic and morphological traits and use the results for selection during nightshade breeding work. This study also revealed that some morphological traits distinguished accessions more effectively than others. The study also revealed that quantitative traits are important in assessing genetic diversity within nightshade accessions and could be used to select relevant accessions that could be incorporated into breeding programs for further crop improvement. However, for all of the traits studied, the phenotypic variance and phenotypic coefficient of variation were greater than the corresponding genotypic variance and genotypic coefficient of variation. Considering all of the traits associated with high yielding and late-maturing accessions, Scabrum, Nshad40, and NigSN18 have the potential to contribute to the development of improved varieties that could become a trademark in South African nightshade. It is reasonable to conclude that there is room for further research with Ncampus and Nfarm, which were the earliest maturing accessions with high leaf yield but low fruit yield due to fruit dropping when ripe.

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Chapter 5:

Analysis of yield and its contributing traits in nightshade accessions

5.1 Abstract

This study was conducted to evaluate the performance of 15 accessions of nightshade over two seasons (data combined) for yield and yield characteristics, in order to assess the presence of variability for desired traits and a significant amount variation for different traits. A field evaluation of selected nightshade germplasm was conducted at the North-West University, Mafikeng, South Africa to identify superior parents for selection. The experiement was laid out in a randomized complete block design (RCBD) with three replications. A correlation was estimated for all traits, and analysis of variance revealed significant differences among traits. Leaf yields of nightshade were lower than their potential, partly due to a lack of improved varieties. Accession Scabrum and Ncampus had significantly high leaf yields of 2684.40 and 2395.40 kg $ha^{-1,}$ respectively. Among the accessions, NigSN18 and ManTown had the highest fruit yields of 5940.10, and 5026.40 kg ha^{-1}, respectively. For example, the number of leaves per $plant^{-1}$ had an insignificant relationship with leaf yield and most of the traits studied, implying that selection with these traits may be possible without sacrificing yield. Therefore, associated selection for more leaves and high leaf yield would be effective for the improvement of the vegetable nightshade. These present results are in line with previous studies, and the selected four were used as parents for selection based on their superior yield. Accession Scabrum assessed for the present study was observed to be late flowering with large succulent stems and leaves, where the accumulation of photoassimilates was key to high leaf yield. While accession Ncampus was early flowering, high branching with more thin, succulent young shoots and leaves, resulting in high leaf yield, however, fruit yield was low due to fruits dropping when ripe.

Keywords: Accessions, Correlation, Fruit yield, Leaf yield, Stem yield, Variation.

5.2 Introduction

Nightshade, also known as Black nightshade or African nightshade, is a member of the Solanaceae family. African nightshades are one of many underutilized and abandoned African indigenous vegetable (AIV) species that, if properly exploited, could improve food, nutrition, and income among rural populations (Abukutsa, 2003; Abukutsa-Onyango, 2007; Dinssa et al., 2016). The crop is primarily used as a leafy vegetable, but it is also used as herbal medicine by some people (Schippers 2002). Despite their potential, most indigenous vegetables remain at the landrace level, with only a few developed cultivars (Afari-Sefa et al., 2012). Leafy vegetables are a valuable part of the diet because of their nutritional value, which plays an important role in the human diet (Prakash & Pal, 1991; Shukla et al., 2003).

In most Sub-Saharan African regions, AIVs are regarded as potential cash crops because they contribute to income generation for individuals and households (Shackleton et al., 2009). For generations, AIVs have been a part of Sub-Saharan Africa's food systems, and their leaves, young shoots and flowers are consumed for a variety of purposes (Ambrose-Oji, 2009; Abukutsa-Onyango, 2010; Yang and Ojiewo, 2013). Nightshades are among the high-priority AIVs with the potential to improve health, nutrition and economics (Edmonds & Chweya, 1997; Yang & Ojiewo, 2013).

Solanum americanum, *Solanum scabrum*, *Solanum nigrum*, and *Solanum villosum* are the most common nightshade species in SSA (Edmonds & Chweya, 1997; Keller, 2004; Yang & Ojiewo, 2013; Yuan et al., 2018). The *Solanum nigrum* complex, also known as the black nightshades complex, consists of eight species: *S. nigrum* L., *S. americanum*, *S. scabrum* Mill, *S. villosum* Mill, *S. grossedentatum* A. Rich., *S. florulentum* Bitter, and *S. tarderemotum* Bitter (Dehmer & Hammer, 2004; Edmonds & Chweya, 1997; Ojiewo et al., 2013; Mwai et al., 2013; Ronoh et al., 2017). *Solanum scabrum* and *S. villosum* are most common in East Africa (Ambrose-Oji, 2009), but they are also grown in other parts of Africa.

However, the leaf yields of these nightshades are relatively low. Early flowering and excessive fruiting are major constraints to leaf expansion, with the latter being significantly reduced after anthesis and nearly stopped at the fruit set. The species in the *Solanum* genus are mostly autogamous, which favours the production of a large number of small fruits and seeds. According to Salisbury (1961 cited in Ojiewo et al., 2005), large plants produce over 3600 fruits, each of which contains around 40 seeds, for a total of nearly 150 000 seeds per plant.

With this level of fruit and seed production, competition for carbohydrates and nutrients between fruits and leaves is obvious.

According to studies, the edible leaves of African nightshade produce the highest yield, ranging between 12 and 50 tons ha^{-1} per season (Edmonds and Chweya, 1997; Ojiewo et al., 2013; Mbwambo et al., 2013; Abukutsa-Onyango, 2015). Despite this, Mbwambo, et al. (2013) and Molina et al. (2020) reported annual yields of 1.5–3.0 and 3.85 tons ha^{-1}, respectively. The average proportion of cultivated nightshade varies by location, with the Arumeru district in Arusha, Tanzania, accounting for 20% of all cultivated land in Africa (Weinberger & Msuya, 2004; Dinssa et al., 2016;).While in South Africa, the production and market for nightshade have not been documented. The species is highly adaptable to a wide range of soil and agro-climatic conditions, and it has a high level of genetic variability and plasticity (Dusgupta & De, 2007; Routray et al., 2012).

Nightshade genetic improvement is highly reliant on germplasm collection and assessment of the magnitude of genetic variability existing in the material for quantitative and qualitative characters, as well as their interrelationship, which is a necessary prerequisite for formulating and accelerating the conventional breeding program to achieve higher yield with better quality. The analysis of correlation coefficients aids in determining the relative contribution of component traits to yield (Panse, 1957). However, the correlation between yield and a component trait can be deceptive at times. It has been difficult to improve the fresh yield of nightshade through knowledge of the variability and linkage among yield-contributing traits. As a result, the study's objectives were to analyze the yield variability and yield contributing traits on the yield of 15 nightshade accessions, as well as to determine the contribution of the component traits to yield, and finally to find appropriate selection traits for improvement of leafy vegetable nightshade.

5.3 Soil analysis, experimental design, and crop husbandry.

These have been described in Chapter 4, section 4.3, and subsections 4.3.1–4.3.2.

5.4 Data collection

5.4.1 Quantitative characteristics

To avoid bias, quantitative data for all agronomic characters were collected in the field, including plant height, number of leaves, number of primary branches, small leaf area (the third leaf from the tip of the main stem), biggest leaf area, number of days to 50% flowering, yield (viz., leaf, stem, and fruit) in kg ha^{-1}, total biomass in kg ha^{-1}, and harvest index in percentage. Measurements and counts of a given trait were done on the same day in the field-grown nightshade accessions.

5.4.2 Growth components

These have been described in Chapter 4, section 4.4, subsection 4.4.1

5.4.2.1 Yield and yield components

The number of leaves, primary branches, and stem diameter per plant was counted and measured for six randomly selected plants per plot at maturity, and the mean was calculated. The number of days to 50% flowering was recorded when half of the plants in each plot had flowers. Leaf, stem, and fruit yield per plot were determined by taking the fresh mass of each trait of the harvested plants from the net plot and calculating the mean in kg ha^{-1}.

5.5 Data analyses

The yield and its contributing characteristics data were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedures of SAS Version 9.4 at 5% probability level. Tukey's test was used to compare mean values. A correlation analysis was performed in SAS to estimate yield and yield-related linkage among the characters and to identify key agronomic and yield-related characters important in nightshade breeding studies.

5.6 Results and Discussion

5.6.1 Analysis of variance

The current study was carried out to determine the variability among 15 nightshade accessions, and data were collected on various characters. The data were statistically analyzed, and the results are presented in Table 5.1.

Table 5.1: Analysis of Variance for 11 yield and yield associated traits

		Mean squares											
Source of variance	**DF**	**PH cm**	**NLS**	**NBPr**	**SD mm**	**SLerea cm^2**	**BLerea cm^2**	**ND_F**	**Leaf_Y kg ha^{-1}**	**Stem_Y kg ha^{-1}**	**Fruit_Y kg ha^{-1}**	**TBiomass kg ha^{-1}**	**Harvest Index (%)**
SEASON	**1**	37500.00	386082.81	18.51	941.84	22.97	998.11	4611.26	13604049.80	64176325.34	156094817.50	585607154.40	785.23
Rep	**2**	2272.20	126599.01	31.57	13.59	38.43	2009.37	270.06	713477.79	13566123.65	6063425.70	46803093.80	289.51
Access	**14**	1541.61	28148.89	56.33	152.50	154.27	23268.50	481.20	1004255.55	4659295.56	21601299.30	43305722.60	224.56
SEASON*Access	**14**	972.97	6127.77	13.14	105.78	17.61	5545.11	148.12	928013.67	3758740.36	11144908.30	28926221.70	101.47
Residual Error	**508/58**	170.12	3822.37	5.70	57.79	4.14	433.21	12.29	477853.01	1512005.90	1640018.00	7713300.00	31.45

DF- degree of freedom, Rep-replicate, Access-accession, S*Access- season by accession interaction,PH- plant height in centimeter, NLS- number of leaves, NPBr- number of primary branches, SD- stem diameter in millimeters, SLarea- smallest leaf area , BLarea- biggest leaf area, ND_F- number of days to 50% flowering, Leaf_Y- leaf fresh yield in kg ha^{-1}, Stem_Y -stem fresh yield in kg ha^{-1}, Fruit_Y -fruit fresh yield in kg ha^{-1}, TBiomass in kg ha^{-1}, and HrvstIndex- harvest index in per cent.

5.6.2 Variation among yield and yield-related traits

The analysis of variance revealed significant differences ($p<.0001$) in yield and yield-related parameters among the accessions. These differences indicated the presence of a wide range of variability and significant room for improvement in the accessions studied. The edible parts of the nightshade plant are the leaves, while the stems, particularly the tender young shoots, are sometimes consumed alongside the leaves. Scabrum had the highest leaf yield in kg ha^{-1} (2684.40), followed by Ncampus (2395.40), with Sharon having the lowest yield of 1977.33 kg ha^{-1}. In terms of stem yield, N5547 had the highest yield (4589.50 kg ha^{-1}), followed by Scabrum (4173.40 kg ha^{-1}), and Sharon had the lowest yield (1555.00 kg ha^{-1}) with an average of 3243.31 kg ha^{-1}. Furthermore, high yields were recorded for NigSN18, ManTown and Nshad5 (Table 4.2). In comparison to the study findings, a previous report by Ojiewo et al. (2013) reported low values of fruit yield in kg ha^{-1}, while they reported higher leaf yields in t ha^{-1}. Stoilova et al. (2015) reported a low yield of African nightshade accessions ranging from 224.9 g/plant to 512.2 g/plant, which was the highest compared to amaranth and spider plant in their study.

Total biomass among evaluated nightshades ranged from 11160.00 kg ha^{-1} (ManTown) to 10636.00 kg ha^{-1} (Scabrum), to 3481.00 kg ha^{-1} (Sharon), with an average of 8126.33 kg ha^{-1}, while harvest index as a percentage ranged from 38.87 per cent (Nfarm) to 37.67 per cent (Ncampus), to 19.59 per cent (SRetrflx), with a mean of 27.85 per cent.

The leaves, tender young shoots, and ripe fruit of nightshade are the most commonly used parts for food, traditional medicine and the production of jam and dye. The study found significant variation in yield and yield contributing characteristics among evaluated accessions. The grand means for yield and yield-related traits in the study were 55.54 cm, 122.78 , 7.72 , 10.82 mm, 5.79 cm^2, 31.43 cm^2, 30.32 days, 1977.33 kg ha^{-1}, 3243.31 kg ha^{-1}, 2903.90 kg ha^{-1}, 8126.3 kg ha^{-1}, and 27.84 per cent, respectively. The study findings agree with previous reports by Ronoh et al. (2018) for leaf area mean value of 30.75 cm^2 for *S. villosum* entries, while Ravi et al. (2013) reported a higher mean value for leaf area in their report. The study showed significant variability in leaf yield of evaluated nightshade accessions.

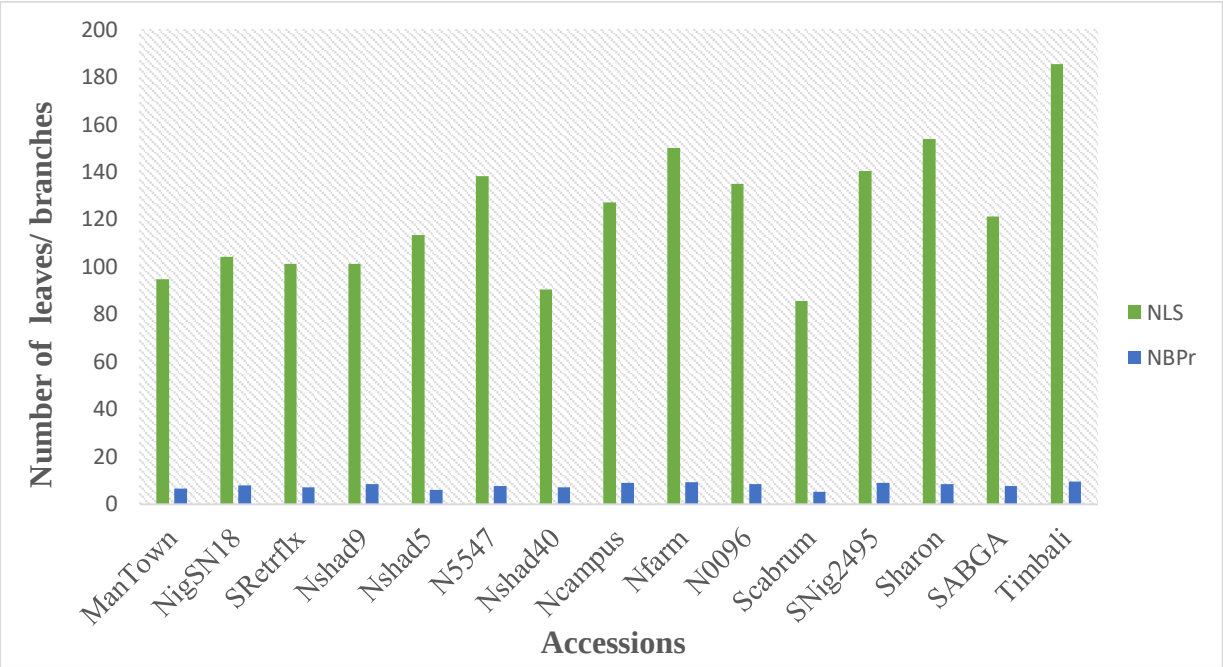

Figure 5.1: Mean performance for number of leaves (NLS) and number of primary branches (NPBrR plant^{-1}) of the 15 nightshade accessions under the study

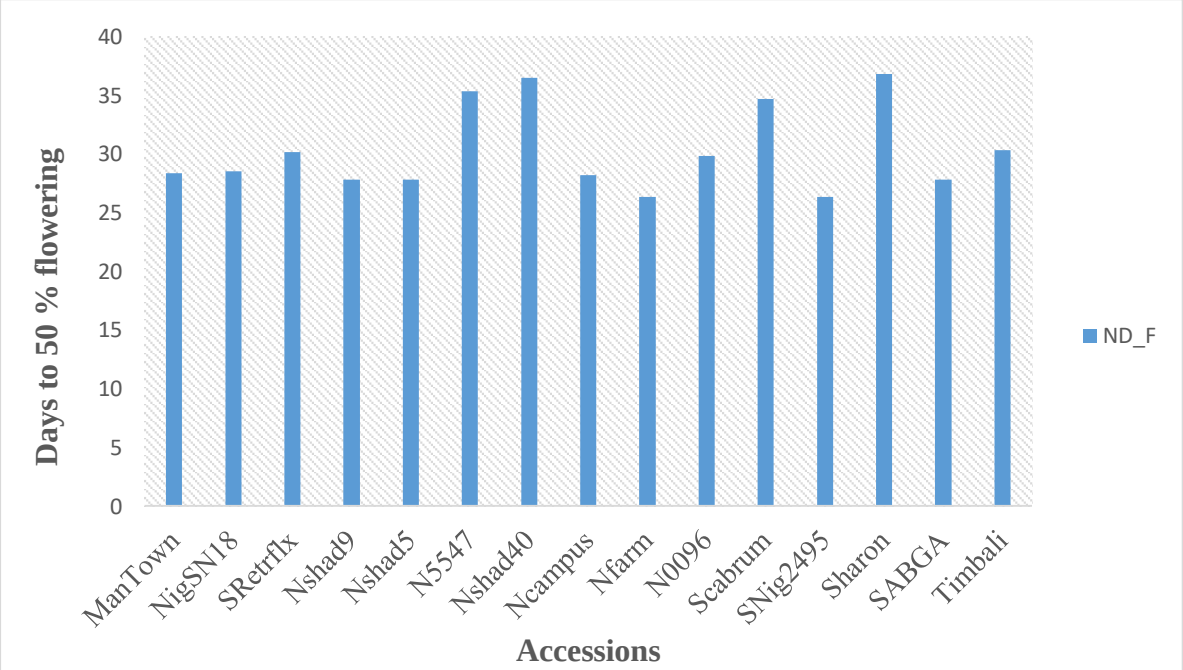

Figure 5.2: Mean performance of number of days to 50 % flowering of the evaluated nightshade accessions under study.

Table 5.2: Yield and yield associated variation among nightshade accessions evaluated

Access	PH (cm)	NLS	NBPr	SD (mm)	SLarea (cm^2)	BLarea (cm^2)	ND_F	Leaf_Y kg ha^{-1}	Stem_Y kg ha^{-1}	Fruit_Y kg ha^{-1}	TBiomass kg ha^{-1}	HrvstIndex (%)
ManTown	61.33ab	94.67df	6.41de	12.16ab	6.10b	51.61c	28.33cf	2173.20ab	3957.70ac	5026.40ab	11160.00a	20.77de
NigSN18	43.50d	104.14de	7.78ad	11.58ab	6.50b	22.36de	28.50bc	1775.30ab	2550.40ac	5940.10a	10268.00ac	21.86ce
SRetrflx	56.11bc	101.28de	6.97be	11.57ab	6.68b	33.26d	30.16b	2039.10ab	4022.10ac	4514.00ac	10578.00ac	19.59e
Nshad9	59.83ac	101.19de	8.31ac	10.82ab	6.24b	24.66e	27.83bc	2041.70ab	3623.00ac	4545.70ac	10213.00ab	21.28de
Nshad5	49.61cd	113.31be	5.97de	10.18b	6.14b	23.19e	27.83bc	1934.00ab	3186.90ac	4779.10c	9902.00ac	26.29be
N5547	66.83a	138.22ae	7.61ad	10.94ab	6.64b	23.40de	35.33a	2285.00ab	4589.50a	2190.30ce	9067.00ad	26.47be
Nshad40	57.89ac	90.39ef	7.08cd	16.42a	9.72a	70.31b	36.50a	2263.50ab	3975.80ac	2932.60be	9174.00ac	29.41ae
Ncampus	51.83bd	127.08be	8.86ab	9.83b	4.14c	20.49de	28.17bc	2395.40ab	3038.80ac	760.50e	6196.00ad	37.67ab
Nfarm	53.67bd	150.08ac	9.31a	9.84b	4.00c	14.30e	26.33c	2040.00ab	2856.00ac	578.10e	5475.00bd	38.87a
N0096	61.63ab	135.06be	8.33ac	8.03b	3.38c	10.17e	29.83b	1419.40ab	2051.10bc	1016.00e	4487.00cd	30.40ae
Scabrum	56.81ac	85.47f	5.06e	12.54ab	9.77a	101.84a	34.67a	2684.40a	4173.40ab	3775.80ad	10636.00ab	30.18ae
SNig2495	50.39cd	140.33ad	8.81ab	9.39b	4.41c	20.63de	26.33c	1911.20ab	2934.50ac	966.00e	5813.00ad	33.21ac
Sharon	45.28d	153.92ab	8.39ab	9.33b	3.16c	9.16e	36.83a	1005.20b	1555.00c	919.70e	3481.00d	29.38ae
SABGA	56.14bc	121.14be	7.53ad	11.63ab	6.53b	35.13cd	27.83bc	2074.30ab	3871.60ac	4439.00ac	10387.00ab	20.99de
Timbali	62.31ab	185.36a	9.42a	8.18b	3.52c	10.99e	30.33b	1618.30ab	2263.80ac	1175.20de	5058.00bd	31.32ad
HSD	**	***	***	**	**	***	*	**	***	***	***	***
Fpr	<.0001	<.0001	<.0001	0.0010	<.0001	<.0001	<.0001	0.0250	<.0001	<.0001	<.0001	<.0001
StD	6.54	27.29	1.25	2.06	2.07	25.42	3.66	409.11	881.21	1897.42	2686.54	6.11
GM	55.54	122.77	7.72	10.82	5.80	31.43	30.32	1977.33	3243.31	2803.90	8126.33	27.84

Access- accession, PH cm- plant height in centimeter, NLS- number of leaves, NPBr- number of primary branches, SD- stem diameter in millimeters, SLarea- smallest leaf area, BLarea- biggest leaf area, ND_F- number of days to 50% flowering, Leaf_Y- leaf fresh yield in kg ha $^{-1}$, Stem_Y -stem fresh yield in kg ha $^{-1}$, Fruit_Y -fruit fresh yield in kg ha $^{-1}$, TBiomass in kg ha $^{-1}$, and HrvstIndex- harvest index in per cent. Fpr- F probability for accessions, HSD- Honest Significant difference at the 0.05 probability level, StD- standard deviation, and GM- grand mean. * - $p<0.05$, ** - $p<0.01$;*** -$p<0.001$. Tukey pairwise comparisons analysed at $p < 0.05$ significant level. Means within columns that do no share a letter are significantly different.

5.6.3 Yield variation among nightshade accessions

During the initial evaluation for the two combined successive seasons, yield and its contributing traits showed considerable relative proportions. Figure 5.3 is an illustration of the relative proportions for leaf, stem and fruit yields. The term yield in this study was used to include all the aboveground parts of the plant that are usually harvested and consumed as a vegetable, thus it included 4 to 5 leaves and young tender shoots (with a length of about 5 to 8 cm), depending on the genotype stem and branches attributes (Figure 5.4).

Yield and yield associated parameters that were measured varied with accessions. Of the 15 accessions assessed Scabrum and Nshad40 were noted to have plants with the longest and broadest leaves, however, the accessions attained fewer leaves and branches compared to other accessions. Whereas accessions Ncampus, Nfarm, and SNig2495, had plants with numerous primary branches resulting in plants with smaller leaves in size but more leaves per plant in number. Figure 5.3 is demonstrative of the relative proportion of yield characteristics relative to the yield contributing parameters towards the yield. Accessions Ncampus and Nfarm were noted to be relatively high in leaf fresh yield with the distribution frequency between 35-40%, and the stem yields for these accessions were also high ranging between 38-89%, while the fruit yields were low. These observations are due to the nature of the plants, as they are found to have plants with numerous leaves, highly branching resulting in various tender young shoots contributing to leaf fresh yield measured and reported under a study. The least fruit fresh yields were due to these accessions having fruits dropping from calyxes and birds feeding on the fruits when ripened. A similar trend was noted for accessions N0096, Timbali and Shoran, however, at the time of harvest, these three accessions had woody young shoots unlike Ncampus, Nfarm, and SNig2495 which had tender shoots. Furthermore, all these accessions attained a high leaf yield proportion irrespective of the size of the leaves and stems (stem diameter).

The accessions Nshad40, N5547 and Scabrum leaf yield relative proportion was low (varying between 25 and 30%) compared to stem and fruit fresh yield proportion. This proportion was due to yield associated traits (viz., the number of leaves, primary branches, leaf size (area) and stem diameter), contributing to the frequency distribution. Scabrum accession had plants with fewer primary branches, few but broadest of leaves, and larger succulent stems which became woody once the plant had completed its life cycle and late-flowering while the plants themselves were intermediate to tall in height. Thus, few numbers of leaves resulted in a

relatively low to moderate leaf fresh yield distribution. Leaf yield in African nightshades can be considered in terms of fresh leaf mass as well as leaf number (Masinde et al., 2010), however, the number of branches should also be taken into consideration for selection and improvement of high yielding genotypes. The evaluated yield contributing traits is evidence that the number of branches is key to the improvement of nightshades. For instance, accession Ncampus recorded high leaf fresh yield, followed by Scabrum (Table 5.2), regardless of their differences in the number of primary branches and leaf size. This means selection of these accessions for crossing might result in plants with larger stems, numerous branches, and intermediate to late flowering and broadest leaf varieties.

Fortuin and Omta (1980), reported a similar biomass distribution for their study of *Solanum nigrum* L, Yulianti et al. (2018), also reported dry weight proportion for *Solanum nigrum* Linn, and these previous findings are comparable to the current study findings.

The leaf fresh yield obtained from Ncampus, Nfarm, SNig2495, Scabrum, and Nshad40 can be considered for selection and improvement, due to their marketable quality. All the accessions under study produced reasonable yield regardless of the soil status, however, literature has indicated that nightshades require fertile soils. Comparable ranges of leaf yield in nightshades and amaranth genotypes have been reported in several trials (Manoko, 2007; Manoka et al., 2007; Mwai, 2007; Mwai et al., 2007; Manoko et al., 2008, 2012; Ondiek et al., 2011; Ravi et al., 2013; Sarker et al., 2014). Preceding reports on high leaf yields in some of the *Solanum scabrum* lines have stated that these high leaf yields may be attributed to their yield associated characteristics such as late flowering and fruiting, larger leaves, larger succulent stems and branches especially before anthesis and fruit set (Schippers, 2000; Mwai et al., 2007, 2009a, 2009b; Ondiek et al., 2011; Ojiewo et al., 2013; Rono, 2018). As expected, the study noted the Scabrum accession which had all the attributes of yield contributing traits.

Accessions NigSN18, ManTown, SRetrflx, Nshad9, Nshad5, and SABGA had a relatively low leaf fresh yield proportion compared to all other accessions evaluated. These six accessions had relatively higher fresh fruit yields, but a low stem yield distribution than other accessions. However, these accessions had plants with woody stems, and harder young shoots which resulted in the harvesting of only leaves at the time of trial termination. Though harvesting was only done at maturity when farming for fresh vegetable production and marketing purposes, these stems and tender shoots could be harvested at vegetative growth stages when the stems

and young shoots are still tender and consumable since harvesting of leafy vegetables has been known to initiate more branches, thus resulting in continuous growth and harvesting. Although in South Africa, marketing data for nightshades has not been documented, the crop is sold and consumed by a relatively low population and mostly adults. Muthoni et al. (2012), stated that this genus has an important economic value with many cultivated species as sources of edible vegetables and fruits, thus, suggesting the importance of leaf and fruit yields.

Based on the fruit fresh yield, NigSN18 attained a relatively higher fruit yield, followed by the accession ManTown. The accession NigSN18 was characterized by dwarf plants, with small leaves (size), more branches and intermediate flowering type of plants, large fruits remaining on the plant when ripe, while ManTown was characterized by tall plants that were high yielding after NigSN18. These attributes are important criteria in participatory selection, Jagatheeswari et al. (2013) indicated that both leaves and fruits are used as a source of dyes. Nzioka (1994) and Boik (1996) also reported that ripe fruits (purple/black) are used as a source of ink. Thus, suggesting the importance of fruits for parents' selection in breeding programs. Breeding for accessions that are high fruit yielding cannot only benefit the individual and/or consuming fruits of this species, but it will also benefit companies that use the fruits to produce jam, dyes, and ink. Unlike, previous reports on nightshade fruit yield, this present study recorded NigSN18 (5940.10 kg ha^{-1}), followed by Nshad5 (5026.40 kg ha^{-1}) to be higher than those of Scabrum (3775.80 kg ha^{-1}) (Table 5.2), respectively.

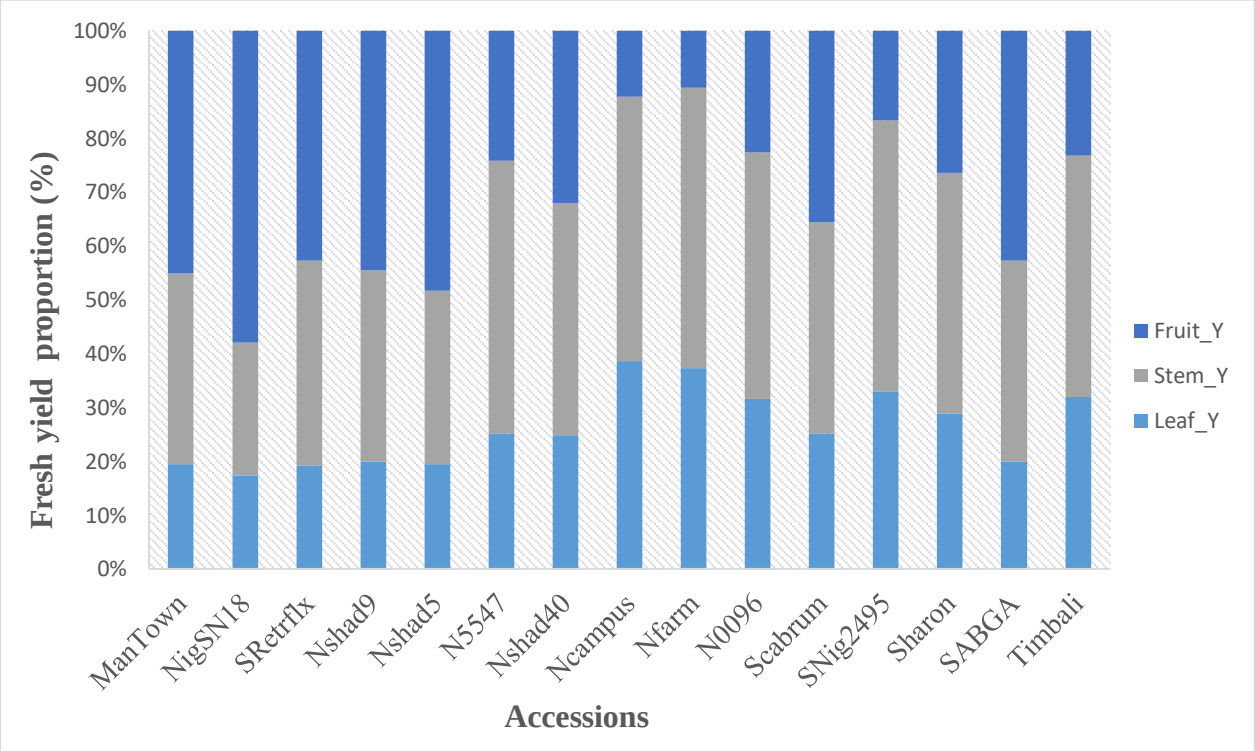

Figure 5.3: Relative proportion of yield contributing traits towards fresh leaf, stem, and fruit fresh yield of the assessed nightshade accessions

The results for most of the yield and yield contributing characteristics under evaluation were comparable to those of other studies (Abukutsa-Onyango, 2007; Mwai et al., 2007; Ojiewo et al., 2013; Ravi et al., 2013). The time between transplanting or sowing and flowering severely limits leaf productivity and yield (Ojiewo et al., 2013). Deflowering was performed on accessions Ncampus, Nfarm, SNig2495, N0096, and Timbali just a day before transplanting. In a study conducted by Mwafusi (1992), defoliation was found to increase leaf yields by 40%. Flower removal as soon as they appear increased leaf yield and prolonged harvesting periods, indicating the importance of selecting late-flowering accessions, according to Ojiewo et al. (2013) and Wangolo et al. (2015). Ferre et al. (2009) and Masabni et al. (2016) reported delayed flower emergence due to shade treatments, which Kartika et al. (2015) also reported in other Solanaceae species (*Lycopersicum esculentum* Mill).

The current study observed early flowering in nightshade, however, flower removal was not used as a treatment. As a result, this present study could not confirm that deflowering resulted in increased leaf yields, as observed for Ncampus and Nfarm, which could be the result of flower removal before transplanting. Late flowering accessions produced a lot of leaves

(Scabrum and Nshad40), but not much fruit. However, intermediate flowering accessions (NigSN18 and ManTown) produced the most fruit. According to Ojiewo et al. (2005), early flowering and excessive fruiting are major constraints to leaf expansion, with the hindmost being markedly reduced after flowering and almost stopping at the fruit set. For all of these traits, the study found significant variation among accessions indicating a potentiality for breeding superior populations (Awas et al. 2015).

Figure 5.4: Harvested nightshade accessions leaves with tender young shoots

5.6.4 Correlation studies among yield and yield contributing traits

The phenotypic correlation among yield and yield contributing characteristics is presented in Table 5.3. From these associations, it is seen that a higher number of leaves per plant was highly significant and positively associated with the number of primary branches (r=0.7679), and harvest index (r=0.5137). On the other hand, the number of leaves and number of primary branches showed a strong negative correlation with yield and yield contributing characters except for harvest index (Table 5.3), respectively.

The association between plant height, leaf yield and stem yield kg ha^{-1} showed significant positive association values (r=0.3217 and 0.4939, respectively), whereas other yield and yield-related parameters showed weak positive and negative associations (Table 5.3). Ronoh et al. (2019) reported similar trends for a weak correlation of traits (plant height and number of branches) in African nightshades. Sarker et al. (2014) revealed similar patterns in amaranth

genotypes. Wasonga et al. (2015) observed a positive and negative relationship between the number of leaves on a Spider plant and the number of primary branches and plant height. Ravi et al. (2013) revealed a positive association between similar Makoi (*Solanum nigrum*) traits.

Leaf area for both small, and biggest leaves had significant positive (r= 0.8745, 0.6970, and 0.6904, 0.7737 and 0.6398, 0.7946 and 0.5970, correspondingly) association with the biggest leaf area, leaf yield, stem yield and total biomass (Table 5.3). The traits (small and biggest leaf area) showed a considerable association with the number of days to 50 per cent flowering while noting a negative correlation with harvest index. A significant positive correlation was noted between stem diameter and all the other traits except for harvest index. Dube (2016), reported comparable results for *Corchorus*. The number of days to 50 per cent flowering displayed both positive and negative links with leaf, stem, and fruit yields as (r=-.0422, 0.1211, and -.1295), nonetheless these correlations were not significant. Wasonga (2014), reported a similar trend for the African Spider plant.

The leaf yield had a significant positive relationship with stem yield (r=0.8452), total biomass (r=0.6357), and a considerable association with fruit yield (r=0.2916). Fruit yield presented a strongly significant correlation (r=0.9086) with total biomass, while a strong significant negative link for this trait with harvest index (r=-.8528) was also exhibited. Additionally, a strong negative association between total biomass and harvest index (r=-.7299) was exhibited (Table 5.3).

This study's phenotypic association analysis (Table 5.3) revealed some interesting findings. The yield-associated traits demonstrated a significant moderate to high significant positive correlation with yield (leaf, stem and fruit) traits, implying that selection based on moderate to high positive correlation values of the yield contributing characteristics may lead to the development of high yielding nightshade accessions. The negative correlation indicates that selecting one trait may jeopardize the selection of the other trait. For example, the significant negative relationship between the number of leaves per plant and leaf area suggests that increasing the number of leaves may not be possible without compromising leaf size.

As reported in eggplant by Kaushik et al. (2018), positively correlated traits may have a common genetic and physiological basis. The negative correlation between the number of leaves and primary branches and other yield and yield contributing characteristics may not occur concurrently with either of the negatively linked traits. The current study found that yield

(i.e., leaf, stem and fruit) traits had a significant positive relationship with stem diameter and both small and largest leaf areas. The number of leaves had a strong positive relationship with the number of primary branches, as evidenced by significant correlation coefficient values. Thus, for the improvement of nightshade accessions, selection based on high yield associated traits (i.e., plant, stem diameter, small and largest leaf area) could be carried out without compromising yield traits (i.e., leaf, stem and fruit).

While analysing correlation coefficient values and interpreting the results, a great deal of variability in yield and yield contributing traits was observed among accessions. Breeders may use the current findings to develop high-yielding varieties in the future. Further research may be conducted to confirm the study in various locations throughout the North West Province for the reproducibility of our findings.

Table 5.3: Correlation estimation of yield and yield associated traits among nightshade accessions under study

Components	PH (cm)	NLS	NBPr	SD (mm)	SLarea (cm^2)	BLarea (cm^2)	ND_F	Leaf_Y kg ha^{-1}	Stem_Y kg ha^{-1}	Fruit_Y kg ha^{-1}	TBiomass kg ha^{-1}	HrvstIndex (%)
PH (cm)	1											
NLS	0.0329	1										
NBPr	-.0569	**0.7679****	1									
SD (mm)	0.0706	-.7558	-.5796	1								
SLarea (cm^2)	0.1659	**-.8043****	-.7726	**0.8879****	1							
BLarea (cm^2)	0.1838	-.7202	-.7719	**0.7607****	**0.8745****	1						
ND_F	0.1665	-.0707	-.2989	0.3938*	0.3794	0.3924*	1					
Leaf_Y kg ha^{-1}	**0.3217***	-.5766	-.4762	**0.5675****	**0.6970****	**0.6904****	-.0422	1				
Stem_Y kg ha^{-1}	**0.4939***	-.6251	-.5928	**0.6646****	**0.7737****	**0.6398****	0.1211	**0.8452****	1			
Fruit_Y kg ha^{-1}	-.0802	-.7417	-.6717	**0.5110****	**0.6151****	0.3990*	-.1295	0.2916	0.4807*	1		
TBiomass kg ha^{-1}	0.1543	-.8169**	-.7416	**0.6656****	**0.7946****	**0.5970****	-.0582	**0.6357****	**0.7964****	**0.9086****	1	
HrvstIndex (%)	-.1194	**0.5137****	**0.4779***	-.3344	-.3921	-.1603	-.0039	0.0071	-.3916*	-.8528**	-.7299**	1

PH- plant height, NLS- number of leaves, NPBr- number of primary branches, SD- stem diameter, SLarea- smallest leaf area, BLarea- biggest leaf area, ND_F- number of days to 50% flowering, Leaf_Y- leaf fresh yield in kg ha $^{-1}$, Stem_Y -stem fresh yield in kg ha $^{-1}$, Fruit_Y -fruit fresh yield in kg ha $^{-1}$, TBiomass in kg ha -1, and HrvstIndex- harvest index in per cent. **= Significant at 1% level; * = Significant at 5% level.

5.7 Conclusion

The yield contributing traits, viz., the number of leaves per plant stem diameter, small and biggest leaf area, as well as fresh leaf yield from the 15 leafy vegetable nightshade accessions evaluated can be used to select breeding parents and or cultivars. The correlation study revealed that selecting vegetable nightshade genotypes based on the number of leaves per plant, leaf area, and stem diameter could result in increased leaf yield. Selection of plant height, stem diameter and leaf area based on mean, range and correlation coefficient values would significantly improve the leaf yield of nightshade leafy vegetables. Associated selection based on high leaf and fruit yield would be effective for vegetable nightshade improvement. By analyzing and interpreting correlation coefficient results, significant variability was discovered among different yields and yield-contributing traits in the studied germplasm. Plant breeders can use this variability to create high-yielding varieties. The study was conducted in a single location, but future research may be conducted in multi-location trials to confirm the current findings and develop stable vegetable nightshade varieties. The association of yield-related traits revealed that breeders can increase fresh leaf yield while maintaining high leaf size and stem diameter.

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Chapter 6:

Variability in mineral nutrient content of nightshade accessions (*Solanum* Sect. *solanum*)

6.1 Abstract

This study aimed to evaluate nutrient composition variability in 15 nightshade accessions. The trials were in the field of the North-West University Mafikeng Campus Gardern for consecutive two seasons (2020 and 2021) implementing Randomized Complete Block Design (RCBD) with three replications. Food insecurity, poor livelihoods, and malnutrition crises have remained major challenges for many centuries in sub-Saharan Africa. Therefore, African/Indigenous leafy vegetables (A/ILVs) are key to the affordable and sustainable solution to the hunger and malnutrition menace in South Africa. The A/ILVs contain higher nutritional benefits than the exotic ones and can withstand both biotic and abiotic stresses. The nightshade vegetable is very rich in calcium, iron, magnesium, phosphorus and zinc. The leaves of nightshade accessions were analyzed for their nutritive value (nitrogen, calcium, magnesium, potassium, sodium, zinc, copper, manganese, iron, phosphorus, and aluminium). There were significant ($P< 0.05$) differences among the nightshade accessions studied with regards to mineral nutrient composition. The average nutrient composition for accessions was 2.97, 3.44, 0.78, 2.94, 0.20, 2341.54, 69.05, 30.94, 143.93, 1333.82, and 1841.75 mg/kg for N, Ca, Mg, K, P, Na, Zn, Cu, Mn, Fe, and Al, respectively. In this study, the PCV values were relatively higher than the GCV values for all traits. The first two major components (PC 1 and 2) accounted for 74.61% of the total variation in nutritional traits, with Ca, Mg, Na, Zn, Fe, Al, N, K, and P content contributing more to variability. The principal component biplots and cluster analysis among accessions revealed a significant level of variability. Using dendrogram cluster analysis, the accessions were differentiated based on nutritional features resulting into 14 clusters. Moderate heritability estimates for Fe (32.35%) and Al (34.78%) are accompanied by increasing genetic progress of 277.12 and 435.73, indicating the presence of additive gene effects that can be exploited through heterosis breeding. Meanwhile, moderate broad sense heritability together with moderate genetic advance was observed for Zn (32.48% and 11.24) and Mn (30.32% and 15.91), which is indicative of non-additive gene effect, which suggests slow progress during selection.

Keywords: Genetic advance, Heritability, Mineral Nutrient, Traits, Variation

6.2 Introduction

African indigenous leafy vegetables are an underutilized source of minerals and vitamins in South Africa (Jansen Van Rensburget al., 2004; Uusiku et al., 2010; Njeme et al., 2014). They can play a huge role in addressing the issues of poverty, food insecurity, and malnutrition. Many underutilized African leafy vegetables have high levels of micronutrients and could significantly contribute to nutritional security if eaten as part of a daily diet (Keatinge et al., 2011). Most of the population suffering from food and nutritional insecurity in South Africa resides in rural areas and informal settlements (Oldewage-Theron et al., 2006). The renewed interest in wild vegetables has also created awareness among the people of their numerous benefits. They are slowly becoming targets for nutrient-intervention programmes.

Food insecurity, poor livelihoods, and malnutrition have been major challenges in Sub-Saharan Africa (SSA) for centuries (FAO, IFAD, UNICEF, WHO, 2017). African indigenous leafy vegetables (ALVs) are crucial to finding a cost-effective and long-term solution to the hunger and malnutrition crisis in Sub-Saharan Africa (Abukutsa-Onyango et al., 2010; Kamga et al., 2013; Grivetti & Ogle, 2017). ALVs have more nutritional benefits than exotics and can withstand both biotic and abiotic stresses (Abukutsa-Onyango, 2007). In humans, daily consumption of phytonutrient-rich green vegetables lowers the risk of chronic diseases, such as cardiovascular disease and cancer, and boosts immunity (Smith & Eyzaguirre, 2007). Some of the major indigenous vegetables that are consumed on the African continent include African nightshades (*Solanum spp*), leafy amaranths (*Amaranthus* spp), spider plant (*Cleome gynandra*), African eggplant (*Solanum ethiopicum*), cowpea (*Vigna unguiculata*), Jute mallow (*Corchorus olitorius*), slender leaf (*Crotalaria brevidens*), pumpkin leaves (*Cucurbita muschata*) and African kale (*Brassica carinata*) (Abukutsa-Onyango, 2007).

African indigenous leafy vegetables have the potential to make a significant contribution to addressing the problem of household food insecurity and malnutrition (Uusiku et al., 2010). It has been reported that African indigenous leafy vegetables contain significant amounts of minerals and vitamins (van Rensburget al., 2004; Kamga et al., 2013).

Human consumption of the leaves is usually due to a scarcity of other preferred foods (Glew et al., 2013). According to research conducted in various parts of the world (Afolayan & Jimoh, 2009; Hussain et al., 2009), it is a rich source of nutrients, antioxidants and important dietary elements. However, despite their nutritional richness and potential to contribute to healthier diets in the country, many indigenous leafy vegetables, including *S. nigrum*, are neglected and despised in South African urban areas. Empirical data shows that African nightshades are high in micronutrients and easy to cultivate (Kamga et al., 2013; Luoh et al., 2014), and thus have the potential to provide nutritional security and a source of income for resource-poor rural communities. The vegetable is high in calcium, iron and zinc, which are micronutrients that are typically lacking in the diets of most South Africans (van Rensburget al., 2004).

Although the literature contains reports on the content of specific nutrients in nightshade, it should be noted that nutrient data for a specific crop from one region may not be representative of the same plant grown in another region with different soil conditions, climate and farming practices. Despite its popularity as a food crop or priority crop for Africa and the availability of informative reports on the nutrient composition of nightshade (Akubugwo et al., 2007; Kouadio et al., 2019; Ogundola et al., 2020; Sivakumar et al., 2020), there is still a need for more information on its mineral content. Furthermore, the nutrient content of a specific crop can vary significantly between countries and even regions within a country due to temperature, rainfall, fertilizer and soil nutrient content is the view of Greenfield and Southgate (1992 cited in Glew et al., 2013).

According to FAO (1996) and Schippers (2000), nightshade has a higher nutrient content than cabbage in terms of iron, calcium, phosphorus and beta-carotene. Several other observations support the notion that ILVs are highly nutritious or comparable to commonly consumed vegetables such as spinach, cabbage, lettuce and kale (Flyman & Afolayan, 2006; Afolayan & Jimoh, 2009; Legwaila et al., 2011). This emphasizes ILVs' potential to improve household food security and reduce nutrient deficiencies. The purpose of this study was to evaluate the nutritional composition and heritability of nutritional traits of 15 nightshade accessions sourced from gene banks and other parts of South Africa, as well as to identify lines with superior nutrient content for use in a nightshade crop improvement program.

6.3 Materials and Methods

6.3.1 Preparation of samples and determination of mineral nutrient content.

The nightshade leaves and young shoots were collected at harvest maturity from the same trial as reported in Chapter 4. Ten grams of leaf sample per accession, each replicated three times, were prepared for analysis. Leaves were washed in deionized water to remove soil and dust and oven-dried at 65 ℃ (Oven series 2000 Scientific) for four days. Samples were ground to a fine powder and stored in air-tight bags, and they were sent for nutrient analysis. The mineral elemental constituents (N, Ca, Mg, K, P, Zn, Cu, Mn, Fe, Na, and Al) were analyzed by Cedara prior to the validated protocol and working conditions. Their laboratory analytic instruments used was the ICP-OES for mineral analysis, a Dumas CNS analyser for carbon, nitrogen, and sulphur determination, temperature controlled ovens for sample drying and a hammer mill (http://www.kzndard.gov.za/quick-links/167-soil-analysis).

6.4 Data Analysis

Statistical analysis (ANOVA) with a statistical significance level set at $p < 0.05$ and means separated using the Tukey test was carried out using SAS Version 9.4 (proc. glm-general linear model procedure). Multivariate analyses were conducted to identify the most significant descriptors in capturing the nutritional variation in the accessions using SAS Software (Version 9.4). Principal component (PCA) and cluster analysis (CA) were used to discriminate and group accessions based on their similarities, respectively. A Pearson correlation analysis was used to decide on the associations between selected traits. Genetic parameters were calculated using relevant formulas as described in Chapter 4, section 4.5.

6.5 Results and Discussion

6.5.1 Variation among the mineral nutrient composition of nightshade accessions

Analysis of variance revealed a significant difference ($p<.0001$) among accessions for all mineral elements studied (Table 6.1). The variation observed among these accessions suggests a wide range of diversity in nutrient levels and offers potential genetic material to improve the mineral nutrient composition through breeding. A non-significant difference was observed for nitrogen content (Table 6.1). The Mg content among the evaluated accessions varied between 0.54% and 0.96% with an average of 0.78%. The K concentration ranged from 2.48% (Nshad40) to 3. 55% (Nfarm) with a mean of 2.94%, indicating a slightly significant difference. Another non-significant difference was observed for phosphorus content, where the mean was 0.20%. The study revealed that there was no significant difference for primary macro minerals (Nitrogen and Phosphorus) content, while K, Ca and Mg showed a significant variation among accessions.

The accession Nshad5 outperformed the rest of the accessions, recording 88.58 mg/kg of Zn, followed by Nshad9 with 84.86 mg/kg and SNig2495, recording the lowest Zn content of 51.37 mg/kg. The mean Zn content was 69.05 mg/kg. The mean copper content was 30.94 mg/kg. Accession Nfarm recorded the highest Cu content of 38.65 mg/kg, followed by Ncampus, recording 37.33 mg/kg, whereas accession Sharon recorded the lowest Cu value of 22.93 mg/kg. The best performer for Mn was Nshad5 with 164.87 mg/kg, followed by Nshad9 with 163.66 mg/kg. The accession with the lowest Mn content was N0096, recording 115.94 mg/kg and the mean Mn value was 143.93 mg/kg. Variation was observed in the iron content. N0096 had the lowest Fe content of 849.10 mg/kg, while SRetrflx had the highest Fe content of 1793.20 mg/kg. The mean for Fe content was 1333.82 mg/kg. Accessions SRetrflx, Nshad9, and ManTown excelled in iron concentration and can be used to improve the iron content in other accessions. There was considerable variance in sodium content among accessions, with concentrations varying from 1631.70 to 3006.10 mg/kg (Sharon and ManTown), with an average of 2341.54 mg/kg. The average concentration of potentially toxic elements in mg/kg of aluminium content for all accessions was 1841.75, with a range of 1122.20 –2512.20 mg/kg (N0095-SRetrflx) of aluminium content recorded.

The analysis of variance revealed significant differences ($p< 0.05$) in mineral composition among the nightshade accessions studied. Similar significant differences in nutrient

composition in African nightshades have been reported, indicating that nightshade is a rich source of essential minerals that are required to address the mineral deficiency crisis in Sub-Saharan Africa (which includes South Africa) (Jimoh et al., 2010; Masinde et al., 2010; Padmashree et al., 2014; Akubugwo et al., 2017; Kirigia et al., 2019). Some mineral elements found in soils are well known in the literature to be essential for plant growth and nutrition (Nemba et al., 2012). Meanwhile, trace mineral attributes are important in some biological metabolisms, and deficiencies or excesses of these elements in both plants and humans can result in physiological disorders according to Harper (1995 cited in Kirigia et al., 2019). According to FAO/WHO (2004 cited in Omondi et al., 2017), mineral nutrients are required for normal and healthy growth and development in both children and adults. The edible leaves of nightshade contain trace minerals that can help to compensate for mineral deficiencies in the human body. The average nutrient composition for accessions was 2.97, 3.44, 0.78, 2.94, 0.20, 69.05, 30.94, 143.93, 1333.82, 2341.54 and 1841.75 mg/kg for N, Ca, Mg, K, P, Zn, Cu, Mn, Fe, Na, and Al, respectively.

Table 6.1: Mean variation for mineral nutrient composition (dry leaf samples in 100mg/kg) of nightshade accessions under study

Access	N %	Ca%	Mg%	K%	P%	Zn mg/kg	Cu mg/kg	Mn mg/kg	Fe mg/kg	Na mg/kg	Al mg/kg
ManTown	2.90a	3.85ab	0.87ac	2.21ab	0.20a	76.22ac	32.77ac	158.07ac	1722.10a	3006.10a	2448.50a
NigSN18	2.79a	4.07a	0.96a	2.60b	0.20a	74.69ac	29.83ad	159.18ac	1665.90ab	2407.40ad	2348.30ab
SRetrflx	3.11a	3.79ab	0.85ac	2.72ab	0.24a	80.91ac	32.03ac	160.36ac	1793.20a	2963.30ab	2512.20a
Nshad9	3.25a	3.83ab	0.89ab	2.94ab	0.22a	84.86ab	32.85ac	163.66ab	1722.90a	2053.00ad	2388.50a
Nshad5	3.14a	3.88ab	0.89ab	2.90ab	0.21a	88.58a	29.43bd	164.87a	1371.10ab	2428.00ad	1871.10ab
N5547	2.87a	3.24ab	0.76ad	3.07ab	0.21a	63.78ac	27.05cd	135.01ae	1077.80ab	2189.50ad	1567.70ab
Nshad40	2.77a	3.79ab	0.92a	2.48b	0.19a	69.72ac	31.36a-d	149.27ae	1300.10ab	2841.80ac	1782.80ab
Ncampus	2.92a	2.93ab	0.64cd	3.14ab	0.17a	58.86ac	37.33ab	140.45ae	1181.90ab	2231.80ad	1595.50ab
Nfarm	3.16a	2.65b	0.54d	3.55a	0.20a	55.02bc	38.65a	125.93be	1150.20ab	1882.90cd	1540.70ab
N0096	2.89a	2.71b	0.63cd	3.08ab	0.20a	54.95bc	24.39cd	115.94e	849.10b	1855.20cd	1122.20b
Scabrum	2.82a	3.80ab	0.86ac	3.08ab	0.21a	74.80ac	31.78ac	154.96ad	1488.20ab	2293.30ad	2053.70ab
SNig2495	2.83a	2.78b	0.59d	3.09ab	0.18a	51.37c	36.34ab	127.11ae	1074.20ab	2441.30ad	1456.20ab
Sharon	2.85a	3.40ab	0.68bd	3.18ab	0.23a	59.79ac	22.93d	118.16de	1235.20ab	1631.70d	1676.10ab
SABGA	3.14a	3.82ab	0.89ab	2.92ab	0.20a	82.73ab	31.70ad	162.76ab	1381.80ab	2948.70ab	1926.50ab
Timbali	3.16a	3.08ab	0.68bc	3.12ab	0.20a	59.42ac	25.63cd	123.25ce	993.60ab	1949.10bd	1336.20ab
Fpr	0.0619	0.0002	<.0001	0.0078	0.6246	<.0001	<.0001	<.0001	0.0027	<.0001	0.0016
HSD	ns	*	***	*	ns	**	***	***	*	**	*
StD	0.16	0.50	0.14	0.33	0.02	12.23	4.57	18.14	293.05	438.69	431.39
GM	2.97	3.44	0.78	2.94	0.20	69.05	30.94	143.93	1333.82	2341.54	1841.75

Access-accession; N-nitrogen; Ca-calcium; Mg-magnesium; K-potassium; P-phosphorus; Zn-zinc; Cu-copper; Mn-manganese; Fe-iron; Na-sodium; Al-aluminium; Fpr- F probability for accession, HSD- Tukey honest significant differences; StD-standard deviation; GM- grand mean.Tukey pairwise comparisons analysed at $p < 0.05$ significant level. Means within columns that do no share a letter are significantly different. * -$p<0.05$, ** - $p<0.01$, *** - $p<0.001$, ns-not significant.

6.5.2 Nutrient correlation for mineral composition

The correlation matrix revealed many significant ($p < 0.05$) positive and negative correlations among nutrients studied (Table 6.2). A significant positive correlation was observed between calcium and magnesium (r = 0.97), zinc and manganese (r = 0.93), as well as iron and aluminium (r = 0.99) (Table 5.2). Positive and significant associations were observed between calcium and zinc, manganese, iron and aluminium (r = 0.89, 0.86, 0.83, and 0.83), respectively. Another positive correlation was observed between magnesium and zinc (r = 0.88), magnesium and manganese (r = 0.87), magnesium and iron (r = 0.75), magnesium and aluminium (r = 0.77), sodium and manganese (r = 0.71), while zinc c (r = 0.79) was positively correlated with iron and aluminium and manganese (r = 0.84) was positively correlated with iron and aluminium. Calcium and potassium had a negative significant correlation (r = -0.71), potassium and sodium had a negative significant correlation (r = -0.75), and magnesium and potassium had a negative significant correlation (r = -0.75).

Positive and moderate associations were observed between calcium and sodium (r = 0.57) and sodium and iron (r = 0.56), while a negative and moderate correlation (r = -0.54) was observed between potassium and zinc. Weak and positive associations were observed between nitrogen and zinc (r = 0.37), magnesium and phosphorus (r = 0.31), sodium and copper (r = 0.34), phosphorus and aluminium (r = 0.45), and copper and aluminium (r = 0.23), respectively. A weak and negative correlation was observed between copper and phosphorus (r = -0.45), calcium and copper (r = -0.12), and magnesium and copper (r = -0.12).

It is essential to understand correlation in order to obtain the expected response of traits. Correlation estimates allow for the evaluation of one or more traits based on the behaviour of the other. In the correlation matrix Zn and Mn, Fe and Al, Mg and Zn, Mn, Fe, Ca, and Mn were found to have a positive and significant ($p < 0.05$) correlation. The strong positive correlation suggests that indirect selection is possible and that selecting for one trait would result in the selection of another trait that is strongly associated with the selected trait. However, a negative and significant association was found between Ca and K (r = -0.71) and Mg and K (r = -0.75), implying that high calcium and/or magnesium levels may not be achieved concurrently with potassium selection. The current study found magnesium to be positively correlated with iron (r = 0.75), which is consistent with a report by Kehinde et al. (2015), who reported a strong positive and significant correlation between magnesium and iron (r = 0.97)

in bush Okra, while Dube (2017) reported a negative correlation between magnesium and iron (r = -0.79) in *Corchorus* species. Negative associations between traits (nutrient elements) pose a challenge to breeders when attempting to improve a specific nutrient in a species without taking into account the associated effect on other nutrients (Burlingame et al., 2009).

Table 6.2: Estimation of mineral nutrient correlation among nightshade accessions evaluated

Ts	N	Ca	Mg	K	Na	Zn	Cu	Mn	Fe	P	Al
N	1										
Ca	0.01	1									
Mg	-0.02	**0.97****	1								
K	0.28	-0.71**	**-0.75****	1							
Na	-0.06	0.57	**0.62****	**-0.75**	1						
Zn	0.37*	**0.89****	**0.88****	-0.54	0.56**	1					
Cu	0.16	-0.12	-0.12	0.06	0.34	0.01	1				
Mn	**0.23**	**0.86****	**0.87****	**-0.61****	**0.71****	**0.93****	**0.25**	**1**			
Fe	0.16	**0.83****	**0.75****	-0.60**	0.56**	**0.79****	0.25	**0.84****	1		
P	0.32*	0.42*	0.31*	-0.05	-0.06	0.45*	-0.45	0.20	0.45*	1	
Al	0.13	**0.83****	**0.77****	-0.64**	0.58**	**0.79****	0.23	**0.84****	**0.99****	0.45*	1

*TS-traits; N-nitrogen; Ca-calcium; Mg-Magnesium; K-potassium; P-phosphorus; Na-sodium; Zn-zinc; Cu-copper; Mn-Manganese; Fe-iron; Al-aluminium, **= Significant at 1% level; * = Significant at 5% level.

6.5.3 Principal component analysis (PCA)

The eleven nutrient traits were grouped into 11 principal components by the principal component analysis, which accounted for the entire genetic variability among the nightshade accessions studied (Table 6.3). For all traits studied, the first three principal components (PC1, PC2, and PC3) with eigenvalues greater than 1.00 explained 88.03 per cent of the total variation. The first principal component, with an eigenvalue of 6.45, accounted for 58.71 per cent of the total variation. The nutrients that contributed the most to variation in the PC1 were Ca, Mg, Na, Zn, Fe, and Al, in that order.

The second major component had an eigenvalue of 1.74 and accounted for 15.90% of the total variation. The traits that contributed to this component were N, K, and P, with correlations of 0.34, 0.24, and 0.64, (Table 6.3). Na and Cu, on the other hand, had a weak to moderate

negative correlation with the second principal component. Principal component 3, which was strongly associated with nitrogen, potassium and copper, explained only 13.43 per cent of the total variation. The remaining principal component had an eigenvalue of less than l.

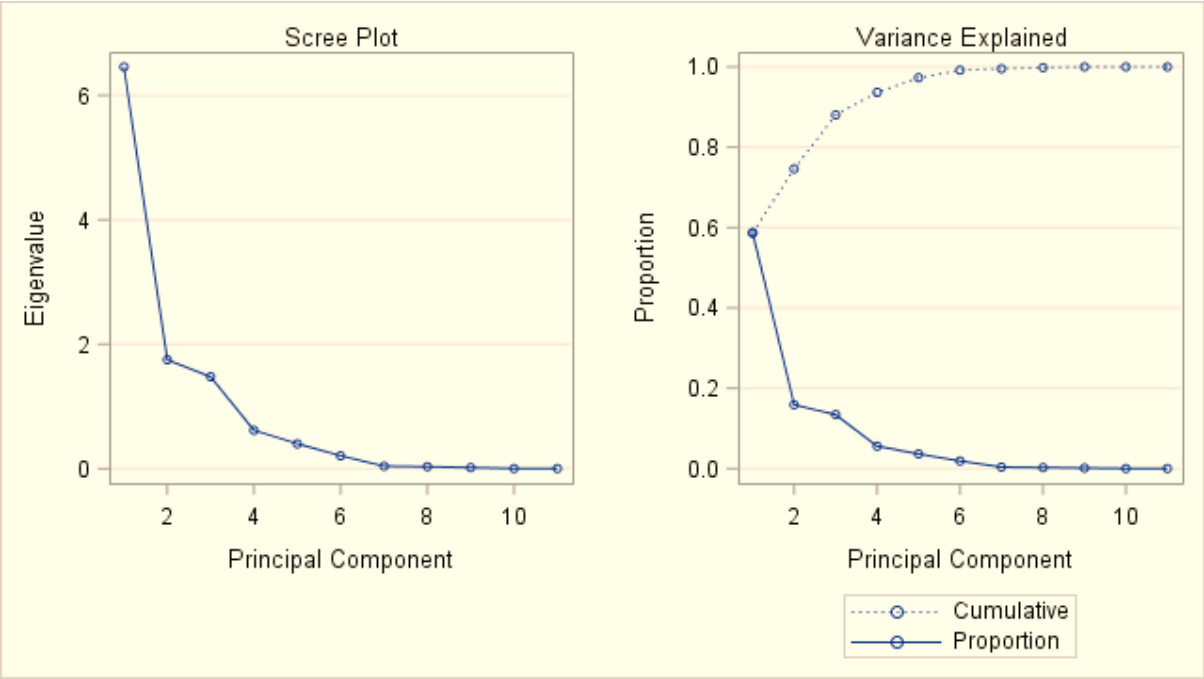

Figure 6.1: A presentation of both the scree plot and variance explained

6.5.4 Principal component biplot

The principal component biplot distinguished nightshade accessions based on their nutritional traits explained by PC1 and PC2 (Table 6.3 and Figure 6.2). Accessions in the first quadrant, Scabrum, NigSN18, Nshad9 and SRetrflx, were associated with high P, Fe, Al, Zn, Ca, Mg and Mn content, but low in N and Cu content. Accessions in the fourth quadrant (Nshad5, Nshad40, SABGA and ManTown) were associated with high content of N, P and K, but relatively low in Mg, Fe and Al. Sharon, Nfarm and SNig2495 all had high K, N and Cu content but low Na and P content. The nutritional traits grouped in the right quadrants (I and IV) were positively correlated with each other and negatively correlated with the opposite side (II and III) quadrants (Figure 6.2 and Table 6.3). The principal component scattered the accessions in a way that it is easy to visualize the distance between them (Figure 6.2).

Multivariate analysis was used to determine the patterns of diversity among accessions. PC1 accounted for 79.62 per cent of the total variance, and PC2 accounted for 20.28 per cent. Ca, Mg, Na, Zn, Mn, Fe, and Al contributed the most to variability in the first principal component.

These findings are consistent with those of Arivalagan et al. (2013) in *Solanum melongena*, who discovered that the first two principal components explained 63.17 per cent of the variation, with PC1 accounting for 42.27 per cent and P, Mg, and Cu contributing the most to the variability. In Dube's (2017) study, the first two PCs explained 70.93 per cent of the variance, with PC1 accounting for 43.98 per cent and PC2 accounting for 26.95 per cent of the variation, with Mn, Cu, Mg and Ca contributing most of the variation in the *Corchorus* study.

Table 6.3: Principal component analysis for mineral nutrient traits of nightshade accessions studied

	PC Axis		
Trait	**PC1**	**PC2**	**PC3**
Eigenvalue (explained variation)	6.46	1.74	1.47
Variability % (proportion variance)	58.71	15.90	13.43
Cumulative % (cumulative variance)	58.71	74.60	88.03
	Factor loading		
N	0.05	**0.34**	**0.64**
Ca	**0.37**	0.09	-0.16
Mg	**0.37**	0.03	-0.19
K	-0.30	**0.24**	**0.36**
Na	**0.28**	**-0.38**	-0.01
Zn	**0.36**	0.16	0.12
Cu	0.04	**-0.50**	**0.57**
Mn	**0.37**	-0.09	0.17
Fe	**0.36**	0.02	0.13
P	0.15	**0.62**	-0.01
Al	**0.36**	0.02	0.10

*PC1-3: Principal components 1-3; N-nitrogen; Ca-calcium; Mg-Magnesium; K-potassium; P-phosphorus; Na-sodium; Zn-zinc; Cu-copper; Mn-Manganese; Fe-iron; Al-aluminium

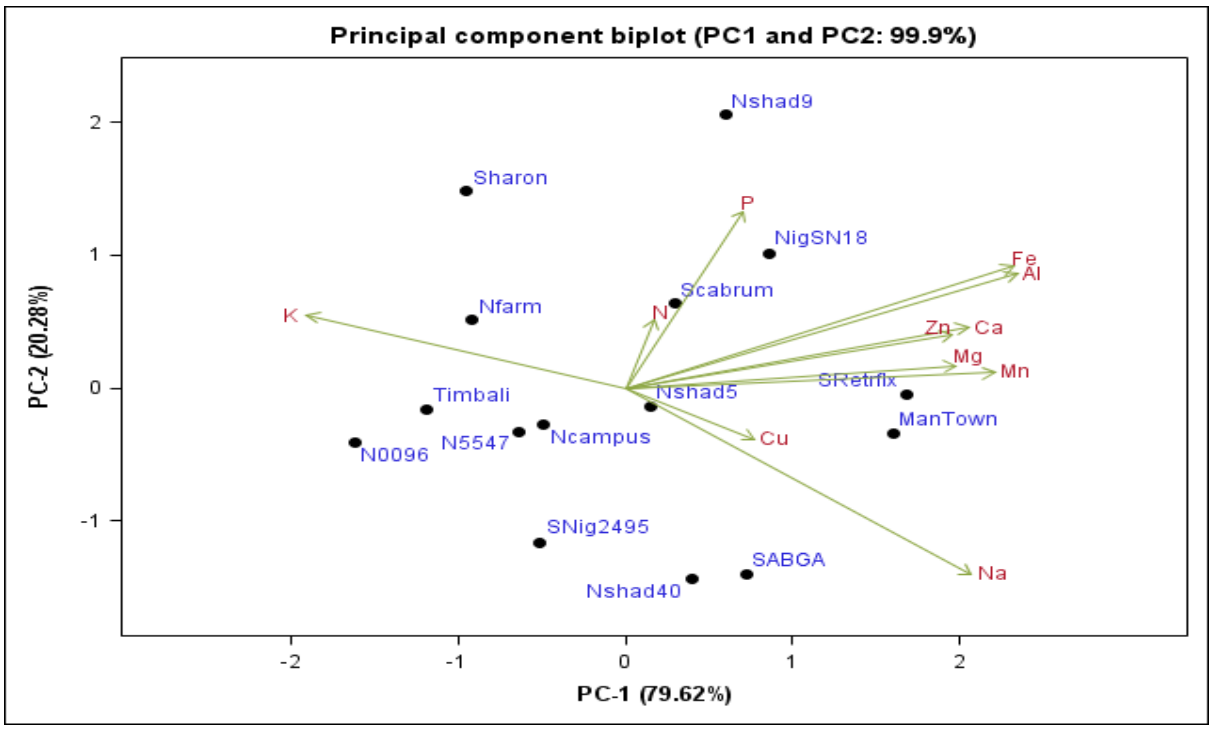

Figure 6.2: Principal component biplot showing the variation of nightshade accessions by mineral nutrient traits

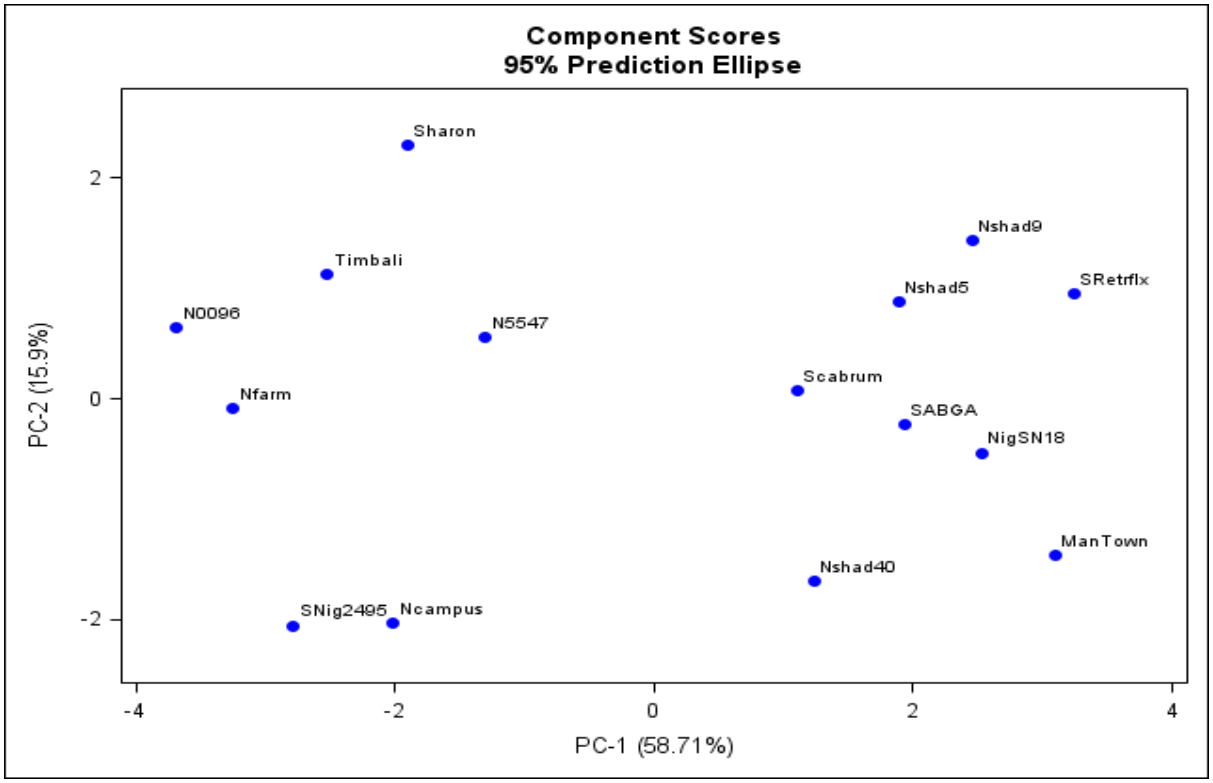

Figure 6.3: A representation biplot of the cumulative variance from principal components 1 and 2

6.5.5 Cluster analysis

The dissimilarity and similarity indices were used to group accessions using the SAS procedure single method (proc.cluster). To examine the nutritional variability among accessions, the dendrogram in Figure 6.4 UPGMA clustering method was used to summarize the nutritional relationships among the accessions and categorize them into distinct nutritional groups. Cluster 1 was a component of clusters 2 and 14, where CL14 contained accessions SRetrflx and ManTown. These accessions were characterized by relatively low Ca, Mg, Mn and Al content but relatively high N and K content. Cluster 2 was a component of accessions in CL12 (Nshad40 and SABGA), which were high in mineral values of Cu, N and K, but relatively low in phosphorus and sodium content.

Cluster 4 was a component of accessions contained in cluster 5 (Nshad9 and NigSN18) and cluster 11 (Scabrum and Nshad5), which were characterized by low nitrogen, phosphorus and copper content, but high in Ca, Mg, Mn, Zn, Fe, and Al concentration. Cluster 3 was a component of CL4 and CL6, with CL6 containing accessions with a low K content but a high correlation with Fe, Al, and P concentrations. Accessions Sharon and SNig2495 were observed to be singletons while joining with clusters 13 and 8. Singleton Sharon was characterized by low mineral concentrations of Al, Fe, Mg, and Ca but relatively high P and N content, whereas singleton SNig2495 was relatively high in phosphorus and low in sodium content. High levels of similarity were observed in cluster 2, which contained 13 accessions grouped together. Accessions Nshad40 and SABGA, SNig2495, Nshad5, Scabrum and Nshad9 and NigSN18 were placed in different clusters and are more diverse, hence they can be used as parental lines for further breeding programs. Accessions placed separately in a different cluster are considered superior and more diverse than other accessions according to Choudhary et al. (2013 cited in Dube 2017).

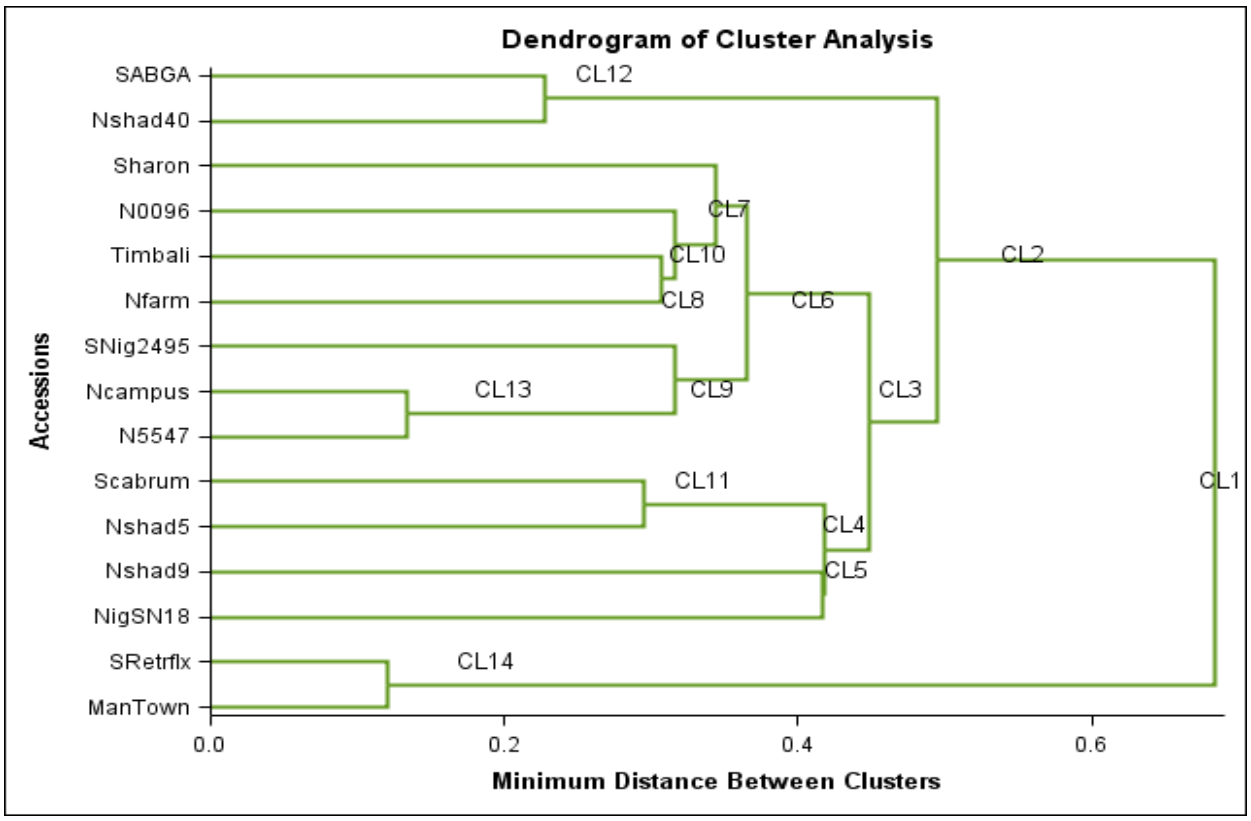

Figure 6.4: UPGMA dendrogram of nightshade accessions based on nutritional distance. CL-cluster

6.6 Genetic parameters

The genetic parameters revealed a significant amount of genetic variability in nutritional traits. Phenotypic variance values for macronutrients excluding phosphorus differed little from genotypic variance values, whereas phenotypic variance for micro elemental values differed little from genotypic variance values (Table 6.4). High PCV values compared to GCV are indicative of environmental factors.

Magnesium and aluminium had genotypic variance ranging from 0.01 to 128651.50, while phosphorus and sodium had phenotypic variance ranging from 0.00133 to 432209.90. In all traits studied, there were significant differences between GCV and PCV. For all mineral nutrient values studied, the estimates of the phenotypic coefficient of variation (PVC) were higher than those of the genotypic coefficient of variation (GCV). PCV had the highest mineral nutrient values for Al, Fe, Na, Mg, Zn, Ca, and Cu, with values of 33.02, 31.18, 28.08, 25.63,

24.33, 23.44, and 21.29 per cent, respectively. Al, Fe, Zn, Na, Mg, and Cu (Table 6.4) had 19.47, 17.73, 13.87, 12.79, 11.44, and 10.80 per cent, respectively.

According to Desmukh et al. (1986 cited in Nyadanu & Dikera, 2014); PCV and GCV are high when > 20%, referred to as medium between 10-20%, and low < 10%. High values of PCV and GCV suggest sufficient genetic variability to facilitate improvement through a selection of the desirable trait (Shukla et al., 2006).

The broad-sense heritability (H^2) ranged from a low to moderate heritability, with aluminium having the highest moderate heritability, followed by Zn, Fe, and Mn (Table 6.4). The proportion of total nutrient variance attributed to genotypic differences was estimated using broad-sense heritability. The results were generally low to moderate, with heritability estimates exceeding 30% in 4 of the 11 nutrient traits studied, and 30% in 7 of the 11 traits. The study found that all macro mineral values had low heritability, whereas micronutrient values were greater than 30% but less than 50%. Sodium, on the other hand, had a low heritability percentage. Moderate heritability coupled with high genetic advance indicates non-additive gene action effects that could be exploited through the use of heterosis breeding, as was observed for Fe and Al. Low heritability combined with low genetic advance indicates a non-additive gene effect (Osman & Khirdir, 1974), implying a low genetic gain.

The study found that N, Ca, Mg, K, P and Na values had low heritability and low genetic advance values, indicating slow progress through selection for these traits. The genetic advance ranged from 0.01 to 435.73 (N–Al), while the genetic advance as a percentage of the mean (GAM per cent) ranged from 1.16 to 23.66 per cent (Table 6.4). Heritability estimates show which traits are likely to be passed down from parent to offspring (Usman et al., 2014; Khan et al., 2015). However, the effectiveness of selection is ascertained not only by heritability but also by genetic advances. A combination of high GCV, broad-sense heritability (H^2), and genetic advance (GA) is ideal for selection (Tefera et al., 2003). According to the genetic parameters used in this study, a moderate broad-sense heritability combined with a moderate GCV and a high GA will be optimal for selecting Fe and Al content among evaluated accessions. Johnson et al. (1955) classified heritability estimates as low (0–30%), moderate (30–60%), and high (> 60%). In addition to heritability, genetic advancement is an important selection parameter that can predict the degree of target trait improvement (Shukla et al., 2004). According to Johnson et al. (1955 cited in Taneva et al., 2019) classification of genetic advance

as a per cent mean is low (0–10%), moderate (10–20%), and high (20–50%). Low genetic advance, according to Teich (1984) as well as Chaturvedi and Gupta (1995), indicates slight changes in target trait improvement in subsequent generations.

The genetic parameters were estimated, and there was a significant amount of genetic variability in mineral nutrient concentration among the accessions studied. According to the findings of the study, moderate heritability combined with high genetic advancement results in low and/or moderate genetic gain. For instance, when moderate (32.35 per cent) heritability is combined with high (277.12 per cent) genetic advance at 5% selection intensity, Fe composition can be improved by 20.78 per cent, whereas moderate heritability (32.48 per cent) combined with low genetic advance (11.24) results in a low genetic gain (16.28 per cent) for Zn.

Table 6.4: Estimates of genetic parameters for the mineral concentration of nightshade accessions

		Mean squares										
Source of variance	DF	N %	Ca %	Mg %	K %	P %	Zn mg/kg	Cu mg/kg	Mn mg/kg	Fe mg/kg	Na mg/kg	AL mg/kg
SEASON	1	0.05232	42.1617	0.88605	0.16129	0.03640	137.17	4936.24	2332.33	1948363.14	25658913.86	4243705.99
Rep	2	1.32592	0.08494	0.07879	1.06317	0.01710	3445.15	26.07	718.60	148404.42	307452.15	366585.39
Access	14	0.17548	1.42680	0.10720	0.43545	0.00190	859.27	125.27	1964.84	509724.33	1134468.67	1129694.08
SEASON*Access	14	0.14468	1.04236	0.05945	0.23402	0.00190	309.24	58.25	785.15	174039.30	596083.11	357785.21
Residual Error	58	0.09790	0.38800	0.01377	0.17506	0.00227	215.45	18.65	355.69	179970.13	266624.35	374339.73
GM		2.97	3.44	0.78	2.94	0.20	69.05	30.94	143.93	1333.82	2341.54	1841.75
$\sigma^2 g$		0.005	0.06	0.01	0.03	1.16	91.67	11.17	196.62	55947.50	89730.93	128651.50
$\sigma^2 p$		0.09	0.65	0.04	0.18	0.0013	282.20	43.40	648.47	172962.20	432209.90	369934.00
H^2 (%)		5.47	9.86	19.91	18.68	0.09	32.48	25.73	30.32	32.35	20.76	34.78
GCV (%)		2.41	7.36	11.44	6.23	0.54	13.87	10.80	9.74	17.73	12.79	19.47
PCV (%)		10.31	23.44	25.63	14.42	18.24	24.33	21.29	17.69	31.18	28.08	33.02
GA at 5%		0.03	0.16	0.08	0.16	6.52	11.24	3.49	15.91	277.12	281.17	435.72
GAM (%)		1.16	4.76	10.57	5.55	0.03	16.28	11.29	11.05	20.78	12.01	23.66

DF-degree of freedom, Rep-replication; Access-accession; SEASON*Access-season by accession interaction; GM-grand mean; $\sigma^2 g$-genotypic variance; $\sigma^2 p$-phenotypic variance; GCV-genotypic coefficient of variation; PCV-phenotypic coefficient of variation; H^2 –broad sense heritability; GA-genetic advance; GAM (%)-genetic advance as mean percent; N-nitrogen; Ca-calcium; Mg-magnesium; K-potassium; P-phosphorus; Zn-zinc; Cu-copper; Mn-manganese; Fe-iron; Na-sodium; Al-aluminium.

6.7 Conclusion

In the present study, there was a significant variation in mineral traits, showing the availability of genetic variability amoung the nightshade accessions that can be used in nutrient improvement. In all traits assessed, PCV is larger than GCV, indicating that there was an environmental influence. Moderate to high GCV, PCV, heritability, and high GAM for traits, Zn, Mn, Fe, and Al indicated indirect selection for higher mineral concentration. The leafy vegetable has a moderate to high mineral nutrient content, and accessions Nshad5, SRetrflr, ManTown, NigSN18 and Scabrum were identified as good sources of the mineral traits studied, and thus could become parental lines in further germplasm improvement through selection.

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Chapter 7:

General discussion, conclusions and recommendations

7.1 General discussion

The Solanaceae family, to which the vegetable nightshade belongs, contains approximately 1500 species, the largest and most complex of which is Solanum, which is found in temperate and tropical regions around the world (Jennifer & James, 1997). There is currently little information available on the levels and genetic diversity of nightshade accessions in South Africa, as well as their utilization. As a result, the nightshade accessions used in the study were characterized using agronomic-morphological, genetic and nutritional parameters in order to identify suitable lines for further use in breeding programs for developing high-yielding and nutrient-rich nightshade lines. Morphological characters were an important source for identifying *Solanum* L. species.

The study revealed low levels of polymorphism ($H' < 0.277$) for the qualitative morphological assessment, which is indicative of an extremely unbalanced frequency for individual traits and a lack of diversity under qualitative morphological evaluation, and this may be due to the study using fewer germplasms than previous authors on Solanum characterization. In all of the traits evaluated, there were significant differences between the accessions. Variations in quantitative yield and its contributing characteristics and nutrient traits were observed in the nightshade accessions studied. The morphological, yield, component and nutrient traits revealed variations in the nature of stem diameter (mm), leaf fresh yield (g/plant and kg ha^{-1}), nitrogen (%), calcium (%), potassium (%), phosphorus (%), iron (mg/kg) and aluminium (mg/kg) at $p = 0.0010$, 0.0250, 0.0013, 0.0619, 0.0002, 0.0078, 0.6246, 0.0027, 0.0016 and.0001, respectively. The variation observed for plant growth characteristics, the desirable late flowering, optimal plant architecture, plant height, number of leaves per plant, number of primary branches per plant, stem diameter (mm), leaf area (cm^2) and days to 50% flowering were significantly different among the studied accessions and it offers potential genetic material for the improvement of nightshades.

Late flowering accessions (excluding Sharon) produced plants with fewer larger leaves, and relatively large succulent stems; these characteristics are essential in indigenous leafy vegetables and should be considered during selection. High yielding accessions should be

considered in the selection and improvement of ILVs; the study also revealed accessions that are high-yielding in fruits. Breeding for high yields may be important for farmers aiming to produce jam, dye, and ink companies, whereas breeding for high leaf yields may be important for human consumption and fresh produce market purposes.

The demand for higher-yielding varieties with better fruit and leaf quality could be met by utilizing the heterosis effect in the F_1 generation after crossing different lines (Yordanov, 1983). Heterosis is increasingly being recognized as a fundamental, highly effective breeding method used in an ever-increasing number of crops. Heterosis offers numerous benefits ranging from early flowering, to high-yielding uniform cultivars, which also include several nightshade plant accessions evaluated in the field and they varied significantly; Ncampus, Nfarm, SNig2495, N0096, and Timbali were early flowering and produced small fruits, whereas Sharon was late flowering and produced small fruits. Scabrum had few leaves and primary branches but had a high leaf fresh yield compared to Ncampus, which had many primary branches, young tender shoots and more leaves. The significant positive correlation results in chapter 4 agree with the fresh yield for the Scabrum and Ncampus accessions, implying that nightshade fresh yield can be improved without affecting the number of leaves per plant or the number of primary branches per plant. The significant positive correlation between stem diameter (mm) and all yield component traits suggests that when selecting high-yielding accessions, these traits should be emphasized as the best selection criteria. Magnesium (Mg) was negatively correlated with all other mineral coefficient values, and this negative correlation with other desirable yields and nutritional traits for the 15 accessions studied. According to Ganguly and Bhat (2012), this suggests that increasing one trait will result in a decrease in the other. The negative correlation between the number of primary branches per plant and other yield-contributing traits indicated that identifying genotypes with more primary branches cannot be done while maintaining a high fresh yield without compromising the other trait (Mazid et al., 2013). Since complex plant attributes like yield are quantitatively inherited and influenced by genetic effects as well as the interaction between genotype and environment, indirection selection using highly correlated characteristics would be preferable (Ahmadikhah et al., 2008).

At least half of the drivers of diversity among the accessions would be captured by just selecting variables leading to loadings in the first two PCs. To this end, it is suggested that chlorophyll content, plant spread (mm), fruit fresh mass g/plant, leaf fresh mass g/plant, leaf dry mass g/plant, stem fresh mass g/plant, leaf area (cm^2), days to 50% flowering, days to fruit forming

and stem yield g/plant contributed to variation captured by the first two PCs. Meanwhile Ca, Mg, Na, Zn, Mn, Fe, P, and Al contributed to the variation captured by the first two PCs in mineral content. Because the goal of PCA is two-fold: reducing redundancy and retaining variables that explain as much variation as possible (Coghlan, 2017), a closer look using scree plots revealed that the first 10 PCs explain up to 95% of the diversity revealed by quantitative morphological hierarchical clustering, while the first 7 PCs explain up to 95% of the diversity revealed by mineral nutrient hierarchical clustering. However, it is noteworthy that the PCA was based on quantitative morphological and nutrient variables only. Thus, chlorophyll content, plant spread (mm), fruit fresh mass g/plant, leaf fresh mass g/plant, leaf dry mass g/plant, stem fresh mass g/plant, leaf area (cm^2), days to 50% flowering, days to fruit forming and stem yield g/plant account for a diversity (say about 99%), while Ca, Mg, Na, Zn, Mn, Fe, P, and Al content were accounted for (say about 99%). For instance, accessions in cluster 2 and cluster 4 can generally be distinguished based on varieties, stem yield, number of leaves per plant, days to 50% flowering, Ca, Mg, Zn, P, and Al.

In quantitative morphological and nutritional parameters, there was a significant amount of genetic variability. When attempting to determine the extent to which variation in yield components and nutritional traits is responsible for differences in yield and nutrient content among the studied accessions, it is worth noting that overall variability is influenced by both heritable and non-heritable attributes. While coefficients of variation measure the magnitude of variability in a population (Al-Tabbal & Al-Fraihat, 2012), estimates of heritability and genetic advances are crucial introductory steps in any breeding program as they provide the information needed in designing the most effective breeding program and the relative practicability of phenotypic variance, genotypic variance, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), broad-sense heritability (H^2), genetic advance (GA) and genetic advance as a mean percentage (GAM) for 22 quantitative agro-morphological and 11 nutritional characteristics of 15 nightshade accessions presented in Table 4.5 and Table 5.4.

In small leaf areas, high heritability estimates, genetic advance, and genetic advance expressed as a mean percentage was observed, indicating the role of an additive gene in the inheritance of these traits. A moderate broad sense heritability, genetic advance, and GAM was observed in the number of leaves, the number of primary branches, largest leaf area, Fe, and Al indicating that these parameters were influenced by additive genes and that selection of these traits may

be desirable and should be considered. A moderate increase in yield and nutrient content of nightshade accessions could result from moderate heritability combined with moderate genetic advancement. Zinc exhibited a moderate H^2 and GA value. In general, it is considered that if a trait is governed by non-additive gene action, it may have high heritability but low genetic advance, whereas if a trait is governed by additive gene action, it will have high heritability and genetic gain. Low heritability coupled with low genetic advance, on the other hand, is an indication of non-additive gene actions, and this was observed in plant height, stem diameter, chlorophyll content, and leaf yield, N, Ca, and P, resulting in a low genetic gain.

7.2 Conclusion and recommendations

Considering all the morphological (both qualitative and quantitative traits) and nutritional traits viz., plant growth habit, plant height (cm), number of leaves per plant, number of primary branches per plant, stem diameter (mm), days to 50% flowering, leaf, fresh yield kg ha^{-1}, stem fresh yield kg ha^{-1}, fruit fresh yield kg ha^{-1}, Ca, Mg, P, Zn, Fe, Mn, and Al, would be selected for the improvement of 15 leafy vegetable nightshade accessions. Direct selection based on mean values, mean range, PCA, cluster, and correlation coefficient values would significantly improve the fresh yields of nightshade. Associative selection based on high nutrient, yield, and yield component traits would also be effective for nightshade improvement. By analyzing and interpreting genetic parameters, correlation, cluster and principal component analysis results, tremendous variability in yield and yield-contributing nutrient traits in the studied germplasm was observed. In the future, breeders can use this variability to develop high-yielding accessions with high nutrient content. Based on the variability under research, more emphasis should be placed on the top-performing accessions with high values in traits during selection and improvement of superior accessions for higher yield, late flowering and nutrient density. Overall, Scabrum, Ncampus, Nshad40, N5547, SRetrflx, NigSN18, ManTown, and Nshad9 outperformed the other nightshade accessions in terms of fresh leaf yield, stem yield, fruit yield, late flowering, and nutrient content.

The study was conducted in a single location, but future research may be conducted in multiple locations to confirm the current study's findings in order to develop stable nightshade variants. The interaction of traits (nutrient, yield contributing characters and yield) revealed that breeders can improve fresh yield and nutrient content without compromising high fresh yields and nutrient content. Molecular characterization of nightshade accessions is also recommended to

support this current study by elucidating differences that are not influenced by environmental factors. Nightshade accessions with the lowest genetic similarity and traits of interest can be chosen, as it can result in varieties with higher nutrient content. A subsequent molecular diversity analysis is required to support the findings of this morphological analysis. To avoid storing the same genotypes with different names in gene banks, additional morphological and molecular characterization of accessions and within accessions may be necessary.

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APPENDICES
ANALYSIS OF VARIANCE (ANOVA) TABLES

Chapter 4: Combined analysis for the two growing seasons (2020/2021)

Appendix A: Analysis of variance (ANOVA) tables for morphological, yield and yield associated traits, and nutritional traits

Table A1: ANOVA table for plant height (cm)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	37500.00000	37500.00000	220.43	<.0001
Rep	2	4544.40000	2272.20000	13.36	<.0001
Access	14	21582.60000	1541.61429	9.06	<.0001
SEASON*Access	14	13621.66667	972.97619	5.72	<.0001

Table A2: ANOVA table for number of leaves

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	386082.8167	386082.8167	101.01	<.0001
Rep	2	253198.0259	126599.0130	33.12	<.0001
Access	14	394084.5815	28148.8987	7.36	<.0001
SEASON*Access	14	85788.8778	6127.7770	1.60	0.0742

Table A3: ANOVA table for number primary branches

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	18.5185185	18.5185185	3.25	0.0722
Rep	2	63.1444444	31.5722222	5.53	0.0042
Access	14	788.7222222	56.3373016	9.88	<.0001
SEASON*Access	14	184.0925926	13.1494709	2.31	0.0045

Table A4: ANOVA table for stem diameter (mm)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	941.846640	941.846640	16.30	<.0001
Rep	2	27.189247	13.594624	0.24	0.7905
Access	14	2135.057691	152.504121	2.64	0.0010
SEASON*Access	14	1480.956432	105.782602	1.83	0.0318

Table A5: ANOVA table for leaf chlorophyll content (nm)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	171.92980	171.92980	1.43	0.2324
Rep	2	1364.39270	682.19635	5.67	0.0037
Access	14	12002.32548	857.30896	7.13	<.0001
SEASON*Access	14	5257.91104	375.56507	3.12	0.0001

Table A6: ANOVA table for plant spread (mm)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	180950.4167	180950.4167	21.26	<.0001
Rep	2	665016.7593	332508.3796	39.06	<.0001
Access	14	733481.7593	52391.5542	6.15	<.0001
SEASON*Access	14	534928.0556	38209.1468	4.49	<.0001

Table A7: ANOVA table for number of fruit plant^{-1}

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	1803360.067	1803360.067	101.05	<.0001
Rep	2	33295.078	16647.539	0.93	0.3941
Access	14	2032482.289	145177.306	8.13	<.0001
SEASON*Access	14	1050767.600	75054.829	4.21	<.0001

Table A8: ANOVA table for fruit fresh mass g plant^{-1}

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	471865.098	471865.098	97.82	<.0001
Rep	2	49465.230	24732.615	5.13	0.0062
Access	14	1026209.550	73300.682	15.20	<.0001
SEASON*Access	14	440860.296	31490.021	6.53	<.0001

Table A9: ANOVA table for leaf fresh mass g/plant

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	41018.36773	41018.36773	28.59	<.0001
Rep	2	6334.30744	3167.15372	2.21	0.1110
Access	14	67292.25563	4806.58969	3.35	<.0001
SEASON*Access	14	37103.34010	2650.23858	1.85	0.0297

Table A10: ANOVA table for leaf dry mass g/plant

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	4411.951834	4411.951834	48.13	<.0001
Rep	2	539.034236	269.517118	2.94	0.0538
Access	14	4723.056208	337.361158	3.68	<.0001
SEASON*Access	14	1527.724944	109.123210	1.19	0.2786

Table A11: ANOVA table for stem fresh mass g/plant

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	209762.4928	209762.4928	59.73	<.0001
Rep	2	109961.0144	54980.5072	15.66	<.0001
Access	14	219218.1464	15658.4390	4.46	<.0001
SEASON*Access	14	234528.1043	16752.0074	4.77	<.0001

Table A12: ANOVA table for stem dry mass g/plant

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	16668.55561	16668.55561	92.79	<.0001
Rep	2	5437.14742	2718.57371	15.13	<.0001
Access	14	6397.37385	456.95527	2.54	0.0016
SEASON*Access	14	8643.30330	617.37881	3.44	<.0001

Table A13: ANOVA for Small Leaf area (cm^2)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	22.971094	22.971094	5.54	0.0190
Rep	2	76.875950	38.437975	9.27	0.0001
Access	14	2159.844248	154.274589	37.20	<.0001
SEASON*Access	14	246.593969	17.613855	4.25	<.0001

Table A14: ANOVA for Biggest Leaf area (cm^2)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	998.1109	998.1109	2.30	0.1297
Rep	2	4018.7462	2009.3731	4.64	0.0101
Access	14	325759.0939	23268.5067	53.71	<.0001
SEASON*Access	14	77631.6068	5545.1148	12.80	<.0001

Table A15: ANOVA for number of days to 50% flowering

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	4611.266667	4611.266667	375.17	<.0001
Rep	2	540.133333	270.066667	21.97	<.0001
Access	14	6736.933333	481.209524	39.15	<.0001
SEASON*Access	14	2073.733333	148.123810	12.05	<.0001

Table A16: ANOVA table for number of days to fruit forming

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	10454.40000	10454.40000	889.71	<.0001
Rep	2	262.80000	131.40000	11.18	<.0001
Access	14	5002.00000	357.28571	30.41	<.0001
SEASON*Access	14	1279.60000	91.40000	7.78	<.0001

Table A17: ANOVA table for number of days to fruit ripening

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	1092.266667	1092.266667	164.63	<.0001
Rep	2	337.600000	168.800000	25.44	<.0001
Access	14	4398.400000	314.171429	47.35	<.0001
SEASON*Access	14	1579.733333	112.838095	17.01	<.0001

Table A18: ANOVA table for leaf fresh yield g/plant

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	1224365.416	1224365.416	28.47	<.0001
Rep	2	128426.022	64213.011	1.49	0.2332
Access	14	1265363.195	90383.085	2.10	0.0250
SEASON*Access	14	1169295.173	83521.084	1.94	0.0400

Table A19: ANOVA table for stem fresh yield g/plant

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	5775868.267	5775868.267	42.44	<.0001
Rep	2	2441900.900	1220950.450	8.97	0.0004
Access	14	5870716.496	419336.893	3.08	0.0013
SEASON*Access	14	4736013.861	338286.704	2.49	0.0078

Table A20: ANOVA table for fruit fresh yield g/plant

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	14048539.10	14048539.10	95.18	<.0001
Rep	2	1091416.59	545708.29	3.70	0.0308
Access	14	27217628.42	1944116.32	13.17	<.0001
SEASON*Access	14	14042577.92	1003041.28	6.80	<.0001

Table A21: ANOVA table for total biomass g/plant

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	52677869.68	52677869.68	75.92	<.0001
Rep	2	8420087.32	4210043.66	6.07	0.0040
Access	14	54535658.51	3895404.18	5.61	<.0001
SEASON*Access	14	36427893.91	2601992.42	3.75	0.0002

Table A22: ANOVA table for harvest index (%)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	785.409521	785.409521	24.96	<.0001
Rep	2	579.175082	289.587541	9.20	0.0003
Access	14	3145.233782	224.659556	7.14	<.0001
SEASON*Access	14	1420.817129	101.486938	3.23	0.0008

Chapter 5: Yield and its contributing traits ANOVA Tables

Table A23: same as presented in chapter 4 ANOVA tables (Appendix A1 – A4)

Appendix A.24: same as presented in chapter 4 ANOVA tables (Appendix A13 – A14)

Table A25: ANOVA table for leaf fresh yield kg ha^{-1}

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	13604049.80	13604049.80	28.47	<.0001
Rep	2	1426955.58	713477.79	1.49	0.2332
Access	14	14059577.75	1004255.55	2.10	0.0250
SEASON*Access	14	12992191.32	928013.67	1.94	0.0400

Table A26: ANOVA table for stem fresh yield kg ha^{-1}

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	64176325.34	64176325.34	42.44	<.0001
Rep	2	27132247.31	13566123.65	8.97	0.0004
Access	14	65230137.84	4659295.56	3.08	0.0013
SEASON*Access	14	52622365.10	3758740.36	2.49	0.0078

Table A27: ANOVA table for fruit fresh yield kg ha^{-1}

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	156094817.5	156094817.5	95.18	<.0001
Rep	2	12126851.3	6063425.7	3.70	0.0308
Access	14	302418189.9	21601299.3	13.17	<.0001
SEASON*Access	14	156028715.9	11144908.3	6.80	<.0001

Table A28: ANOVA table for total biomass kg ha $^{-1}$

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	585607154.4	585607154.4	75.92	<.0001
Rep	2	93606187.7	46803093.8	6.07	0.0040
Access	14	606280115.7	43305722.6	5.61	<.0001
SEASON*Access	14	404967103.8	28926221.7	3.75	0.0002

Table A29: ANOVA table for harvest index (%)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	785.232284	785.232284	24.96	<.0001
Rep	2	579.028127	289.514063	9.20	0.0003
Access	14	3143.886507	224.563322	7.14	<.0001
SEASON*Access	14	1420.663049	101.475932	3.23	0.0008

Chapter 6: Nutrition concentration ANONA tables for combined seasons

Table A30: ANOVA table for nitrogen (N)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	0.05232111	0.05232111	0.53	0.4677
Rep	2	2.65185317	1.32592659	13.54	<.0001
Access	14	2.45685978	0.17548998	1.79	0.0619
SEASON*Access	14	2.02562889	0.14468778	1.48	0.1488

Table A31: ANOVA table for calcium (Ca)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	42.16177778	42.16177778	108.66	<.0001
Rep	2	0.16988603	0.08494302	0.22	0.8040
Access	14	19.97530004	1.42680715	3.68	0.0002
SEASON*Access	14	14.59305556	1.04236111	2.69	0.0043

Table A32: ANOVA table for magnesium (Mg)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	0.88605444	0.88605444	64.31	<.0001
Rep	2	0.15759574	0.07879787	5.72	0.0054
Access	14	1.50082306	0.10720165	7.78	<.0001
SEASON*Access	14	0.83229556	0.05944968	4.31	<.0001

Table A33: ANOVA table for potassium (K)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	0.16129000	0.16129000	0.92	0.3411
Rep	2	2.12634416	1.06317208	6.07	0.0040
Access	14	6.09640132	0.43545724	2.49	0.0078
SEASON*Access	14	3.27622667	0.23401619	1.34	0.2150

Table A34: ANOVA table for phosphorus (P)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	0.03640111	0.03640111	16.02	0.0002
Rep	2	0.03420234	0.01710117	7.53	0.0012
Access	14	0.02671251	0.00190804	0.84	0.6246
SEASON*Access	14	0.02661556	0.00190111	0.84	0.6277

Table A35: ANOVA table for zinc (Zn)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	137.17147	137.17147	0.64	0.4282
Rep	2	6890.30970	3445.15485	15.99	<.0001
Access	14	12029.71348	859.26525	3.99	<.0001
SEASON*Access	14	4329.31258	309.23661	1.44	0.1666

Table A36: ANOVA table for copper (Cu)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	4936.247121	4936.247121	264.71	<.0001
Rep	2	52.141035	26.070518	1.40	0.2553
Access	14	1753.724638	125.266046	6.72	<.0001
SEASON*Access	14	815.555729	58.253981	3.12	0.0011

Table A37: ANOVA table for manganese (Mn)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	2332.33984	2332.33984	6.56	0.0131
Rep	2	1437.20592	718.60296	2.02	0.1418
Access	14	27507.81945	1964.84425	5.52	<.0001
SEASON*Access	14	10992.15419	785.15387	2.21	0.0182

Table A38: ANOVA table for iron (Fe)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	1948363.141	1948363.141	10.83	0.0017
Rep	2	296808.833	148404.417	0.82	0.4435
Access	14	7136140.549	509724.325	2.83	0.0027
SEASON*Access	14	2436550.199	174039.300	0.97	0.4970

Table A39: ANOVA table for sodium (Na)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	25658913.86	25658913.86	96.24	<.0001
Rep	2	614904.31	307452.15	1.15	0.3228
Access	14	15882561.34	1134468.67	4.25	<.0001
SEASON*Access	14	8345163.48	596083.11	2.24	0.0167

Table A40: ANOVA table for aluminium (Al)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEASON	1	4243705.99	4243705.99	11.34	0.0014
Rep	2	733170.78	366585.39	0.98	0.3817
Access	14	15815717.12	1129694.08	3.02	0.0016
SEASON*Access	14	5008992.89	357785.21	0.96	0.5079