UNIVERSITY OF ZULULAND

A SURVEY OF TRADITIONAL LEAFY VEGETABLES AND STUDIES OF GENETIC DIVERSITY OF *CUCURBITA* LANDRACES IN NORTHERN KWAZULU-NATAL, SOUTH AFRICA

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A survey of traditional leafy vegetables and studies of genetic diversity of *Cucurbita* landraces in northern KwaZulu-Natal, South Africa

by

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DECLARATION

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

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I certify that the above statement is correct.

Professor A.M. Zobolo

ABSTRACT

Traditional leafy vegetables (TLVs) are commonly herbaceous plants with relatively high nutrition and health significance. They have formed part of food security in rural-based communities for generations. The utilization of TLVs; the diversity in morphology, yield and genetics of highly preferred vegetable species was investigated. The objectives were to identify the known and preferred TLVs, and determine morphology, yield and genetic diversity of most preferred *Cucurbita* landraces in northern KwaZulu-Natal. In a total of 72 recorded species, the following were recorded for the first time as TLVs: *Commelina erecta*, *Deinbollia oblongifolia*, *Erythroxylum delagoense*, *Galinsoga ciliata*, *Ipomoea wightii*, *Limeum sulcatum*, *Priva meyeri* var. *meyeri*, *Trachyandra asperata* var. *asperata* and *Trachyandra* cf. *saltii* var. *saltii*. High species diversity was recorded at Umkhanyakude district when compared with others. Most vegetables were collected from the wild, during rainy seasons, and were consumed daily to every two days. *Cucurbita* species were predominantly grown in spring and summer, from the seeds that were saved from the previous harvest. Leafy shoots were harvested in less than two months from seeding.

Diversity in morphology and yield was evident in landraces within the same and among different *Cucurbita* species. *Cucurbita pepo* landraces had the highest shoot, fruit and seed yields. In all *Cucurbita* landraces, harvest-pruning initiated at six weeks from planting resulted in longer vines and higher numbers of: lateral branches; leaves; pistillate and staminate flowers. Dendrogram from molecular analysis using random amplified polymorphic DNA (RAPD) markers primarily grouped *C. pepo* landraces according to the fruit colour change at maturity, while both RAPD and simple sequence repeat (SSR) markers also grouped them according to their agro-ecological origin. Some unique RAPD and SSR bands discriminated the self-pollinated *C. pepo* landraces from the unselfed ones.

The newly documented TLVs have formed the basis for future research of their growth, yield as well as improved palatability. Also the results of diversity in morphology and genetics of *Cucurbita* landraces can be used to improve these vegetable species.

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ABBREVIATIONS

A:	Number of polymorphic bands
ANOVA:	Analysis of variance
Bd:	Boiled
Bl:	Bulb
C:	Cultivated
CB:	*Cucurbita*
Cl:	Climber
cm:	centimetre
cm^2:	square centimetre
CPSP:	Unselfed *Cucurbita pepo* landrace with green ripe fruits from Umkhanyakude district
Cr:	Corm
D:	Nei's genetic distances
D_{ST}:	Gene diversity between populations
Fd:	Fried
Fl:	Flowers
Fr:	Fruits
g:	grams
G_{ST}:	Coefficient gene differentiation between populations
H:	Nei's gene diversity
Hr:	Herb
H_S:	Gene diversity within the population
H_T:	Total gene diversity
I:	Shannon's information index
I_N:	Nei's genetic identity
kg:	kilograms
LAN:	Limestone Ammonium Nitrate fertilizer
Le:	Leaves
M:	Umkhanyakude district

m: metre

M-IN: *Cucurbita pepo* from Umkhanyakude district, with variegated leaves and green mature fruit.

M-IS: *Cucurbita argyrosperma* from Umkhanyakude district, with green leaves and mature fruit.

M-IT: *Cucurbita pepo* from Umkhanyakude district, with variegated leaves and yellow/orange mature fruit.

mm^2: square millimetre

MNS: Unselfed *C. pepo* landrace with orange/yellow ripe fruits from Umkhanyakude district

MS: Selfed *C. pepo* landrace with orange/yellow ripe fruits from Umkhanyakude district

M-UM: *Cucurbita maxima* from Umkhanyakude district, with green leaves and mature fruit.

Ne: Effective number of alleles per loci

Nm: Gene flow

NPK: Nitrogen Phosphorus Potassium fertilizer

P: Percentage of polymorphic bands

PCR: Polymerase Chain Reaction

R: Raw

RAPD: Random Amplified Polymorphic DNA

RCBD: Randomized complete block design

RW: Reverted to a wild state

SD: Semi-domesticated

Se: Seeds

SE: Standard error

Sh: Shoots

Sr: Shrub

SSR: Simple Sequence Repeat

T: uThungulu district

Tb: Tubers

T-IT: *Cucurbita pepo* from uThungulu district, with variegated leaves and yellow/orange mature fruit.

Tl: Trailer

TLVs: Traditional Leafy Vegetables

TNS: Unselfed *C. pepo* landrace with orange/yellow ripe fruits from uThungulu district

Tr: Tree

TS: Selfed *C. pepo* landrace with orange/yellow ripe fruits from uThungulu district

T-UM: *Cucurbita maxima/pepo* from uThungulu district, with variegated leaves and yellow/orange mature fruit.

UPGMA: Unweighted Pair Group Method of Arithmetic Average

W: Wild

Z: Zululand district

Z-IT: *Cucurbita pepo* from Zululand district, with variegated leaves and yellow/orange mature fruit.

ZNS: Unselfed *C. pepo* landrace with orange/yellow ripe fruits from Zululand district

ZS: Selfed *C. pepo* landrace with orange/yellow ripe fruits from Zululand district

Z-UM: *Cucurbita maxima* from Zululand district, with green leaves and mature fruit.

LIST OF APPENDICES

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Chapter 1

Introduction

Traditional leafy vegetables (TLVs) are an important part of farming systems and consumption throughout Africa (Duodu et al., 1999; Jansen van Rensburg et al., 2004) and in many countries of the world (Negi and Roy, 2001). They are important sources of micronutrients including vitamin A and C, iron, zinc, folate and other nutrients and minerals needed for increased resistance against infections (Jansen van Rensburg et al., 2004; Glew et al., 2005; Flyman and Afolayan, 2006).

These vegetables are crucial to food security, particularly during famine and natural disasters (Jansen van Rensburg et al., 2004; Glew et al., 2005). Many plants grow in the wild or as weeds in cultivated areas, but have also been domesticated through semi-cultivation or cultivation. When domesticated, they require few inputs and tend to grow and thrive in areas where cultivation of exotic vegetables is difficult (Jansen van Rensburg et al., 2004).

An estimate of about 1000 plant species in sub-Saharan Africa are leafy vegetables (Odhav et al., 2007). However, Flyman and Afolayan (2006) considered this as an underestimate, since local people utilize many unreported plants for food purposes. South Africa is endowed with a great plant biodiversity (Lewu and Mavengahama, 2010), where more than 100 different traditional vegetables have been reported in South Africa alone (Dweba and Mearns, 2011). In sub-Saharan Africa, people intensify the consumption of TLVs when cereal harvest is inadequate (Cook et al., 1998).

The TLVs are thus of great importance to sub-Saharan Africa, but have been displaced and neglected as a result of: inadequate research and development (Odhav et al., 2007); their classification as weeds by some researchers

(Abukutsa Onyango and Onyango, 2005); excessive cultivation of field crops, which includes chemical elimination of wild vegetables and habitat change (Odhav et al., 2007); their principal association with women; primitiveness; famine foods (Vorster et al., 2007; Dweba and Mearns, 2011); and lack of appreciation of their nutritional and intrinsic value by researchers and development experts (Jansen van Rensburg et al., 2004; Madulu and Chalamila, 2005). Unlike exotic vegetables that have been selected and improved by breeders for improved palatability, most TLVs have not benefited from such research (Gockowski et al., 2003). Current research still appears to focus on the popular or commonly used species, some of which have already been fully or partially domesticated (Flyman and Afolayan, 2006).

Traditional knowledge of plant uses is generally possessed by people who are 40 years and older, and is passed on orally to younger generations in Africa (Jacobs, 2002; Zobolo and Mkabela, 2006). It is therefore vital that more research on collection and databasing of traditional knowledge is conducted on potentially exploitable wild species, thus addressing dietary deficiencies in impoverished African rural communities by promoting their increased utilization (Flyman and Afolayan, 2006). Having a database of the nutrient content of wild and cultivated edible vegetables available in the region would be of value to educators and public health officials in a position to provide dietary advice to the food-stressed populations (Glew et al., 2005).

Pumpkins are widely cultivated traditional vegetables in South Africa (Vorster et al., 2007; Lewu and Mavengahama, 2010; Dweba and Mearns, 2011) and in Zimbabwe (Ndoro et al., 2007) for the consumption of their leaves, flowers, fruits and seeds. Communities usually set aside the best seeds for cultivation the following year (Ndoro et al., 2007; Jury et al., 2008). They store seeds in various containers including glass bottles, plastics, gourds, clay pots and tins (Ndoro et al., 2007; Amutha et al., 2009) until they can be sown without any protective measures (Dovie et al., 2003; Jury et al., 2008), as they know very little about

these measures. Few people in rural communities know how to use smoke in the kitchen, ash, chemical, and tightly sealed containers (Ndoro et al., 2007; Amutha et al., 2008) to protect the seeds during storage.

Pumpkins are subtropical to tropical vegetable crops that are usually grown in spring and summer when there is sufficient rainfall (Dahlberg and Burlando, 2009; Shaffer, 2010). They grow well in soils with high organic matter (Ghaly and Alkoaik, 2010), which is maintained by the major use of domestic animal manure (Vorster et al., 2007; Schönfeldt and Pretorius, 2011), and to a lesser extent the inorganic fertilizers (Ndoro et al., 2007; Vorster et al., 2007). Depending on the cultivation implements that farmers have, pumpkin seeds are either planted in rows or broadcasted (Ndoro et al., 2007), where bean-maize-pumpkin intercropping or mixed cropping systems are common (Dovie et al., 2003 and 2007; Ndoro et al., 2007).

Local varieties of crops, including TLVs, maintained by traditional farming systems, called landraces, are important genetic resources (Pujol et al., 2007). Their diversity helps farmers in low-input systems maintain relative stability of yield despite their ability to control environmental variation (Kishinevsky et al., 1996; Pujol et al., 2007). Landraces have potential resistance to various diseases (Sousa et al., 1997). Landraces of TLVs and other crops are also a source of genes for scientific plant breeding (Pujol et al., 2007).

Molecular markers are a powerful device for determining genetic variability within and among plant populations (Khan et al., 2009). These markers include amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), sequence characterized amplified regions (SCAR), simple sequence repeats (microsatellites) (SSR), single nucleotide polymorphism (SNP) and sequence tagged site (STS), to name a few (Collard et al., 2005).

Random amplified polymorphic DNA (RAPD) is used to measure genetic variation within germplasm collections and between the closely related individuals without requiring their existing germplasm information (Loeffler and Morden, 2003; Bhutta et al., 2006). Polymerase chain reaction (PCR) based RAPD markers are dominant markers that are extensively used in genetic mapping and identification of loci linked with different traits (Bhutta et al., 2006). The RAPD method is simple and quick and has therefore been used for diversity analysis, ecological characterization and spatial distribution in many crop plants (Bhutta et al., 2006; Liu et al., 2007). The genetic diversity among *Cucurbita moschata* landraces from Korea, southern Africa and other geographical origins was determined using RAPD markers (Ferriol et al., 2004a).

Simple Sequence Repeat (SSR) or microsatellite are co-dominant markers which are also valuable for detecting variation among closely related varieties and studying intra- and interspecific relationships, however its application depends on the difficult-to-obtain suitable microsatellite markers (Sestili et al., 2008; El-Domyati et al., 2011; Formisano et al., 2012; Kalia et al., 2011; Gong et al., 2012). Microsatellite markers have been used in genetic analysis of *C. pepo* (Esteras et al., 2008; Gong et al., 2008; 2012; Formisano et al., 2012).

1.1 Problem statement

Northern KwaZulu-Natal forms the core part of the Maputaland Centre of Plant Diversity and Endemism, but no comprehensive list of the TLVs occurring in this area have been documented. Some agronomic measures of TLVs are practiced at low scale farming systems without any documentation relating to different landraces available, their yield and their genetic diversity in this region.

1.2 Objectives

- To identify and document the different TLV landraces available and utilized in northern KwaZulu-Natal.
- To determine variation among the yield of plant parts used for vegetable purposes in *Cucurbita* landraces.
- To characterize *Cucurbita* landraces from different districts by means of morphological features.
- To study the molecular diversity among *Cucurbita pepo* landraces and to determine if the self-pollination and/or the differences in agro-ecological origins have influence on the genetic diversity of these landraces.

1.3 Research questions

- What are the TLVs available in northern KwaZulu-Natal and how are they utilized?
- What are the differences in the yield of plant parts used as vegetables among *Cucurbita* landraces from different districts?
- Can the morphological features show differences among *Cucurbita* landraces from different districts?
- Can molecular study reveal the genetic diversity among selfed and unselfed *Cucurbita pepo* populations and/or the differences in their agro-ecological origins?

1.4 Hypotheses

- The northern part of KwaZulu-Natal is endowed with high diversity of TLVs which are extensively consumed in rural communities in particular.
- The *Cucurbita* landraces obtained from different districts differ in yield when grown under the same experimental conditions.

- The morphological features can show differences among *Cucurbita* landraces from different districts.
- Molecular study can reveal the genetic diversity among selfed and unselfed *Cucurbita pepo* populations and/or the differences in their agro-ecological origins.

1.5 Structure of the Thesis

Chapter 1 contains the general introduction, problem statement, research objectives, research questions and hypotheses. **Chapter 2** has the literature review on traditional leafy vegetables; growth and yield assessment of different *Cucurbita* landraces; morphology and molecular characterization of different *Cucurbita* landraces. Chapters three, four and five have their own introduction, materials and methods, results and discussion.

Chapter 3 deals with the survey of identifying and documenting traditional leafy vegetables in northern KwaZulu-Natal. **Chapter 4** focuses on the growth experiments which determine the diversity in the morphology and yield traits of *Cucurbita* landraces. **Chapter 5** presents the growth and self pollination of *Cucurbita* landraces from different districts, as well as their molecular analysis using the Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeat (SSR) markers.

Chapter 6 presents the conclusions and recommendations made regarding the survey on traditional leafy vegetables; yield traits of different landraces; morphology, agronomic and molecular characterization of different *Cucurbita* landraces.

Chapter 2

Literature Review

2.1 Introduction

This review describes the consumption of TLVs particularly by communities in rural areas. The knowledge and preference of these vegetables by different gender at different age groups are also described. This review further mentions vegetation where these vegetables are collected, the collecting season in case they occur in the wild, as well as their extent of domestication through cultivation. The consumption frequency, the growth stage of vegetable collection and parts consumed for TLVs are also explained.

The origin, distribution, domestication and usage of *Cucurbita* species are discussed. The review also describes the cultivation of *Cucurbita* species in South Africa and other African countries; their seed sourcing and growth season; as well as the time spent from seeding to leafy shoot removal of these species. The agronomic characters such as growth habit, leaf size and number, fruit size and number; as well as pollination and gene flow in *Cucurbita* species are also described.

This review also discusses the importance of landraces in plant breeding programs. It further explains the use of molecular markers in determining diversity in genotypes that are of different agro-ecological origins as well as the impact of self-pollination on plants' genetic diversity.

2.2 Traditional leafy vegetables

Leafy vegetables are mostly herbaceous plant species whose leafy shoots (or only leaves), flowers, fruits, seeds and/or roots are eaten as vegetables (Reddy

et al., 2007). Such vegetables may be originally from Africa, thus referred to as indigenous (African) leafy vegetables (Abukutsa Onyango and Onyango, 2005) or have been known and utilized over generations in Africa although they may not have originated in Africa, and therefore are known as traditional vegetables (Jansen van Rensburg et al., 2004). Leafy vegetables can either be cultivated or collected from the wild (Lewu and Mavengahama, 2010; Dweba and Mearns, 2011). In the current research, traditional leafy vegetables refer to indigenous, non-indigenous, cultivated and wild leafy vegetables. Wild leafy vegetables are either collected from grasslands, forests, along the roads and footpaths, along river banks, or found as weeds in moist areas of the cultivated and open fields (Dovie et al., 2007; Odhav et al., 2007; Reddy et al., 2007; Faber et al., 2010).

Traditional leafy vegetables are an essential constituent of people's diet throughout sub-Saharan Africa (Vainio-Mattila, 2000; Dovie et al., 2007), where poor households, in particular, more extensively rely on them for consumption (Twine et al., 2003). They are important sources of macronutrients and micronutrients such as vitamin A and C, iron, zinc, folate and other nutrients and minerals needed to maintain health and promote immunity against infections (Kinyuru et al., 2012). Leafy vegetables are inexpensive, easy to cook and provide roughage (Wallace et al., 1998). They are usually eaten as a sidedish stew to staple foods such as maize porridge (Vorster et al., 2007; Lewu and Mavengahama, 2010). The majority of people use wild vegetables which are regarded as easily accessible and more palatable than cultivated ones (Vainio-Mattila, 2000).

Early researchers have for a long time described some naturalized wild vegetables as weeds (Vainio-Mattila, 2000; Abukutsa Onyango and Onyango, 2005). The following wild leafy vegetable species were declared by Henderson (2007) as naturalized and casual alien plants in southern Africa: *Amaranthus hybridus; Bidens bipinnata; Bidens biternata; Bidens pilosa; Chenopodium*

album; Commelina benghalensis; Hypochaeris radicata; Malvastrum coromandelianum; Nasturtium officinale; and *Sonchus oleraceus*.

2.3 Knowledge of and preference for traditional leafy vegetables

2.3.1 Gender and age effect on the knowledge of and preference for traditional leafy vegetables

Knowledge of the utilization and preservation practices of traditional leafy vegetables is generally possessed by elderly women in rural societies (Dweba and Mearns, 2011). For instance in the Usambara mountains, Tanga Region, North Eastern Tanzania; in Wayanad District, India; and in Andhra Pradesh, wild vegetables are largely collected by women and children along their way to forests (Vainio-Mattila, 2000; Narayanan and Kumar, 2007; Reddy et al., 2007). In northern KwaZulu-Natal, South Africa and in Wayanad District, India, gender research shows a majority of plant species and varieties used for food and medicine are known, conserved and managed by women at household level (Zobolo and Mkabela, 2006; Narayanan and Kumar, 2007). In the study conducted in the Eastern Cape, Limpopo and KwaZulu-Natal provinces of South Africa, the knowledge of leafy vegetables is shown to be in the domain of women only, where girls tend to know only the common and abundant traditional vegetables (Vorster et al., 2007; Faber et al., 2010). Faber et al. (2010) reported that older women collected *Amaranthus spp*. and *Bidens pilosa* in the rural areas of KwaZulu-Natal, and, *Cleome gynandra* and *Citrullus lanatus* in the rural areas of Limpopo.

There is a great deal of gender variation in knowledge of and preference for TLVs among different communites and cultural groups. In Wayanad, the *Paniya* and *Kumar* men-folk of settled communities perceive the collection of wild greens as beneath their dignity as it is considered to be a woman’s role (Narayanan and Kumar, 2007). In South Africa: the *Zulu, Shangaan, Swazi, Tshonga, Pedi* and

Ndebele men do not always prefer the consumption of leafy vegetables as a relish for the stiff porridge, though they were all eating it, while the *Xhosa* males see the leafy vegetables as a woman's food and they prefer to eat meat (Vorster et al., 2007).

The difference in gender preference is also based on the taste and plant choice. In South Africa no gender differences are noted with amaranth and cucurbit consumption, and the wide variety of cucurbits help to increase the variety in taste, and also men generally prefer the bitter taste of *Bidens pilosa*, though they are used in the mix of leaves to help add taste to the dish (Vorster et al., 2007). In Wayanad, men are seldom spotted collecting rare but delicious leafy vegetables such as *Ophioglossum reticulatum* and *Adenia hondala* for their leaf and flower consumption (Narayanan and Kumar, 2007).

Local exposure to vegetation/biomes with high vegetable species diversity also influences the knowledge and preference by different genders. According to Vainio-Mattila (2000), in Usambara Mountains, Tanzania, communities living next to public forest land or forest reserve use more forest derived and rare species than the communities distant to the reserve.

Several authors report a sharp decline from older to younger generations in the knowledge and preference of the utilization of plants species for food and medicinal purposes (Faber et al., 2010; Dweba and Mearns, 2011). This decline is probably caused by the time limitations the youth experience in the form of long schooling hours and homework thereafter, thus hardly perform livestock tending duties in the veld (Vorster et al., 2007). Also, younger generation prefer to access vegetables from local markets than through the collection of wild plants in faraway habitats (Reddy et al., 2007; Dweba and Mearns, 2011). A lack of information on the various cooking methods that would make the vegetables more appealing to the youth will also cause this decline (Dweba and Mearns, 2011). Further, a lack of awareness of the leafy vegetables' nutritional status

(Vorster et al., 2007; Dweba and Mearns, 2011) as well as their association with poverty and primitiveness are some of the reasons that discourage the youth from learning about TLVs (Narayanan and Kumar, 2007; Faber et al., 2010; Dweba and Mearns, 2011). The lack of TLVs documentation is one of the major problems.

However, some elderly people have a desire to pass on knowledge of food and medicinal plants to their young ones: verbally (Jacobs, 2002); through documentation for future use (Zobolo and Mkabela, 2006); and by accessing assistance from their children, especially girls, during collection and preparation, which is an essential part of learning about traditional knowledge and ethnic foods (Narayanan and Kumar, 2007).

2.3.2 Relationship between knowledge of and preference for traditional leafy vegetables

The extent of the use of wild green leafy vegetables varies with differences in climate, soil and vegetation; and varies in accordance with different lifestyle and indigenous traditions of different ethnic groups across Africa. Therefore these variances reflect differences in availability and use of edible plants (Vainio-Mattila, 2000). In general, of the leafy vegetables people know, they prefer to use only a proportion of them. In the Limpopo Province, South Africa, as many as 21 species are known per household but between five and seven species are consumed by individual households (Shackleton, 2003; Dovie et al., 2007). Also, Narayanan and Kumar (2007) in India recorded a total of 102 wild edible leaves in *Paniya*, *Kattunaikka* and *Kuruma* tribes, but families consume 88, 43 and 21, respectively.

The preferences and wide use of traditional leafy vegetables depend on: their accessibility; abundance; ease of preparation; taste and plant choice; association

with primitiveness, poverty and women; and ethnicity (Jansen van Rensburg et al., 2007; Vorster et al., 2007; Dweba and Mearns, 2011). Odhav et al. (2007) also reported the consumption of some traditional leafy vegetables only during famine; where the *Amaranthus dubius, Amaranthus hybridus, Amaranthus spinosus, Bidens pilosa, Chenopodium album, Galinsoga parviflora,* and *Momordica balsamina* are consumed regularly, but *Justicia flava*, occasionally, while *Asystasia gangetica* is only consumed during famine. Poor households in rural areas more frequently eat wild herbs than richer households (Jansen van Rensburg et al., 2007; Lewu and Mavengahama, 2010).

The *Amaranthus spinosus*, *Amaranthus hybridus*, *Bidens pilosa* and *Galinsoga parviflora* are among the most important and highly favoured leafy vegetables in the East and West Usambaras, Tanzania, because of their local abundance, easy access and good taste (Vainio-Mattila, 2000). In South Africa, Vorster et al. (2007) recorded the following species: *Amaranthus* spp.; *Bidens pilosa*; *Chenopodium album*; *Corchorus* spp.; *Cucurbita* spp.; *Momordica balsamina*; and *Vigna unguiculata* as some of the more popular leafy vegetables in areas where they are widespread.

2.3.3 The well known and preferred traditional leafy vegetables

Several authors reported some of the following cultivated and wild plants as the most popular and/or well favoured leafy vegetable species in different areas: *Alternanthera sessilis*; *Amaranthus hybridus*; *Amaranthus hypochondriacus*; *Amaranthus spinosus*; *Amaranthus* spp.; *Amaranthus thunbergii*; *Bidens pilosa*; *Chenopodium album*; *Citrullus lanatus*; *Colocasia esculenta*; *Commelina benghalensis*; *Corchorus* spp.; *Cucurbita maxima*; *Cucurbita pepo*; *Cucurbita* spp.; *Galinsoga parviflora*; *Momordica balsamina* and *Vigna unguiculata* (Vainio-Mattila, 2000; Jansen van Rensburg et al., 2007; Narayan and Kumar, 2007; Ndoro et al., 2007; Vorster et al., 2007; Faber et al., 2010; Nabulo et al., 2010).

In the research conducted in KwaZulu-Natal, Limpopo and Eastern Cape provinces of South Africa, the following are the most popular traditional leafy vegetables: *Cucurbita* spp. (96.7%); *Cleome gynandra* (64.4%); *Vigna unguiculata* (58.3%); *Amaranthus* spp. (45.8%); *Corchorus* spp. (44.4%) and *Momordica balsamina* (43.3%) (Vorster et al., 2007).

2.4 Scientific validation of traditional leafy vegetables that have diverse local names

The local names of traditional leafy vegetables vary greatly from place to place and between communities in northern KwaZulu-Natal as well as South Africa (Jansen van Rensburg et al., 2007; Lewu and Mavengahama, 2010). Both wild and cultivated vegetables are collectively known as *imifino* in isiZulu (Jansen van Rensburg et al., 2007; Faber et al., 2010; Lewu and Mavengahama, 2010).

It is important that traditional leafy vegetables are properly identified by their scientific and local names (Lewu and Mavengahama, 2010). The use/collection of voucher specimens ensures an appropriate scientific identification of vegetable species in the case of a great variation in their local nomenclature. Voucher specimens have been widely collected where local names are used during community surveys of vegetable research (Malla and Chhetri, 2009; Faber et al., 2010; Molebatsi et al., 2010; Dweba and Mearns, 2011).

2.5 Family diversity of traditional leafy vegetables

The differences in climate, soil and vegetation influences the differences in the availability and use of traditional leafy vegetables (Vainio-Mattila, 2000). In East and West Usambaras, Tanzania, Vainio-Mattila (2000) documented Acanthaceae, Amaranthaceae, Asteraceae, and Brassicaceae as the most

important families of wild green leafy vegetables, among 26 reported families. In Kenya and other parts of East Africa traditional leafy vegetables are used by both rural and urban communities and include several families such as Amaranthaceae, Bacellaceae, Brassicaceae, Capparaceae, Cucurbitaceae, and Tiliaceae (Abukutsa Onyango and Onyango, 2005). Further, in Andhra Pradesh, India; Reddy et al. (2007) report 69 families of wild food plants, where four families: Amaranthaceae (11 species); Rubiaceae (9 species); Euphorbiaceae (8 species) and Papilionaceae (7 species); have a high number of species, with Amaranthaceae family having the highest number of species. The following families of TLVs were reported in South Africa: Amarantharaceae, Asteraceae, Brassicaceae, Capparaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae, Malvaceae, Solanaceae, Tiliaceae, (Jansen van Rensburg et al., 2007; Venter et al., 2007).

2.6 Consumption frequency of preferred vegetables

The pattern at which traditional leafy vegetables are consumed is highly variable in South African households (Jansen van Rensburg et al., 2007) and other countries (Vainio-Mattila, 2000; Narayanan and Kumar, 2007); depending on poverty conditions, urbanization status, accessibility, abundance and time of year.

According to Vainio-Mattila (2000), the most used vegetable species are available all year round and are eaten on average four days a week. In rainy seasons such as spring and summer, traditional leafy vegetables are generally eaten daily to five times a week (Dovie et al., 2007; Jansen van Rensburg et al., 2007; Reddy et al., 2007). However, their use is less in winter than in summer (Shackleton, 2003). During the rainy season: 27% of households eat wild herbs daily; 19% weekly, while another 19% on two or three days a week, in Limpopo province, South Africa (Dovie et al., 2007).

Faber et al. (2010) documented that in summer, leaves of: *Amaranthus* spp. and *Bidens pilosa* are picked weekly in KwaZulu-Natal; while in Limpopo, leaves of *Amaranthus* spp. are collected every two to five days and those of *Citrullus lanatus* are picked daily for consumption purposes. In India, *Alternanthera sessilis* and *Colocasia esculenta* are reported to be consumed almost every day of the week; and species like *Amaranthus spinosus* are eaten on an average of three times a week; and *Bidens pilosa* is eaten two to three times a month, which is a category of less frequently eaten leafy vegetables (Narayanan and Kumar, 2007), while in Zimbabwe, pumpkins (*Cucurbita maxima/moschata*) are consumed three to four times a week during rainy seasons (Ndoro et al., 2007). The people of Nigeria eat *Cucurbita* species (*C. maxima, C. moschata* and *C. pepo*) daily, either as vegetables from leaves or pulp from the cooked fruits (Agbagwa et al., 2007).

2.7 Stage of vegetable collection and parts consumed

Plant species are usually collected for consumption purposes at their vegetative stage, when leaves are young and tender, or when they have very young fruits (Vainio-Mattila, 2000; Jansen van Rensburg et al., 2007). Different parts of wild collected plants can be used, such as: roots; tubers; stems; rhizomes; leaves; flowers; fruits and seeds (Vorster et al., 2007).

In Nigeria, both leaves and fruits of *Abelmoschus esculentus* are cooked as vegetables (Olasantan and Salau, 2008). According to Ghaly and Alkoaik (2010), leaves of *Ipomoea batatas* are traditionally eaten when they are still nutritious at a young stage. Young leaves with stems (tender shoots) or leaves only of *Amaranthus* spp. and *Bidens pilosa*; leaves of *Citrullus lanatus*; are picked for consumption in various parts of South Africa (Faber et al., 2010). In India, the leaves of many home garden species, including *Cucurbita maxima* and *Vigna*

unguiculata, are used for vegetable purposes (Narayanan and Kumar, 2007). In South Africa all cucurbits provide fruit, leaves and sometimes flowers for the diet (Vorster et al., 2007).

2.8 Collecting season for wild vegetables

Some wild leafy vegetables are easily accessible all the year round (Vainio-Mattila, 2000); or they are collected after the first rains; during the wet season; in spring; and in summer (Dovie et al., 2007; Narayanan and Kumar, 2007; Ndoro et al., 2007; Faber et al., 2010). Wild vegetables: *Amaranthus hybridus*, *A. thunbergii, Amaranthus* spp., *Bidens pilosa*; and *Citrullus lanatus* are commonly harvested in rainy seasons especially spring and summer (Dovie et al. 2007; Faber et al., 2010). The leaves of *Bidens pilosa* are harvested in winter from the plants growing next to the rivers (Faber et al., 2010).

2.9 *Cucurbita* species

2.9.1 Origin, distribution and domestication of *Cucurbita* species

The genus, *Cucurbita* is a member of the Cucurbitaceae family which consists of approximately 130 genera and 900 species mainly distributed in warm climatic regions, especially in the tropics and subtropics (Agbagwa et al., 2007; Aruah et al., 2010; Ahamed et al., 2011). It is composed of 12 to 20 species that are of American origin (Hadia et al., 2008; Formisano et al., 2012). About five different *Cucurbita* species: *Cucurbita argyrosperma*; *C. ficilifolia*; *C. maxima*; *C. moschata*; and *C. pepo*, are among the earliest cultivated and domesticated plants in the New World (Formisano et al., 2012; Simon, 2011).

2.9.2 Usage of *Cucurbita* species

Cucurbita species play a significant role in human nutrition and are of great economic importance (Kathiravan et al., 2006; Ghobary and Ibrahim, 2010). In South Africa *Cucurbita* species' leaves, flowers and young fruits are cooked as vegetables while seeds are roasted and eaten as a snack (Jansen van Rensburg et al., 2007). Fruits, flowers and young shoots of *C. maxima* (Villaseñor et al., 1996) and; mature and young fruits, male flowers, seeds and young tips of vines of *C. moschata* (Ferriol et al., 2004a) are widely consumed as vegetables. The fruits and young shoots are good sources of calcium, phosphorus, iron and vitamin B (Villaseñor et al., 1996). Seeds are eaten to remove intestinal worms and to control nausea during pregnancy (de Queiroz-Neto et al., 1994).

2.9.3 Cultivation of *Cucurbita* species in South Africa and other African countries

Cucurbita maxima and *C. pepo* are some of the most popular cultivated cucurbit species in South Africa (Jansen van Rensburg et al., 2007), and the genus is also represented by these species in Nigeria (Agbagwa and Ndukwu, 2004; Agbagwa et al., 2007). *Cucurbita* species are grown either in homegardens around homesteads; in vegetable gardens or in the fields in South Africa (Molebatsi et al., 2010; Torquebiau et al., 2010) and other countries (Ndoro et al., 2007; Shaffer, 2008). In Nigeria, *Cucurbita* species are rarely found growing in the wild (Agbagwa and Ndukwu, 2004; Agbagwa et al., 2007).

Several authors reported the cultivation of *Cucurbita* species in South Africa (Jansen van Rensburg et al., 2007; Vorster et al., 2007; Lewu and Mavengahama, 2010; Dweba and Mearns, 2011) and in Zimbabwe (Ndoro et al., 2007), without their precise description of taxa. In the survey conducted by Vorster et al. (2007) in KwaZulu-Natal; Limpopo and Eastern Cape provinces of

South Africa, a high percentage (98.3%) of the interviewed households grow *Cucurbita* species.

2.9.4 Seed sourcing and growth season of *Cucurbita* species

The majority of farmers, particularly in the rural areas, reserve the best fruits and/or seeds for cultivation the following year, with very few individuals accessing seeds from local seed retailers and thus they maintain the ownership of *Cucurbita* landraces (Ndoro et al., 2007; Jury et al., 2008; Balkaya et al., 2009; 2010).

Cucurbita species are known to be summer or warm weather crops (Ghaly and Alkoaik, 2010). The majority of farmers sow them in spring and summer (Jansen van Rensburg et al., 2007; Ndoro et al., 2007) when there is adequate rainfall (Ndoro et al., 2007; Vorster et al., 2007; Shaffer, 2008; 2010). A minority sow them in autumn, winter and all year round: with irrigation during dry seasons (Vorster et al., 2007; Lewu and Mavengahama, 2010); and cultivation in vlei areas (Ndoro et al., 2007; Shaffer, 2008).

2.9.5 Time from seed sowing to leafy shoot removal of *Cucurbita* species

Jansen van Rensburg et al. (2007) reported the general consumption of leafy parts of vegetables with inclusion of young succulent stems and very young fruits. Also, young shoots *Cucurbita pepo* and *C. maxima* (Pieroni et al., 2002) and *C. moschata* (Ferriol et al., 2004a.) are harvested for vegetable purposes.

2.9.6 Growth habit of *Cucurbita* species

Growth habit in *C. maxima* and *C. pepo* is among the variable vegetative traits (Wu et al., 2007; 2008; Wu and Cao, 2008). Most cultivars of *Cucurbita* species are traditionally large, indeterminate, trailing plants (Paris and Brown, 2005; Wu et al., 2008), whose vines may spread up to 15 m from the crown of the plant (Wu et al., 2007; Wu and Cao, 2008) or over 43 m in some instances (Ahamed et al., 2011). Some cultivars of *C. pepo* and *C. maxima* species are bushy since they have short vines (Wu et al., 2007; 2008; Wu and Cao, 2008).

At 60 days after germination, bush plants of *C. moschata* have shorter vines (14.5 ± 4.9 cm) than vine plants (175.6 ± 28.2 cm) (Wu et al., 2007). In their study of *C. moschata* genotypes in Bangladesh, Ahamed et al. (2011) found the vine length at fruit harvest to range from 169.6 to 400.1 cm. Bush-type pumpkin plants have fewer lateral shoots than vine plants (Loy, 2004; Wu et al., 2008). Ahamed et al. (2011) reported a range from 2.02 to 4.7 branches per plant among *C. moschata* genotypes.

The bush growth habit is controlled by a single dominant gene *Bu* which confers reduction in internode length (Wu et al., 2007; Wu and Cao, 2008), and is dominantly expressed in early development of *C. pepo* and *C. maxima* plants (Paris and Brown, 2005; Wu et al., 2007).

Loy (2004) reported that in temperate climates, semi-determinate *Cucurbita* plants can reach the end point of their exponential growth phase within six to seven weeks from seeding. According to Paksoy and Aydin (2004), the vegetative growth period of *C. pepo* is almost 100 days.

2.9.7 Leaves of *Cucurbita* species

Leaf area has essential function in light interception, water and nutrient use, photosynthesis, crop growth and potential yield (Cho et al., 2007). Leaf areas are determined either by measuring leaf length, leaf width, petiole length, or a combination of these variables (Loy, 2004; Cho et al., 2007). The net photosynthetic capacity of a plant is determined throughout the growth cycle by measuring its net assimilation rate and the rate of increase in both fresh and dry weight per unit leaf area per unit time, because of a strong relationship between leaf development and crop growth (Loy, 2004; Cho et al., 2007). In their study, Cho et al. (2007) estimate the leaf area of individual cucumber leaves by using models of measuring leaf length and width.

Mean leaf size (area) increases rapidly from about 100 to 300 cm^2 in early development to over 1500 cm^2 in leaves of large-leaved *Cucurbita* cultigens (Loy, 2004; Amer, 2011). However, at maturity stage the leaf area decreases because of senescing leaves in the lower part of the canopy (Amer, 2011). Loy (2004) reports the existence of genetic variability for both the number of axillary shoots and size of leaves, where in comparative cumulative leaf area development between large- and small-leaved *C. maxima* cultivars, it was found that even though the small-leaved cultivar initiated more lateral branches and had more leaves than large-leaved cultivar, the large-leaved cultivar had significantly greater leaf area during the first 40 days of growth.

Under wide plant spacing, vining *C. maxima* cultigens may produce more than 300 leaves on a single plant (Loy, 2004). NeSmith (1997) found the number of leaves per plant 40 days after sowing for four *C. pepo* cultivars over five sowing dates, showing a range from 8 to 28 leaves per plant. According to NeSmith (1997) and Loy (2004), crop leaf area depends on the degree of branching or number of shoot meristems, the leaf appearance rate, the leaf expansion rate, the duration of leaf expansion, the final leaf size and leaf senescence rate. Both

branching habit and leaf size can vary considerably among *Cucurbita* cultivars (Loy, 2004), and the environmental factors affecting the leaf appearance rate and ultimately the leaf numbers include temperature, photoperiod, radiation, water stress and nutrient supply (NeSmith, 1997).

2.9.8 Flowering in *Cucurbita* species

Cucurbita pepo is monoecious and has unisexual flowers that form solitarily in the axils of the leaves (Ercan and Kurum, 2003; de Menezes et al., 2005). In *Cucurbita* species staminate flowers are generally more numerous and bloom earlier than the pistillate flowers (Aruah et al., 2010). According to the report by Vidal et al. (2010), in the period from 39 to 66 days after planting, the number of staminate flowers per plant increased from 15 to 34 and pistillate increased from 0.3 to 2.2 flowers.

Flowering of *C. pepo* and *C. maxima* cultivars begins about six to eight weeks from seeding, but in early maturing cultivars of *C. pepo*, pistillate flowering commences as early as 30 to 40 days from seeding (Loy, 2004). However, Agbagwa and Ndukwu (2004) reported that the time of flowering of the three species from the date of planting varies from eight weeks in *C. moschata*, to 11 and 13 weeks in *C. maxima* and *C. pepo*, respectively. Bush-type *Cucurbita* plants are characterized by earlier flowering and a higher ratio of pistillate to staminate flowers than vine-type plants (Loy, 2004; Wu et al., 2007; 2008; Wu and Cao, 2008). Vine plants of *C. moschata* produced more staminate flowers (21.1 ± 4.3) than bush plants (13.7 ± 3.3) (Wu et al., 2007; Wu and Cao, 2008).

Flowering in several plants including *Cucurbita* is genetically controlled by flowering locus T (FT) gene (Zeevaart, 2008; McGarry and Kragler, 2013). This FT gene produces florigen, the phloem-mobile signal that is produced by leaves

and induces floral initiation at the shoot apex (Kragler, 2010; McGarry and Kragler, 2013).

2.9.9 Pollination and fruit set in *Cucurbita* species

Pollination is the transfer of pollen from the anther of one flower to the stigma of another or the same flower (Sukprakarn et al., 2005). The various wild specialist bees are potential pollinators for *Cucurbita* species (Aruah et al., 2010; Vidal et al., 2010).

Pollinators' (particularly bees) arrival on flowers almost coincides with the resumption of early morning sun and opening of both staminate and pistillate flowers, as fertilization occurs in the early hours of the day (Ercan and Kurum, 2003; Agbagwa et al., 2007; Vidal et al., 2010). This also corresponds with the hours of peak pollen viability, which ensures a high percentage of pollen germination, as fertilization decreases in the heat of the day (Agbagwa et al., 2007; Vidal et al., 2010). The most efficient visits are the first ones because they result in the deposition of a large number of highly viable pollen grains on the stigma (Agbagwa et al., 2007; Vidal et al., 2010).

Nepi and Pacini (1993) reported that in *C. pepo* the stigma is receptive (pollen grains germinate on it) for four days (from one day before anthesis to two days past anthesis), whereas the ovules are receptive (fertilized and produce fruits) for two days (from one day before anthesis to the day of anthesis). One day after pollination, the grains emit pollen tubes that reach the ovary but do not penetrate the ovules, while two days after pollination, the grains emit tubes that fail to grow beyond the stigma (Nepi and Pacini, 1993).

The coordinated action of growth hormones provided and/or regulated by the pollen grains, pollen tubes and developing seed triggers fruit development after

pollination and fertilization (De Menezes et al., 2005). In *Cucurbita* species, the low fruit set, low fruit yield and reduced fruit size can be due to insufficient or absence of pollinators, thus low pollen loads; or to some biotic or abiotic stresses, where unfavourable environmental conditions such as high temperatures, drought and low irradiance can cause abortion of flower buds (De Menezes et al., 2005; Vidal et al., 2010).

The need for many pollen grains per ovule is due to reduction in pollen viability at low temperatures, rains, low activity of the bees and no germinating pollen left on the stigma (Vidal et al., 2010). Pumpkins require 12 to 16 bee visits per flower to transfer enough pollen that will ensure a higher percentage of fruit set (Vidal et al., 2010). Although pumpkin fruit set will occur with natural pollinators, the addition of honey bee colonies maximizes the fruit size (Pacini et al., 1997; Vidal et al., 2010).

The successful production of parthenocarpic fruits in some cultivars of *C. pepo* have been reported, where their production is crucial for indoor (greenhouse) production (off season) when pollinators are absent or outdoor production in large areas with a low population of pollinating insects (Kurtar, 2003; De Menezes et al., 2005; Nogueira et al., 2011). Out of 40 pistillate flowers of *Telfairia occidentalis* that opened among nine plants (4.4 flowers per plant), only 10 set fruits (1.1 fruits per plant) (Akoroda et al., 1990).

2.9.10 Hand pollination of *Cucurbita* species

Both staminate and pistillate pumpkin flowers that are ready for anthesis the following morning can be noticed in the late afternoon as they begin to show yellow/orange colour along the seams of the petals and the tips look like they are just about to break apart (Fike, 2011). To exclude the effect of cross pollination particularly by bees, both staminate and pistillate flowers are either bagged,

covered with a gelatin capsule (Robinson, 2000; Sukprakarn et al., 2005; Hoehn et al., 2008) or have flower tips closed with masking tape (Fike, 2011) until hand pollination is conducted.

According to Thralls and Treadwell (2008), hand pollination is best done in the morning when both staminate and pistillate flowers are open and when humidity helps to activate the pollen. They recorded one method of hand pollination where staminate flowers are picked and both the staminate and pistillate petals are removed to expose the stamens and stigma, respectively, and the pollen is either transferred by gently touching the stigma with the anthers or by using a paint brush to pick up pollen from the anther and apply to the stigma. In this method where both pistillate and staminate flowers have their petals removed, re-bagging of female flowers is therefore essential, to avoid bee effect (Sukprakarn et al., 2005). However, Sukprakarn et al. (2005) and Fike (2011) mentioned either turning over or removal of petals only on the staminate flowers to expose anthers, and gently rolling or rubbing the pollen onto the stigma of the flower, with subsequent re-tapping or re-bagging of the pistillate flowers afterwards in order to prevent random bee pollination. One staminate flower can be used to hand pollinate several pistillate flowers (Thralls and Treadwell, 2008). To improve pollination, the use of more than one (e.g two to three) staminate flower per pistillate flower is recommended (Hoehn et al., 2008; Fike, 2011).

Thralls and Treadwell (2008) also explained a method of cutting staminate flowers like that of a cut flower, and keeping them in water over night to pollinate the available pistillate flowers the next morning. Staminate flowers with removed petals may also be stored in a refrigerator for three to four days, by laying them on moist paper towels, with caution taken to prevent the pollen from directly contacting the moist paper towel (Thralls and Treadwell, 2008).

2.9.11 Self-pollination (inbreeding) in *Cucurbita* species

Self-pollination is common among flowering plants (Ferrari et al., 2006). It increases plant mean homozygosity, which is not the natural genetic state of cross-pollinated species (Ercan and Kurum, 2003; Cardoso, 2004; Du et al., 2008). In the wild, *Cucurbita pepo* subsp. *texana* shows mixed mating systems, producing both selfed and outcrossed progeny (Ferrari et al., 2006). Studies on *C. foetidissima*, a closely related species, which grows in ecologically similar habitats to *C. pepo*, showed a production of 73% of seeds through self-fertilization (Ferrari et al., 2006).

Inbreeding resulting from self-fertilization is generally associated with a significant loss of or reduction in fitness, where inbreeding reduces heterozygosity (Ercan and Kurum, 2003; Cardoso, 2004; Ferrari et al., 2006; Du et al., 2008). Inbreeding also has a negative impact on pollination by reducing the blossom volatiles which attract pollinators (Ferrari et al., 2006); reduces pollen quality and performance (Cardoso, 2004); and causes production of smaller and fewer flowers and fruits (Ferrari et al., 2007). It also increases susceptibility to pathogens and herbivorous insects (Ferrari et al., 2006 and 2007). It further reduces plant size, vigour (Du et al., 2008; Ghobary and Ibrahim, 2010) and seed yield (Ercan and Kurum, 2003).

According to Ferrari et al. (2006), population genetic theory predicts that the magnitude of inbreeding depression should decrease over time with continuously high rates of selfing, as deleterious recessive alleles are purged from the population by selection against homozygotes. Further, Cardoso (2004) reported that the vigour loss during self-pollination did not occur in *C. pepo*, and self-pollination for ten generations of *C. maxima* did not affect vigour or reproductive capacity. Inbreeding with selection for three generations was used to improve the variety “Eskandarani” of *C. pepo* in Egypt, where selected population for three generations (P_3) had a decrease in the number of days to first pistillate flower

than the original population (P_0); and also the average fruit weight, total and marketable number of fruits per plant, total and marketable weight of fruits per plant were increased in the P_3 population (Ghobary and Ibrahim, 2010).

2.9.12 Gene flow in *Cucurbita* species

Studies on selectively neutral genetic markers in squash and sunflowers reveal that long-term and continuing genetic exchange occurs between crops and their wild relatives, which illustrates that gene flow, hybridization, and introgression, have occurred in the past (Spencer and Snow, 2001). Thus, plants that display phenotype traits of both the domesticated and wild species occur, suggesting gene flow between species (Cuevas-Marrero and Wessel-Beaver, 2008).

The great potential of pollen dispersal is likely to contribute to the high rates of gene flow among cultivated and weedy *Cucurbita* populations. There is evidence for this in a form of crop/weed gene flow among populations of: *C. pepo* and *C. texana*; and *C. argyrosperma* and *C. fraterna* (Montes-Hernandez and Eguiarte, 2002); *C. argyrosperma* subsp. *argyrosperma* and *C. argyrosperma* ssp. *sororia*; *C. moschata* and *C. argyrosperma* (Cuevas-Marrero and Wessel-Beaver, 2008). Therefore, the pollen-mediated crop-to-weed gene flow requires cross-compatibility and overlapping flowering periods within a crop-weed complex (Jenczewski et al., 1999). Gene flow can also be facilitated by informal seed exchange among farmers of *Cucurbita* species (Montes-Hernandez and Eguiarte, 2002; Ferriol et al., 2004a, 2004b) and *Citrullus* species (Mujaju et al., 2010).

The frequent occurrence of hybridization in *Cucurbita* and other genera suggests that most reproductive barriers are imperfect and that striking morphological differences are often irrelevant to reproductive affinity (Jenczewski et al., 1999; Spencer and Snow, 2001). Long-distance gene dispersal has been reported

where pollinating bees transferred pollen over distances exceeding 1 km in *C. pepo* (Spencer and Snow, 2001) and five percent of outcrossing was found in *C. pepo* and *C. texana*, at a distance of 1300 m (Montes-Hernandez and Eguiarte, 2002).

2.9.13 Fruit colour intensity in *Cucurbita* species

Cucurbita pepo is highly polymorphic with respect to colour, thus fruit colour is amenable to qualitative genetic analysis by means of developmental approach (Paris, 2000, Paris and Brown, 2005). Out of the 11 identified loci which affect developmental fruit colour in *C. pepo*, three genes of major effect – D, l-1, and l-2 – account for a considerable portion of the genetic variation in intensity of fruit colouration that is observed in this species (Paris, 2000).

Fruit colouration of *Cucurbita pepo* can change dramatically over time in response to fruit development (Paris, 2009). The fruits of most pumpkin cultivars of *Cucurbita pepo* subsp. *pepo* that are grown in the United States are intense orange at maturity (Paris, 2000; 2009). However, when young, several days past anthesis, these fruits are light green, except for some darkening of the main carpellary veins, becoming blackish-green by 2-3 weeks past anthesis, and then turning intense orange on ripening, six weeks after anthesis (Paris, 2000; 2009). This developmental colouration is conferred by genotype D/D l-1/l-1 L-2/L-2 (Paris, 2000).

The fruits of L-1/ — L-2/ — plants are intense green throughout development. In contrast to plants homozygous recessive for either or both l genes, the fruits of L-1/— L-2/ — plants retain their black-green colour through maturity, not turning orange or yellow when ripe (Paris, 2000; Paris and Brown, 2005).

The other important locus affecting fruit colour in *C. pepo* is the one with Gene Y, which is incompletely dominant over y; when homozygous, it confers a yellow colour to the young fruit, which remains yellow or turns yellow-orange or orange as it matures; when heterozygous, the yellow-orange color is conferred beginning when the fruit is intermediate in age (Paris and Brown, 2005).

2.9.14 Fruit number, size (length, diameter and area) and weight; seed number and seed weight of *Cucurbita* species

Cucurbita species are extremely diverse in fruit characters (Aruah et al., 2010). The number of fruits per plant may range from 4.0 to 22.0 among *C. maxima* cultivars (Loy, 2004); and from 2.0 to 15.7 among *C. moschata* genotypes (Ahamed et al., 2011). The vining, small-fruited *C. maxima* cultivars may set more than 20 fruits on a single plant (Loy, 2004). The bush plant of *C. moschata* has a higher rate of fruit abortion than vine plants, but they have a larger total number of fruit than vine plants on a plant basis (Wu et al., 2007; Wu and Cao, 2008).

The fruit weight varies from 1.5 to 4.2 kg among *C. moschata* genotypes (Ahamed et al., 2011); and from 3.2 to 11.8 kg among *C. maxima* populations (Balkaya et al., 2010). The fruit length and fruit diameter differ widely, from 26.0 to 49.8 cm and from 35.1 to 56.5 cm, respectively, among *C. maxima* populations (Balkaya et al., 2010). The bush-type *C. moschata* plants have smaller fruits than vine-type plant (Wu et al., 2008; Wu and Cao, 2008).

The size and shape of the mature *Cucurbita* fruits can be influenced by the number of developing seeds (Ercan and Kurum, 2003). Fruits with a below-average number of seeds are prone to abortion (Ercan and Kurum, 2003). In *Cucurbita pepo* which has a double sigmodial growth curve, fruits with fewer seeds tend to grow more rapidly, even though the final volume is less than fruits

with greater seed numbers (Ercan and Kurum, 2003). Fruits with low seed numbers are smaller than fruits with high seed numbers (Ercan and Kurum, 2003). In the experiment by Nerson (2005) among *C. pepo* cultivars, seed yield per fruit increased with increasing fruit weight, where in most cases the increase was due to the increase in both seed number and seed weight. However, minor exceptions were also found in this experiment, where the seed weights were unaffected by fruit weight (Nerson, 2005). According to Nerson (2007), seed yield in all cucurbit crops grown in the field is greatly affected by environmental conditions such as light intensity or temperature.

Loy (2004) reported that seeds of *Cucurbita* range in size from about 50 mg for small-fruited gourds to over 250 mg in some large-fruited cultigens. However, Nerson (2005) reported a general compensation tendency where *C. pepo* cultivars with high seed numbers have relatively small seeds. Also the mean seed weight per fruit (g) in *C. pepo* is closely related to the seed number per fruit (Nerson, 2005). Dry weight of 100-seed range from 6.4 to 13.6 g among *C. moschata* genotypes (Ahamed et al., 2011) and from 20.1 to 66.4 g among *C. maxima* populations (Balkaya et al., 2009).

2.10 Landraces and plant breeding

A landrace is an early cultivated form of a crop species, evolved from a wild population, and generally composed of a heterogeneous mixture of genotypes (Salazar et al., 2007; Mujaju et al., 2010) or traditional and local crop varieties particularly used by local farmers (Montes-Hernandez and Eguiarte, 2002; Modi, 2004). A world concerned with the loss of genetic variability prioritizes the maintaining of their genetic resources (Malvar et al., 2007). Thus, substantial genotypic variation in traditional landraces makes them essential genetic resources for plant breeders (Sari et al., 2008; Balkaya et al., 2009; 2010).

Landraces are often cultivated in marginal production areas such as small gardens, and in subsistence farming (Sari et al., 2008; Balkaya et al., 2010); rooted in local communities' culture and are often identified as a part of cultural heritage (Salazar et al., 2007). They can grow and reproduce under low external input conditions and diverse as well as adverse local environments (Modi, 2004; Salazar et al., 2007). However, to a large extent most *Cucurbita* landraces have long been replaced by new cultivars which ensure higher yields and income and meet the requirements of processors and consumers, as production systems moved from subsistence cultivation to intensive and market-oriented systems (Balkaya et al., 2009; 2010). The new plant varieties that are being developed by farmers are not necessarily from the landraces but are from different sources and therefore serve as an addition to landraces and local varieties from farmers' communities (Salazar et al., 2007).

According to Modi (2004), co-operative programmes encouraging small farmers to continue growing landraces (*in situ* landrace conservation) will be a worthwhile investment for high-tech agriculture, because the pool of genetic diversity in landraces is an important resource for breeders trying to develop high-yield cultivars. Therefore, allowing landraces to disappear undermines the foundation of genetic diversity of domesticated species (Modi, 2004). Morphological and molecular characterization of diverse germplasm for landraces increases breeders' and farmers' use of them (Ferriol et al., 2004a; 2004b; Balkaya et al., 2010).

Indigenous leafy vegetables have many unanswered research questions, ranging from germplasm conservation to sustainable production strategies, which limit the efforts towards exploiting their nutritional and production potentials (Opabode and Adebooye, 2005). Research on these vegetables has focused mainly on their value in ethnobotany, the collection, preservation and the assessment of food value and chemical composition but no serious breeding and seed production research has been conducted on them (Adebooye et al., 2005).

2.11 Genetic diversity within and between populations

Information on genetic diversity of plant germplasm, within and between accessions, is the important basis of conservation biology and genetic improvement (Yuan et al., 2007; Mujaju et al., 2010). Genetic diversity information offers the opportunity for production diversification and the development of new farming systems and new quality products (Mujaju et al., 2010). Gene differentiation and gene flow are important indices to evaluate a population's genetic structure (Yuan et al., 2007; Mujaju et al., 2010). According to Yuan et al. (2007), the gene flow, the movement of genes within and between populations, is negatively correlated with gene differentiation. The coefficient of genetic differentiation (G_{ST}) approaches zero when gene diversity is high, even if sub-populations are completely differentiated (Mujaju et al., 2010).

The population genetic structure is mainly affected by long distance diffusion of pollen owing to inbreeding and outcrossing (Yuan et al., 2007; Mujaju et al., 2010). However, in the case of long geographical distances where populations are distributed in several provinces and local districts, the main way of gene exchanges can be occasional introduction of seeds and seedlings (Yuan et al., 2007), as well as informal seed exchanges practiced by farmers (Balkaya et al., 2009; Mujaju et al., 2010). It is also possible that unique local populations are formed by variable genotype, which is produced by the interaction effect between gene and environment to adapt to special environment during long-term natural and artificial selection (Yuan et al., 2007).

Mujaju et al. (2010) reported on a variety of locally sourced seeds which are usually agro-ecologically and socio-economically adapted to local conditions. A high genetic diversity maintained within populations is influenced by characteristics of the species and is encouraging from a conservation

perspective (Han et al., 2007). This high level of population differentiation may be explained by several factors, including the species' breeding system, genetic drift or genetic isolation of populations, where the breeding system of a species is an important determinant of variability at both the species and population levels (Han et al., 2007).

Han et al. (2007) reported that according to population genetic theory, genetic drift is the predominant factor which shapes genetic structure in species with low levels of gene flow ($Nm < 1.0$). Therefore, for species with $Nm > 4.0$, gene flow overwhelms the effect of genetic drift. While for species exhibiting intermediate levels of gene flow ($1.0 < Nm < 4.0$), gene flow and genetic drift interact to produce the observed genetic structure.

2.12 Genetic markers

Genetic markers are signs or flags which occupy specific genomic positions called loci within chromosomes, are located in close proximity to genes, and represent genetic differences between individual organisms or species (Collard et al., 2005).

Three types of genetic markers include: morphological (classical or visible) markers which themselves are phenotypic traits or characters; biochemical markers, which include allelic variants of enzymes called isozymes; and molecular (DNA) markers, which reveal sites of variation in DNA (Collard et al., 2005; Gajera et al., 2010). Morphological markers are usually visually characterized phenotypic traits such as flower colour; fruit size and shape; seed size and shape; growth habit or pigmentation (Aruah et al., 2010; Ahamed et al., 2011). Isozyme or allozyme markers are differences in enzymes that are detected by electrophoresis and specific staining (El-Domyati et al., 2011). However, despite being limited in number and influenced by environmental

factors or plant developmental stages (Khan et al., 2009; El-Domyati et al., 2011), morphological and biochemical markers have been extremely useful to plant breeders due to the availability of an extensive literature for several crop species, and allozymes sometimes give concordant results with those from DNA markers (Collard et al., 2005; Djè et al., 2006).

Molecular markers are abundant and are not affected by environmental factors and/or the developmental stage of the plant, thus are the most widely used type of markers (Collard et al., 2005; El-Domyati et al., 2011). They arise from different classes of DNA mutations such as substitution mutations (point mutations), rearrangements (insertions or deletions) or error in replication of tandemly repeated DNA and they are selectively neutral because they are usually located in non-coding regions of DNA (Collard et al., 2005). Molecular markers are used in the construction of linkage maps (Fukino et al., 2008; Gong et al., 2008), and they have numerous applications in plant breeding such as assessing the level of genetic diversity within germplasm; cultivar identity as well as a general guide in the selection of the parents for hybridization to maximize expression of heterosis (Hadia et al., 2008; El-Domyati et al., 2011; Formisano et al., 2012; Gong et al., 2012).

Three classes of molecular markers: hybridization-based, polymerase chain reaction (PCR)-based and DNA sequence-based; may be broadly divided based on their detection (Collard et al., 2005). Molecular markers that reveal differences between individuals of the same or different species are called polymorphic markers, whereas markers that do not discriminate between genotypes are called monomorphic markers (Collard et al., 2005). Polymorphic markers can be described as codominant or dominant, based on whether they can discriminate between homozygotes and heterozygotes (Collard et al., 2005). Codominant markers indicate differences in size and may have many different alleles whereas dominant markers are either present or absent and only have two alleles (Collard et al., 2005).

2.13 Molecular markers for analyzing genetic diversity among landraces

Molecular genetic markers have been developed into powerful tools for genetic relationships and genetic diversity within and between plant populations (Hadia et al., 2008; Khan et al., 2009), and can aid in management and conservation of biodiversity (Gajera et al., 2010). They provide a quick and reliable method for estimating genetic relationships among genotypes of any organism (Khan et al., 2009). Molecular markers such as random amplified polymorphism DNA (RAPD) and simple sequence repeats (SSR) or microsatellite markers can be used to determine the genetic variation among landraces (Gajera et al., 2010; Formisano et al., 2012; Gong et al., 2012).

2.13.1 Random Amplified Polymorphic DNA

Random Amplified Polymorphic DNA (RAPD) marker is also known as Arbitrarily Primed-Polymerase Chain Reaction (AP-PCR) (Power, 1996). It can be used in molecular biology to: determine taxonomic identity; assess kinship relationships; analyse mixed genome samples; create specific probes; analyze interspecific gene flow and hybrid speciation (Khan et al., 2009); and has proven to be quite efficient in detecting genetic variations in many plants (Zhang et al., 2008; Gajera et al., 2010). The RAPD technology has the following main advantages: suitability for work on anonymous genomes; applicability to problems where only limited quantities of DNA are available; able to generate a nearly unlimited number of markers; an efficient or simple technique; and low expense (Behera et al., 2008; Zhang et al., 2008; Khan et al., 2009; Gajera et al., 2010). A large set of primers that are used in RAPD markers have the advantage of screening the entire genome (Navajas and Fenton, 2000).

Random Amplified Polymorphic DNA markers also have some limitations. Poor reproducibility/repeatability of results (the ability to achieve the same results whenever and wherever the same individuals or populations are tested) is one of its major pitfalls and is influenced by PCR conditions (Bagley et al., 2001; De Wolf et al., 2004; Mujaju et al., 2010). However, even if RAPD reproducibility can be achieved, replicates are always necessary to verify the reproducibility of the results and bands that fail to be reproduced consistently should not be considered (Barracosa et al., 2008). The RAPD bands are dominant, thus heterozygotes cannot be distinguished from homozygotes of the dominant allele (Navajas and Fenton, 2000; Morimoto et al., 2006; Mujaju et al., 2010). The nature of the genomic change that is scored is not known (De Wolf et al., 2004).

In the Cucurbitaceae family, RAPD method has been utilized to analyze the genetic diversity of: *Cucurbita maxima*; *C. moschata* and *C. pepo* (Ferriol et al., 2003; 2004b; Hadia et al., 2008; Tsivelikas et al., 2009; Du et al., 2011; Formisano et al., 2012); *Lagenaria siceraria* (Morimoto et al., 2006); *Momordica charantia* (Dey et al., 2006); *Trichosanthes dioica* (Khan et al., 2009). A molecular analysis performed in *C. maxima*; *C. moschata* and *C. pepo* using RAPD markers, revealed a high level of polymorphism (Hadia et al., 2008; Tsivelikas et al., 2009).

2.13.2 Simple Sequence Repeat

Simple sequence repeats (SSRs) are variously known as microsatellites, short tandem repeats (STRs) or simple sequence length polymorphism (SSLP) (Kalia et al., 2011). They are tandemly repeated, very short stretches of DNA sequences of 1-6 base pairs long (El-Domyati et al., 2011; Kalia et al., 2011; Gong et al., 2012). Simple sequence repeat markers have a frequent occurrence in all prokaryotic genomes and are abundant in most eukaryotic species (Fukino et al., 2008; Formisano et al., 2012; Kalia et al., 2011). They are highly

polymorphic as a result of variations in the number of repeats (El-Domyati et al., 2011; Formisano et al., 2012; Kalia et al., 2011; Gong et al., 2012).

By virtue of their uniqueness, microsatellite loci are most valuable for detecting variation among closely related varieties and studying intra- and interspecific relationships (Kalia et al., 2011; Gong et al., 2012). Depending on the number of nucleotides per repeat unit, SSR markers have been classified as mono-, di-, tri-, tetra-, penta- or hexanucleotides (El-Domyati et al., 2011; Kalia et al., 2011) that have 4 to 10 repeat units side-by-side (El-Domyati et al., 2011). Microsatellite markers are classified as perfect, imperfect and compound, based on the arrangement of nucleotides in the repeat motifs (Kalia et al., 2011). Further, SSRs can be classified as nuclear (nuSSR), mitochondrial (mtSSR) or chloroplastic (cpSSR), based on their location in the genome (Kalia et al., 2011).

Simple sequence repeat markers have many desirable attributes, including: hypervariability, informativeness, reliability (reproducibility), codominant inheritance, multiallelic nature, relative abundance, and high throughput genotyping, which has resulted in them gaining considerable importance in plant genetics and breeding (El-Domyati et al., 2011; Formisano et al., 2012; Kalia et al., 2011; Gong et al., 2012). However, the application of SSR techniques to plants depends on the availability of suitable microsatellite markers and these markers are difficult to obtain in species for which genetic and genomic tools are lacking (Garcia et al., 2004; Formisano et al., 2012).

In Cucurbitaceae family the SSR technique has been successfully used in the genetic diversity analysis of: *Citrullus lanatus* (Jarret et al., 1997); *Cucumis melo* (Szabo et al., 2008; Escribano et al., 2012); *Cucumis sativus* (Esteras et al., 2008; Sestili et al., 2008); and *C. pepo* and *C. moschata* (Ferriol et al., 2003; 2004a; Stift et al., 2004; Esteras et al., 2008; Gong et al., 2008; 2012; Formisano et al., 2012).

2.14 Genetic variation of landraces from different geographic regions

The genetic diversity among landraces or accessions of cucurbits (Du et al., 2011; Barboza et al., 2012) and Bambara groundnuts (Amadou et al., 2001) from diverse agro-ecological conditions is probably due to the genetic variability of the species which allows them to adapt specifically to these conditions, especially when the plants were introduced to such areas many years ago. This therefore reflects that these landraces were developed in isolated zones (Du et al., 2011). However the close relationship (grouping) among landraces from diverse micro- and macro-geographic origins could be due to the existence of seed exchange among farmers (Yuan et al., 2007; Du et al., 2011; Barboza et al., 2012). Relationships may also be because they shared a common origin.

A study conducted on 16 individuals of *C. moschata* from a wide distributional range extending from Mexico to Bolivia in South America, shows a wide genetic variation among them, which may be a result of adaptation to different ecological zones (Sanjur et al., 2002). Random amplified polymorphic DNA markers were used to analyze the genetic diversity among landraces of *C. moschata* species from Korea, southern Africa, and other geographical origins, where in all cases the accessions grouped together according to the agroclimatic regions of origin and not according to morphological traits (Ferriol et al., 2004a).

The RADP markers used to investigate the intraspecific variation between *Lagenaria siceraria* plants demonstrate that landraces from Africa, Asia, and the New World are genetically differentiated from each other (Morimoto et al., 2006). Similar markers (RAPD) indicate that there is a trend of micro-geographical variation among both cultivated *Lagenaria siceraria* and wild *L. sphaerica* landraces, with their accessions collected from different provinces of Kenya (Morimoto et al., 2006). The cultural isolation caused by different ethnic groups inhabiting different areas could be reflected in the geographical differentiation in the cultivated species *L. siceraria*, whereas the geographic differentiation might

have progressed only by the true ‘isolation by distance’ or by random drift, without any natural or artificial selection in the wild species *L. sphaerica* (Morimoto et al., 2006).

2.15 Conclusion

This literature review has stated the wide consumption of TLVs that are collected from the wild or as weeds among the cultivated crops, with few cultivated vegetable species. These vegetables are mostly neglected in crop improvement research because they are considered as weeds and associated with poor communities. The *Cucurbita* species were reported as highly polymorphic species both in their morphological and reproductive characters. Landraces were reported to have diversity in their genetic make-up; are adapted to local environment and thus are essential for plant breeding. It has shown that genotypes from different agro-ecological origins have wide genetic diversity. Also, self-pollination causes great genetic diversity among the populations of the same species.

The following chapters record the types of TLVs that are known and preferred in northern KwaZulu-Natal and the assessment of morphological diversity in agronomic traits of *Cucurbita* landraces. The study of genetic diversity in *C. pepo* landraces that are of different agro-ecological origins as well as the impact of self-pollination on these landraces, are reported.

Chapter 3

Identification and utilization of traditional leafy vegetables in northern KwaZulu-Natal

3.1 Introduction

Traditional leafy vegetables are edible plant species that are either cultivated or harvested from the wild for their leafy parts, including leaves; young, succulent stems; flowers; young and mature fruits; and roots (Gockowski et al., 2003; Jansen van Rensburg et al., 2004; 2007). These vegetables are often used multi-contexually, for example as both food and medicine (Dweba and Mearns, 2011), where the bitter tasting leafy vegetables are associated with medicine, and sour tasting ones with food (Pieroni et al., 2002).

Leafy vegetables are important sources of micronutrients including vitamin A and C, iron, zinc, folate and other nutrients, minerals and roughage needed to maintain health and promote immunity against infections (Flyman and Afolayan, 2006; Johns et al., 2006). Despite their high nutritional value, most traditional leafy vegetables are not popular and replaced by exotic vegetables in many countries, and have not been selected and improved by breeders for enhanced palatability, like the introduced vegetables (Jansen van Rensburg et al., 2004; Odhav et al., 2007).

Traditional leafy vegtables are easy to cook (Wallace et al., 1998). The conventional household cooking methods are blanching, boiling and stir-frying for 2-5 minutes. However the boiling method has been found to destroy the amount of vitamin C concentration in all cooked vegetables (Somsub et al., 2008). The traditional cooking method of 90 minutes for cassava leaves and 50 minutes for pumpkin leaves results in significant losses in protein, fats and vitamins. Sun

drying using traditional mats causes losses of vitamin A of 36% and 38% for cassava and pumpkin leaves, respectively (Lyimo et al., 1991).

A great diversity of TLV species occurs in agricultural and agro-pastoral communities because disturbed habitats promote the growth of these weedy species (Vainio-Mattila, 2000). For instance, weedy traditional vegetables such as *Amaranthus hybridus* and *Momordica foetida* are collected and sold at road markets by rural women in northern KwaZulu-Natal (Zobolo et al., 2008). Fleuret (1979) recorded more than 15 species of wild leafy vegetables in her study in the Lushoto district, Tanzania. Woodcook's (1995) studied on indigenous knowledge and forest use in the East Usambaras in Tanzania documents 25 wild leafy vegetable species.

South Africa has an exceptionally high plant biodiversity (Dahlberg and Burlando, 2009), where a portion of the Maputaland-Pondoland-Albany centre of endemism, one of three globally recognized biodiversity hotspots, is in northern KwaZulu-Natal (Meer, 2010). Maputaland hotspot harbours many endemic plants (Torquebiau et al., 2010), where *Albertisia delagoensis* (De Wet and van Wyk, 2008) is an example. The most popular traditional leafy vegetables in South Africa and KwaZulu-Natal are in the genera *Amaranthus, Bidens, Chenopodium, Citrullus, Cucurbita, Corchorus* and *Momordica* (Vorster et al., 2007; Faber et al., 2010).

Pumpkins (*Cucurbita* species) are subtropical to tropical vegetable species that are mostly grown in spring and summer when there is rain availability (Jansen van Rensburg et al., 2007; Ghaly and Alkoaik, 2010). Their harvested seeds are dried and stored in any available and preferably tightly-closed container until they are sown (Ndoro et al., 2007; Amutha et al., 2009).

Traditional knowledge on plant uses is generally possessed by older people (40 years and above) and is passed on orally to younger generations (Zobolo and

Mkabela, 2006). Although a great deal of papers on South African traditional leafy vegetables have already been published (Afolayan and Jimoh, 2009) there is still a lot of tradition to be documented, protected and saved (Guarino, 1997).

Even though the northern KwaZulu-Natal forms the core-area of Maputaland biodiversity hotspot and most people depend on plants for their survival, there is no comprehensive documentation of traditional leafy vegetables utilized in this area. Therefore the objectives of this research were:

- To identify the traditional leafy vegetables utilized by the communities of northern KwaZulu-Natal, South Africa.
- To determine the leafy vegetable types preferred by the communities and their consumption frequency.
- To determine the collecting season of wild preferred leafy vegetables and the seed source, growth season and initiation of leafy shoot removal in domesticated preferred leafy vegetables.

3.2 Materials and methods

3.2.1 Study area

A survey of traditional leafy vegetables was conducted in three district municipalities of northern KwaZulu-Natal, South Africa (Figure 3.1), namely Umkhanyakude, uThungulu and Zululand. Umkhanyakude district (Mseleni: 27^{o}38' S and 32^{o}47' E) covers the areas with coastal sand dune forest and sand dry forests found in Maputaland on deep white sands (Pooley, 2003). It occurs in the Maputaland Centre of Plant Endemism which has many rare, unusual and endemic plant species, and also has very high species diversity (Van Wyk & Smith, 2001). The climate is subtropical, with mean annual rainfall ranging between 707 mm and 721 mm, and a mean maximum summer temperature of 29^{o}C, while in winter it is 17^{o}C (South African Weather Service, 2008).

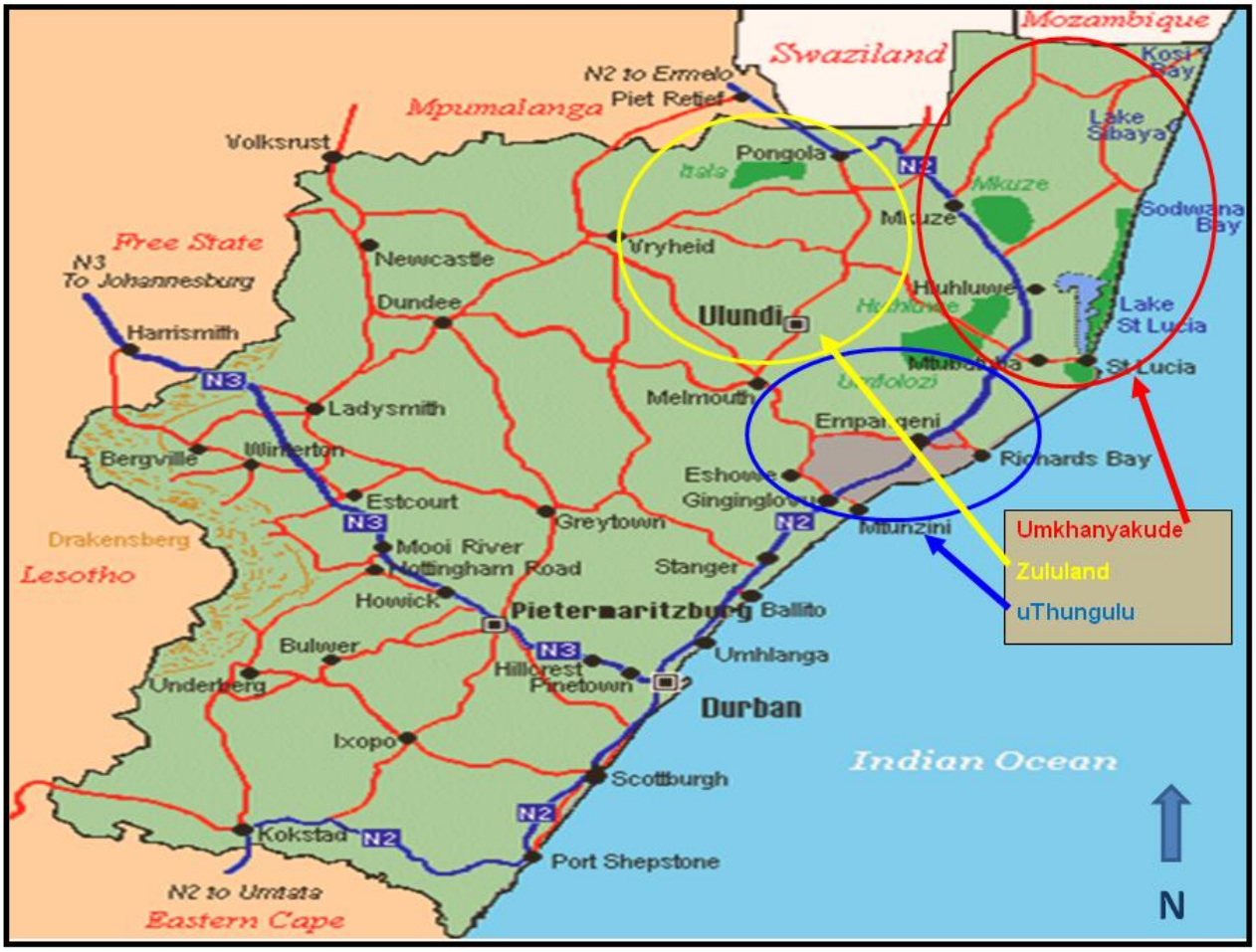

Figure 3.1: The Umkhanyakude, uThungulu and Zululand district municipalities surveyed in northern KwaZulu-Natal, South Africa (http://www.places.co.za/maps/new%20maps/kwazulu_natal_map4.gif).

UThungulu district (Nkandla: 28^{o}37' S and 31^{o}25' E) is situated in the transition of coastal forest merging with mist-belt forest on the mainly south- and east-facing slopes of high escarpments (Pooley, 2003). It is part of the Maputaland-Pondoland-Albany biodiversity hotspots which harbours many endemic plant species (Van Wyk & Smith, 2001). The climate is subtropical with annual rainfall ranging between 1640 mm and 1740 mm, and a mean maximum summer temperature of 32^{o}C while in winter it is 18^{o}C (South African Weather Service, 2008).

Zululand district (Ulundi: 28^{o}32' S and 31^{o}47' E) is characterized by granite outcrops and open grassy glades in cool, inland regions, with its vegetation ranging from mistbelt forest to bushveld (Pooley, 2003). The climate is temperate with annual rainfall ranging between 587 mm and 750 mm, and a mean maximum summer temperature of 32^{o}C while in winter it is 14^{o}C (South African Weather Service, 2008).

3.2.2 Community surveys

The three districts have both peri-urban and rural areas. Peri-urban areas are defined as areas that are closely located to well-developed areas and have facilities and lifestyle that mimics that of urban areas. In each district three to five remote rural villages were identified with the aid of the following traditional leaders (amaKhosi): Umkhanyakude District: Inkosi Hlabisa, Mabaso and Tembe; UThungulu District: Inkosi Biyela, Mkhwanazi, Mzimela, and Shange; and Zululand District: Inkosi Buthelezi, Mbatha and Zungu. The personnel from the Department of Agriculture, in all districts, were also contacted in order to have full access to the vegetable farmers.

The interviews were conducted with structured questionnaires (Appendix I) in isiZulu, the mother tongue of interviewees. Fifty homesteads were randomly sampled per village. Hence, a total of 150 homesteads were sampled in each district, with 450 in total which gives a sampling intensity of 62.5% of the total homesteads. The homesteads sampled in uMkhanyakude district were situated around the villages of Manguzi, Ngwavuma, Mbazwana, Mseleni and Hlabisa. In uThungulu district, the villages visited were at KwaShange, Mahlayizeni and Ongoye, and in the Zululand district, the villages are Exolo, Ewela and Nkonjeni. Among all villages, only Ongoye village at uThungulu district was classified as in the peri-urban area. The information obtained from household interviews was consolidated through focus groups using garden and general community meetings (Table 3.1).

Table 3.1: Number of participants during the indigenous and traditional leafy vegetable survey in northern KwaZulu-Natal

District	Area (Village)	Participants	
		Households	**Focus groups**
Umkhanyakude	Hlabisa	50	10 groups: 3 people/grp.
	Mseleni and Mbazwana	50	10 groups: 10 people/grp.
	Ngwavuma and Manguzi	50	10 groups: 10 people/grp.
UThungulu	Ongoye and KwaMzimela	50	10 groups: 3 people/grp.
	KwaShange	50	10 groups: 3 people/grp.
	Mahlayizeni	50	10 groups: 3 people/grp.
Zululand	Nkonjeni	50	10 groups: 5 people/grp.
	Ewela	50	10 groups: 5 people/grp.
	Exolo	50	10 groups: 5 people/grp.

The gender and age of the eldest and active participant was recorded per household. Informants with a minimum age of 18 years were interviewed and the age ranges were grouped as follows: young-age (18-34 years); middle-age (35-54 years); and old-age (55 years and above).

Interviewees listed the traditional leafy vegetables they knew and the different types of landraces within each vegetable, where possible, and then selected the vegetables they preferred. Aspects of the preferred vegetables which were recorded include: the frequency of their consumption, parts used; and the source of plants, whether cultivated or wild. The frequency of consumption ranged from daily to weekly, where daily consumption was rated as very frequent; every two days was less frequent; while weekly consumption was not frequent consumption.

Growth season(s) and initiation of leafy shoot removal from sowing were determined for the preferred cultivated leafy vegetables. The wild leafy vegetables preferred in all districts were also investigated for their collecting season(s).

3.2.3 Collection and preparation of voucher specimens

Voucher specimens were collected concurrently with interviews and prepared according to Fish (1999). However, in cases where specimens were absent because of unfavourable seasons, follow-up collecting trips were conducted, with reference to the given isiZulu name(s). Where possible a specimen for each isiZulu name given was collected per village in each district. This ensured proper species identification using a very wide local naming system used by the communities. The cultivated vegetable species, whose seeds were the only material available in the communities, were grown at the University of Zululand Ethnobotanic Garden for the preparation of voucher specimens. The identified voucher specimens were scientifically validated at the University of Zululand (ZULU) and KwaZulu-Natal (NH) herbaria.

3.2.4 Statistical analysis

Results were statistically analyzed using One-Way ANOVA and the significancy was determined at 5 % ($P<0.05$). Values in tables are means ± standard error (SE).

3.3 Results

3.3.1 Gender and age of the interviewees

The percentages of interviewed males and females, the age groupings interviewed, and the knowledge and preferences of traditional leafy vegetables, for each district, are presented in Table 3.2. In all three districts three times more females than males were interviewed since they were the ones mostly found in homesteads and were actively participating in the interviews.

There was significantly higher percentage of middle-age than old-age female participants in the Zululand district. There were no differences in knowledge of traditional vegetables between the three age groups. Traditional leafy vegetable preferences did not vary with ages in any district. However, at Umkhanyakude district significantly higher percentage of young-aged male respondents preferred TLVs than middle-aged respondents. There were minor differences in the knowledge and preference of the traditional leafy vegetables in the three districts, as indicated by superscript letters in Table 3.2.

3.3.2 Traditional leafy vegetables reported by informants of northern KwaZulu-Natal: taxonomy, cultivation status, part(s) used and mode of preparation.

This study recorded a total of 72 plant species belonging to 49 genera in 31 families as traditional leafy vegetables in northern KwaZulu-Natal (Appendix II). Among these, 56 plant species were gathered from the wild while 16 were cultivated. All species, except *Ophioglossum polyphyllum* A. Braun (Ophioglossaceae, Pteridophyta) were angiosperms. Out of the angiosperms, four families, Araceae, Asphodelaceae, Commelinaceae and Hyacinthaceae, were monocotyledons; the rest were dicotyledons.

Table 3.2: Gender, age percentage of informants; mean known and preferred leafy vegetables per age group of interviewees (n=3)

Criterion	Age group	Percentage of informants' response					
Interviewees' gender		**Females**			**Males**		
		Umkhanyakude	**UThungulu**	**Zululand**	**Umkhanyakude**	**UThungulu**	**Zululand**
		75.33 ± 3.53	75.36 ± 3.68	84.00 ± 6.11	24.67 ± 3.53	24.64 ± 3.68	16.00 ± 6.11
Informants' age (years)	**Young-age**	14.65 ± 3.49 a	16.17 ± 3.09 a	14.55 ± 2.08 ab	13.48 ± 5.22 a	21.06 ± 4.56 a	25.56 ± 5.30 a
	Middle-age	20.10 ± 3.02 a	17.65 ± 2.89 a	22.38 ± 1.98 b	22.51 ± 3.22 a	19.95 ± 2.56 a	17.22 ± 4.33 a
	Old-age	15.25 ± 3.04 a [ab]	16.18 ± 0.30 a [b]	13.07 ± 0.30 a [a]	14.02 ±2.04 a	8.99 ± 3.02 a	7.22 ± 4.94 a
Known vegetables	**Young-age**	7.33 ± 0.61 a	5.22 ± 1.01 a	5.23 ± 0.71 a	9.64 ± 1.86 a [b]	5.19 ± 0.51 a [b]	3.32 ± 0.21 a [a]
	Middle-age	8.75 ± 0.92 a [b]	5.52 ± 1.07 a [ab]	4.85 ± 0.60 a [a]	6.68 ± 0.58 a [b]	4.92 ± 0.25 a [a]	4.25 ± 1.01 a [ab]
	Old-age	6.58 ± 0.71 a	5.07 ± 1.09 a	5.39 ± 0.66 a	7.44 ± 1.09 a	5.78 ± 0.97 a	4.17 ± 2.32 a
Preferred vegetables	**Young-age**	3.41 ± 0.30 a	2.25 ± 0.34 a	3.27 ± 0.53 a	3.59 ± 0.39 b [b]	1.73 ± 0.03 a [a]	1.71 ± 0.36 a [a]
	Middle-age	3.31 ± 0.20 a [b]	2.41 ± 0.05 a [a]	3.63 ± 0.03 a [b]	2.20 ± 0.20 a	2.77 ± 0.59 a	2.83 ± 0.44 a
	Old-age	3.93 ± 0.45 a	2.73 ± 0.83 a	3.32 ± 0.46 a	3.33 ± 0.51 ab	3.44 ± 0.73 a	1.25 ± 0.63 a

Values are mean ± standard error (SE). The mean values followed by a different letter within a column and a superscript letter within a row, differ significantly (P<0.05).

The largest proportion of traditional vegetable plant species belonged to Amaranthaceae, Asteraceae and Cucurbitaceae with 11.11% each; followed by Commelinaceae and Convolvulaceae, with 5.56% each; and Acanthaceae, Brassicaceae and Fabaceae, with 4.17% each.

A total of 56, 45, and 38 traditional vegetable plant species were recorded at Umkhanyakude, uThungulu and Zululand districts, respectively. Among these species, 30 were common in all districts; three were reported at Umkhanyakude and uThungulu districts; three at Umkhanyakude and Zululand districts; and three at uThungulu and Zululand districts. The number of plant species exclusively reported at Umkhanyakude, uThungulu and Zululand districts, were 20, 9, and 4 respectively.

Traditional leafy vegetables (TLVs) were found to have up to six local (isiZulu) names, either within a district or between two or three districts. However, 82.35% of TLVs consumed in more than one district were found to have at least one commonly used local name among the synonyms.

Different species within a genus were referred to by one local name: *Momordica foetida* and *M. balsamina*, called “iNtshungu” in the uThungulu and Zululand districts; *Galinsoga ciliata* and *G. parviflora*, called “isiShukelana”, “uMaMkhize”, and others, in the uThungulu district; while *Ipomoea plebeia* subsp. *africana* and *I*. cf. *cairica* were both called “umBophamfe” in the Zululand district. Again, different genera had the same local name in one or other districts: “isiHlalakuhle” referred to *Limeum sulcatum* in the Umkhanyakude and Zululand districts; *Boerhavia diffusa* and *Hypochaeris radiata* in the uThungulu district; and *Sisymbrium thellungii* in the uThungulu and Zululand districts; while *Momordica foetida, M. balsamina* and *Coccinia rehmannii* were all called “iNtshungu” in the Zululand district; and “iMbilikicane” referred to both *Chenopodium album* and *Ipomoea plebeia* subsp. *africana* in the uThungulu district. One TLV species was

also found to have different local names in different districts: *Coccinia rehmannii* was called “iHhawulane” and “iNgwili” in the Umkhanyakude district; “amaPholonjane” in the uThungulu district; and “iNtshungu” in the Zululand district, while *Boerhavia diffusa* was called “isiHlalakuhle” in the uThungulu district; “uNkunzana” in the Zululand district; and “imiFino” (did not have a specific name) in the Umkhanyakude district.

The growth habit of vegetable plant species, in order of prevalence was: 54.80% herbs; 17.80% twinners or trailers; 12.30% climbers; 5.50% shrubs; and 5.50% trees. Some herbs have bulbs (2.70%) and corms (1.40%).

The plant parts used as vegetables ranged from, in order of most to least: leaf (62.50%); young shoots (22.50%); fruit (15.00%); seed (6.25%); flower (5.00%); tuber (2.50%); and corm (1.25%). Green aerial parts and young shoot apices were the most commonly gathered plant parts and they were mostly consumed after being cooked. Non-cultivated food plants represented an important part of the daily diet during the spring season and they were consumed mainly in mixtures. Only a few leafy vegetables: *Arachis hypogea; Carica papaya; Citrullus lanatus; Diospyros galpinii;* and *Passiflora incarnata*, were traditionally eaten raw, particularly their fruits. The most common practice was to gather, boil, then fry in olive or sunflower oil with garlic and onion, sometimes adding hot chillies. This vegetable soup was traditionally eaten with cooked maize (*Zea mays*) porridge.

Among the cultivated species, the pumpkins *Cucurbita maxima* and *Cucurbita pepo* were of particular interest because they were cultivated by informants in all of the three surveyed districts. The reliance of the informants on these *Cucurbita* species was due to the fact that many plant parts (young shoots, flowers, fruits and seeds) are edible.

3.3.3 Knowledge percentage of traditional leafy vegetables in northern KwaZulu-Natal

Thirty TLVs were known in all districts, of which twenty-three were wild and seven cultivated (Table 3.3). The well-known (knowledge more than 50%) TLVs in the Umkhanyakude district were: *A. hybridus, A. thunbergii, B. pilosa,* and *C. pepo*; while the least known (knowledge less than 5%) vegetables were *A. schimperi B. diffusa*; *C. murale*; *O. tenax* and *T. tetragonioides*. In the uThungulu district the most known vegetables were: *A. hybridus*, *B. pilosa* and *C. pepo*; whereas the least known were: *B. diffusa, C. murale, C. rehmannii, C. benghalensis, D. marlothii, L. siceraria, M. balsamina, S. retroflexum, T. tetragonioides* and *V. unguiculata*.

In the Zululand district: *A. hybridus*, *B. pilosa*, *C. album* and *C. pepo* were the famous vegetables, but *B. diffusa, C. rehmannii, C. esculenta, C. benghalensis*, *D. marlothii*; *M. balsamina, O. tenax, U. urens* and *V. unguiculata* were unfamiliar vegetables. Therefore the three most popular (knowledge more than 80%) leafy vegetables in all districts of northern KwaZulu-Natal were *A. hybridus*, *B. pilosa* and *C. pepo*, while the most unpopular (knowledge 0.67%) was *B. diffusa*.

In the Umkhanyakude district, a significantly higher percentage of informants knew *A. hybridus*, *B. pilosa* and *C. pepo* over other species, except *A. thunbergii*. These three species were also significantly famous in the uThungulu district when compared with other species except *A. schimperi*. In the Zululand district, knowledge of *A. hybridus* was significantly higher than all other species except *B. pilosa*.

Most people in the uThungulu district knew of *C. pepo* than communities in the Umkhanyakude and Zululand districts. The *I. batatas* and *S. oleraceus* were known by higher percentage of informants in the Umkhanyakude district than in the uThungulu and Zululand districts.

Table 3.3 Knowledge of traditional leafy vegetables in three districts of northern KwaZulu-Natal

Vegetable species	Percentage of respondents that knew the vegetables		
	Umkhanyakude (n=3)	UThungulu (n=3)	Zululand (n=3)
Amaranthus hybridus	84.67 ± 8.67 e	97.33 ± 1.76 d	98.00 ± 2.00 d
Amaranthus retroflexus	20.00 ± 17.01 abcd	15.00 ± 8.89 abc	22.00 ± 6.43 b
Amaranthus spinosus	20.00 ± 8.00 abcd	41.67 ± 14.66 bc	21.33 ± 4.06 b
Amaranthus thunbergii	56.67 ± 18.34 bcde	22.00 ± 8.08 abc	30.67 ± 12.45 ab
Asystasia schimperi	0.67 ± 0.67 a	28.00 ± 25.01 abcd	16.67 ± 9.82 ab
Bidens bipinnata	42.67 ± 10.09 cd	33.33 ± 19.88 abc	21.33 ± 7.69 ab
Bidens biternata	42.67 ± 10.09 cd	33.33 ± 19.88 abc	21.33 ± 7.69 ab
Bidens pilosa	92.67 ± 0.67 e	92.00 ± 5.03 d	84.67 ± 7.42 cd
Boerhavia diffusa	0.67 ± 0.67 a	0.67 ± 0.67 a	0.67 ± 0.67 a
Chenopodium album	34.00 ± 4.16 cd	24.00 ± 2.31 c	52.00 ± 10.00 b
Chenopodium murale	1.33 ± 0.67 ab	2.00 ± 1.16 a	6.00 ± 5.03 ab
Citrullus lanatus [c]	23.33 ± 11.80 abcd	6.67 ± 0.67 b	20.67 ± 11.22 ab
Coccinia rehmannii	20.67 ± 13.48 abcd	4.00 ± 4.00 ab	0.67 ± 0.67 a
Colocasia esculenta [c]	15.33 ± 5.21 bc	12.00 ± 4.16 abc	1.33 ± 0.67 a
Commelina benghalensis	10.67 ± 5.21 abc	0.67 ± 0.67 a	1.33 ± 1.33 a
Corchorus olitorius	28.00 ± 10.07 abcd	10.67 ± 9.68 abc	12.67 ± 7.06 ab
Cucurbita maxima [c]	24.00 ± 4.16 c	34.00 ± 8.33 c	30.00 ± 7.57 b
Cucurbita pepo [c]	88.67 ± 0.67 e [a]	94.67 ± 1.33 d [b]	82.00 ± 3.06 c [a]
Dipcadi marlothii	6.00 ± 6.00 abc	1.33 ± 0.67 a	0.67 ± 0.67 a
Ipomoea batatas [c]	33.33 ± 4.06 cd [b]	7.33 ± 2.40 ab [a]	10.00 ± 3.06 b [a]
Lagenaria siceraria [c]	10.00 ± 5.29 abc	2.67 ± 1.33 ab	9.33 ± 4.06 ab
Momordica balsamina	18.00 ± 12.49 abcd	2.00 ± 2.00 ab	0.67 ± 0.67 a
Momordica foetida	9.33 ± 7.33 abc	20.00 ± 10.39 abc	14.67 ± 7.51 ab
Obetia tenax	4.67 ± 3.71 ab	8.00 ± 5.03 ab	3.33 ± 3.33 ab
Solanum retroflexum	9.33 ± 2.91 b	2.67 ± 1.76 ab	8.67 ± 4.37 ab
Sonchus oleraceus	41.33 ± 2.91 d [b]	15.00 ± 5.57 abc [a]	16.00 ± 6.93 ab [a]
Tetragonia tetragonioides	3.33 ± 3.33 ab	2.67 ± 0.67 a	6.67 ± 2.91 ab
Urtica urens	21.33 ± 20.34 abcd	11.00 ± 4.73 abc	2.67 ± 2.67 a
Vigna unguiculata [c]	18.00 ± 9.02 abcd	1.33 ± 1.33 a	0.67 ± 0.67 a
Zantedeschia aethiopica	14.00 ± 14.00 abcd	12.00 ± 9.17 abc	5.33 ± 2.67 ab

The species names have variety and subspecies names which are not written. Vegetable species with superscript 'c' are cultivated. Mean values followed by a different letter within a column and superscript letter within a row, differ significantly ($p \leq 0.05$). Values are mean ± standard error (SE).

The knowledge percentage of 33 vegetables, out of 37 known in one or two districts, was very low (less than 20%), except for *G. parviflora* (22.33 ± 13.35%) in the uThungulu district; as well as *M. esculenta* (28.67 ± 14.89%), *P. scandens* (46.67 ± 23.33%) and *R. torulosa* (29.33 ± 14.71%) in the Umkhanyakude district. Differences were also not significant among these vegetables.

3.3.4 Traditional leafy vegetable preference in northern KwaZulu-Natal

The communities of northern KwaZulu-Natal preferred 43 traditional leafy vegetables from the total of 72 recorded where 32 were wild and 11 were cultivated. The Umkhanyakude, uThungulu and Zululand districts preferred a total of 37, 19, and 25 traditional leafy vegetables, respectively. Fifteen traditional leafy vegetables (Table 3.4) were preferred in all three districts, where 67% species were wild and 33% were cultivated.

Table 3.4 Traditional leafy vegetables preferred in all districts of northern KwaZulu-Natal

Vegetable species	Percentage of respondents that preferred the vegetables		
	Umkhanyakude (n=3)	uThungulu (n=3)	Zululand (n=3)
Amaranthus hybridus	52.00 ± 19.22 abcd [ab]	74.33 ± 5.61 c [a]	91.33 ± 1.76 e [b]
Amaranthus spinosus	18.67 ± 11.68 abcd	28.33 ± 10.84 ab	22.00 ± 5.03 cd
Amaranthus thunbergii	32.67 ± 10.48 bcd	20.33 ± 5.04 b	49.33 ± 15.07 cde
Bidens bipinnata	21.33 ± 9.41 abcd	11.33 ± 5.21 ab	10.00 ± 3.06 bc
Bidens pilosa	37.33 ± 8.11 d	25.67 ± 7.84 b	33.33 ± 6.36 cd
Chenopodium album	6.67 ± 1.76 bc [ab]	2.00 ± 1.16 a [a]	17.33 ± 3.71 c [b]
Citrullus lanatus [c]	12.67 ± 2.91 bc	3.33 ± 2.40 a	6.67 ± 0.67 b
Corchorus olitorius	10.00 ± 7.02 abcd	1.33 ± 1.33 a	4.67 ± 2.91 abc
Cucurbita maxima [c]	9.33 ± 3.53 abc	15.00 ± 5.86 ab	10.67 ± 4.67 abc
Cucurbita pepo [c]	42.00 ± 6.43 d	35.33 ± 2.40 b	39.33 ± 3.71 d
Ipomoea batatas [c]	10.67 ± 1.33 c [b]	3.33 ± 0.67 a [a]	3.33 ± 2.40 ab [ab]
Lagenaria siceraria [c]	6.67 ± 2.40 abc	3.33 ± 3.33 a	6.00 ± 3.06 abc
Sonchus oleraceus	4.67 ± 0.67 b	2.00 ± 1.16 a	6.67 ± 4.81 abc
Tetragonia tetragonioides	0.67 ± 0.67 a	2.00 ± 1.16 a	1.33 ± 0.67 a
Urtica urens	7.33 ± 7.33 abcd	1.33 ± 1.33 a	0.67 ± 0.67 a

[c] Cultivated species. Values are mean ± S.E. The mean values followed by a different letter within a column and superscript letter within a row, differ significantly (P<0.05).

The most preferred (preference more than 25%) traditional leafy vegetables in the Umkhanyakude district were: *A. hybridus, A. thunbergii, B. pilosa* and *C.*

pepo; whereas *T. tetragonioides* was the least preferred (preference less than 2.5%) vegetable species. In the uThungulu district: *A. hybridus, A. spinosus, B. pilosa* and *C. pepo* most favored, but *C. album*, *C. olitorius*, *S. oleraceus*, *T. tetragonioides* and *U. urens* were the least favored. The most preferred vegetables in the Zululand district were: *A. hybridus, A. thunbergii, B. pilosa* and *C. pepo*; while *T. tetragonioides* and *U. urens* were the least preferred vegetables. Therefore in all districts of northern KwaZulu-Natal *A. hybridus, B. pilosa* and *C. pepo* were the most favoured while *T. tetragonioides* was the least.

In the Umkhanyakude district, *B. pilosa* and *C. pepo* were significantly preferred over other vegetable species, except for *A. hybridus*, *A. spinosus*, *A. thunbergii*, *B. bipinnata*, *C. olitorius* and *U. urens*. In the uThungulu district, *A. hybridus* was significantly favored over all other vegetables. This vegetable species was also significantly preferred in the Zululand district when compared with other species except *A. thunbergii*.

The *A. hybridus* and *C. album* were more preferred in the Zululand district than uThungulu district, while *Ipomoea batatas* was more favoured in the Umkhanyakude district than uThungulu district.

In a total of 27 vegetables preferred in either one or two districts, 22 (81.48%) were collected from the wild and only five (18.52%) were cultivated. Of these vegetables, in their order of prevalence: 15 (55.56%) were favoured only at Umkhanyakude district; five (18.52%) at Umkhanyakude and Zululand districts; three (11.11%) at Zululand district only; two (7.41%) at Umkhanyakude and uThungulu districts; and two (7.41%) at uThungulu and Zululand districts. The percentage of informants preferring these vegetables was very low (ranging between 0.67 to 14%), and differences were not significant among vegetables and between different districts.

3.3.5 Consumption frequency of preferred traditional leafy vegetables

Results presented in Table 3.5 show that in the Umkhanyakude district 100%; 86.67% and 73.33% of the preferred leafy vegetables were consumed daily; every two days and weekly, respectively. In the uThungulu district 73.33%; 73.33% and 53.33% species were consumed daily; every two days and weekly, respectively. The communities of Zululand district consumed 86.67% daily; 86.67% in every two days; and 80.00% weekly.

A significantly higher percentages of people in the Umkhanyakude district consumed *B. pilosa* and *C. pepo* daily compared with other species, except *Amaranthus* species. Significantly higher consumption of *C. pepo* was also recorded at every two day interval than *C. album*, *C. maxima*. In the uThungulu district there was significantly higher consumption of *B. pilosa* daily than *C. album*, *C. lanatus*, *C. olitorius*. In the Zululand district significantly higher percentages of informants consumed *A. hybridus* daily over all other species. At two day intervals, the consumption of *B. pilosa* and *C. pepo* was significantly higher than other species except *A. hybridus*, *A. thunbergii* and *L. siceraria*.

A significantly larger percentage of people in the Umkhanyakude district ate: *C. maxima* every two days rather than weekly; and *C. pepo* daily rather than weekly. In harvesting or wet season, a significantly higher percentage of people in the uThungulu district ate *A. hybridus* every two days rather than weekly. Also in the Zululand district a significantly higher percentage of informants consumed: *A. hybridus* daily rather than in other frequencies; *A. thunbergii* daily rather than weekly; *B. pilosa* and *C. pepo* in every two days rather than weekly.

Table 3.5 Consumption frequency (%) of vegetables preferred in all districts

Dist	Vegetable species	Daily	Every two days	Weekly
M	*Amaranthus hybridus*	30.03 ± 13.59 abc	9.95 ± 4.62 abc	8.69 ± 5.18 a
	Amaranthus spinosus	14.67 ± 12.72 abc	3.99 ± 2.00 abc	0.00 ± 0.00 a
	Amaranthus thunbergii	19.97 ± 8.71 abc	9.94 ± 9.08 abc	9.47 ± 5.89 a
	Bidens bipinnata	18.67 ± 8.90 abc	4.75 ± 2.93 abc	2.73 ± 1.80 a
	Bidens pilosa	31.43 ± 7.76 c	7.33 ± 3.98 abc	11.29 ± 8.58 a
	Chenopodium album	5.98 ± 1.16 b	1.99 ± 1.14 ab	1.99 ± 1.14 a
	Citrullus lanatus	7.99 ± 3.06 ab	6.68 ± 0.68 bc	5.33 ± 5.33 a
	Corchorus olitorius	10.02 ± 4.64 ab	4.03 ± 3.08 abc	2.63 ± 1.73 a
	Cucurbita maxima	7.33 ± 2.91 ab [ab]	4.00 ± 1.16 b [b]	0.00 ± 0.00 a [a]
	Cucurbita pepo	28.13 ± 4.09 c [b]	15.91 ± 4.12 c [ab]	4.67 ± 4.67 a [a]
	Ipomoea batatas	7.97 ± 1.96 b	5.29 ± 2.87 abc	3.29 ± 3.29 a
	Lagenaria siceraria	6.02 ± 1.99 b	3.99 ± 3.06 abc	2.01 ± 2.01 a
	Sonchus oleraceus	7.39 ± 3.50 ab	2.70 ± 2.70 abc	6.67 ± 3.53 a
	Tetragonia tetragonioides	0.67 ± 0.67 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	Urtica urens	7.33 ± 7.33 ab	0.00 ± 0.00 a	0.00 ± 0.00 a
T	*Amaranthus hybridus*	20.43 ± 8.71 ab [ab]	17.93 ± 3.40 b [b]	5.97 ± 2.31 a [a]
	Amaranthus spinosus	11.37 ± 8.37 ab	7.35 ± 3.54 ab	2.68 ± 1.76 a
	Amaranthus thunbergii	7.69 ± 4.12 ab	4.81 ± 2.87 ab	0.00 ± 0.00 a
	Bidens bipinnata	3.31 ± 2.38 ab	4.65 ± 2.39 ab	1.35 ± 1.35 a
	Bidens pilosa	8.04 ± 1.16 b	8.69 ± 2.91 b	6.00 ± 4.00 a
	Chenopodium album	0.67 ± 0.67 a	2.67 ± 2.67 ab	0.00 ± 0.00 a
	Citrullus lanatus	0.67 ± 0.67 a	0.00 ± 0.00 a	0.67 ± 0.67 a
	Corchorus olitorius	1.33 ± 1.33 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	Cucurbita maxima	6.67 ± 5.70 ab	1.36 ± 1.36 ab	1.33 ± 1.33 a
	Cucurbita pepo	9.28 ± 5.39 ab	8.79 ± 4.11 ab	6.62 ± 5.63 a
	Ipomoea batatas	2.67 ± 1.76 ab	0.00 ± 0.00 a	0.00 ± 0.00 a
	Lagenaria siceraria	0.00 ± 0.00 a	0.00 ± 0.00 a	1.32 ± 1.32 a
	Sonchus oleraceus	0.00 ± 0.00 a	0.67 ± 0.67 ab	0.00 ± 0.00 a
	Tetragonia tetragonioides	0.00 ± 0.00 a	1.33 ± 0.67 ab	0.00 ± 0.00 a
	Urtica urens	0.00 ± 0.00 a	0.67 ± 0.67 ab	0.00 ± 0.00 a
Z	*Amaranthus hybridus*	46.07 ± 4.14 c [b]	15.41 ± 3.96 bc [a]	9.93 ± 4.34 ab [a]
	Amaranthus spinosus	12.03 ± 5.98 ab	2.08 ± 1.20 ab	2.65 ± 1.78 ab
	Amaranthus thunbergii	23.43 ± 6.43 b [b]	7.36 ± 4.70 abc [ab]	4.63 ± 1.68 ab [a]
	Bidens bipinnata	8.02 ± 3.49 ab	0.66 ± 0.66 ab	0.69 ± 0.69 ab
	Bidens pilosa	18.77 ± 7.11 ab [ab]	11.29 ± 1.41 c [b]	3.35 ± 2.39 ab [a]
	Chenopodium album	9.39 ± 4.84 ab	3.33 ± 1.76 ab	1.98 ± 1.98 ab
	Citrullus lanatus	0.00 ± 0.00 a	4.66 ± 1.34 b	2.01 ± 1.16 ab
	Corchorus olitorius	2.00 ± 2.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	Cucurbita maxima	3.31 ± 2.38 a	5.31 ± 1.31 b	2.65 ± 0.65 b
	Cucurbita pepo	17.97 ± 6.92 ab [ab]	16.62 ± 2.73 c [b]	8.00 ± 4.11 ab [a]
	Ipomoea batatas	1.32 ± 1.32 a	0.67 ± 0.67 ab	2.68 ± 2.68 ab
	Lagenaria siceraria	0.68 ± 0.68 a	4.67 ± 2.40 abc	0.68 ± 0.68 ab
	Sonchus oleraceus	3.98 ± 1.99 a	1.98 ± 1.98 ab	0.00 ± 0.00 a
	Tetragonia tetragonioides	0.00 ± 0.00 a	0.67 ± 0.67 ab	0.67 ± 0.67 ab
	Urtica urens	0.67 ± 0.67 a	0.00 ± 0.00 a	0.00 ± 0.00 a

Values are mean ± S.E. The mean values followed by a different letter within a column and a different superscript letter within a row, differ significantly (P<0.05). Dist, district; M, Umkhanyakude; T, uThungulu; Z, Zululand.

Among vegetable species consumed daily when available in all districts, the consumption of *B. pilosa*, *C. album* and *C. pepo* was significantly higher in the Umkhanyakude than in the uThungulu district whereas that of *I. batatas* was higher in the Umkhanyakude than in the Zululand district, while vegetable consumption at other frequencies did not differ significantly.

Some communities reported other consumption frequencies: once and twice a month; very seldom; in summer; when desired; and when available.

3.3.6 Seed sourcing, growth season and initiation of shoot removal in *C. pepo*

The results for seed sourcing and growth season of *C. pepo*, one of the most well-known and preferred cultivated TLVs are presented in Table 3.6. The majority of people in all districts saved seeds in their homesteads for future growth; with significantly higher percentages in the Umkhanyakude district than in the Zululand district.

In all districts, significantly higher percentages of people sowed their pumpkin seeds in spring and summer than in other seasons. Some informants noted that: if pumpkin seeds are sown in winter they germinate but have retarded growth; and that the growth of pumpkins is possible throughout the year when it was raining.

In all districts the majority of people started picking pumpkin leafy shoots for vegetable consumption in less than two months from seed sowing, with very few individuals picking them between two and three months.

Table 3.6: Seed sourcing, growth season and initiation of shoot removal in *Cucurbita pepo*

Criterion	Knowledge percentage of pumpkin seed sourcing and storing in northern KwaZulu-Natal		
	Umkhanyakude (n=3)	**uThungulu (n=3)**	**Zululand (n=3)**
Seed source			
Home-saved seeds	90.00 ± 3.06 b [b]	90.67 ± 4.67 b [ab]	77.33 ± 1.76 b [a]
Seed merchants	0.67 ± 0.67 a	0.67 ± 0.67 a	0.00 ± 0.00 a
Growth season			
Spring	42.67 ± 8.19 b	64.00 ± 16.04 b	66.00 ± 4.00 b
Summer	50.67 ± 2.67 b	51.33 ± 11.62 b	48.67 ± 6.36 b
Autumn	10.00 ± 3.06 a	2.00 ± 1.16 a	7.33 ± 5.46 a
Winter	17.33 ± 6.77 a	4.00 ± 4.00 a	3.33 ± 0.67 a
Throughout the year	24.67 ± 11.10 ab	4.00 ± 3.06 a	6.67 ± 3.53 a
Time from seed sowing to leafy shoot removal			
<2 months	72.67 ± 8.11 b	81.33 ± 10.67 b	70.67 ± 7.42 b
>2-3 months	11.33 ± 7.69 a	11.33 ± 5.93 a	6.67 ± 5.70 a

Values are mean ± S.E. The mean values followed by a different superscript letter within a row and letter within a column differ significantly ($P<0.05$).

3.3.7 Collecting seasons for wild traditional leafy vegetables

Results for collecting seasons for 10 wild vegetables preferred in all districts are presented in Table 3.7. The year-round criterion was treated separately because informants mentioned it with other seasons.

In the Umkhanyakude district, in descending order, 100% of the preferred vegetables were collected year-round; 90% were each collected in spring summer and autumn; while only 10% were collected in winter. In the uThungulu district: 100%; 90%; 60% and 40% of the preferred leafy vegetables were collected in summer; spring; year-round and autumn, respectively; whereas none were collected in winter. Also, in the Zululand district: 100%; 90%; 70%; 60% and

20% of species were collected in summer; spring; autumn; year-round and winter respectively.

Many people knew *A. hybridus, B. bipinnata, B. pilosa, C. album* and *S. oleraceus* (Umkhanyakude district) to be collected in spring; *A. thunbergii*, *B. bipinnata* (Umkhanyakude district), and *B. pilosa* (uThungulu district) in summer; and *A. thunbergii* (uThungulu district) throughout the year, when compared with other species.

In the Umkhanyakude district *A. hybridus* and *C. album* were well-known to be collected in spring rather than in winter; *A. thunbergii* in summer rather than winter; *B. bipinnata* in spring and summer rather than winter; while *B. pilosa* and *S. oleraceus* were most known to be collected in spring rather than autumn and winter. Further, a higher percentage of people in the uThungulu district knew that *B. pilosa* was collected in summer rather than in autumn and winter.

The collection of *A. hybridus, A. spinosus, A. thunbergii, B. bipinnata, B. pilosa, C. album, C. olitorius, S. oleraceus* and *U. urens*; in spring and summer in all districts, was compared, and there were significantly more people in the Umkhanyakude district than in the uThungulu district, who knew that *S. oleraceus* was collected in spring.

Communities in districts represented in brackets, noted the availability of the following species year-round only when they grew in wet places particularly near the rivers or in marshland or when it rained for the whole year: *A. hybridus, A. thunbergii* and *B. pilosa* (all districts); *B. bipinnata* and *C. album* (Umkhanyakude and Zululand districts); *A. spinosus* (uThungulu and Zululand districts); *C. olitorius* and *S. oleraceus* (Umkhanyakude district).

Table 3.7 Collecting seasons for wild leafy vegetables preferred in all districts

District	Vegetable species	Spring	Summer	Autumn	Winter	Year-round
Umkhanyakude	*Amaranthus hybridus*	18.67 ± 4.06 b [b]	29.33 ± 15.51 ab [ab]	6.00 ± 3.06 a [ab]	0.00 ± 0.00 a [a]	15.33 ± 8.67 a [ab]
	Amaranthus spinosus	4.67 ± 3.71 ab	14.00 ± 13.01 ab	1.33 ± 0.67 a	0.00 ± 0.00 a	0.67 ± 0.67 a
	Amaranthus thunbergii	12.00 ± 5.03 ab [ab]	13.33 ± 3.33 b [b]	6.00 ± 3.06 a [ab]	0.00 ± 0.00 a [a]	17.33 ± 10.41 a [ab]
	Bidens bipinnata	11.33 ± 2.91 b [b]	11.33 ± 2.67 b [b]	3.33 ± 2.40 a [ab]	0.00 ± 0.00 a [a]	11.33 ± 7.69 a [ab]
	Bidens pilosa	18.67 ± 3.71 b [b]	24.67 ± 11.80 ab [ab]	5.33 ± 2.40 a [a]	0.00 ± 0.00 a [a]	17.33 ± 8.82 a [ab]
	Chenopodium album	8.67 ± 2.91 b [b]	13.33 ± 8.51 ab [ab]	0.67 ± 0.67 a [ab]	0.00 ± 0.00 a [a]	4.67 ± 2.91 a [ab]
	Corchorus olitorius	4.00 ± 2.31 ab	8.67 ± 4.67 ab	0.67 ± 0.67 a	0.00 ± 0.00 a	3.33 ± 1.76 a
	Sonchus oleraceus	8.00 ± 1.16 b [b]	14.00 ± 9.17 ab [ab]	0.67 ± 0.67 a [a]	0.00 ± 0.00 a [a]	6.00 ± 3.06 a [ab]
	Tetragonia tetragonioides	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.67 ± 0.67 a
	Urtica urens	8.00 ± 8.00 ab	15.33 ± 15.33 ab	1.33 ± 1.33 a	0.67 ± 0.67 a	0.67 ± 0.67 a
uThungulu	*Amaranthus hybridus*	22.00 ± 12.00 a	35.33 ± 14.44 ab	0.67 ± 0.67 a	0.00 ± 0.00 a	7.33 ± 3.53 ab
	Amaranthus spinosus	10.67 ± 6.00 a	21.33 ± 9.33 ab	1.33 ± 1.33 a	0.00 ± 0.00 a	2.00 ± 1.16 ab
	Amaranthus thunbergii	8.00 ± 5.03 a	12.00 ± 6.43 ab	0.00 ± 0.00 a	0.00 ± 0.00 a	4.00 ± 1.16 b
	Bidens bipinnata	5.33 ± 2.40 a	8.67 ± 3.53 ab	1.33 ± 1.33 a	0.00 ± 0.00 a	0.67 ± 0.67 ab
	Bidens pilosa	6.67 ± 3.71 a [ab]	12.67 ± 2.91 b [b]	2.00 ± 2.00 a [a]	0.00 ± 0.00 a [a]	3.33 ± 2.40 ab [ab]
	Chenopodium album	1.33 ± 0.67 a	1.33 ± 0.67 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.67 ± 0.67 ab
	Corchorus olitorius	0.00 ± 0.00 a	1.33 ± 1.33 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	Sonchus oleraceus	1.33 ± 1.33 a	1.33 ± 1.33 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	Tetragonia tetragonioides	1.33 ± 1.33 a	2.00 ± 1.16 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	Urtica urens	1.33 ± 1.33 a	1.33 ± 1.33 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
Zululand	*Amaranthus hybridus*	39.33 ± 18.81 a	44.67 ± 21.83 a	10.00 ± 4.00 a	2.00 ± 1.16 a	9.33 ± 5.81 a
	Amaranthus spinosus	11.33 ± 7.69 a	11.33 ± 7.69 a	3.33 ± 1.76 a	0.00 ± 0.00 a	2.67 ± 2.67 a
	Amaranthus thunbergii	21.33 ± 12.72 a	26.67 ± 13.78 a	6.00 ± 2.31 a	0.67 ± 0.67 a	9.33 ± 6.57 a
	Bidens bipinnata	4.67 ± 2.91 a	6.67 ± 4.81 a	2.00 ± 2.00 a	0.00 ± 0.00 a	0.67 ± 0.67 a
	Bidens pilosa	14.00 ± 8.08 a	19.33 ± 11.57 a	3.33 ± 2.40 a	0.00 ± 0.00 a	2.00 ± 2.00 a
	Chenopodium album	7.33 ± 4.06 a	10.00 ± 5.77 a	2.00 ± 1.16 a	0.00 ± 0.00 a	0.67 ± 0.67 a
	Corchorus olitorius	0.67 ± 0.67 a	1.33 ± 1.33 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	Sonchus oleraceus	3.33 ± 3.33 a	5.33 ± 5.33 a	0.67 ± 0.67 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	Tetragonia tetragonioides	0.67 ± 0.67 a	0.67 ± 0.67 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	Urtica urens	0.00 ± 0.00 a	0.67 ± 0.67 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a

Values are mean ± S.E. The mean values followed by a different superscript letter within a row and letter within a column differ significantly (P<0.05).

3.4 Discussion

3.4.1 Gender and age differences in the knowledge of and preference for traditional leafy vegetables in northern KwaZulu-Natal

Young males of Umkhanyakude district knew and preferred higher percentages of leafy vegetables than young males in both uThungulu and Zululand districts; and also middle- and old-aged males of the same district. This could be the result of being brought up as children who are willing to offer assistance to elderly women in collecting and cooking these vegetables, thus sharing knowledge (Vainio-Mattila, 2000; Narayanan and Kumar, 2007; Reddy et al., 2007) and also were exposed to high local vegetable species diversity (Dahlberg and Burlando, 2009; Meer, 2010). This however, contradicts with the reports of Zobolo and Mkabela (2006) and Odhav et al. (2007) that TLVs are known and utilized by older generations of 40 years and above and that they are not accepted by most of younger generations.

3.4.2 Traditional leafy vegetables recorded in northern KwaZulu-Natal and their scientific validation

South Africa has an exceptionally high biodiversity, with many people still using a wide variety of plants for food and medicine (Twine et al., 2003, Jansen van Rensburg et al., 2004; Dahlberg and Burlando, 2009), thus 72 species in 49 genera of 31 families of plant species consumed in northern KwaZulu-Natal showed a wide diversity of plants used as TLVs. This high vegetable species record in this region concurs with our hypothesis which states that the northern part of KwaZulu-Natal is endowed with high diversity of TLVs which are extensively consumed in rural communities in particular. A survey done in Mara Region, Tanzania recorded 38 species of edible wild fruits and leafy vegetables from 51 informants (Johns et al., 1996). The highest total number of traditional

leafy vegetables was recorded (56) in the Umkhanyakude district. This is probably because the district falls under the Maputaland Centre of Plant Endemism and Diversity, which has a high species diversity (Dahlberg and Burlando, 2009; Meer, 2010).

In a total of 31 families of vegetable species recorded in northern KwaZulu-Natal, the Acanthaceae, Amaranthaceae, Asteraceae, Brassicaceae, Commelinaceae, Convolvulaceae, Cucurbitaceae, and Fabaceae formed the largest proportions. This is comparable to Vainio-Mattila (2000) who documented Acanthaceae, Amaranthaceae, Asteraceae, and Brassicaceae as the most important families of wild green leafy vegetables, among 26 reported families in East and West Usambaras, Tanzania. Again, in Andhra Pradesh, India; Reddy et al. (2007) reported 69 families of wild food plants, where Amaranthaceae and Fabaceae families had a high number of species.

In addition to the traditional leafy vegetables cultivated by the communities of northern KwaZulu-Natal, the following wild vegetable species were declared by Henderson (2007) as naturalized and casual alien plants in southern Africa: *A. hybridus; B. bipinnata; B. biternata; B. pilosa; C. album; C. benghalensis; H. radicata; M. coromandelianum; N. officinale; and S. oleraceus*.

The use of voucher specimens ensures an appropriate scientific identification of vegetable species in the case of a great variation in their local naming. Voucher specimens have been widely collected where local names were used during community surveys of vegetable research (Faber et al., 2010; Molebatsi et al., 2010; Dweba and Mearns, 2011).

The following species were recorded for the first time as traditional leafy vegetables: *Commelina erecta; Deinbollia oblongifolia; Erythroxylum delagoense; Galinsoga ciliata; Ipomoea wightii; Limeum sulcatum; Priva meyeri* var. *meyeri; Trachyandra asperata* var. *asperata and Trachyandra* cf. *saltii* var. *saltii*.

3.4.3 Plant parts used for vegetable purposes and the stage of collection

The observations of use of green aerial parts and young shoot apices in the present study are with the reports by Faber et al. (2010) and Dweba and Mearns (2011). Different parts of the same vegetable plant species can be consumed as vegetables in different areas. In northern KwaZulu-Natal, young fruits of *Abelmoschus esculentus* (okra) are consumed, however, in Nigeria both leaves and fruits are cooked as vegetables (Olasantan and Salau, 2008).

Communities in northern KwaZulu-Natal mentioned the shoot collection of: *A. thunbergii*, *B. pilosa*, *C. album*, *G. parviflora* before flowering; *A. spinosus* at seedling stage before thorn formation; young tender shoots and/or leaves of *A. hybridus*, *Cucurbita* spp, *I. batatas* and *M. esculenta*. This confirms reports by Vainio-Mattila (2000) and Jansen van Rensburg et al. (2007) that plants are usually collected and cooked for vegetable purposes at their vegetative stage, when their leaves are young and tender or when they have very young fruits. Also, according to Ghaly and Alkoaik (2010), leaves of *I. batatas* are traditionally eaten when they are still young, as they are more nutritious at this stage.

3.4.4 Vegetable knowledge and preference in northern KwaZulu-Natal

The people of northern KwaZulu-Natal eat or prefer lesser numbers of vegetables than those they know. This corresponds with the findings of Shackleton (2003) and later by Dovie et al. (2007), in the Limpopo Province, South Africa, where individual households were consuming between five and seven species, but as many as 21 species were known per household. Similar reports were recorded by Narayanan and Kumar (2007) in India where there was a great deal of variation in the wild green vegetables preferred by different communities: in a

total of 102 identified species in *Paniya*, *Kattunaikka* and *Kuruma* tribes, families consumed 88, 43 and 21, respectively.

Their low preference for traditional leafy vegetables could have been influenced by at least one of the following reasons: taste and plant choice; labels of backwardness; poverty and famine foods; and ethnicity and gender particularly women's food (Dweba and Mearns, 2011).

The high number of known and preferred traditional vegetables in the present study were collected from the wild, probably because they were easily accessible during rainy seasons (Shackleton, 2003; Narayanan and Kumar, 2007; Ndoro et al., 2007) or throughout the year (Vainio-Mattila, 2000; Faber et al., 2010); and to people with low financial capacity (Dovie et al., 2007).

The highest number of known and preferred vegetables was found in the Umkhanyakude district. This is most likely due to the position of this district in the Maputaland Centre of Plant Endemism and Diversity (Dahlberg and Burlando, 2009; Meer, 2010) which enabled easy access to traditional vegetables as a result of their local abundance (Vainio-Mattila, 2000). The local vegetable abundance in the current study was further shown by a high number of wild vegetable species: *A. delagoensis; A. sessilis; A. gangetica; D. oblongifolia; E. delagoense; H. odorata; I. wightii; P. daemia; P. scandens* and *T. asperata*, which were known and some also preferred in this district and were reported only at Manguzi/Ngwavuma and Mbazwana/Mseleni villages which have deep Quartenary sands (Dahlberg and Burlando, 2009; Gibbon et al., 2010) compared with Hlabisa villages at Umkhanyakude district and all villages from uThungulu and Zululand districts.

The communities of uThungulu district knew a higher number of leafy vegetables, the second highest from Umkhanyakude district, but had the lowest preferenceof these vegetables. Such low preference could have been as a result of the

association of traditional leafy vegetables with elderly people especially women; primitiveness and poverty foods (Faber et al., 2010; Dweba and Mearns, 2011). This may also result from the location of this district in semi-urban area where most of people are employed and may not be interested in TLVs.

Zululand district communities knew the least vegetable species of them all but they preferred the highest percentage (65.79%) of what they had, thus were second in the number of preferred species. This high vegetable species preference may be caused by their ability to satisfy hunger, especially for poor people (Dovie et al, 2007; Odhav et al., 2007); availability year-round particularly when they were growing in wet areas and/or among crops with frequent irrigation (Gibbon et al., 2010; Faber et al., 2010; Lewu and Mavengahama, 2010; Torquebiau et al., 2010). Since surveyed areas of Zululand district were located far from towns, the locals are generally poor and rely exclusively on TLVs.

3.4.5 High knowledge and preference of *Amaranthus* species, *Bidens pilosa* and *Cucurbita pepo*

The A*maranthus* species, *B. pilosa* and *C. pepo* were the three most well-known and most preferred traditional leafy vegetables in the northern KwaZulu-Natal (Tables 3.3 and 3.4). Research conducted in KwaZulu-Natal (Zobolo and Mkabela, 2006; Odhav et al., 2007; Vorster et al., 2007; Lewu and Mavengahama, 2010), South Africa (Vorster et al., 2007), and in Tanzania (Vainio-Mattila, 2000) shows one or all of these species as vegetables. Jansen van Rensburg et al. (2007), Vorster et al. (2007) and Faber et al. (2010) reported *A. hybridus* and *C. pepo* as two of the most widely used leafy vegetables in South Africa. This further corresponds with the findings of Narayanan and Kumar (2007) in India, where out of a total of 102 species identified in *Paniya*, *Kuruma* and *Kattunaikka* communities, only eight, four and three wild edible leaves were

widely used, respectively. The *A. sessilis*, *A. spinosus* and *C. esculenta* were among those leafy vegetables that were frequently used by these Indian tribes.

Jansen van Rensburg et al. (2007) and Vorster et al. (2007) recorded that in KwaZulu-Natal, Limpopo and Eastern Cape, South Africa, cucurbits were highly valued in all regions, and had no gender differences in preference, and the wide variety of cucurbits helped to increase the variety in taste. All cucurbits, ranging from squashes to pumpkins and watermelons, were highly valued as they provided fruit, leaves and sometimes, flowers for the diet. The orange and yellow cucurbits were more preferred by consumers as they tended to alleviate vitamin A malnutrition (Vorster et al., 2007).

Further, according to Jansen van Rensburg et al. (2007) and Vorster et al. (2007), amaranth (*Amaranthus* sp.) were widely used in South Africa, whether alone or mixed with other leaves. Men were reported to prefer the bitter taste of *B. pilosa*, though they are used in the mix of leaves to help add taste to the dish (Vorster et al., 2007). The wild vegetables' preference ranking in West and East Usambaras, Tanzania, was similarly based on good taste: *A. hybridus* and *A. spinosus*; as well as easy access and local abundance: *B. pilosa*, *G. parviflora*, and *A. spinosus* (Vainio-Mattila, 2000). The similar reasons could be true for the preference of these species in northern KwaZulu-Natal.

3.4.6 Consumption frequency of preferred vegetables

Out of a total of 15 preferred leafy vegetable species in all districts, 10 (67%) species: *A. hybridus*; *A. spinosus*; *A. thunbergii*; *B. bipinnata*; *B. pilosa*; *C. album*; *C. olitorius*; *C. maxima*; *C. pepo*; and *I. batatas* were consumed daily, which was the highest number (percentage) compared with other consumption frequencies (Table 3.5). This confirms that leafy vegetables are an important component of the diet of people throughout sub-Saharan Africa (Vainio-Mattila, 2000;

Shackleton, 2003; Dovie et al., 2007). However, in India, Narayanan and Kumar (2007) reported *Bidens pilosa* as the least frequently consumed leafy vegetable, ranging between 2-3 times a month based on its abundance, availability and accessible supply.

Among these most frequently consumed vegetables, seven (70%) were wild species and three (30%) were cultivated species (Table 3.4 and 3.5). Therefore the daily consumption of these wild species can be possible during the rainy seasons in spring and summer (Shackleton, 2003; Dovie et al. 2007; Faber et al., 2010); in winter when they grow as weeds on the irrigated plots of cultivated fields (Lewu and Mavengahama, 2010); when they are easily available throughout the year (Vainio-Mattila, 2000); or when they grow along watercourses (Faber et al., 2010). Again the cultivated species can be consumed daily if: it is during wet season (Ndoro et al., 2007); when irrigated if growing under dryland conditions (Vorster et al. 2007); easy to grow in dry and wet seasons (Shaffer, 2008 and 2010) or when they are grown in soils with high moisture content such as wetlands and riverbanks (Dahlberg and Burlando, 2009; Gibbon et al., 2010; Torquebiau et al., 2010).

The highest percentage and most frequent vegetable consumption was found in the Umkhanyakude district (Table 3.5) probably due to easy accessibility because of high species diversity within this region (Dahlberg and Burlando, 2009; Meer, 2010; Torquebiau et al., 2010). However, in the Zululand district frequent consumption was probably as a result of vegetables' availability during wet seasons (Shackleton, 2003; Ndoro et al., 2007; Faber et al., 2010); collection next to the rivers (Faber et al., 2010); vegetable growth among the irrigated crops (Lewu and Mavengahama, 2010); and hunger satisfaction particularly for poor people (Dovie et al, 2007; Odhav et al., 2007). However, the lowest percentage and least consumption frequency of vegetables was found in the uThungulu district possibly as a result of the association of TLVs with elderly people especially women, primitiveness and poverty foods (Faber et al., 2010; Dweba

and Mearns, 2011). This might also be a result of the location of this district in the peri-urban area where most people are employed and may prefer and afford other food sources particularly from local markets than TLVs. This was evident because some informants in this district (Ongoye/KwaMzimela village) reported the consumption of leafy vegetables only when they desired them and not out of necessity.

Amaranthus hybridus, *Bidens bipinnata*, *B. pilosa* and *Cucurbita pepo*, were the most frequently consumed leafy vegetables in northern KwaZulu-Natal (Table 3.5), probably because they had at least one of the following attributes: a good taste (Vainio-Mattila, 2000; Voster et al., 2007); easily accessible throughout the season and locally abundant near people's homesteads (Dovie et al., 2007; Narayanan and Kumar, 2007) especially in rainy seasons during spring and summer (Ndoro et al., 2007; Faber et al., 2010); grows easily when cultivated or widely grown in southern Africa (Ndoro et al., 2007); easy to cook (Ndoro et al., 2007; Voster et al., 2007; Faber et al., 2010); no gender differences in their preference (Voster et al., 2007). Further, at least one of these attributes may hold true for the higher daily consumption of *B. pilosa*, *C. album* and *C. pepo* in the Umkhanyakude over the uThungulu district and that of *I. batatas* in the Umkhanyakude over the Zululand district.

In all districts, the variation in consumption frequency of *Amaranthus* and *Bidens* species ranged from daily to weekly (Table 3.5). However, *Citrullus lanatus* had a similar consumption frequency only at Umkhanyakude district. Faber et al. (2010) found that in summer leaves of: *Amaranthus* spp. and *B. pilosa* are picked weekly in KwaZulu-Natal; while in Limpopo, leaves of *Amaranthus* spp. are picked every two to five days and those of *C. lanatus* are picked daily for consumption purposes. In the current study, *Alternanthera sessilis* and *Colocasia esculenta* were not preferred TLVs in all districts. However, in India, Narayanan and Kumar (2007) report *Alternanthera sessilis* and *Colocasia esculenta* as being consumed almost everyday of the week. The daily to weekly consumption

interval of *Cucurbita* species in northern KwaZulu-Natal is similar to that in the Manicaland province of Zimbabwe, where pumpkins (*Cucurbita maxima/moschata*) are eaten three to four times a week during rainy season (Ndoro et al., 2007).

The pumpkins *Cucurbita maxima* and *Cucurbita pepo* were of particular interest because they were cultivated in all three surveyed districts, and the latter was highly preferred and the most consumed in all districts. The reliance of the informants on these *Cucurbita* species was because many plant parts (young shoots, flowers, fruits and seeds) are edible, observations also done by Jansen van Rensburg et al. (2007), Vorster et al. (2007) and Faber et al. (2010).

3.4.7 Sourcing and growth season of *Cucurbita pepo* seeds, and initiation of shoot harvesting for vegetable purposes

Home-saved seeds constituted the bulk of seed source in all districts, with very few individuals buying the seeds from seed merchants in the Umkhanyakude and uThungulu districts (Table 3.6). This corresponds with the findings of Ndoro et al. (2007) and Jury et al. (2008) where farmers set aside the best seeds for cultivation the following year, and thus they kept the ownership of pumpkin landraces.

The majority of farmers were growing pumpkins in spring and summer (Table 3.6). Pumpkins are known to be summer or warm weather crops (Ghaly and Alkoaik, 2010). Sowing in spring of pumpkins in South Africa was confirmed by Jansen van Rensburg et al. (2007). Growth in such seasons may be related to moist, sub-tropical climate (Dahlberg and Burlando, 2009) and rain availability (Shaffer, 2010), for this sub-tropical to tropical vegetable species. The minority also grew pumpkins in autumn, winter, and year-round and this may be influenced by: fair winter temperatures (Gibbon et al., 2010); irrigation during dry

seasons (Lewu and Mavengahama, 2010); and cultivation in vlei areas (Shaffer, 2008).

The majority of people started removing three-leaved shoots for vegetable purposes when pumpkin plants were still less than two months from sowing (Table 3.6). Jansen van Rensburg et al. (2007) reported on the general consumption of leafy parts of vegetables including the young succulent stems and very young fruits. However, they report the use of leaves, flowers and young fruits which are picked and cooked for vegetable purposes; but according to the present study three-leaved shoot tips were picked and cooked with flowers and young fruits for vegetable purposes in northern KwaZulu-Natal.

3.4.8 Collecting season for preferred wild vegetables

The year-round collection of the preferred wild leafy vegetables in the three districts, (Table 3.7) concurs with the report by Vainio-Mattila (2000) that the most used wild leafy vegetables are easily available all year round. The highest percentage of species collected throughout the year in the Umkhanyakude district may be influenced by the the area being more coastal and has high rainfall and suitable temperature throughout the year (Dahlberg and Burlando, 2009; Gibbon et al., 2010; Torquebiau et al., 2010). However, the soils are poor (Gibbon et al., 2010) except for the scattered wetlands that provide pockets of productive soil (Dahlberg and Burlando, 2009); but according to Dweba and Mearns (2011) traditional leafy vegetables can survive poor soils.

Amaranthus hybridus, *A. spinosus*, *A. thunbergii*, *Bidens bipinnata*, *B. pilosa* and *Chenopodium album* were reported in all districts as collected throughout the year when rainfall was available throughout the year and/or they were growing in wet places. This correspond with Dovie et al. (2007) that *A. hybridus*, *A. thunbergii* and *B. pilosa*; are commonly harvested and eaten while still fresh, in

rainy seasons. The leaves of *B. pilosa* are further reported by Faber et al. (2010) to be harvested in winter from the plants growing next to the rivers.

The highest collection of *A. hybridus, A. spinosus, A. thunbergii, B. bipinnata, B. pilosa, C. album, C. olitorius, S. oleraceus* and *U. urens* occurred in spring and summer (rainy seasons) in all districts of northern KwaZulu-Natal. This observation concurs with records by: Shackleton (2003); Dovie et al. (2007); Narayanan and Kumar (2007); Ndoro et al. (2007); and Faber et al. (2010), that most wild leafy vegetables are collected after the first rains; in the wet season; in spring; and in summer.

3.5 Conclusion

The present study showed that the people of northern KwaZulu-Natal know and utilize considerable amount of diverse TLV species. Umkhanyakude district had the highest percentage of recorded and preferred TLVs. This research recorded the following species for the first time as traditional leafy vegetables: *Commelina erecta; Deinbollia oblognifolia; Erythroxylum delagoense; Galinsoga ciliata; Ipomoea wightii; Limeum sulcatum; Priva meyeri* var. *meyeri; Trachyandra asperata* var. *asperata* and *Trachyandra* cf. *saltii* var. *saltii*.

Amaranthus hybridus, *B. pilosa* and *C. pepo* were the most popular TLVs in the region. Wild vegetables were harvested during the rainy seasons or year-round if they were growing in moisture-sufficient areas. *Cucurbita* species including *C. pepo* were grown in spring and summer and had their leafy shoot harvested in less than two months from seeding. When available, many people were eating them daily or every two days. Communities were saving seeds from the previous harvest which is a symbol of the ownership of these *Cucurbita* species. Therefore, the supply of preferred TLVs through cultivation, both in wet and dry seasons, is essential.

The findings of this chapter led to further investigation on diversity in growth and yield (shoots, fruits and seeds) of eight landraces in three *Cucurbita* species, as presented in the following chapter.

Chapter 4

Diversity in morphology and yield characteristics of *Cucurbita* landraces from northern KwaZulu-Natal, South Africa

4.1 Introduction

The genus *Cucurbita* is one of the most morphologicaly variable genera on the the entire plant kingdom (Aruah et al., 2010). Most cultivars have indeterminate and trailing growth form (Wu et al., 2008; Ahamed et al., 2011), while some have a bushy growth habit with short vines (Loy, 2004; Wu and Cao, 2008). Reduced internode length is conferred by a single gene, *Bu* (Wu and Cao, 2008), whose expression is dominant in early development of *C. pepo* and *C. maxima* plants (Paris and Brown, 2005; Wu et al., 2007).

Leaf area is highly related to both the fresh and dry weight of a plant because it plays an important role particularly in photosynthesis and crop growth (Cho et al., 2007). Loy (2004) reported a considerable variation in both branching habit and leaf size among *Cucurbita* cultivars which is a result of the existence of genetic variability. A wide range of number of leaves per plant was also reported among *C. pepo* cultivars in Griffin, USA (NeSmith, 1997).

Flowering in different *Cucurbita* species commences between 6 (Loy, 2004) and 13 (Agbagwa and Ndukwu, 2004) weeks from seeding. Aruah et al. (2010) reported that in *Cucurbita* species staminate flowers are generally more numerous and bloom earlier than the pistillate flowers. Cucurbits generally have a very low fruit set compared to opened female flowers, probably due to either insufficient or absence of pollinators; high temperatures; drought; or low irradiance (De Menezes et al., 2005; Vidal et al., 2010).

Different genotypes or populations of the same *Cucurbita* species vary widely with respect to the: number of fruits per plant (Ahamed et al., 2011); fruit weight, length and diameter (Balkaya et al., 2010); number of seeds per fruit (Aruah et al., 2010); total seed weight (Nerson, 2005) and 100-seed weight (Balkaya et al., 2009).

Several authors have reported the growth and consumption of *Cucurbita* species particularly in rural areas of KwaZulu-Natal and South Africa but no work to date has focused on the assessment of morphology and yield of shoots, leaves, flowers, fruits and seeds which are essential for vegetable purposes. Therefore, establishing the level of diversity among *Cucurbita* landraces grown in northern KwaZulu-Natal will form the basis for future research on the breeding and improvement of such income-promising leafy vegetable crops particularly for small-holder farming sectors.

The objectives of this study were:

- To study the morphological and growth type variations among *Cucurbita* landraces grown in northern KwaZulu-Natal.
- To investigate the diversity in the yield of shoots, flowers, fruits and/or seeds among *Cucurbita* landraces.
- To study the effect of time for initiating shoot removal on morphology, growth and yield of *Cucurbita* landraces.

4.2 Materials and methods

Seeds of eight *Cucurbita* landraces (Table 4.1 and Figures 4.1– 4.4) collected from the Umkhanyakude, Uthungulu and Zululand districts of northern KwaZulu-Natal were grown at the University of Zululand Experiment Station (28°51'S, 31°50'E) between September and February for two years (seasons) in a randomized complete block design (RCBD) with three replications. Each plot had 28 plants of one landrace; sown in four rows of seven plants each, where ten (group one) and four (group two) tagged sample plants per plot were in the middle of plots to avoid border effect. Plots were 2 m apart and plants were 1 m apart within and between the rows.

Holes of 10–15 cm depth were made. About 10 g of 2:3:2(22) NPK fertilizer was sprinkled in the hole and mixed with the soil before placing a seed. Three seeds were placed per hole and later thinned to one plant per stand after emergence. At four weeks from seeding, shoot tips with folded leaves only were removed (pruned) and about 10 g of LAN was applied to plants at least 15– 20 cm away from the root stalk or stem, to stimulate foliage formation. Weeding, fungicide and insecticide operations were performed when necessary.

At six and eight weeks from seeding, shoot apexes with three leaves were harvested from plants belonging to group one. The harvested shoot was analyzed for leaf area (mm^2) of all three removed leaves, named from the shoot apex to the base, as leaf one, two, and three; as well as shoot dry weight. Leaf area was determined according to the model used by Cho et al. (2007) with some adaptations, where the length was measured from lamina tip to the intersection of the lamina and petiole along the lamina midrib, while leaf width was measured from edge to edge between the widest lamina lobes. For dry weight determination, shoots were oven-dried at 60°C for a maximum of 48 h, using Term-O-Mat oven (from Labotec).

Table 4.1 Codes of the eight *Cucurbita* landraces belonging to three different species; their landrace name, scientific name and location in northern KwaZulu-Natal as well as leaf variegation and mature fruit colour

Code	Landrace name	Scientific name	Location (district)	Leaf variegation	Mature fruit colour
M-IT	iThanga	*C. pepo*	Umkhanyakude	variegated	yellow/orange
T-IT	iThanga	*C. pepo*	uThungulu	variegated	yellow/orange
Z-IT	iThanga; iPhuzi	*C. pepo*	Zululand	variegated	yellow/orange
M-UM	uMpampini oluhlaza	*C. maxima*	Umkhanyakude	not variegated	green
T-UM	uMpampini ophuzi	*C. pepo/ C. maxima*	uThungulu	variegated	yellow/orange
Z-UM	uMpampini oluhlaza	*C. maxima*	Zululand	not variegated	green
M-IN	iNhlwathi emnyama	*C. pepo*	Umkhanyakude	variegated	green
M-IS	isiPhama	*C. argyrosperma*	Umkhanyakude	not variegated	green

Leaf variegation, green leaves with silvery-white speckles on the veins.

The first remaining axillary bud after shoot removal was analyzed at day seven for the area of three developed leaves; and at day 28 for shoot length (m), stem branching, number of leaves, and number of staminate and pistillate flowers. Two plots of five plants each were used as replicates (n = 10). In plants of group two a total number of set fruits per plant was determined at nine weeks after planting. Two plots of four plants per plot were used as replicates (n = 8).

Fruits were harvested at six months from seeding, after a complete die back of the foliage. Due to low fruit yield per plant in all landraces, all the plants per plot were used to determine the average number of harvested fruit. Twelve fruits (four per plot) per landrace were used to determine fruit weight (kg), fruit length and diameter (cm), total number of seeds per fruit, total seed weight and 100-seed weight (g). The data were subjected to a one-way analysis of variance (ANOVA).

Figure 4.1 Shoots and fruits of *Cucurbita pepo* landraces (photo: NR Ntuli)

Figure 4.2 Shoots and fruit of *Cucurbita maxima/pepo* landrace from uThungulu district (photo: NR Ntuli)

Figure 4.3 Shoots and fruit of *Cucurbita maxima* landraces (photo: NR Ntuli)

Figure 4.4 Shoots and fruits of *Cucurbita argyrosperma* landraces (photo: NR Ntuli)

4.3 Results

4.3.1 Shoot analysis

4.3.1.1 Dry weight of shoots removed from six and eight week-old plants

The dry weights of the shoots removed from six week-old plants were significantly higher than those of the eight week-old plants, within each landrace (Figure 4.5). Shoot dry weights of M-IN and M-IS landraces removed from six week-old plants were significantly higher than T-IT landrace. Among landraces whose shoots were removed from eight-week old plants; M-IT, T-IT, M-UM, M-IN and M-IS landraces had higher dry weights than Z-IT and Z-UM landraces.

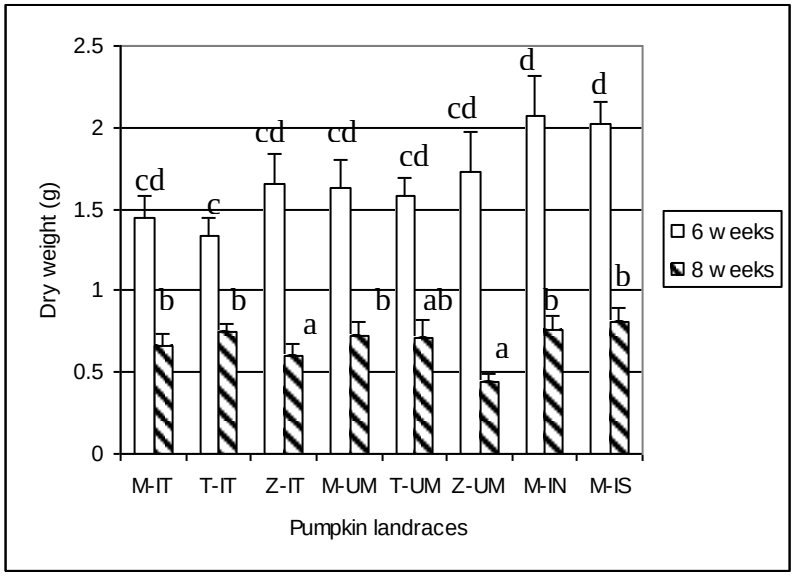

Figure 4.5: Dry weight of three-leaved shoots removed from six and eight week-old plants of different *Cucurbita* landraces (n=10). Bars followed by different letter(s) differ significantly (P<0.05). Landraces: M-IT, *C. pepo* with yellow/orange mature fruit from Umkhanyakude district; T-IT, *C. pepo* with yellow/orange mature fruit from uThungulu district; Z-IT, *C. pepo* with yellow/orange mature fruit from Zululand district; M-UM, *C. maxima* with green mature fruit from Umkhanyakude district; T-UM, *C. maxima/pepo* with yellow/orange mature fruit from uThungulu district; Z-UM, *C. maxima* with green mature fruit from Zululand district; M-IN, *C. pepo* with green mature fruit from Umkhanyakude district; M-IS, *C. argyrosperma* with green mature fruit from Umkhanyakude district.

The differences were not significant among the *C. maxima* landraces originating from the three districts, in shoots removed from six week-old plants. Further, the dry weights of *C. pepo* shoots removed from eight-week old plants were higher in plants whose seeds were originally from both the Umkhanyakude and uThungulu districts than those originating from the Zululand district; while in *C. maxima*, the dry weight was higher in plants originating from the Umkhanyakude district than those from the Zululand district.

4.3.1.2 Leaf area of removed and intact shoots

The results on the leaf area for leaf one, two and three, starting from the shoot apex of the removed three-leaved shoot and the shoot resulting from the first axillary bud at day seven after shoot removal are presented in Table 4.2.

(a) Comparison of leaf area of shoots removed at six and eight weeks with intact shoots, assessed at day seven from shoot removal

The leaf area of the removed shoots ranged from 617 to 2000 mm^2 in leaf one, 1386 to 5175 mm^2 in leaf two, and 2805 to 11521 mm^2 in leaf three; whereas in the intact shoots, the leaf area varied from 382 to 1182 mm^2 in leaf one, 1034 to 3069 mm^2 in leaf two, and 2099 to 10779 mm^2 in leaf three, among different landraces in six and eight week-old plants.

Generally the leaf area of the removed shoots was larger than that of the resulting shoots measured at seven days after shoot removal from six week-old plants. However, the area for leaf three of M-IT, T-UM, M-IN and M-IS landraces did not differ significantly between the removed and intact shoots. Further, the differences in the leaf area for shoots removed from eight week-old plants and their resulting shoots after seven days were not significant, with only the T-IT landrace as an exception where the area of leaf three of the intact shoots was larger than that of the removed shoots.

Table 4.2: The leaf area (mm^2) of the removed and the resulting, intact shoots of *Cucurbita* landraces at six and eight weeks after shoot harvest initiation (n=5)

Plant age	Landrace	Leaf 1		Leaf 2		Leaf 3	
		Removed	Intact	Removed	Intact	Removed	Intact
6 weeks	M-IT	1728 ± 216 c [b]	685 ± 75 b [a]	4195 ± 517 bc [b]	1530 ± 161 ab [a]	10340 ± 2410 b [a]	5744 ± 1256 ab [a]
	T-IT	1553 ± 188 c [b]	552 ± 91 ab [a]	3381 ± 248 b [b]	1950 ± 483 ab [a]	8790 ± 856 b [b]	5203 ± 1039 ab [a]
	Z-IT	1630 ± 299 bc [b]	545 ± 82 ab [a]	3880 ± 730 bc [b]	1333 ± 117 a [a]	10195 ± 2035 b [b]	3543 ± 520 a [a]
	M-UM	2000 ± 212 c [b]	1100 ± 129 c [a]	3847 ± 550 bc [b]	1910 ± 191 b [a]	7770 ± 1080 b [b]	3983 ± 162 a [a]
	T-UM	1970 ± 206 c [b]	415 ± 139 ab [a]	5175 ± 481 c [b]	2365 ± 618 ab [a]	11215 ± 1110 b [a]	8795 ± 1878 b [a]
	Z-UM	1495 ± 194 bc [b]	846 ± 180 bc [a]	3270 ± 422 b [b]	1752 ± 461 ab [a]	9250 ± 782 b [b]	4684 ± 1309 ab [a]
	M-IN	1653 ± 273 bc [b]	498 ± 66 ab [a]	4340 ± 694 bc [b]	1900 ± 502 ab [a]	11521 ± 2504 b [a]	10779 ± 4102 ab [a]
	M-IS	1734 ± 296 c [b]	382 ± 68 a [a]	3244 ± 495 b [b]	1034 ± 259 a [a]	8147 ± 1090 b [a]	5463 ± 1725 ab [a]
8 weeks	M-IT	632 ± 47 a [a]	1182 ± 287 abc [a]	1521 ± 188 a [a]	3069 ± 955 ab [a]	4110 ± 588 a [a]	5167 ± 2252 ab [a]
	T-IT	881 ± 66 b [a]	787 ± 191 abc [a]	2138 ± 353 ab [a]	1937 ± 264 ab [a]	5525 ± 874 ab [a]	2099 ± 1114 ab [b]
	Z-IT	622 ± 78 a [a]	761 ± 292 abc [a]	1587 ± 281 a [a]	2775 ± 1445 ab [a]	4084 ± 887 a [a]	6381 ± 2948 ab [a]
	M-UM	1068 ± 171 b [a]	802 ± 162 bc [a]	1705 ± 388 a [a]	1273 ± 401 ab [a]	3428 ± 754 a [a]	2172 ± 1136 a [a]
	T-UM	909 ± 155 ab [a]	1049 ± 250 bc [a]	2442 ± 428 ab [a]	1553 ± 750 ab [a]	6070 ± 1124ab [a]	4379 ± 1834 ab [a]
	Z-UM	617 ± 111 ab [a]	982 ± 499 abc [a]	1386 ± 218 a [a]	1777 ± 948 ab [a]	3298 ± 834 a [a]	2865 ± 1656 a [a]
	M-IN	745 ± 89 ab [a]	898 ± 166 bc [a]	1659 ± 162 a [a]	2278 ± 560 ab [a]	3872 ± 593 a [a]	5135 ± 1434 ab [a]
	M-IS	827 ± 65 b [a]	702 ± 200 abc [a]	1509 ± 104 a [a]	1311 ± 364 ab [a]	2805 ± 326 a [a]	2799 ± 781 a [a]

Values that are followed by a different letter within a column and different superscript letter within a row differ significantly ($P<0.05$). Landraces: M-IT, *C. pepo* with yellow/orange mature fruit from Umkhanyakude district; T-IT, *C. pepo* with yellow/orange mature fruit from uThungulu district; Z-IT, *C. pepo* with yellow/orange mature fruit from Zululand district; M-UM, *C. maxima* with green mature fruit from Umkhanyakude district; T-UM, *C. maxima/pepo* with yellow/orange mature fruit from uThungulu district; Z-UM, *C. maxima* with green mature fruit from Zululand district; M-IN, *C. pepo* with green mature fruit from Umkhanyakude district; M-IS, *C. argyrosperma* with green mature fruit from Umkhanyakude district.

(b) Leaf area of shoots removed from six and eight week-old plants

Comparison within a landrace revealed that shoots removed from six week-old plants had significantly larger leaves than shoots removed from eight week-old plants in all three leaves of all *Cucurbita* landraces, except leaf one of Z-UM and M-IN landraces; and leaf three of T-IT and T-UM landraces.

Comparison of leaf area in shoots removed from six week-old plants among different landraces revealed that only leaf two of T-UM landrace was significantly larger than those of T-IT, Z-UM and M-IS landraces, while leaf one and leaf three did not show significant differences. Therefore, leaf two further shows the *C. maxima*/*pepo* landrace from the uThungulu district as having significantly larger leaves than *C. maxima* landrace from the Zululand district. However, in eight week old plants: leaf one of T-IT, M-UM and M-IS landraces were significantly larger than leaf one of M-IT and Z-IT landraces; which also shows that *C. pepo* landrace from the uThungulu district had significantly larger leaves than *C. pepo* landraces from the Umkhanyakude and Zululand districts.

(c) Leaf area of new shoots resulting after removal from six and eight week-old plants

Variations in leaf area, within each landrace, were not significant in new vines after removal from six and eight week-old plants, in all leaves.

In comparisons between landraces where new vines grew from six week-old plants, the M-UM landrace recorded the largest leaf one area (1100 mm^2) over all other landraces except the Z-UM; followed by the M-IT landrace, whose leaf one area was significantly larger than the M-IS landrace. Results for leaf one also reflect the *C. maxima* landrace from the Umkhanyakude district as having larger leaves than the *C. maxima*/*pepo* landrace from the uThungulu district. Leaf two

area of the M-UM landrace was significantly larger than leaf two area of the Z-IT and M-IS landraces, while area of leaf three of the T-UM landrace was significantly larger than that of the Z-IT and M-UM landraces. Leaf three area further revealed the *C. maxima*/*pepo* landrace from the uThungulu district as having larger leaves than *C. maxima* from the Umkhanyakude district. Variations were not significant among landraces with regards to the areas of leaf one, two and three, in new vines which grew from eight week-old plants.

4.3.1.3 Number of leaves in new vines from six and eight week-old plants of *Cucurbita* landraces

The results of the number of leaves per plant determined for vines resulting after three-leaved shoot apex removal at an age of six and eight weeks are presented in Table 4.3. Only the M-IT landraces had significantly higher numbers of leaves on new vines resulting from six rather than eight week-old plants.

The number of leaves varied from 12.80 to 42.60 and 9.80 to 23.00 in new vines from six and eight week-old plants, respectively. The differences in the number of leaves among landraces and of *C. pepo* and *C. maxima* landraces whose seeds were from different districts were not significant, in both vines from six and eight week-old plants.

4.3.1.4 Length of new vines from six and eight week-old plants of *Cucurbita* landraces

The new vines of the M-IT, M-UM and Z-UM landraces from six week-old plants were significantly longer than vines from eight week-old plants (Table 4.3). The landraces differed significantly for vine length from six week-old plants, which ranged from 0.418 to 1.802 m. The M-IT, M-UM and Z-UM landraces had significantly longer vines than the T-IT, T-UM and M-IS landraces. The vine length in new vines from eight week-old plants ranged from 0.432 m (T-UM) to 1.304 m (M-IS), but the differences were not significant among landraces.

Table 4.3 Variation in some quantitative characters, at day 28, in new vines after shoot apex removal from six and eight week-old plants of eight *Cucurbita* landraces (n=5)

Shoot removal	Landrace	No. of leaves	Vine length (m)	No. of branches	Branches' length (cm)	No. of pistillate flowers	No. of staminate flowers
6 weeks	M-IT	25.00 ± 6.21 b	1.746 ± 0.370 b	2.80 ± 1.74 ab	3.91 ± 2.41 a	0.80 ± 0.58 ab	15.60 ± 4.07 b
	T-IT	14.60 ± 2.66 ab	0.788 ± 0.256 a	0.20 ± 0.20 a	1.20 ± 1.20 a	1.20 ± 0.58 ab	8.40 ± 2.34 ab
	Z-IT	16.80 ± 4.19 ab	1.364 ± 0.417 ab	1.75 ± 1.11 a	5.42 ± 2.87 a	0.80 ± 0.37 ab	10.20 ± 2.85 ab
	M-UM	42.20 ± 4.36 b	1.664 ± 0.428 b	8.40 ± 1.83 b	17.33 ± 3.54 b	2.80 ± 0.97 b	50.20 ± 12.48 c
	T-UM	12.80 ± 2.87 ab	0.418 ± 0.103 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.60 ± 0.25 b	8.60 ± 2.66 ab
	Z-UM	42.60 ± 7.65 b	1.802 ± 0.158 b	3.80 ± 1.16 ab	10.88 ± 2.56 b	3.00 ± 1.30 ab	35.60 ± 8.02 bc
	M-IN	17.40 ± 4.64 ab	1.070 ± 0.307 ab	2.60 ± 1.03 a	3.35 ± 0.58 a	3.80 ± 1.72 ab	8.00 ± 2.00 ab
	M-IS	12.80 ± 3.40 ab	0.720 ± 0.260 a	2.60 ± 1.40 a	2.35 ± 0.65 a	0.00 ± 0.00 a	13.40 ± 5.48 ab
8 weeks	M-IT	9.80 ± 1.85 a	0.454 ± 0.162 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.40 ± 0.25 a	5.00 ± 1.23 a
	T-IT	11.80 ± 2.87 ab	0.662 ± 0.292 a	0.00 ± 0.00 a	0.00 ± 0.00 a	1.00 ± 0.32 a	6.20 ± 2.08 ab
	Z-IT	12.20 ± 2.27 ab	0.920 ± 0.222 a	0.20 ± 0.20 a	0.60 ± 0.60 a	0.60 ± 0.40 ab	8.60 ± 2.42 ab
	M-UM	23.00 ± 8.67 ab	0.512 ± 0.126 a	3.00 ± 1.18 a	9.44 ± 4.10 ab	2.40 ± 0.51 b	19.40 ± 8.10 abc
	T-UM	10.00 ± 1.92 a	0.432 ± 0.155 a	0.00 ± 0.00 a	0.00 ± 0.00 a	1.00 ± 0.32 b	5.20 ± 1.53 a
	Z-UM	18.20 ± 7.19 ab	0.446 ± 0.169 a	1.40 ± 0.75 a	5.78 ± 3.58 ab	1.20 ± 0.37 b	13.00 ± 5.51 ab
	M-IN	16.20 ± 3.79 ab	1.064 ± 0.299 ab	1.00 ± 0.78 a	2.20 ± 1.49 a	2.60 ± 1.08 b	8.40 ± 2.27 ab
	M-IS	21.00 ± 5.77 ab	1.304 ± 0.321 ab	1.80 ± 1.36 a	3.30 ± 2.83 a	1.00 ± 0.55 ab	20.20 ± 9.49 abc

Values are mean ± standard error. Mean values that are followed by different letters within a column differ significantly (P<0.05). Landraces: M-IT, *C. pepo* with yellow/orange mature fruit from Umkhanyakude district; T-IT, *C. pepo* with yellow/orange mature fruit from uThungulu district; Z-IT, *C. pepo* with yellow/orange mature fruit from Zululand district; M-UM, *C. maxima* with green mature fruit from Umkhanyakude district; T-UM, *C. maxima/pepo* with yellow/orange mature fruit from uThungulu district; Z-UM, *C. maxima* with green mature fruit from Zululand district; M-IN, *C. pepo* with green mature fruit from Umkhanyakude district; M-IS, *C. argyrosperma* with green mature fruit from Umkhanyakude district.

It was evident from the length of new vines from six week-old plants that *C. pepo* landrace from the Umkhanyakude district (M-IT) had significantly longer vines than that from the uThungulu district (T-IT), and also *C. maxima* landraces from the Umkhanyakude (M-UM) and Zululand (Z-UM) districts had significantly longer vines than *C. maxima/pepo* from the uThungulu district (T-UM).

4.3.1.5 Branching in new vines from six and eight week-old plants of *Cucurbita* landraces

The results for the number and length of branches of the vines resulting after shoot removal from six and eight week-old plants are presented in Table 4.3. The T-UM (six and eight week-old plants); the M-IT and T-IT landraces (eight week-old plants) did not produce branches. Only the M-UM landrace had significantly more branches in new vines from six week-old plants than from eight week-old plants.

Significant variations were observed among landraces with respect to the number and length of branches per new vine from six week-old plants only. Among those landraces that formed branches in the new vines from six and eight week-old plants, the following were the lowest to highest ranges in the number of branches (0.20 to 8.40 and 0.20 to 3.00 cm) and length of branches (1.20 to 17.33 cm and 0.60 to 9.44 cm) per vine, respectively. In new vines from six week-old plants, the M-UM landrace had significantly more branches than the T-IT, Z-IT, T-UM, M-IN and M-IS landraces, whereas the M-UM and Z-UM landraces had significantly longer branches than all other landraces. In the new vines from eight week-old plants, the most numerous (3.00) and longest (9.44 cm) branches were recorded in the M-UM landraces, while the fewer in number (0.20) and shorter (0.60 cm) branches were recorded in the Z-IT landrace.

Among *C. pepo* landraces from different districts, there were no significant differences in the number and lengths of branches of new vines from six and eight week-old plants, as well as the total number of lateral branches. In new vines formed after shoot removal from six and eight week-old plants, the *C. maxima/pepo* from the uThungulu district (T-UM landrace) did not form branches. The number of branches of the new vines from six week-old plants was significantly higher in *C. maxima* from the Umkhanyakude district than that from the Uthungulu district, both landraces from the Umkhanyakude and Zululand districts had significantly longer vines than the landrace from the uThungulu district.

4.3.2 Flower analysis in new vines from six and eight week-old plants of *Cucurbita* landraces

The results of the number of staminate and pistillate flowers of the new vines after shoot tip removal from six and eight week-old plants are shown in Table 4.3. In all *Cucurbita* landraces staminate flowers were significantly more numerous than pistillate flowers.

4.3.2.1 Number of pistillate flowers in new vines from six and eight week-old plants of *Cucurbita* landraces

The *Cucurbita* landraces had significant differences in the number of pistillate flowers developing in new vines formed after shoot removal from six and eight week-old plants, which ranged from 0.00 to 3.80 and 0.40 to 2.60, respectively (Table 4.3).

The M-IS landrace did not produce pistillate flowers in new vines from six week old plants but did in eight week-old plants. In new vines from six week-old plants, the M-UM and T-UM landraces had significantly more pistillate flowers than the

M-IS landrace; while in new vines from eight week-old plants, the M-UM, T-UM, Z-UM and M-IN landraces also had significantly more pistillate flowers than the M-IT and T-IT landraces. Differences in new vines from eight week-old plants also revealed that *C. pepo* landrace from the Umkhanyakude district (M-IN) had more pistillate flowers than other landraces from the Umkhanyakude (M-IT) and the uThungulu (T-IT) districts.

4.3.2.2 Number of staminate flowers in new vines from six and eight week-old plants of *Cucurbita* landraces

The M-IT landrace had significantly higher numbers of staminate flowers on the new vines from six week-old plants than eight week-old plants.

Significant variations were observed among landraces for numbers of staminate flowers in new vines formed after shoot removal from six week-old plants, where they ranged from 8.00 to 50.20 (Table 4.3). Differences were not significant in the number of staminate flowers borne in new vines from eight week-old plants; however the range of 5.00 (M-IT) to 20.20 (M-IS) staminate flowers was recorded.

In new vines from six week-old plants the M-UM landrace had significantly higher numbers of staminate flowers than all other landraces except the Z-UM landrace, which however did not (Z-UM) differ significantly from the others. Further, in new vines from six week-old plants, the *C. maxima* landrace from the Umkhanyakude district had more staminate flowers than the landrace from the uThungulu district. The M-IS landrace had significantly higher total numbers of staminate flowers than the Z-UM and M-IN landraces.

4.3.3 Fruit and seed analysis

The number of set fruits at week nine from seeding; number of harvested fruits; the size and weight of fruits; the number and weight of seeds for each pumpkin landrace are recorded in Table 4.4.

4.3.3.1 Fruit set in *Cucurbita* landraces

Only the M-IT, T-IT, Z-IT, T-UM and M-IN landraces had set fruits at nine weeks from planting, with a range of 0.25 to 1.13 fruits per vine and did not differ significantly from each other.

4.3.3.2 Number of harvested fruits per plant

The number of harvested fruits per plant in the M-UM (0.67 ± 0.33), Z-UM (0.33 ± 0.33) and M-IS (1.00 ± 0.58) landraces were significantly lower than other landraces. Only the M-IT, T-IT, Z-IT, T-UM and M-IN landraces, with a range of 5.67 to 17.00 mean fruit yield per plant, did not differ significantly from each other, and were used for: size and weight of fruits; and number and weight of seed analyses.

Table 4.4 Number of set fruits at week nine from seeding (n=8), number of harvested fruits per plant (n=3); fruit size and weight; number of seeds and seed weight in different *Cucurbita* landraces (n=12)

Pumpkin landraces	No. of set fruits	No. of harvested fruits	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (kg)	No. of fully developed seeds per fruit	Percentage of fully developed seeds	100-seed weight (g)	Total seed weight (g)
M-IT	1.13 ± 0.40 a	11.00 ±2.08 a	38.60 ± 1.99 c	68.48 ± 3.63 c	4.95 ± 0.41 b	401.83 ± 29.15 ab	85.17 ± 2.34 a	14.73 ± 1.01 b	64.73 ± 10.68 ab
T-IT	0.50 ± 0.27 a	17.00 ± 3.61 a	30.38 ± 1.01 b	51.83 ± 2.09 a	3.47 ± 0.09 a	330.00 ± 27.45 a	78.31 ± 3.79 a	12.93 ± 0.84 b	48.25 ± 5.42 a
Z-IT	0.50 ± 0.27 a	6.67 ± 2.40 a	28.42 ± 1.08 ab	59.41 ± 1.96 b	3.65 ± 0.13 a	421.83 ± 27.23 b	92.40 ± 1.74 b	10.68 ± 0.44 a	50.70 ± 4.46 a
T-UM	0.25 ± 0.16 a	5.67 ± 2.60 a	27.40 ± 0.82 a	65.81 ± 2.08 c	3.76 ± 0.14 a	375.58 ± 24.58 ab	92.50 ± 2.44 b	13.01 ± 0.88 b	48.47 ± 4.38 a
M-IN	0.38 ± 0.26 a	9.00 ± 0.58 a	36.25 ± 1.35 c	66.33 ± 3.25 bc	4.93 ± 0.38 b	422.58 ± 36.89 ab	92.04 ± 1.27 b	14.48 ± 0.70 b	70.19 ± 4.82 b

Values are mean ± standard error. Mean values that are followed by different letters within a column, differ significantly (P<0.05). Landraces: M-IT, *C. pepo* with yellow/orange mature fruit from Umkhanyakude district; T-IT, *C. pepo* with yellow/orange mature fruit from uThungulu district; Z-IT, *C. pepo* with yellow/orange mature fruit from Zululand district; T-UM, *C. maxima/pepo* with yellow/orange mature fruit from uThungulu district; M-IN, *C. pepo* with green mature mature fruit from Umkhanyakude district.

4.3.3.3 Size and weight of fruits

Significant variations among the landraces were observed for fruit size and weight. The following were the lowest to highest range of fruit: length (27.40 to 38.60 cm); diameter (51.83 to 68.48 cm) and weight (3.47 to 4.95 kg). The analysis of fruit length and diameter showed that the fruit of M-IT and M-IN landraces were the longest followed by those of T-IT landrace, and the one from T-UM landrace was the shortest, while the widest fruit landraces were from M-IT and T-UM landraces, followed by those from Z-IT landrace and then the T-IT landrace had the smallest. This showed that fruits of the T-IT landrace were more oblong and less wide; fruits of the T-UM landrace were flattened and wide; and those of the Z-IT landrace varied between oblong-slender and flattened-wide type fruits. The M-IT and M-IN landraces had significantly higher fruit weight than the T-IT, Z-IT and T-UM landraces.

4.3.3.4 Number and weight of seeds

The landraces differed significantly for number and weight of fully developed seeds. The number of fully developed seeds per fruit ranged from 330.00 to 422.58; the percentage of fully developed seeds per fruit (78.31 to 92.50%); the 100-seed weight (10.68 to 14.73 g); and the total seed weight (48.25 to 70.19 g). The number of seeds per fruit was higher in the Z-IT landrace than the T-IT landrace, whereas the percentage of seeds per fruit was higher in the Z-IT, T-UM and M-IN landraces than in the M-IT and T-IT landraces. Measurement of 100-seed weight per landrace revealed a higher weight in all landraces than the Z-IT landrace. However, the weight of the total fully developed seeds per fruit was higher in the M-IN landrace and lower in the T-IT, Z-IT and T-UM landraces.

4.4 Discussion

4.4.1 Shoot analysis

4.4.1.1 Differences in measured variables due to changes in the plants from vegetative growth to flowering

Three-leaved shoots removed from six week-old plants had significantly higher dry weight (Figure 4.5) and larger leaf area (Table 4.2) than those removed from eight week-old plants, in all landraces. Also, the new vines resulting after shoot removal from six week-old plants had significantly numerous leaves and longer vines than new vines from eight week-old plants (Table 4.3), in some landraces. These differences were probably caused by plants channeling a greater proportion of photosynthetic products for shoot growth at six weeks, which was then altered at an age of eight weeks due to the change from vegetative growth to flowering (reproduction). According to Loy (2004), in temperate climates, semi-determinate *Cucurbita* plants can reach the end of their exponential growth phase within six to seven weeks from seeding.

The results on the leaf area of the removed shoots (Table 4.2) also concur with the report by Loy (2004) and Amer (2011) where the mean leaf area increased rapidly from about 100 to 300 cm^2 (10 000 to 30 000 mm^2) in early development to over 1500 cm^2 (150 000 mm^2) in leaves of large-leaved *Cucurbita* cultigens. However, those landraces that had some larger leaves at an age of eight weeks can be referred to as late season landraces, having prolonged vegetative growth with later flowering initiation that deters leaf expansion. Late season landraces were earlier reported in other *Cucurbita* studies (Agbagwa and Ndukwu, 2004; Ahamed et al., 2011). Probably because of high growth rates at week six (to seven) (Loy, 2004) and fast rates of leaf size increase (NeSmith, 1997; Loy, 2004; Amer, 2011), *Cucurbita* landraces from northern KwaZulu-Natal did not differ significantly in the area of removed leaf one and three, even though leaf two of the T-UM landrace was significantly bigger than that of the T-IT, Z-UM and M-IS landraces (Table 4.2). Also the variations observed in eight week-old plants

can be considered as minor, where only leaf one of the T-IT, M-UM and M-IS landraces was significantly larger than leaf one of the M-IT and Z-IT landraces.

The insignificant variations in leaf area within each landrace in new vines after removal from six and eight week-old plants in all leaves (Table 4.2) was possibly caused by: low rate of leaf expansion in the newly developing vine after shoot removal, regardless of plant age; or the initiation of flowering which also relates to the end of exponential growth within week six to seven from seeding in some *Cucurbita* plants. According to Loy (2004) and Amer (2011), the plant leaf area depends on the growth vigor and the rate of leaf expansion which is mostly deterred at flower onset. This detention of leaf expansion at flower onset was probably a reason for insignificant difference between the leaf area of shoots removed from eight week-old plants and that of the new shoots resulting thereafter. However, those landraces that had some larger leaves at an age of eight weeks can be referred to as late season landraces, having prolonged vegetative growth with later flowering initiation that deters leaf expansion. Late season landraces were earlier reported in other *Cucurbita* studies (Agbagwa and Ndukwu, 2004; Ahamed et al., 2011).

The same reason of the retardation of vegetative growth and altered channeling of photo-assimilates as plants change from the vegetative growth to flowering (Akoroda et al., 1990; Loy, 2004), would probably hold true also for the absence of branches in new vines of the M-IT and T-IT landraces from eight week-old plants (Table 4.3). However, the lack of branches in new vines of the T-UM landraces resulting from six and eight week-old plants was probably caused by the bushy growth habit in this landrace. This correlates to the reports of Loy (2004) and Wu et al. (2008) that bush-type pumpkin plants have fewer lateral shoots than vine plants.

The higher dry weight and larger leaves in shoots removed from six week-old plants would probably mean that people may have more food to consume at this growth stage than at eight weeks.

4.4.1.2 Leaf area of removed and intact shoots

(a) Comparison of leaf area of shoots removed at six and eight weeks with intact shoots, assessed at day seven from shoot removal

Generally the leaf area of shoots removed from six week-old plants was significantly larger than that of the resulting shoots measured at seven days after shoot removal (Table 4.2). This may be due to the differences in the leaf expansion rate and duration of leaf expansion (NeSmith, 1997; Loy, 2004); where in the present study the main vine of the removed shoots may have a high leaf expansion rate in a shorter period in comparison to newly formed vines.

It was evident in the present study that if three-leaved shoots are removed from six week-old plants, the resulting new vine will have significantly smaller leaves after a week (day seven) than removed shoots, thus weekly harvests from the same vine may result in the consumption of smaller leaf quantity in a form of dry weight. Asiegbu (1983) reported that the removal of edible shoot apex (harvest-pruning) in a period of 3-4 weeks between harvests provided a better regularity of timing, within which a greater proportion of the leaves that had expanded or been produced since the previous harvest was still acceptable for harvest. Further, the frequent harvest schedules (3-4 weeks) in *Telfairia occidentalis* gave a greater number of branches, and presumably also a greater number of leaves were produced, than the infrequent schedules (6-8 weeks) (Asiegbu, 1983). Thus the frequency of shoot harvesting by people in northern KwaZulu-Natal, which ranges from daily to weekly, is possible in bigger gardens where shoot harvest is altered between the vines so that the previously harvested vines are given enough time to attain acceptable leaf expansion for consumption.

It was essential to study the leaf area in *Cucurbita* landraces of northern KwaZulu-Natal because according to NeSmith (1997) and Cho et al. (2007) leaf area is essential for photosynthesis, crop growth and yield potential. Thus the estimation of leaf area may be useful to determine its relationship with the growth rate of *Cucurbita* landraces. Cho et al. (2007) also reported close relationships between leaf area and both fresh and dry weights.

(b) Leaf area of new shoots resulting after removal from six and eight week-old plants

The M-UM, T-UM and Z-UM landraces had significantly bigger leaves than the M-IS landrace, as expressed in different leaves (Table 4.2); which concurs with Loy (2004) that leaf size can vary considerably among *Cucurbita* cultivars; and also the existence of genetic variability for leaf size between the small- and large-leaved *C. maxima* cultivars. It is therefore evident that the M-UM, T-UM and Z-UM landraces had a higher rate of leaf expansion than other landraces.

4.4.1.3 Number of leaves in new vines from six and eight week-old plants of *Cucurbita* landraces

The variation in the number of leaves from 12.80 to 42.60 and 9.80 to 23.00 in new leaves from six and eight week-old plants, respectively (Table 4.3), concurs with the report by NeSmith (1997) who found the number of leaves per plant 40 days after sowing for four *C. pepo* cultivars as ranging from 8 to 28 leaves per plant. However, this range was less than the range of 97.70 to 210.00 leaves per plant among ten *Cucurbita* landraces at ten weeks after planting as reported by Aruah et al. (2010), probably because in the current research the number of leaves was determined only in the new vines and not the entire plant. The insignificant differences between landraces in the number of leaves per new vine

from both six and eight week-old plants, concur with the insignificant differences found by Aruah et al. (2010) among some Nigerian *Cucurbita* landraces.

4.4.1.4 Length of new vines from six and eight week-old plants of *Cucurbita* landraces

The variation in vine length from 0.418 to 1.802 m and 0.432 to 1.304 m in new vines from six and eight week-old plants, respectively (Table 4.3), corresponds with the findings of Ahamed et al. (2011) among *C. moschata* genotypes where their vine length at harvest varied from 0.170 to 0.400 m. Further, Aruah et al. (2010) reported a variation from 0.383 to 0.707 m in vine length among *Cucurbita* landraces at ten weeks after seeding.

The new vines of the M-IT, M-UM and Z-UM landraces resulting from six week-old plants were significantly longer than new vines of the T-IT, T-UM and M-IS landraces (Table 4.3). The differences between the landraces can be attributed to their variable growth habit where some landraces have a spreading, viny growth habit, with long, thin internodes but others have a compact, bush growth habit with short, thick internodes. Wu et al. (2007) reported the existence of both bush and vine growth habits in *Cucurbita* species.

The significant differences in the length of new vines from six week-old plants could be attributed to genetic differences existing between landraces. Wu et al. (2007), and Wu and Cao (2008) reported that the reduced internode length in Cucurbita plants is conferred by a single dominant gene for bush growth habit called *Bu*, whose expression is dominant in early development stages.

It was evident from the length of new vines from six week-old plants that the *C. pepo* landrace from the Umkhanyakude district (M-IT) had significantly longer vines than that from the uThungulu district (T-IT). Also the *C. maxima* landraces

from the Umkhanyakude and Zululand districts had significantly longer vines than the *C. maxima/pepo* from the uThungulu district (Table 4.3), which revealed the agro-morphological diversity among these landraces in northern KwaZulu-Natal. This finding concurs with Aruah et al. (2010) who reported an enormous agro-morphological diversity among *Cucurbita* landraces in Nigeria, although in their findings the vine length character did not differ significantly between landraces, as in the current study.

4.4.1.5 Branching in new vines from six and eight week-old plants of *Cucurbita* landraces

Significant variations observed between landraces with respect to the number and length of branches per new vine from six week-old plants (Table 4.3), agrees with Ahamed et al. (2011), who reported a significant variation in the number and length of branches per plant among *Cucurbita moschata* genotypes.

The variation from 0.20 to 8.40 and 0.20 to 3.00 in the number of branches produced by new vines from six and eight week-old plants, respectively, between landraces (Table 4.3) is somehow comparable to a range from 2.02 to 4.7 branches per plant among *C. moschata* genotypes reported by Ahamed et al. (2011) in the whole plant. This profuse branching might reflect the *Cucurbita* landraces of northern KwaZulu-Natal as more viny thus had more branches than reported *C. moschata* genotypes. It also confirms that in Southern Africa, farmers maintain seeds from pumpkin landraces for future pumpkin production (Ndoro et al., 2007; Ahamed et al., 2011); and that the more recently developed cultivars of *Cucurbita* species have fewer lateral shoots with short internodes, which gives them a bushy appearance (Wu et al., 2008; Ahamed et al., 2011).

The range from 1.20 to 17.33 cm and 0.60 to 9.44 cm, of the length of branches per new vine from six and eight week-old plants, respectively (Table 4.3), was far

lower than the range from 169.9 to 400.1 cm reported at harvest and in the main stem among *C. moschata* genotypes by Ahamed et al. (2011). Branching at a tertiary stage (from newly developing vines after shoot apex removal) might indicate these landraces as having an indeterminate, viny growth form concurring with the report of Wu et al. (2008). The production of numerous and long branches in new vines from six and eight week-old plants of the M-UM and Z-UM landraces, may render them as more viny and vigorous growers than other landraces (Loy, 2004; Wu et al., 2008; Ahamed et al., 2011).

Significant variation in the number and length of branches from the new shoots among the *C. maxima* landraces from different districts of northern KwaZulu-Natal was probably caused by differences in their agro-ecological areas, which concurs with the report by Aruah et al. (2010) among Nigerian *Cucurbita* landraces. Further, the *C. maxima* landrace from the Umkhanyakude district had significantly more and longer branches in the new vines than a landrace from the uThungulu district. This shows the landraces included in this study from the Umkhanyakude district as more vigorous, viny growing landraces than bushy landraces of the uThungulu district, while landraces in the Zululand district are semi-bushy. This also reveals high phenotypic diversity within populations of *Cucurbita* species which may result from their genetic diversity as corresponding with earlier reports of Aruah et al. (2010) and Balkaya et al. (2010). The close relationship between the Umkhanyakude and Zululand district landraces may result from the seed exchange of local populations of *C. maxima* with surrounding areas, as reported by Balkaya et al. (2009; 2010) in Turkey, or their leaf and fruit phenotypic relationship where they are both not variegated as compared to the variegated landrace from the uThungulu district, thus they might be genetically related.

Growth form (bushy or viny), number of branches per vine and the earliness of branching are the main components of the vegetable yield, since they will

determine the frequency of three-leaved shoot harvested by local people for vegetable purposes.

4.4.2 Number of flowers in new vines from six and eight week-old plants of Cucurbita landraces

In all *Cucurbita* landraces staminate flowers were significantly more numerous than pistillate flowers (Table 4.3), which correspond with the report by Vidal et al. (2010). Significant variations in the number of pistillate and staminate flowers on the new vines from six week-old plants, among the studied *Cucurbita* landraces, may be an indication of genotype effect existing among these landraces, in concordance with the report by Aruah et al. (2010). The M-IS landrace did not produce pistillate flowers in new vines from six week-old plants but did in eight week-old plants, probably because it is a long season crop thus flowering later than the others, and possibly requires an early planting, as reported by Ahamed et al. (2011) in other *Cucurbita* landraces.

The number of pistillate flowers in new vines from six and eight week-old plants ranged from 0.00 to 3.80 and 0.40 to 2.60, respectively (Table 4.3). Earlier studies reported for the entire plant: the variation from 6.00 to 8.67 female flowers among 10 *Cucurbita* landraces (Aruah et al., 2010); from 2.80 to 4.71 among 10 hybrid lines of *Cucurbita pepo* (Ercan and Kurum, 2003); and 4.5 to 10.6 among 41 half-sib families of *C. pepo* landraces (Kasrawi, 1995).

The number of staminate flowers borne in new vines from six and eight week-old plants varied from 8.00 to 50.20 and 5.00 to 20.20, respectively (Table 4.3). Other researchers reported staminate flowers in the entire plants of: 41 half-sib families of *C. pepo* landraces; ten *Cucurbita pepo* hybrid lines and ten *Cucurbita* landraces, as ranging from 22 to 75 (Kasrawi, 1995); 5.33 to 7.54 (Ercan and Kurum, 2003) and from 33.70 to 66.00 flowers (Aruah et al., 2010), respectively. These earlier reports were more comparable to the variation in staminate flowers

of the new vines of the current research although the range of 5.33 to 7.54 among *C. pepo* hybrid lines (Ercan and Kurum, 2003) was lower; which possibly indicates that these northern KwaZulu-Natal landraces are not as improved as these *C. pepo* hybrid lines, thus no reduction in male flower production.

The significant variation between landraces in the number of pistillate and staminate flowers (Table 4.3) was probably an indication of varietal differences existing among the *Cucurbita* species in northern KwaZulu-Natal, which concurs with research by Aruah et al. (2010). The significant variations in the number of pistillate and staminate flowers in landraces originating from different localities might be an indication of agro-ecological variations among landraces, thus agreeing with the report by Du et al. (2011). Also, significant differences in the number of pistillate flowers in different *C. pepo* landraces from the Umkhanyakude district (M-IT and M-IN) probably shows the genotypic differences between these landraces, which correspond with the report by Aruah et al. (2010).

The number of female flowers per plant; early flowering and their low abortion rate are some of the important criteria in ensuring high immature and mature fruit, as well as seed yield for consumption and future cultivation purposes. Therefore, *Cucurbita* landraces possessing one or both such attributes can be beneficial to local farmers and also for future improvement.

Apart from being essential for pollination purposes, staminate flowers are eaten in KwaZulu-Natal and the rest of South Africa (Jansen van Rensburg et al., 2007; Vorster et al., 2007), thus their abundance enhances the harvested amount for vegetable purposes. Collection of these flowers will not affect pollination if they are collected late during the day or in the afternoon when pollination has taken place and any remaining pollen grains are no longer viable. According to Vidal et al. (2010), pollen viability declines with the heat increase of the day.

4.4.3 Fruit and seed analysis

4.4.3.1 Fruit set

Although the M-UM landrace had significantly higher numbers of pistillate flowers in new vines from both six and eight week-old plants (Table 4.4), it had no fruits probably due to a high rate of flower bud abortion. According to Vidal et al. (2010), the abortion of flower buds in pumpkins is due to unfavourable environmental conditions such as high temperatures, drought and low irradiance.

Only the *C. pepo* landraces and the *C. maxima/pepo* landrace (T-UM) had set fruits at week nine, but other *C. maxima* and *C. argyrosperma* landraces did not set fruit at this stage. The absence of fruit set in *C. maxima* and *C. argyrosperma* landraces was probably caused by the abortion of female flower buds or mature female flowers as a result of low numbers of pollinators or unfavourable environmental conditions, which concurs with the reports by Akoroda et al. (1990) and Vidal et al. (2010). The fruit set was probably limited by the inefficiency of these plants to fill them with photo-assimilates, as corresponding with the report of Akoroda et al. (1990) in *Telfaria occidentalis*. According to Loy (2004), normally the number of pistillate flowers produced on a *C. maxima* and *C. pepo* plant greatly exceeds the potential for fruit set. Akoroda et al. (1990) further report that although a female plant of *Telfairia occidentalis* may produce five open flowers, only two to three set fruit and only one or two are retained and develop fully.

4.4.3.2 Number, size and weight of harvested fruits

The average number of harvested fruits per plant in the current study ranged from 0.33 to 17.00 among the *Cucurbita* landraces (Table 4.4). In previous research the number of fruits per plant among: *C. pepo* landraces varied from 2.8

to 9.0 (Kasrawi, 1995); *C. pepo* hybrid lines ranged from 2.43 to 4.21 (Ercan and Kurum, 2003); *Cucurbita maxima* cultivars varied from 4 to 22 (Loy, 2004); *C. moschata* genotypes ranged from 2.0 to 15.7 (Ahamed et al., 2011); and *Cucurbita* landraces varied from 4.67 to 8.00 (Aruah et al., 2010). The significantly low number of fruit production per plant in the M-UM, Z-UM and M-IS landraces makes them prone to extinction if no work to preserve them is initiated. This may also explain why the knowledge and occurrence of the M-IS landrace is lower in areas of the Umkhanyakude district because some communities may have lost its seed bank over time.

Cucurbita landraces of northern KwaZulu-Natal had fruit weight varying significantly with a range from 3.47 to 4.95 kg (Table 4.4), which was higher than the mean fruit size of 1.3 kg reported by Loy (2004) among *C. maxima* cultivars. However the fruit size range among: *Cucurbita* cultivars [3 to 7 kg] (Loy, 2004); *C. maxima* landraces [0.5 to 18.7 kg] (Ferriol et al., 2004b); *C. moschata* hybrids [1.8 to nearly 5 kg] (Loy, 2004); *C. moschata* genotypes [1.5 to 4.2 kg] (Ahamed et al., 2011); *C. pepo* landraces [1.122 to 3.471 kg] (Nerson, 2005); and *Cucurbita* landraces [2.21 to 8.70 kg] (Aruah et al., 2010), somehow concur with the findings of the present study.

The following were the lowest to highest range of fruit: length (27.40 to 38.60 cm) and diameter (51.83 to 68.48 cm) among *Cucurbita* landraces in northern KwaZulu-Natal (Table 4.4). The fruit length and fruit diameter among: *C. pepo* hybrid lines varied from 13.64 to 15.31 cm and 3.70 to 4.55 cm, respectively (Ercan and Kurum, 2003); *C. maxima* populations varied from 8.2 to 66.0 cm and 13.6 to 32.6 cm (Ferriol et al., 2004b) and also from 26.0 to 49.8 cm and 35.1 to 56.5 cm, respectively (Balkaya et al., 2010); and *Cucurbita* landraces ranged from 30.33 to 61.33 cm and 56.93 to 90.67 cm, respectively (Aruah et al., 2010).

The variation in the number of fruits per plant, fruit length and fruit diameter could be attributed to genetic differences existing among the landraces. Aruah et al.

(2010) and Balkaya et al. (2010) reported that *Cucurbita* genotypes produce fruits of various sizes as dictated by genetic constitution. Fruit number per plant and fruit size are some of the main components of yield since fruits are harvested both at immature and mature states for consumption purposes, while the size of the larger mature fruits are mostly preferred by local farmers as the best selection for local commercialization and future cultivation.

4.4.3.3 Number and weight of seeds

In the present study, the number of fully developed seeds per fruit ranged from 330.00 to 422.58 (Table 4.4). This corresponds with the report of Loy (2004) that seed numbers per fruit of *Cucurbita* generally range from 100 to about 500 seeds. Nerson (2005) reported the number of fully developed seeds per fruit of *C. pepo* cultivars as ranging from 102 to 513. Also, Aruah et al. (2010) reported the number of seeds per fruit among *Cucurbita* landraces as ranging from 214.70 to 523.70 seeds. The number of seeds per fruit is the main component for future cultivation and maintenance of these landraces, since according to reports by Ndoro et al. (2007) and Jury et al. (2008), the majority of local farmers set aside the best fruits and/or seeds from the previous harvest for future cultivation.

The weight of the total fully developed seed per fruit varied from 48.25 to 70.19 g among *Cucurbita* landraces in northern KwaZulu-Natal (Table 4.4). Nerson (2005) reported a wide range of seed yield per fruit from 12 to 69 g among 16 tested *C. pepo* cultivars. The dry weight of 100-seed ranged from 10.68 to 14.73 g in *Cucurbita* landraces of northern KwaZulu-Natal (Table 4.4). However, a broader range from 6.4 to 13.6 g (Ahamed et al., 2011) and 6.3 to 20.9 g (Wu et al., 2011), was reported among *C. moschata* genotypes. Further, seed weight of 100 seeds among *C. maxima* landraces varied from 14.15 to 59.08 g (Ferriol et al., 2004a) and from 20.1 to 66.4 g (Balkaya et al., 2009), and from 10.10 to 53.00 g among *Cucurbita* landraces (Aruah et al., 2010), which was a higher and broader range than reported in KwaZulu-Natal.

The Z-IT landrace had significantly lower 100-seed weight than other landraces (Table 4.4) possibly due to the smallest seed size found in this landrace. Loy (2004) reported that seeds of *Cucurbita* range in size from about 50 mg for small-fruited gourds to over 250 mg in some large-fruited cultigens. Further, Nerson (2005) reported a general tendency for compensation where *C. pepo* cultivars with high numbers of seeds tended to have relatively small seeds.

It was evident in M-IT and M-IN landraces that fruits containing more seeds with bigger size achieved greater fruit size, where they both had significantly higher fruit length, area and weight; and 100-seed weight than other landraces; and they did not differ significantly in fruit diameter and total seed weight from each other (Table 4.4). These findings agree with Ercan and Kurum (2003) and Aruah et al. (2010) who reported that fruits containing more seeds grew faster and achieved greater size. Nerson (2005) reported the mean seed yield per fruit (g) in each fruit type group of *C. pepo* to be closely related to the seed number per fruit. The close relationship between the M-IT and M-IN landraces, which made them different from the T-IT and Z-IT landraces in terms of fruit and seed analysis, was probably caused by genetic variations based on differences in geographical origin among these landraces, as reported by Ferriol et al. (2004b) and Wu et al. (2011). Thus, similarities in *C. pepo* landraces from different geographic origins showed indications of seed exchange between farmers, which agrees with Wu et al. (2011) who reported on Chinese *C. moschata* germplasm that could not be divided into groups based on geographic locations in China. Ferriol et al. (2004b) and Balkaya et al. (2010) reported that farmers have maintained local populations of *C. maxima* and exchanged seeds with surrounding areas, mainly at local markets.

The variation in the total number and percentage of fully developed seeds per fruit, 100-seed weight and the total seed weight per fruit between *Cucurbita* landraces in northern KwaZulu-Natal indicates that these characteristics are

genetically controlled, as reported earlier by Aruah et al. (2010); Balkaya et al. (2009; 2010) and Wu et al. (2011).

4.5 Conclusion

This study served as the first report on the diversity in morphology and yield traits of *Cucurbita* landraces grown for vegetable purposes in northern KwaZulu-Natal, and also about *Cucurbita argyrosperma* in South Africa. The *C. pepo* (M-IT, T-IT, Z-IT, M-IN) and *C. maxima/pepo* (T-UM) landraces were the high shoot, pistillate flower, fruit and seed yielding landraces, thus they showed potential for hunger alleviation, future breeding and improvement for commercialization. The *C. maxima* (M-UM, Z-UM) and *C. argyrosperma* (M-IS) landraces are recommended for shoot use as leafy vegetables, therefore this necessitates future research for enhancing their fruit production in this area. The ideal time for shoot harvest that enabled high production in all landraces was six weeks after seeding.

The significant variation in the dry weight of the three-leaved shoots; leaf area; vine length; number and length of branches per vine; number of fruits per plant, fruit length, fruit diameter, fruit weight, number and percentage of fully developed seeds per fruit, 100-seed weight and total seed weight could be attributed to genetic differences existing among the landraces. This however needs verification through future genetic studies among these landraces.

The most interesting finding in this study was the existence of diversity both between and within the *Cucurbita* landraces collected from a relatively limited number of districts in northern KwaZulu-Natal. The variation within landraces (either *C. maxima* or *C. pepo* landraces) and also those originating from the same district (M-IT and M-IN landraces), indicated that these landraces were highly heterozygous populations. The similarity between *Cucurbita maxima* landraces from the Umkhanyakude (M-UM) and Zululand (Z-UM) districts

probably reflects the effect of seed exchange among the farmers in these districts.

The use of molecular markers is therefore essential to ascertain the genetic diversity in these landraces. Thus, Chapter 5 analyzes genetic diversity among selfed and unselfed *C. pepo* landraces from three districts in northern KwaZulu-Natal.

Chapter 5

Population genetic diversity of pumpkin (*Cucurbita pepo*) landraces revealed by RAPD and SSR markers

5.1 Introduction

Cucurbita pepo is one of the most nutritionally and economically important species in the genus *Cucurbita* L. of Cucurbitaceae family that is cultivated worldwide and is of American origin (Tsivelikas et al., 2009; Ghobary and Ibrahim 2010; Formisano et al., 2012). It is a highly polymorphic vegetable species, both in vegetative and reproductive characteristics, with a wide range of genetic variability (Paris, 2000; Paris and Edelstein, 2001; Ferriol et al., 2003; Kathiravan et al., 2006; Formisano et al., 2012).

In traditional agriculture, genetic diversity is created by a diverse array of local varieties called landraces, which are well-adapted to local environmental conditions and inputs (Modi 2004; Mujaju et al., 2010). Maintenance of landraces through *in situ* conservation is a preferred option for traditional small scale farmers (Modi, 2004). For example, part of the genetic variability of the first American summer squash cultigens remains intact in diverse landraces that are still cultivated for self-consumption and sale in local markets (Formisano et al., 2012).

In South Africa and other countries, communities and small scale farmers grow pumpkins as intercrop stands, whether with other plants, or with wild as well as cultivated forms of other cucurbits (Daniel-Kalio and Braide, 2006; Cuevas-Marrero and Wessel-Beaver, 2008; Jury et al., 2008; Mujaju et al., 2010; Molebatsi et al., 2010; Torquebiau et al., 2010). This intercropping practice enhances the geneflow among the cucurbit species due to random bee pollination (Cuevas-Marrero and Wessel-Beaver, 2008; Mujaju et al., 2010). Gene exchange among plant populations located in distant geographical areas

can be influenced by the occasional introduction of seeds and seedlings as well as informal seed exchanges between farmers (Yuan et al., 2007; Mujaju et al., 2010; Du et al., 2011; Barboza et al., 2012).

Self-pollination increases plant mean homozygosity, which is not the natural genetic state of cross-pollinated species and reduces the proportion of heterozygosity in the population thus reducing the vigor of plants (Ercan and Kurum, 2003; Cardoso, 2004). Generally, selfing and sib mating are practiced for inbreeding purposes (Ercan and Kurum, 2003). Traits studies by Ghobary and Ibrahim (2010) in selfed *C. pepo* showed that the phenotypic expression of these traits were indicative of their genetic behaviour.

The genetic variability within *C. pepo* has previously been assessed using allozymes and different DNA marker systems including restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter simple sequence repeats (ISSRs) and simple sequence repeats (SSRs) (Ferriol et al., 2003; 2004a; 2004b; Formisano et al., 2012).

The potential applications of RAPD fingerprinting in molecular biology include: determination of taxonomic identities; detection of interspecific gene flow; assessment of kinship relationships; analysis of mixed genome samples; and production of specific probes and gene mutations (Hadrys, et al., 1992; De Wolf et al., 2004). However the RAPD markers are the least informative of all known DNA markers. They are dominant markers and information on heterozygosity is missing; and also have poor reproducibility or repeatability of results (Navajas and Fenton, 2000; De Wolf et al., 2004; Morimoto et al., 2006; Barracosa et al., 2008; Mujaju et al., 2010; Formisano et al., 2012). It is therefore important to maintain strictly constant PCR conditions and use high-molecular-weight (non-degraded) DNA in order to achieve reproducible results (Hadrys et al., 1992; K'Opondo et al., 2009). Inspite of some limitations RAPD markers have been

used extensively and effectively to analyze genetic diversity in cucurbits (Ferriol et al., 2003; 2004a; 2004b; Dey et al., 2006; Morimoto et al., 2006; Hadia et al., 2008; Khan et al., 2009; Tsivelikas et al., 2009; Du et al., 2011). These markers are also beneficial because they: can be applied on unknown genomes and to limited DNA quantities; are a simple technique; can produce a nearly unlimited number of markers; use a large set of primers which can screen the entire genome (Navajas and Fenton, 2000; Khan et al., 2009; Gajera et al., 2010).

Simple sequence repeat (SSR) or microsatellite markers occur frequently in most eukaryotic genomes and can be very informative, reliable (reproducible), codominant, multiallelic and highly polymorphic, making them well suited for detecting variation among closely related varieties (Garcia et al., 2004; Formisano et al., 2012). However, the application of SSR techniques to plants is only possible when the microsatellite markers suitable for that plant are available (Garcia et al., 2004; Formisano et al., 2012). These markers are available for *Cucurbita* species (Gong et al., 2008; 2012; Formisano et al., 2012). Although genetic diversity among *Cucurbita pepo* landraces or genotypes has been done earlier in other countries using RAPD (Hadia et al., 2008) and SSR (Formisano et al., 2012), but none of these studies were conducted in South Africa.

The aim of the present work was to analyze the polymorphism and genetic relationship among and within pumpkin landraces from the Umkhanyakude, uThungulu and Zululand districts of northern KwaZulu-Natal using the random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers. This work further analyzed the effect of self pollination in genetic variation of these landraces, as they are traditionally intercropped with other *Cucurbita* landraces, thus enhancing gene flow between different landraces.

5.2 Materials and methods

Molecular characterization of pumpkin landraces was carried out at the University of Zululand in the Agriculture and Botany Departments. The genetic diversity within these pumpkin landraces was analyzed using random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers.

5.2.1 Plant material

The seeds of *Cucurbita pepo* collected from the uThungulu, Umkhanyakude and Zululand districts were grown at the Ethnobotany Garden and Agriculture Farm at the University of Zululand, and were used as the source of plant material. Two sets of plants were used to harvest leaf material for DNA extraction: One set was from the seeds that were freshly from the communities of the three districts, and another set was from the seeds that were initially from the communities of these districts but the fruits were self-pollinated to ensure the fixing of traits, where the natural pollinators (bees) were suspected to mix some pollen as the communities are practicing intercropping with other Cucurbitaceae species in their fields. Young (folded to semi-folded) leaves were picked, freeze-dried and stored at 4°C for future use.

5.2.2 Self pollination procedure

The *Cucurbita pepo* from the Umkhanyakude, Uthungulu and Zululand districts were grown in different areas that were about two kilometers away from each other to prevent the incidence of pollen transference among plants from different districts. Both pistillate and staminate flowers that were to be selfed the following morning were covered with a light fine-porous cloth (curtain fabric), mimicking a cheesecloth bag (used by Winsor et al., 2000), in the afternoon prior to flower anthesis, recognisable by the appearance of a slight touch of yellow or orange at

the apex of the corolla tube or rather when the yellow/orange colour of the petals (corolla) was clearly seen or intensified from the outside, as described by Ercan and Kurum (2003). At flower anthesis, soon after dehiscence of pollen sacs (pollen anthesis), self pollination (pollen transference from the staminate flower to the stigma of the pistillate flower, in the same plant) was initiated from 04h00 until about 08h30 in the morning, when the viability and germination potential of pollen grains was still high (Nepi and Pacini, 1993; Agbagwa et al., 2007) and both flower types were wide open.

During selfing, the staminate flowers were picked and had their corolla tube removed to expose the pollen-laden stamens and the pollen was gently rubbed on the stigma lobes of the pistillate flower in the same plant (Thralls and Treadwell, 2008; Fike, 2011). One male flower was used for each female recipient (Spencer and Snow, 2001) due to high levels of irregularities in anthesis of both staminate and pistillate flowers of one plant. To prevent uncontrolled bee pollination, after self pollination the pistillate flowers were re-covered for the whole day, and the cover was removed the following day, since the female flowers are receptive on the ovules for only one day (day of flower anthesis) (Nepi and Pacini, 1993; Agbagwa et al., 2007).

5.2.3 DNA extraction protocol

The DNeasy Plant Mini Kit (from QIAGEN®, Valencia, CA, USA) was used to extract DNA from the leaves according to the manufacturer's instructions. Twenty plants per accession were used to source leaf material. A mortar and pestle was used to grind the freeze-dried leaves. For each plant per accession, 20 mg of ground leaf tissue was placed in 1.5 µl Eppendorf Safe-Lock Tubes (microcentrifuge tubes), with an addition of 400 µl of Buffer AP1 and 4 µl RNase A stock solution (100 mg/ml). This mixture was vortexed vigorously to remove any tissue clumps that might have formed.

To lyse the cells, the mixture was incubated for 10 min at 65°C pre-heated water bath, with mixing two or three times during incubation by inverting tubes.

The detergent, proteins and polysaccharides were precipitated by adding 130 µl Buffer AP2 to the lysate, mixed, and incubated for 5 min on ice. To eliminate the effect of the DNA shearing that could result from the very viscous lysate and large amounts of precipitates that might be generated during this step, the lysate was centrifuged (using Eppendorf Mini Spin plus) for 5 min at 14,000 rpm, and the supernatant transferred (by pipette) into the QIAshredder Mini spin column placed in a 2 ml collection tube, and centrifuged for 2 min at 14,000 rpm.

The flow-through fraction was transferred to a new 1.5 ml microcentrifuge tube, without disturbing the cell-debris pellet. Usually 450 µl of the lysate is recovered, thus 1.5 volumes (675 µl) of Buffer AP3/E was added to the cleared lysate and mixed by pipetting immediately. Some precipitate was formed during this step.

The 650 µL of the mixture, including any formed precipitate, was transferred by pipette into the DNeasy Mini spin column placed in a 2ml collection tube and centrifuged for 1 min at 8000 rpm, and the flow-through was discarded. The collecting tube was re-used and this step was repeated using the remaining sample.

The DNeasy Mini spin column was placed into a new 2 ml collection tube, and 500 µl of Buffer AW was added and centrifuged for 1 min at 6000 x g (8000 rpm). The flow-through was discarded and the collection tube was re-used. Another 500 µl of Buffer AW was added, and centrifuged at 14,000 rpm to dry the membrane.

The DNeasy Mini spin column was transferred to a 1.5 ml or 2 ml microcentrifuge and 100 µl of Buffer AE was added directly onto the DNeasy membrane. This was incubated for 5 min at room temperature (15-25°C), and then centrifuged for

1 min at 8000 rpm to elute. This step was repeated on a separate microcentrifuge tube to have two similar sets of DNA with the same purity but different concentrations.

5.2.4 Amount and purity of DNA

The yield of DNA in ng/µl was measured using the Nano Drop ND-1000 Spectrophotometer (software ND-1000 V3.5.1; USA). The DNA purity was calculated at 260/280 nm wavelengths, where the DNA with an absorbance ranging between 1.7 and 1.9 were considered pure and were used for Polymerase Chain Reactions (PCR).

5.2.5 RAPD amplification

Approximately 50 ng of DNA was amplified through the PCR using 25 µl reactions under the following conditions: 1X of GoTaq® Green Master Mix, 2X (Promega Corporation); 0.4 µM random 10-mer oligonucleotide primer (Inqaba Biotechnical Industries (Pty) Ltd), and Nuclease-Free Water (Promega Corporation).

Amplification was performed in a MJ Mini Personal Thermal Cycler (from BIO-RAD) programmed for an initial denaturation at 95°C for 5 min followed by 40 cylces of 95°C for 30 s, 36°C for 30 s, and 72°C for 1 min, and final extension of 72°C for 4 min. Amplified products were separated in 1% agarose in 1x Tris-Borate-EDTA (TBE) buffer with 125 ng ethidium bromide per liter, using gel electrophoresis run at 70V for 1 hour. The nucleic acid markers 100bp (Promega Corporation) and 1kb (Fermentas, Inqaba Biotechnical Industries (Pty) Ltd) were used to compare the amplification product sizes.

Of thirty six primers tested, nine primers producing distinct polymorphic bands were selected for further analysis (Table 5.1). Each primer producing constituent amplification of well defined, brightly staining bands was used in further amplification of DNA from all individuals. Amplification was repeated to ensure reproducibility of scored products. Random Amplified Polymorphic DNA markers were scored for presence or absence, and each marker was identified by primer and marker size.

The polymorphism rates of RAPD primers were evaluated using seven *Cucurbita pepo* landraces' populations: Umkhanyakude unselfed (MNS); Umkhanyakude selfed (MS); Umkhanyakude green ripe fruits (CPSP); uThungulu unselfed (TNS); uThungulu selfed (TS); Zululand unselfed (ZNS); and Zululand selfed (ZS).

5.2.6 Data analysis for RAPD

The data for Random Amplified Polymorphic DNA (RAPD) was analysed using the Population Genetic Analysis (POPGENE version 1.31).

The following genetic diversity parameters were determined: 1) the number of polymorphic bands (A) and the percentage of polymorphic bands (P); 2) gene diversity (H) and Shannon's information index (I); 3) Nei's genetic distances (D) and genetic identity (I_N), which were evaluated using the cluster analysis that was performed with the Unweighted Pair Group Method of Arithmetic Average (UPGMA); and 4) the coefficient gene differentiation among the populations within species, which was determined using Nei's gene diversity method. The formula was $G_{ST} = D_{ST}/H_T$, $H_T = H_S + D_{ST}$, where, H_T is the total gene diversity, H_S is the gene diversity within the population, and D_{ST} is the gene diversity between populations. The gene flow was determined as $Nm = 0.5\ (1\text{-}G_{ST})/G_{ST}$ (Yuan et al., 2007).

5.2.7 Genotyping using SSR markers

Genotyping using SSR markers was conducted by INCOTEC South Africa (Pty) Ltd. Company at Pietermaritzburg, South Africa. All samples were used in bulked amplification, using DNA extracted from all the leaf samples of each entry submitted. Ten SSR markers were used (Table 5.4). Polymerase chain reaction products were fluorescently labeled and separated by capillary electrophoresis on an ABI 3130 automatic sequencer (Applied Biosystems, Johannesburg, South Africa); analysis was performed using GeneMapper 4.1. The program GGT 2.0 (Van Berloo, 2008) was used to calculate the Euclidean distances between bulked samples, the matrix of the genetic distances were used to create an UPGMA dendrogram of the results. POPGENE was used to calculate: Nei's gene diversity (H); Shannon's information index (I); genetic diversity index within populations (H_S); genetic differentiation coefficient between populations (G_{ST}) and gene flow (Nm).

5.3 Results

5.3.1 Random amplified polymorphic DNA (RAPD) analysis

5.3.1.1 Polymorphism of RAPD amplified bands by different primers

The analysis of seven *C. pepo* populations with the nine RAPD primers that were polymorphic identified a total of 100 reproducible fragments (Table 5.1). Among them, 94 were polymorphic (94%), ranging in size from 75 to 1800 bp. A range between nine and 14 fragments were amplified per primer, with an average of 11.11 bands. The maximum number (14) of polymorphic fragments was obtained with the primer CB19. The number of polymorphic fragments for each primer varied from eight and 14, with an average of 10.44 bands.

Table 5.1 Sequence, produced band size range and polymorphism of different RAPD primers, as well as genetic variability within seven *Cucurbita pepo* populations

RAPD Primer	Sequence (5' – 3')	Band size range (bp)	N	A	P	H	I	H_S	G_{ST}	Nm
CB9	GGTGACGCAG	100-1300	10	9	90	0.1084	0.2201	0.1081	0.0027	183.3149
CB12	AGTCGACGCC	100-1300	9	8	89	0.1417	0.2707	0.1412	0.0031	160.5899
CB13	ACGCATCGGA	100-1100	10	10	100	0.1334	0.2584	0.1331	0.0022	223.7226
CB15	GGCTGGTTCC	75-1400	12	11	92	0.1783	0.3227	0.1772	0.0060	82.5414
CB17	GTAACCAGCC	100-1400	12	11	92	0.1781	0.3225	0.1774	0.0039	127.1654
CB19	GGTGCTCCGT	75-1400	14	14	100	0.1335	0.2585	0.1330	0.0035	143.1361
CB21	CAGCACTGAC	100-1800	12	12	100	0.1623	0.3004	0.1611	0.0070	70.7242
CB23	CTGGGCACGA	200-1400	11	9	82	0.1865	0.3340	0.1847	0.0100	49.4545
CB27	AAGTGCGACC	200-1300	10	10	100	0.1335	0.2586	0.1329	0.0047	106.6980
Total	_	_	100	94	94					
Average	_	_	11.11	10.44	94	**0.1506**	**0.2829**	**0.1499**	**0.0051**	**97.7840**

CB: Cucurbita; N: total number of bands; A: number of polymorphic bands; P: percentage of polymorphism; H: Nei's gene diversity; I: Shannon's information index; H_S: Genetic diversity index within populations; G_{ST}: Genetic differentiation coefficient between populations; Nm: Gene flow.

5.3.1.2 Population genetic diversity, differentiation (G_{ST}), and gene flow (Nm)

The Nei's gene diversity index (H) varied from 0.1084 (CB9) to 0.1865 (CB23), with an average of 0.1506 ± 0.0267. The Shannon's information index (I) ranged from 0.2201 (CB9) to 0.3340 (CB23), with an average of 0.2829 ± 0.0386. The genetic diversity index within populations (H_S) varied from 0.1081 (CB9) to 0.1847 (CB23), with an average of 0.1499 ± 0.0007.

The genetic differentiation coefficient between populations (G_{ST}) ranged between 0.0022 (CB13) and 0.0100 (CB23), with an average of 0.0051, which showed that the genetic variation between populations accounted for between 0.22% and 1.00%, with an average of 0.51% of the total variation. The gene flow (Nm) ranged between 49.4545 (CB23) and 223.7226 (CB13), with an average of 97.7840, according to the genetic differentiation coefficient between populations, which indicated that there was a high exchange between *C. pepo* populations.

The Nei's gene diversity index (H) and Shannon's information index (I) were conducted to further understand the genetic diversity among the selfed and unselfed populations of *C. pepo* originating from three different districts (Table 5.2). The Nei's gene diversity index and Shannon's information index were the highest in ZS population (H=0.1677; I=0.3060) and the lowest in TNS population (H=0.1301; I=0.2518). Comparisons between selfed and unselfed populations within a district revealed that selfed populations of the uThungulu and Zululand districts had higher Nei's gene diversity index and Shannon's information index than their analogous unselfed populations, while the opposite was evident in populations from the Umkhanyakude district.

Table 5.2 Genetic variation among *C. pepo* populations based on RAPD analysis

Population	Nei's gene diversity (H)	Shannon's information index (I)
MNS	0.1643 ± 0.0523	0.3003 ± 0.0733
MS	0.1393 ± 0.0469	0.2645 ± 0.0681
TNS	0.1301 ± 0.0349	0.2518 ± 0.0512
TS	0.1461 ± 0.0302	0.2760 ± 0.0449
ZNS	0.1427 ± 0.0391	0.2704 ± 0.0556
ZS	0.1677 ± 0.0438	0.3060 ± 0.0613
CPSP	0.1587 ± 0.0318	0.2941 ± 0.0467

Values are Mean ± standard deviation. Populations (district and population name): MNS, Umkhanyakude unselfed; MS, Umkhanyakude selfed; TNS, uThungulu unselfed; TS, uThungulu selfed; ZNS, Zululand unselfed; ZS, Zululand selfed; CPSP, *C. pepo* species with dark green mature fruits.

5.3.1.3 Specific RAPD marker production per primer per landrace(s)

Specific RAPD markers for CPSP population only were produced by: primers CB9 and CB12 (700 bp); primers CB13, CB19 and CB21 (1000 bp); and primer CB27 (1100 bp). Also primers CB15 and CB17 produced exclusive markers 100 bp and 500 bp, respectively, in all populations except CPSP population. The CPSP population had fruits that did not change their colour to orange or yellow at maturity.

Primer CB9 also showed the effect of selfing by identifying a unique band of 400 bp in unselfed populations from all districts including CPSP population, while primer CB21 identified marker 900 bp for only unselfed populations from all districts excluding CPSP population. Also CB15 produced specific band (200 bp) for only selfed populations from all districts, but also including the CPSP

population. Primer CB9 produced unique band 600 bp in MS and ZS populations only, which were both selfed but from different districts. Primer CB23 produced specific band 1000 bp in MNS and MS populations, both from one eco-geographic region, the Umkhanyakude district.

The following specific RAPD markers were only amplified in each of the following populations: MNS population [CB9 (800 bp), CB12 (900 bp), CB27 (800 bp)]; TNS population [CB23 (900 bp), CB27 (1000 bp)]; MS population [CB9 (1200 bp)] and TS population [CB13 (900 bp)].

5.3.1.4 Genetic identity and genetic distance between *C. pepo* populations, based on RAPD markers

To further elucidate the gene differentiation between *C. pepo* populations, Nei's original measure of genetic identity (I_N) and genetic distance (D) was evaluated (Table 5.3). The genetic identity ranged from 0.9985 to 0.9996, while the genetic distance varied from 0.0004 to 0.0015. The TS and TNS populations were the highest in genetic identity (I_N = 0.9996) and the closest in the genetic distance (D = 0.0004). The CPSP and MNS populations as well as CPSP and TNS populations were the lowest in genetic identity (I_N = 0.9985) and the furthest in genetic distance (D = 0.0015).

The phylogenetic relationship between populations was further illustrated by a dendrogram (Figure 5.1) using the UPGMA algorithm based on Nei's genetic distance (1972). The dendrogram grouped the populations into two main clusters, where cluster two had CPSP population (landrace with green and white fruit variegation at maturity) which was distant from a group with all other six populations. As with the results obtained in cluster one, which had two sub-clusters, a clear grouping according to geographical origin was observed. Sub-cluster one grouped the populations from the Umkhanyakude district (MNS and

MS). Sub-cluster two included populations from the uThungulu (TNS and TS) and Zululand (ZNS and ZS) districts, where populations from the former district formed a cluster and then assembled with ZNS and ZS populations, both from the Zululand district.

Table 5.3 Nei's original measure of genetic identity and genetic distance among seven *C. pepo* populations with RAPD markers

Population ID	MNS	MS	TNS	TS	ZNS	ZS	CPSP
MNS	****	0.9995	0.9988	0.9992	0.9986	0.9989	0.9985
MS	0.0005	****	0.9988	0.9991	0.9987	0.9991	0.9992
TNS	0.0012	0.0012	****	0.9996	0.9994	0.9990	0.9985
TS	0.0008	0.0009	0.0004	****	0.9994	0.9990	0.9993
ZNS	0.0014	0.0013	0.0006	0.0006	****	0.9990	0.9988
ZS	0.0011	0.0009	0.0010	0.0010	0.0010	****	0.9989
CPSP	0.0015	0.0008	0.0015	0.0007	0.0012	0.0011	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Populations (district and population name): MNS, Umkhanyakude unselfed; MS, Umkhanyakude selfed; TNS, uThungulu unselfed; TS, uThungulu selfed; ZNS, Zululand unselfed; ZS, Zululand selfed; CPSP, *C. pepo* species with dark green mature fruits.

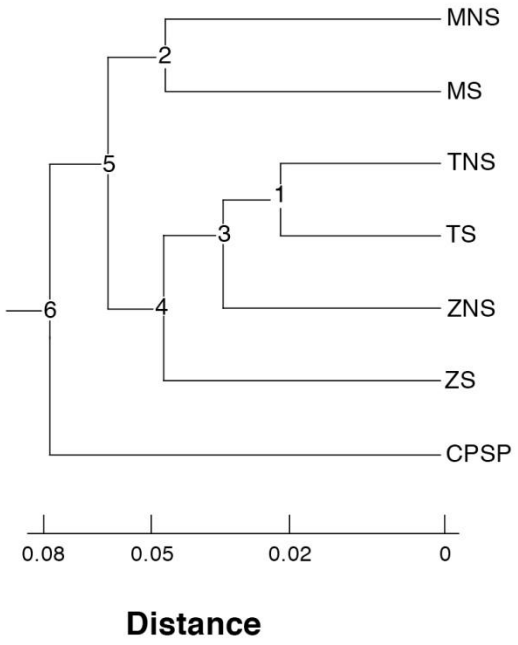

Figure 5.1 Unweighted Pair Group Method of Arithmetic Mean (UPGMA) dendrogram based on Nei's (1972) genetic distance, summarizing data on differentiation in seven populations of *C. pepo* eco-geographical populations with RAPD markers

Populations (district and population name): MNS, Umkhanyakude unselfed; MS, Umkhanyakude selfed; TNS, uThungulu unselfed; TS, uThungulu selfed; ZNS, Zululand unselfed; ZS, Zululand selfed; CPSP, *C. pepo* species with dark green mature fruits.

5.3.2 Simple Sequence Repeat (SSR) analysis

5.3.2.1 SSR polymorphism

A total of 56 alleles were observed with a range from one (CUTC002746 and CUTC04645) to 12 (PKCT122) alleles per SSR marker (Table 5.4). Thirty-eight alleles were polymorphic, where zero (CUTC002746; CMPMC21 and CUTC04645) to 11 (PKCT122) were detected for each SSR marker. The SSR markers detected 67.86% polymorphism, which varied from 0% (CUTC002746; CMPMC21 and CUTC04645) to 100% (CUTC009607). The allele size ranged from 124 to 251 bp. Microsatellite markers: CUTC002746; CMPMC21 and CUTC04645 were found to be monomorphic primers by giving similar bands: 195 bp; 127 and 129 bp; and 195 bp, respectively in all accessions.

5.3.2.2 Unique SSR alleles per population

Unique alleles specific for CPSP population were detected by SSR marker: CMTP9 (151 bp); CMTP132 (134 bp) and PKCT111 (200 bp and 202 bp) (Table 5.4). Also, the following markers revealed alleles that are unique to TNS population: CMTP9 (174 bp); CMPTM39 (180 bp and 182 bp); CUTC009607 (167 bp) and PKCT111 (225 bp). Marker CMTP9 (with 187 bp) and CUTC009607 (with 157 bp and 160 bp) revealed a relationship in ZNS, ZS and CPSP populations by the presence of alleles unique to them only. The CMTP132 revealed the uniqueness of TS population by the absence of allele 136 bp compared with other populations. The CUTC009607 marker revealed allele 169 bp as unique to uThungulu populations and CPSP population. The following SSR markers revealed alleles unique to populations from the uThungulu [CUTC009607 (158 bp and 171bp)] and Zululand [CUTC009607 (155 bp) and PKCT111 (217 bp)] districts only.

Table 5.4 Polymorphism and sizes of alleles detected by ten SSR markers in seven *C. pepo* populations

SSR Marker	CMTP9	CMTP132	CUTC002746	CMPMC21	CUTC004399	CUTC04645	CMPTM39	CUTC009607	PKCT122	PKCT111	**TOTAL**	**AVERAGE**
Size Range (bp)	155-195	115-140	190-201	122-135	170-190	185-206	165-185	150-175	210-255	199-213		
Detected Alleles	**7**	**4**	**1**	**2**	**4**	**1**	**4**	**10**	**12**	**11**	**56**	**5.6**
Polymorphic Alleles	**3**	**2**	**0**	**0**	**3**	**0**	**2**	**10**	**11**	**7**	**38**	**3.8**
Percentage Polymorphism	**42.86**	**50.00**	**0**	**0**	**75.00**	**0**	**50.00**	**100**	**91.67**	**63.64**	**67.86**	
Allele Sizes per Populations												
MNS	159, 162, 168,171	124,125,136	195	127,129	175,181	195	174,176	154,156	208,210,233,235,241, 243,245,247,249,251	204,206,208,210		
MS	159,162,168,171	124,125,136	195	127,129	175,178, 179,181	195	174,176	154,156	210,212,233,235,237, 239,247,249,251	204,206,208,210		
TNS	159,162,168,171,174	124,125,136	195	127,129	175,178, 179,181	195	174,176, 180, 182	154,156,158, 167,169,171	208,210,212,233,235,237, 239,243,245,247,249,251	204,206,208,210, 219,221,223,225		
TS	159,162,168,171	124,125	195	127,129	175	195	174,176	152,154,156, 158,169,171	210,212,243,245, 247,249,251	204,206,208,210		
ZNS	159,162,168,171,187	124,125,136	195	127,129	175,181	195	174,176	154,155,156, 157,160	208,210,212,233,235, 243,245,247,249,251	204,206,208,210, 217,219,221,223		
ZS	159,162,168,171,187	124,125,136	195	127,129	175	195	174,176	155,157,160	208,210,212,233, 235,237,239	204,206,208,210, 217,219,221,223		
CPSP	151,159,162,168,171,187	124,125,134,136	195	127,129	175,181	195	174,176	152,154,156, 157,160,169	208,210,212,233,235,237, 239,245,247,249,251	200,202,204,206, 208,210		

5.3.2.3 Unselfed and selfed populations from the same district

In populations from the Umkhanyakude district, the SSR marker CUTC004399 showed the presence of alleles 178 bp and 179 bp in selfed population only; while marker PKCT122 showed the presence of 208 bp, 241 bp, 243 bp and 245 bp in unselfed population while selfed population had 212 bp, 237 bp and 239 bp (Table 5.4). In addition to unique alleles found in the TNS population when compared to other populations (as mentioned earlier); the following markers revealed alleles presented in brackets only in unselfed population from the uThungulu district: CUTC004399 (alleles 178 bp, 179 bp and 181 bp); PKCT122 (alleles 208 bp, 233 bp, 235 bp, 237 bp and 239 bp) and PKCT111 (alleles 219 bp, 221 bp and 223 bp); while marker CUTC009607 showed allele 152 bp only in selfed population. Differences in populations from the Zululand district were shown by marker CUTC004399 where allele 181 bp was only detected in unselfed population; marker CUTC009607 detected alleles 154 bp and 156 bp only in unselfed population; while marker PKCT122 revealed alleles 243 bp, 245 bp, 247 bp, 249 bp and 251 bp only in unselfed population but alleles 237 bp and 239 bp only in selfed populations.

5.3.2.4 Population genetic diversity, differentiation (G_{ST}) and gene flow (Nm) with SSR markers

The SSR markers CUT002746 and CUTC04645 had the lowest genetic variability (H=0.0191; I=0.0544; Hs=0.0191), while marker PKCT122 had the highest (H=0.1726; I=0.3148; Hs=0.1719); where the averages were: H=0.0675; I=0.1454 and Hs=0.0672 (Table 5.5). The genetic differentiation coefficient between populations (G_{ST}) ranged from zero (CUT002746; CMPMC21 and CUTC04645) to 0.0076 (CUTC009607) with an average of 0.0038. Gene flow (Nm) varied between 65.4118 (CUTC009607) and 2000.00 (CUT002746; CMPMC21 and CUTC04645) with an average of 132.0931.

Table 5.5 Genetic variability within seven *C. pepo* populations based on SSR markers

SSR Marker	H	I	H_S	G_{ST}	Nm
CMTP9	0.0886	0.1879	0.0885	0.0011	442.4883
CMTP132	0.0569	0.1323	0.0568	0.0010	506.5375
CUTC002746	0.0191	0.0544	0.0191	0.0000	2000.0000
CMPMC21	0.0381	0.0958	0.0381	0.0000	2000.0000
CUTC004399	0.0436	0.1068	0.0433	0.0060	82.6283
CUTC04645	0.0191	0.0544	0.0191	0.0000	2000.0000
CMPTM39	0.0435	0.1067	0.0434	0.0022	225.8178
CUTC009607	0.0809	0.1750	0.0803	0.0076	65.4118
PKCT122	0.1726	0.3148	0.1719	0.0040	123.0468
PKCT111	0.1122	0.2260	0.1115	0.0064	77.7294
Average	**0.0675**	**0.1454**	**0.0672**	**0.0038**	**132.0931**

H: Nei's gene diversity; I: Shannon's information index; H_S: Genetic diversity index within populations; G_{ST}: Genetic differentiation coefficient between populations; Nm: Gene flow.

Genetic diversity analysis among unselfed and selfed *C. pepo* populations from different districts revealed that the Nei's gene diversity index and Shannon's information index were the highest in TNS population (H=0.0853; I=0.1742) and the lowest in TS population (H=0.0564; I=0.1260) (Table 5.6). Comparisons between selfed and unselfed populations within a district revealed that unselfed populations of the uThungulu and Zululand districts had a higher Nei's gene diversity index and Shannon's information index than their corresponding selfed populations, while the opposite was apparent in populations from the Umkhanyakude district.

Table 5.6 Genetic variation among *C. pepo* populations with SSR markers

Population	Nei's gene diversity (H)	Shannon's information index (I)
MNS	0.0580 ± 0.0478	0.1283 ± 0.0801
MS	0.0600 ± 0.0428	0.1329 ± 0.0737
TNS	0.0853 ± 0.0611	0.1742 ± 0.1002
TS	0.0564 ± 0.0399	0.1260 ± 0.0724
ZNS	0.0726 ± 0.0558	0.1528 ± 0.0942
ZS	0.0618 ± 0.0467	0.1348 ± 0.0823
CPSP	0.0762 ± 0.0575	0.1589 ± 0.0966

Values are Mean ± standard deviation. Populations (district and population name): MNS, Umkhanyakude unselfed; MS, Umkhanyakude selfed; TNS, uThungulu unselfed; TS, uThungulu selfed; ZNS, Zululand unselfed; ZS, Zululand selfed; CPSP, *C. pepo* species with dark green mature fruits.

5.3.2.5 Genetic distances and genetic relationship among *C. pepo* populations based on SSR markers

Genetic distance between *C. pepo* populations ranged from 0.2542 to 0.5157 (Table 5.7). The closest distance (highest degree of similarity) was observed between MNS and MS populations which are both from the coastal region in the Umkhanyakude district, while the furthest distance (lowest degree of similarity) was observed between MS and ZS populations, a selfed Umkhanyakude district and a selfed Zululand district (inland region) population, respectively.

The UPGMA dendrogram grouped the populations into two main clusters (Figure 5.2). Cluster one had two sub-clusters where the first sub-cluster grouped the populations according to their geographic origin (MNS and MS populations from the Umkhanyakude district) and second sub-cluster grouped selfed populations from different origins (TS and ZS populations from the uThungulu and Zululand

districts, respectively). The unselfed population from the Zululand district (ZNS) and *C. pepo* population from the Umkhanyakude district which retains its green fruit colour at maturity formed cluster two and were assembled with unselfed population from the uThungulu district (TNS).

Table 5.7 Pairwise Jaccard's genetic distances between *C. pepo* populations based on SSR markers

Population ID	MNS	MS	TNS	TS	ZNS	ZS
MS	0.2542					
TNS	0.3901	0.4259				
TS	0.2750	0.2887	0.4509			
ZNS	0.3698	0.4173	0.4337	0.3736		
ZS	0.4810	0.5157	0.5127	0.3783	0.3838	
CPSP	0.3640	0.4078	0.4413	0.3687	0.4052	0.4556

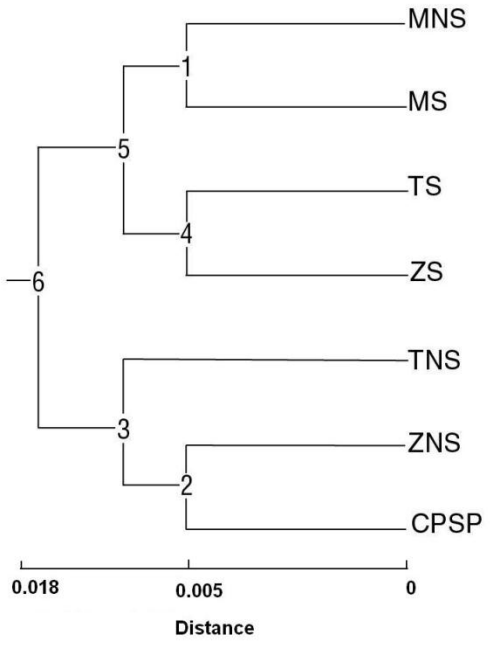

Figure 5.2 Unweighted Pair Group Method of Arithmetic Mean (UPGMA) dendrogram based on Nei's (1972) genetic distance, summarizing data on differentiation in seven populations of *C. pepo* eco-geographical populations with SSR markers

Populations (district and population name): MNS, Umkhanyakude unselfed; MS, Umkhanyakude selfed; TNS, uThungulu unselfed; TS, uThungulu selfed; ZNS, Zululand unselfed; ZS, Zululand selfed; CPSP, *C. pepo* species with dark green mature fruits.

5.4 Discussion

5.4.1 Polymorphism in *C. pepo* based on RAPD and SSR analysis

The level of polymorphism among the *C. pepo* population was relatively high with RAPD marker, ranging between 82% and 100% with an average of 94%. This high level of RAPD markers polymorphism in *C. pepo* genotypes is in

accordance with the results of Kathiravan et al. (2006); Hadia et al. (2008); and Tsivelikas et al. (2009), who reported that *C. pepo* is a highly polymorphic species. The degree of RAPD polymorphism detected in the present study was slightly higher than that reported in other studies of *Cucurbita maxima* 84%, *Cucurbita moschata* 87% and *Cucurbita pepo* 89% (Hadia et al., 2008); *Trichosanthes dioica* 79% (Khan et al., 2009), and *Citrullus lanatus* 88% (Mujaju et al., 2010); *Momordica charantia* 37% (Dey et al., 2006; Behera et al., 2008), *Lagenaria siceraria* 30% (Morimoto et al., 2006), *Lagenaria sphaerica* 24% (Morimoto et al., 2006), and *Cucumis melo* 72% (Yildiz et al., 2011).

A total of 56 SSR alleles, with a size range from 124 bp to 251 bp, as detected by 10 SSR markers were found among *Cucurbita pepo* landraces of northern KwaZulu-Natal (Table 5.4). In Zimbabwe, Mujaju et al. (2010) reported a total of 43 SSR alleles, with 123 bp to 285 bp size range, as revealed by nine SSR markers among *Citrullus lanatus* landraces. The percentage polymorphic alleles varied from 0% to 100% with an average of 67.86% (Table 5.4). Mujaju et al. (2010) reported a percentage polymorphic alleles range from 55.6% to 88.9% among watermelon landraces. The monomorphism of SSR markers: CUTC002746; CMPMC21 and CUTC04645 (Table 5.4) concurs with Tantasawat et al. (2010) who reported monomorphism in SSR marker VM 21 which gave a single band of 179 bp in all accessions of *Vigna* species.

5.4.2 Population genetic structure and geographic diversity of northern KwaZulu-Natal *C. pepo* landraces

Gene differentiation and gene flow are important indexes to evaluate the population genetic structure. The gene differentiation coefficients of *C. pepo* landraces in northern KwaZulu-Natal were 0.0051 and 0.0038, when analyzed with RAPD and SSR markers, respectively. Ferriol et al. (2003) reported the gene differentiation coefficients of *C. pepo* landraces, mainly from Spain, as 0.25

and 0.18, when analysed with SRAP and AFLP, respectively. Further, the gene differentiation coefficients of Spanish *C. moschata* landraces as determined by AFLP and SRAP analyses were 0.28 and 0.17, respectively (Ferriol et al., 2004b). The average values of G_{ST} were 0.0051 and 0.0038, based on the RAPD and SSR markers for *C. pepo* in northern KwaZulu-Natal, indicate that the gene differentiation was higher within populations (99.49% and 99.62%) than between populations (0.51% and 0.38%), respectively. Similar findings were reported by Yuan et al. (2007) among *Punica granatum* populations in China.

Based on the RAPD and SSR analysis of *C. pepo* landraces of northern KwaZulu-Natal, the average values of the gene flow were 97.7840 and 132.0931, respectively. According to Han et al. (2007), this gene flow overwhelmed the effect of genetic drift. Yuan et al. (2007) and Mujaju et al. (2010) reported that the movement of genes within and between populations, the gene flow, is negatively correlated with the gene differentiation, and is transferred by pollen and seed between populations for seed plants. Also, the population genetic structure is mainly affected by a long distance diffusion of pollen and diffusion capability of pollen offspring owing to inbreeding and outcrossing propagation (Yuan et al., 2007). However, *C. pepo* distributed in different districts of KwaZulu-Natal with long geographical distance, had a very low possibility of the pollen spread by insects (particularly bees) between populations. This agrees with a report among Chinese Pomegranate accessions (Yuan et al., 2007). Several authors have reported the evidence of gene flow and hybridization between several interplanted *Cucurbita* species (Decker-Walters et al., 1990; Wessel-Beaver, 2000; Montes-Hernandez and Eguiarte, 2002; Cuevas-Marrero and Wessel-Beaver, 2008). The possible gene flow among *Cucurbita* species was also possible for long distances pollen transfer by bees ranging between 800m and 1300m (Montes-Hernandez and Eguiarte, 2002; Spencer and Snow, 2001). Therefore the main way of gene exchanges can be the occasional introduction of seeds as enhanced by seed exchanges between farmers of different districts in

KwaZulu-Natal, which agrees with the reports of Mujaju et al. (2010); Du et al. (2011) and Barboza et al., 2012).

The Nei's gene diversity index (H=0.1506 and 0.0675), Shannon's information index (I=0.2829 and 0.1454) and the genetic diversity index within populations (H_S=0.1499 and 0.0672), as revealed by RAPD and SSR markers, respectively, also indicate molecular genetic diversity for the *C. pepo* populations studied herein. The gene diversity obtained from RAPD and SSR analysis among different *C. pepo* landraces from northern KwaZulu-Natal was lesser than that obtained from AFLP markers among different *C. moschata* accessions (Wu et al., 2011); from RAPD among *Trichosanthes dioica* accessions (Khan et al., 2009); and also from ISSR, SRAP and RAPD markers among Turkish and Foreign *Cucumis melo* genotypes (Yildiz et al., 2011).

The RAPD analysis produced specific RAPD marker by primer CB23 (1000 bp) in both MNS and MS populations from the Umkhanyakude district while SSR analysis showed SSR alleles unique to populations from the uThungulu [CUTC009607 (158 bp and 171bp)] and Zululand [CUTC009607 (155 bp) and PKCT111 (217 bp)] districts only. This probably indicated the effect of eco-geographic differences in gene diversity of plants, where landraces from the same geographic area are closely related. A similar observation was reported among *Cucurbita* species studied in Spain (Ferriol et al., 2004a; 2004b) and in China (Du et al., 2011).

5.4.3 Genetic variation between unselfed and selfed *C. pepo* populations

The Nei's gene diversity index and Shannon's information index revealed by RAPD markers was higher in selfed populations from uThungulu and Zululand districts than their corresponding unselfed population. This probably means that selfing enabled an easier detection of rare alleles, which can increase their effectiveness of selection and the amount for genetic improvement in a breeding

program, which concurs with the report by Ghobary and Ibrahim (2010). However, the detection of higher genetic diversity in unselfed populations from these districts than their analogous selfed populations, as shown by SSR markers, probably revealed the heterozygosity in unselfed than selfed populations. Ercan and Kurum (2003); Cardoso (2004) and Ferrari et al. (2006; 2007) reported that self pollination enhances homozygosity while reducing the heterozygosity in plant genomes. The production of specific RAPD markers and SSR alleles for either selfed or unselfed populations only, also confirmed that selfing had changed the genetic state of these *C. pepo* landraces from different districts in northern KwaZulu-Natal. Ferrari et al. (2006; 2007) reported the change in a plant's genetic state with selfing.

5.4.4 Specific RAPD markers and unique SSR alleles per landrace(s)

With both RAPD and SSR markers, some fragments were uniquely amplified from single populations, indicating the genotype variation among *C. pepo* landraces in northern KwaZulu-Natal. Similar results were observed for some South American and Spanish *C. moschata* accessions using SRAP and AFLP markers (Ferriol et al., 2004a). Hadia et al. (2008) have identified the specific RAPD markers that showed genotype variation among *C. maxima*, *C. moschata* and *C. pepo* species, as well as those showing differences within their populations. Again, Barracosa et al. (2008) in their study of *Ceratonia siliqua*, made use of unique RAPD markers that were cultivar-specific to differentiate the Portuguese cultivars.

Six RAPD primers (CB9; CB12; CB13; CB19; CB21 and CB27) and three SSR markers (CMTP9; CMTP132 and PKCT111) produced unique bands for the CPSP population only, while two RAPD primers (CB15 and CB17) produced unique bands for all other six populations except CPSP. The presence and absence of unique bands in CPSP populations only indicated that these primers were probably marking the genes or loci that are affecting fruit colour

development or change at maturity among these landraces, where the CPSP maintains its green fruit variegation at maturity. Paris (2000) reported 11 loci that have been identified as affecting developmental fruit colour in *C. pepo*, and of these, three genes of major effect – D, l-1, and l-2 – account for a considerable portion of the genetic variation in intensity of fruit colouration that is observed in this species. The developmental fruit colouration from light green fruits, several days past anthesis, except for some darkening of the main carpillary veins, becoming blackish-green past anthesis, and then turning intense orange on ripening (Paris, 2000; 2009), as observed in other landraces, was conferred by genotype D/D l-1/l-1 L-2/L-2 (Paris, 2000). However, fruits of L-1/ — L-2/ — plants are intense green throughout development, as was the case with the CPSP landrace, where, in contrast to plants homozygous recessive for either or both l genes, the fruits of L-1/— L-2/ — plants retain their black-green colour through maturity, not turning orange or yellow when ripe, as earlier reported by Paris (2000).

5.4.5 Genetic diversity and relationship among *C. pepo* populations

The range of genetic identity from 0.9985 to 0.9996 (with RAPD) and genetic distance range from 0.0004 to 0.0015 (with RAPD) and from 0.2542 to 0.5157 (with SSR) indicated the presence of variability among the seven populations of *C. pepo* in northern KwaZulu-Natal. With both RAPD and SSR markers, populations originating from the same districts (uThungulu and Umkhanyakude districts, respectively) were the closest in genetic distance, thus had the highest degree of similarity, while those from distant districts were the farthest (Tables 5.3 and 5.5), probably because most of the farmers have maintained the production of these landraces for many years within each district. The same was reported among *Punica granatum* cultivars (Yuan et al., 2007), *Trichosanthes dioica* accessions (Khan et al., 2009) and *Cucurbita moschata* accessions (Du et al., 2011; Barboza et al., 2012). However, the lowest genetic identity and farthest

genetic distance between CPSP and MNS populations, both from the Umkhanyakude district (determined with RAPD markers), was possibly because the CPSP population did not change fruit colour to orange or yellow at maturity whereas the MNS population did change. Therefore differences in genes that are responsible for fruit colour formation at maturity as explained by Paris (2000; 2009) might have influenced this high diversity between these populations.

The farthest distance between MS population from the Umkhanyakude district and ZS population from the Zululand district (Table 5.5) using SSR markers was probably the effect of geographic origins and ecotype variations of these populations, since the Umkhanyakude district is more coastal while the Zululand district is inland. Cortese et al. (2010) reported the same among *Panicum virgatum* populations using SSR markers.

The genetic diversity among studied *C. pepo* populations based on RAPD and SSR markers through dendrograms was very small (Figures 5.1 and 5.2). This was possibly due to the seed sourcing of these populations from three districts of northern KwaZulu-Natal (which is only one province), which was probably a small scale to show high genetic diversity. Earlier studies reported genetic diversity among *Cucurbita* species from different provinces of the same country (Tsivelikas et al., 2009) and also different countries (Ferriol et al., 2003; 2004a; 2004b). This low genetic diversity might also have resulted from the investigation of one *Cucurbita* species, the *C. pepo*. Hadia et al. (2008) in their research reported a higher genetic diversity among populations of *C. maxima*, *C. moschata* and *C. pepo*, which are three different species. Further, this low genetic diversity was probably due to the very high gene flow which was detected by these markers (Tables 5.1 and 5.5).

The dendrograms showed that populations from the Umkhanyakude district clustered together both on RAPD and SSR markers (Figures 5.1 and 5.2). Different factors could have led to this grouping. Probably there was no seed

exchange between the farmers from other investigated districts, in contrast to the reports by (Barboza et al., 2012). Also, the deep sand coastal ecotype of the Umkhanyakude district (Gibbon et al., 2010) as compared to the uThungulu and Zululand districts might have changed the genetic make-up of its *Cucurbita pepo* populations. This clustering further showed that these populations are grouped according to their agro-ecological regions rather than selfing effect. This concurs with the findings of Ferriol et al. (2004a; 2004b) in their study of *Cucurbita maxima* and *C. moschata*, respectively, where they obtained the results with both sequence-related amplified polymorphism (SRAP) and amplified fragment length polymorphism (AFLP) markers, where accessions were grouping according to their geographical origin. Also, Amadou et al. (2001) in their genetic diversity analysis of *Vigna subterranea* using RAPD markers, found the highest similarity on accessions that were originating from the same country. Further, Tsivelikas et al. (2009) reported the genetic diversity analysis of *C. moschata* landraces using RAPD markers where accessions were grouped according to the agro-climatic regions and not according to the morphological traits.

A clear grouping, first according to fruit colour change at maturity, and secondly according to geographical origin, was obtained with RAPD markers. The separation of CPSP population from a group of six populations that had changed in their fruit colour at maturity may support the wide genetic variation in these landraces with reference to their fruit colour formation as recorded by Paris (2000; 2009).

Further, the grouping of populations from the uThungulu and Zululand districts with both markers was probably due to existence of seed exchanges among farmers of these districts. The same was reported earlier by other researchers in cucurbits (Montes-Hernandez and Eguiarte, 2002; Ferriol et al., 2004a; 2004b; Barboza et al., 2012) and other species (Yuan et al., 2007). In populations from these districts, the SSR markers also revealed the effect of self pollination in

changing the gene make-up (Ghobary and Ibrahim, 2010), where the selfed populations made a separate cluster from the unselfed populations.

Unselfed and selfed *C. pepo* landraces from different districts had similar morphological features. Therefore, genetic differences obtained among them using RAPD and SSR markers agree with our hypothesis that the landraces with similar morphological features have different genetic constitution. Further, the clustering of landraces from the same agro-ecological conditions also concurs with our hypothesis which states that landraces from different districts have different genetic components.

5.5 Conclusion

Both RAPD and SSR markers have revealed high polymorphism and genetic diversity among the *C. pepo* landraces with different agro-ecological origins. They further revealed the effect of selfing on the change of genetic make-up in *C. pepo* landraces. RAPD marker revealed the genetic diversity in landraces with differences in fruit colour change at maturity to a higher extent. *Cucurbita pepo* landraces from Umkhanyakude district (MNS and MS landraces) clustered together, with both RAPD and SSR markers.

Chapter 6

Conclusion and Recommendations

Traditional leafy vegetables are important sources of micronutrients which are essential for human health. Many of these vegetables are collected from the wild, particularly by old women. When domesticated they need few inputs and can grow and reproduce well in adverse conditions which are not conducive for modern crops. Landraces are local crop varieties which are maintained by the farmers and have high genetic diversity, thus serve as sources of genes for plant breeding. In this thesis, the objectives were to identify different landraces of traditional leafy vegetables that are utilized in northern KwaZulu-Natal; to grow and determine the yield of plant parts used for vegetable purposes in *Cucurbita* landraces, one of the best preferred leafy vegetable species in this region. Diversity in morphology and genetics of these *Cucurbita* landraces was also investigated.

The first phase of this research revealed northern KwaZulu-Natal as having considerable vegetable species diversity, where a total of 72 vegetable species were recorded from three districts. The highest percentage of utilized vegetable species was collected from the wild. The Umkhanyakude district had the highest percentage of recorded and preferred traditional leafy vegetables. The following wild species were recorded for the first time as traditional leafy vegetables: *Commelina erecta; Deinbollia oblongifolia; Erythroxylum delagoense; Galinsoga ciliata; Ipomoea wightii; Limeum sulcatum; Priva meyeri* var. *meyeri; Trachyandra asperata* var. *asperata* and *Trachyandra* cf. *saltii* var. *saltii*. These findings agree with our hypothesis that the northern part of KwaZulu-Natal is endowed with high diversity of traditional leafy vegetables which are extensively utilized particularly in rural communities. Further research on improved palatability, agronomy, and breeding particularly for crop improvement of these vegetables is essential for food security and economy in South Africa.

Among the recorded vegetable species in the region, *Amaranthus hybridus*, *B. pilosa* and *C. pepo* were the most well-known and favoured traditional leafy vegetables. Wild vegetables were accessed during the rainy seasons or year-round if they were growing in moisture-sufficient areas.*Cucurbita* species including *C. pepo* were grown in spring and summer and had initiation of their leafy shoot harvest in less than two months from seeding. When available, many people eat them daily or every two days. Communities were saving seeds from the previous harvest which is a symbol of the ownership of these *Cucurbita* species. Therefore, the supply of preferred traditional leafy vegetables both in wet and dry seasons, through cultivation is essential.

The second part of this study also served as the first report on the diversity in morphology and yield traits of *Cucurbita* landraces grown for vegetable purposes in northern KwaZulu-Natal, and also in reporting about *Cucurbita argyrosperma* in South Africa. Landraces differed significantly in yield, namely: shoot dry weight; numbers of leafy shoots, of set and harvested fruits, and of seeds; size of harvested fruits; and weights of fruits and seeds. These differences between landraces agree with our hypothesis that the landraces obtained from different districts differ in yield when grown under the same experimental conditions. However, insignificant leaf yield between landraces disagrees with our hypothesis. The *C. pepo* (namely, M-IT, T-IT, Z-IT and M-IN) and C. *pepo*/*maxima* (T-UM) landraces were the high shoot, pistillate flower, fruit and seed yielding landraces, thus have potential for hunger alleviation, future breeding and improvement for commercialization. The *C. maxima* (M-UM and Z-UM) and *C. argyrosperma* (M-IS) landraces had significantly lower fruit production and are recommended for shoot use as leafy vegetables. This therefore necessitates future research on enhancing their fruit production in this area. Six weeks after seeding was the proper time for shoot harvest that enabled the highest production of utilized plant parts in all *Cucurbita* landraces.

The significant variation between landraces in the dry weight of the three-leaved shoots; leaf area; vine length; number and length of branches per vine; number of fruits per plant, fruit length, fruit diameter, fruit weight, number and percentage of fully developed seeds per fruit, 100-seed weight and total seed weight, could be attributed to genetic differences existing between the landraces.

The existence of diversity both between and within the *Cucurbita* landraces collected from a relatively limited number of districts in northern KwaZulu-Natal was the most remarkable finding in this study. Heterozygosity was also expressed by significant variation within landraces (either *C. maxima* or *C. pepo* landraces) and also those originating from the same district (M-IT and M-IN landraces). The similarity between *Cucurbita maxima* landraces from the Umkhanyakude (M-UM) and Zululand (Z-UM) districts probably reflected the effect of seed exchange among the farmers of these districts. These findings are also in agreement with our hypothesis that the morphological features can show differences among *Cucurbita* landraces from different districts.

In the last part of this research, unselfed and selfed *C. pepo* populations, with differences in fruit colour change at maturity, from the Umkhanyakude, uThungulu and Zululand districts of northern KwaZulu-Natal were assessed using random amplified polymorphic DNA (RAPD) and simple sequence repeat(SSR) markers.Both RAPD and SSR markers have proved to be useful for analyzing population genetic diversity among the *C. pepo* landraces with different agro-ecological origins. They further revealed the effect of selfing on the change of gene make-up in *C. pepo* landraces. The RAPD marker revealed genetic diversity in landraces with differences in fruit colour change at maturity to a higher extent.

The landraces from Umkhanyakude district (MNS and MS) showed uniqueness because in both RAPD and SSR dendrogramme they clustered together. It is therefore recommended to preserve seeds of this landrace through seed banking

and also to advise the farmers to avoid seed exchange between districts, thus they can maintain the *C. pepo*genetic diversity within the province. As in agreement with our hypothesis, the molecular study revealed the genetic diversity among selfed and unselfed *C. pepo* populations and also the differences in their agro-ecological origins.

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APPENDIX I: Form of consent of using ethnobotanial information in English and isiZulu

FORM OF CONSENT OF USING ETHNOBOTANICAL INFORMATION

Researchers

Prof. A.M. Zobolo; Prof. P. Tongoona, Dr. R.M. Madakadze and Mrs. N.R. Ntuli

Institution

University of Zululand - Department of Botany

Research Project

This study sought to document the traditional knowledge of traditional leafy vegetables that are utilized by communities at Umkhanyakude, uThungulu and Zululand districts of northern KwaZulu-Natal. The information will be gathered using structured questionnaires. Voucher specimens will be collected for identification and further scientific studies. This study is for academic purposes and is of no commercial value. Results from this study will be presented at conferences and published in academic journals. The data will also be used towards the completion of a Doctoral Degree in Botany by the above mentioned student.

Please take note of the following:

You are under no obligation to share any secrets or personal information which you do not feel comfortable in sharing with us.

Follow up visit

We undertake to reveal the main results of this study to every homestead visited on completion of this project.

Signature of interviewee

_____

IFOMU LOKUCELA IMVUME YOKUSEBENZISA ULWAZI OLUMAYELANA NOKUSETSHENZISWA KWEZITSHALO ZESINTU

Abacwaningi
USolwazi A.M. Zobolo; uSolwazi P. Tongoona; uDokotela R.M. Madakadze kanye noNkosikazi N.R. Ntuli
Isikhungo semfundo
Inyuvesi yakwaZulu - Umnyango wezezitshalo

Ucwaningo

Inhloso yalolu cwaningo ukuqopha ulwazi lwemifino yesintu edliwa abantu baseMkhanyakude; oThungulu kanye naseZululand, enyakatho nesifundazwe sakwaZulu-Natal. Lolu lwazi luzoqoqwa ngokusebenzisa imibuzo ehlelwe ngokulandelana. Izingxenge-ngqangi zalemifino zizoqoqwa, zicutshungulwe ukuze kutholakale ulwazi ngazo futhi ziphinde zisetshenziswe ekuqhubekeni kwezinye izifundo zocwaningo. Lolu cwaningo luyingxenye yemfundo kuphela, aluyona ingxenye yezokuhweba. Imiphumela yalolu cwaningo izokwethulwa kwizinkomfa iphinde futhi ishicilelwe emabhukwini ezemfundo. Imiphumela izophinde isetshenziselwe ekutholeni iziqu zobudokotela kumkhakha wezezitshalo kumfundi obalulwe ngenhla.

Qaphelisisa lokhu okulandelayo:

Awuphoqelekile ukuthi usitshele izimfihlo noma iluphi ulwazi lwakho ozizwa ungakhululekile ukusitshela lona.

Ukuqhubeka kohambo

Siyathembisa ukuthi imiphumela yalesi sifundo sizoyethula kubona bonke ababhlali ababeyingxenye ekunikezeleni ulwazi lwabo ekupheleni kwalolu cwaningo.

Igama lomuntu obuzwayo

_____

APPENDIX II

Traditional leafy vegetables research questionnaires (informal survey)

1. Area particulars and socio-demographic data

1.1 Date:

1.2 Enumerator:

1.3 District:

1.4 Village:

1.5 Respondent:

a) Male

b) Female

1.6 Age group of the respondent (years):

a) young-age (18-34 years)

b) middle-age (35-54 years)

c) old-age (55 years and above)

2. What traditional leafy vegetables do you grow and/or collect?

2.1 Regarding vegetables listed in 2, how many types (landraces) do you use or know?

3. Vegetables' preference

3.1 Which traditional vegetables do you use the most (prefer)?

3.2 How many times do you eat them?

a) daily

b) every two days

c) every three days

d) weekly

e) other specify

3.3 Which plant parts do you use?

a) Leaves

b) young shoots

c) fruits

d) seeds

e) roots

4. Seed sourcing and storage

4.1 What is the seed source of your leafy vegetable?

a) home-saved seeds

b) seed merchants

c) soil seed bank

4.2 How do you store your leafy vegetable seed?

a) clay pots

b) eaves

c) bottles

d) plastics

e) jute bags

f) other- specify

4.3 For how long do you store your leafy vegetable seeds?

a) 1 season

b) 2 seasons

c) 3 seasons

d) more than 3 seasons

4.4 What do you use to protect the leafy vegetable seeds during storage?

a) no protection

b) smoke in kitchen

c) ash
d) chemicals
e) other – specify

4.5 What storage problems do you face with leafy vegetable seeds?
a) no problem
b) pest and diseases
c) loss of quality
d) no refrigeration

5. Agronomic characterization of cultivated vegetables

5.1 When do you grow these vegetables?
a) spring (August-October)
b) summer (November-January)
c) autumn (February-April)
d) winter (May- July)
e) throughout the year

5.2 What is the soil type where you grow them?
a) sandy
b) loam
c) humus-rich
d) clayey

5.3 What is the soil moisture regime of the land you allocate leafy vegetables?
a) permanent wetland
b) intermittent wetland
c) dryland

5.4 What planting procedure do you use?

a) seed broadcasting
b) seed dribbling in a row
c) per station
d) seedling transplanting
e) volunteer crops

5.5 What cropping system do you use?
a) intercrop stands
b) monocrop stands

5.6 What type of fertilizer do you apply to leafy vegetables?
a) no fertilizer
b) inorganic fertilizer
c) animal manure
d) compost manure

5.7. When do you apply the fertilizer?
a) before planting (during soil preparation)
b) during planting
c) after planting (during growth)

5.8 What is the time interval from seed sowing to first leafy shoot removal for vegetables?
a) less than 2 months
b) more than 2 to 3 months
c) more than 3 to 4 months
d) more than 4 months

5.9 What are the pests or diseases that affect the leafy vegetables?

5.10 What is the vegetable response towards pests and diseases?

a) tolerant

b) moderate

c) susceptible

d) other - specify

APPENDIX III: The local name, locality, voucher number, growth habit, cultivation status, part(s) used and preparation mode of leafy vegetables of northern KwaZulu-Natal

Species	Family	isiZulu and/or isiThonga name	Locality	Voucher no.	Growth habit	Cultivation status	Part(s) used	Preparation mode
Abelmoshus esculentus (L.) Moench	Malvaceae	amaNdwandwa	M	NRN 501	Sr	C	fr	Bd and Fd
Albertisia delagoensis (N.E.Br.) Forman	Menispermaceae	uNgandingandi	M	NRN 390	Cl; Tl	W	le	Bd and Fd
Alternanthera sessilis (L.) DC.	Amaranthaceae	iMfingwane, uNgudla luphongolo	M	NRN 396	Hr	W	sh	Bd and Fd
Amaranthus hybridus L. subsp. *hybridus* var. *hybridus*	Amaranthaceae	iSheke elikhulu; iMbuya; iMbuya enkulu (eluhlaza, ebomvu); iBhedelele; umBhido; uGobolo; uGwabuzela; uMagwabugwabu	M; T; Z	NRN 459	Hr	W	sh	Bd and Fd
Amaranthus retroflexus L.	Amaranthaceae	iMbuya eluhlaza	M; T; Z	NRN 497	Hr	W; SD	sh	Bd and Fd
Amaranthus spinosus L.	Amaranthaceae	iMbuya enameva; iMbuyabathwa; iMbuyatsheke; uMabalabala; uMahlaba; uHlabahlaba; uPhululu; uQhuthululu; umQhuthu	M; T; Z	NRN 405	Hr	W; SD	le	Bd and Fd
Amaranthus thunbergii Moq.	Amaranthaceae	iSheke elincane; iMbuya encane (ecwebezelayo)	M; T; Z	NRN 361	Hr	W; SD	le	Bd and Fd
Amaranthus dubius Mart. ex Thell.	Amaranthaceae	iMbuya ebomvu	T	NRN 406	Hr	W; SD	sh	Bd and Fd
Amaranthus hypochondriacus L.	Amaranthaceae	iMbuya ebomvu	M	NRN 357	Hr, Sr	W; SD	sh	Bd and Fd
Aneilema aequinoctiale (P. Beauv.) Loudon	Commelinaceae	iTleletlele lesilisa; iDangabane lesilisa (lendoda)	M	NRN 325	Hr	W	le	Bd and Fd
Arachis hypogea L.	Fabaceae	amaKinati; amaNtongomane	M; T	NRN 504	Hr	C	se	Bd; Fd and R

APPENDIX III: The local name, locality, voucher number, growth habit, cultivation status, part(s) used and preparation mode of leafy vegetables of northern KwaZulu-Natal (Continued)

Species	Family	isiZulu and/or isiThonga name	Locality	Voucher no.	Growth habit	Cultivation status	Part(s) used	Preparation mode
Asystasia gangetica (L.) T. Anderson	Acanthaceae	isiHhobo; umLomo wenyoni	M	NRN 378	Hr	W	le	Bd and Fd
Asystasia schimperi T. Anders.	Acanthaceae	iMbobela	M; T; Z	NRN 455	Hr, Sr	W	le	Bd and Fd
Bidens bipinnata L.	Asteraceae	uQadolo oluhlaza (ontsakantsaka)	M; T; Z	NRN 413	Hr	W	sh	Bd and Fd
Bidens biternata (Lour.) Merr. and Sherff	Asteraceae	uQadolo oluhlaza (ontsakantsaka)	M; T; Z	NRN 462	Hr	W	sh	Bd and Fd
Bidens pilosa L.	Asteraceae	uQadolo; uQadolo omnyama (obomvu); uCadolo; uCucuza	M; T; Z	NRN 400	Hr	W	sh	Bd and Fd
Boerhavia diffusa L.	Nyctaginaceae	imiFino engenagama; isiHlalakuhle; uNkunzana	M; T; Z	NRN 412	Hr	W	le	Bd and Fd
Carica papaya Linn.	Caricaceae	uPhopho	M	NRN 505	Tr	C	le; fr	Bd; Fd and R
Chenopodium album L.	Chenopodiaceae	iMbilikicane; isiDwaba samaSwazi; iMbindla; isiKigi; isiKigi sesalukazi; uBhici lwesalukazi; isiGcozi; uDekane	M; T; Z	NRN 318	Hr	W	le	Bd and Fd
Chenopodium murale L. var. *murale*	Chenopodiaceae	iMbilikicane enkulu	M; T; Z	NRN 414	Hr	W	le	Bd and Fd
Citrullus lanatus (Thunb.) Matsum. and Nakai	Cucurbitaceae	amaBhece; umHhense; iKh(a)ebe	M; T; Z	NRN 360	Tl; Cl	C; RW	sh; fr	Bd; Fd and R
Coccinia rehmannii Cogn.	Cucurbitaceae	iHhawulane; iNgwili; amaPholonjane; iNtshungu	M; T; Z	NRN 441	Tl; Cl	W	le, fr	Bd and Fd
Colocasia esculenta L. Schott	Araceae	amaDumbe; imiDebeza	M; T; Z	NRN 506	Cr	C	cr, le	Bd and Fd
Commelina africana L.	Commelinaceae	iTleletlele lesifazane; iDangabane lesifazane	M	NRN 324	Tl	W	le	Bd and Fd

APPENDIX III: The local name, locality, voucher number, growth habit, cultivation status, part(s) used and preparation mode of leafy vegetables of northern KwaZulu-Natal (Continued)

Species	Family	isiZulu and/or isiThonga name	Locality	Voucher no.	Growth habit	Cultivation status	Part(s) used	Preparation mode
Commelina benghalensis L.	Commelinaceae	iTleletlele; iDangabane	M; T; Z	NRN 352	Tl	W	le	Bd and Fd
Commelina erecta L.	Commelinaceae	iDangabane	T	NRN 415	Tl	W	le	Bd and Fd
Corchorus olitorius L.	Tiliaceae	iGusha(e); uNtaba ziyadilika; uDekane; uShelele; uShibilika	M; T; Z	NRN 313	Hr	W	le	Bd and Fd
Cucurbita argyrosperma C. Huber	Cucurbitaceae	isiPhama	M	NRN 507	Tl; Cl	C	fr	Bd and Fd
Cucurbita maxima Duch.	Cucurbitaceae	uMpampini; uZulu; uGubungu	M; T; Z	NRN 508	Tl; Cl	C	fl; fr; se; sh	Bd and Fd
Cucurbita pepo L.	Cucurbitaceae	iThanga; iPhuzi; iNhlwathi emnyama (emhlophe)	M; T; Z	NRN 353	Tl; Cl	C	fl; fr; se; sh	Bd and Fd
Deinbollia oblongifolia (E.Mey. ex Arn.) Radlk.	Sapindaceae	umGontsi	M	NRN 366	Tr	W	sh	Bd and Fd
Diospyros galpinii (Hiern) De Winter	Ebenaceae	amaBhontsi	M	NRN 374	Hr; Sr	W	fr, le	Bd, Fd and R
Dipcadi marlothii Engl.	Hyacinthaceae	isiKhwa	M; T; Z	NRN 476	Hr	W	fl; le	Bd and Fd
Dipcadi viride (L.) Moench	Hyacinthaceae	uNconti; uNcodi; uNgcomungcomu	Z	NRN 464	Hr	W	le	Bd and Fd
Erythroxylum delagoense Schinz	Erythroxylaceae	umBhontsi; amaBhontsi	M	NRN 371	Hr; Sr	W	le	Bd and Fd
Galinsoga ciliata (Raf.) S.F.Blake	Asteraceae	uMamsangweni; uGobuhlanya; isiShukelana; uMaMkhize; uMaMhlongo, uMasuku onoboya	M; T	NRN 478	Hr	W	sh	Bd and Fd
Galinsoga parviflora Cav.	Asteraceae	uMamsangweni; uGobuhlanya; isiShukelana; uMaMkhize; uMaMhlongo, uMasuku	M; T	NRN 398	Hr	W	sh	Bd and Fd
Hermbstaedtia odorata (Burch.) T. Cooke	Amaranthaceae	isiGamfumane	M	NRN 330	Hr	W	sh	Bd and Fd

APPENDIX III: The local name, locality, voucher number, growth habit, cultivation status, part(s) used and preparation mode of leafy vegetables of northern KwaZulu-Natal (Continued)

Species	Family	isiZulu and/or isiThonga name	Locality	Voucher no.	Growth habit	Cultivation status	Part(s) used	Preparation mode
Hypochaeris radicata L.	Asteraceae	isiHlalakuhle; umKopoloto	T	NRN 423	Hr	W	le	Bd and Fd
Ipomoea batatas (L.) Lam.	Convolvulaceae	uBhatata, uNtende, amaSchembu, amaTsimbu, amaThimbu	M; T; Z	NRN 358	Tl; Tw	C	le; tb	Bd and Fd
Ipomoea cf. *cairica* (L.) Sweet	Convolvulaceae	umBophamfe	Z	NRN 452	Tl; Tw	W	le	Bd and Fd
Ipomoea plebeia R. Br. subsp. *africana* A. Meeuse	Convolvulaceae	umBophamfe; umKhokha, isaNdla sonwabu, uNyawo lwenkukhu; iMbilikicane	T; Z	NRN 485	Tl; Tw	W	le	Bd and Fd
Ipomoea wightii (Wall.) Choisy	Convolvulaceae	iNcumbisane; iMvumbisa	M	NRN 393	Tl; Tw	W	le	Bd and Fd
Justicia flava (Vahl) Vahl	Acanthaceae	imbobela	Z	NRN 444	Hr	W	le	Bd and Fd
Lagenaria siceraria (Molina) Standl.	Cucurbitaceae	amaSwela	M; T; Z	NRN 376	Tl; Cl	C	fr; le	Bd and Fd
Limeum sulcatum (Klotzsch) Hutch.	Molluginaceae	isiHlalaka(u)hle; isiHlalakamnandi; isiGamfumane sesilisa	M; Z	NRN 329	Hr	W	le	Bd and Fd
Malvastrum caromandelianum (L.) Garcke	Malvaceae	uVemvane; uVemvane olunoboya	T	NRN 410	Hr	W	sh	Bd and Fd
Manihot esculenta Crantz	Euphorbiaceae	umDumbula; amaThapha; uKhulanaye	M	NRN 364	Sr	C	le; tb	Bd and Fd
Momordica balsamina L	Cucurbitaceae	umKaka; umKakane; iNkakha; iNtshungu	M; T; Z	NRN 307	Tl; Cl	W	fr; le	Bd and Fd
Momordica foetida Schumach.	Cucurbitaceae	iNtshungu	M; T; Z	NRN 491	Tl; Cl	W	le	Bd and Fd
Morus australis Poir.	Moraceae	umJikijolo; uJingijolo	M	NRN 377	Tr	C; RW	le	Bd and Fd
Nasturtium officinale R.Br.	Brassicaceae	uGelekula; uWata	Z	NRN 466	Hr	W	sh	Bd and Fd

APPENDIX III: The local name, locality, voucher number, growth habit, cultivation status, part(s) used and preparation mode of leafy vegetables of northern KwaZulu-Natal (Continued)

Species	Family	isiZulu and/or isiThonga name	Locality	Voucher no.	Growth habit	Cultivation status	Part(s) used	Preparation mode
Obetia tenax (N. E. Br.) Friis	Urticaceae	uZi; uBa(u)bazi; iMpongozembe	M; T; Z	NRN 490	Tr	W	le	Bd and Fd
Ophioglossum polyphyllum A. Braun	Ophioglossaceae	isi(a)Nkuntshane; isiNdletshane	M; Z	NRN 385	Hr	W	le	Bd and Fd
Passiflora incarnata Linn.	Passifloraceae	amaGranadilla	M	NRN 365	Tl; Cl	C; RW	fr, le	Bd; Fd and R
Pergularia daemia (Forssk.) Chiov.	Apocynaceae	uNquntane	M	NRN 304	Tl; Tw	W	fr; le	Bd and Fd
Priva meyeri Jaub. and Spach var. *meyeri*	Verbenaceae	iNamathela	T	NRN 416	Hr	W	le	Bd and Fd
Pyrenacantha scandens Planch. ex Harv.	Icacinaceae	uM(a)khokhothwane	M	NRN 302	Tl; Tw	W	le	Bd and Fd
Riocreuxia torulosa Decne.	Apocynaceae	uFuthane; uMfuthane; isiFuthane	M; Z	NRN 392	Tl; Tw	W	le	Bd and Fd
Senecio madagascariensis Poir.	Asteraceae	umThithimbili	T	NRN 486	Hr	W	sh	Bd and Fd
Sisymbrium officinale (L.) Scop.	Brassicaceae	imiFino yamaNdiya	T	NRN 320	Hr	SD	le	Bd and Fd
Sisymbrium thellungii O.E.Schulz	Brassicaceae	isiHlalaku(a)hle	T; Z	NRN 488	Hr, Sr	W	le	Bd and Fd
Solanum americanum Mill.	Solanaceae	umSobo	T	NRN 321	Hr	W	le	Bd and Fd
Solanum retroflexum Dunal	Solanaceae	umSobo; umSobobo	M; T; Z	NRN 314	Hr	W	le	Bd and Fd
Sonchus oleraceus L.	Asteraceae	iGabegabe; i(isi)Klabeklabe; iHlabe; iKlabi; iHogo(gwe)	M; T; Z	NRN 401	Hr	W	le	Bd and Fd

APPENDIX III: The local name, locality, voucher number, growth habit, cultivation status, part(s) used and preparation mode of leafy vegetables of northern KwaZulu-Natal (Continued)

Species	Family	isiZulu and/or isiThonga name	Locality	Voucher no.	Growth habit	Cultivation status	Part(s) used	Preparation mode
Tetragonia tetragonioides (Pall.) Kuntze	Aizoaceae	isiPinashi semvelo (esenabayo, sentaba, sehlathi); amaZambanyana; isiBhalamangongo	M; T; Z	NRN 332	Hr	W	le	Bd and Fd
Trachyandra asperata Kunth. var. *asperata*	Asphodelaceae	uNjwati	M	NRN 391	Bl	W	fl; le	Bd and Fd
Trachyandra cf. *saltii* (Baker) Oberm. var. *saltii*	Asphodelaceae	uDoda; uNjeza	T; Z	NRN 421	Bl	W	le	Bd and Fd
Urtica urens L.	Urticaceae	iMbati; iMbati yomhlanga	M; T; Z	NRN 498	Hr	W	le	Bd and Fd
Vigna subterranea (L.) Verdc.	Fabaceae	iziNdlubu	T	NRN 509	Hr	C	se	Bd
Vigna unguiculata (L.) Walpers	Fabaceae	iMbumba(e); iMbumba esheshayo (ephuzayo); iNyangani	M; T; Z	NRN 355	Tl; Tw	C	le; se	Bd and Fd
Zantedeschia aethiopica (L.) Spreng.	Araceae	iNtebe	M; T; Z	NRN 493	Cr	W	le	Bd and Fd

Locality: M, Umkhanyakude district; T, uThungulu district; Z, Zululand district. Growth habit: Bl, bulb; Cl, climber; Cr, corm; Hr, herb; Sr, shrub; Tl, trailer; Tr, tree. Cultivation status: C, cultivated; RW, reverted to a wild state; SD, semi-domesticated; W, wild. Part(s) used: cr, corms; fl, flowers; fr, fruits; le, leaves; se, seeds; sh, shoots; tb, tuber. Preparation mode: Bd, boiled; Fd, fried; R, raw.