Food security potential of bottle gourd [*Lagenaria siceraria* (Molina Standly)] landraces: an agronomic perspective

by

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PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Crop Science, School of Agricultural, Earth and Environmental Sciences, of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa. The research was financially supported by the South African Department of Agriculture, Forestry and Fisheries (DAFF) Zero Hunger Project.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

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Signed: Professor Albert T. Modi

Date: 30 June, 2014

DECLARATION

I, Nkanyiso Justice Sithole, declare that:

(i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii) this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

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a) their words have been re-written but the general information attributed to them has been referenced;

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Signed: Nkanyiso J Sithole

Date: 30 June 2014

ABSTRACT

Issues surrounding food security of rural households in sub-Saharan Africa have become topical in the recent years due to low food production and poverty combined with increasing population which often leads to malnutrition. The marginal nature of agricultural land in these areas, combined with the predicted effects of climate change, challenges the existence of major crops and their potential to ensure food security in future. This has led to renewed efforts to re-instate neglected underutilised species (NUS) such as bottle gourd, because of their likely adaptability to marginal areas of agricultural production. The objective of the study was to evaluate the potential of bottle gourd [*Lagenaria siceraria* (Molina Standly)] as a future food security crop, focussing on the agronomic perspective. Four separate experiments on seed quality, controlled environment determination of water stress, field trials to determine yield and laboratory determination of nutritional value were conducted. Bottle gourd landraces were compared with two commercial pumpkin cultivars and one cucumber in an effort to benchmark the crop with popular related conventional crops. Seeds of landraces were collected from farmers' fields and those of commercial varieties were sourced from a local seed company. Results of seed quality showed variability with respect to viability and vigour. Despite this variability, it was found that seed quality of landraces was comparable to that of commercial hybrids. Under controlled environment conditions, for all water treatments, stomatal conductance (SC) was observed to be significantly ($P < 0.05$) lower in landraces than commercial varieties. This led to the conclusion that landraces demonstrated a characteristic of potentially efficient water use, which might be associated with drought tolerance. Under field conditions, the yield of all varieties was found to be significantly ($P < 0.05$) higher during summer than winter season. Landraces had higher ($P<0.05$) yield than hybrid varieties in summer. The results of nutritional analyses revealed that bottle gourd was well endowed with most of the nutrients required for good health. Hybrid varieties contained more ($P < 0.05$) nutrients than landraces. Although landraces were found to have lower levels of nutrients than hybrids, they were found to contribute significantly higher percentages to Daily Recommended Allowances (RDA). Sequential harvesting showed that the best time to harvest leaves was before the onset of flowering. The study concluded that although bottle gourd landraces were often inferior to hybrids, they remain an important germplasm resource with potential to contribute to future food security in marginal production areas of South Africa.

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DEDICATION

This thesis is dedicated to my son Mali Sithole and the late Sambulo Sithole. God loves His kids and even though I may not be with you physically, I am with you every moment of my life.

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CHAPTER 1

INTRODUCTION

A recent review by Oelofse and van Averbeke (2012) on the nutritional status of rural South Africans indicated that under – and over–nutrition co-existed within the same communities and often in the same household. Increased production and intake of fruits and leafy vegetables was identified as one of the many potential solutions for addressing poor food security and nutrition imbalances in rural communities (Chweya and Eyzaguirre 1999; Schippers 2002; Laker, 2007). In contrast to urban and semi-urban communities, people in rural areas have access to land which they can use to cultivate crops, thus contributing to their food security. However, this is usually hampered by a lack of adequate resources in these areas. Information, expertise and low capital to buy agricultural inputs such as seeds, fertilizers, herbicides and insecticides as well as infrastructure needed to produce these crops is often limiting in these areas. Under these circumstances, it has been suggested that traditional crop species can play a vital role in ensuring food security under such low input systems because of their likely suitability to these areas (Nieuwoudt and Groenewald, 2003, Modi *et al*., 2006; Schonfeldt and Pretorius, 2011; Odhav *et al*., 2007). However, most traditional crops remain underutilized despite reports that they may be better suited to low input systems (Mabhaudhi *et al*., 2013). This could be due to lack of clear policy instruments encouraging cultivation of these crops, lack of research interest from agricultural scientists and low yields due to poor agronomic practices. Therefore, to promote the use of traditional crop species, there is a need to conduct research that will contribute to the documentation of optimum agronomic practices. This would contribute significantly to food security through increasing productivity of these crops, thus promoting balanced diets. Bottle gourd (*Lagenaria siceraria* (Molina) Standley) is a good example of an underutilized traditional crop.

Bottle gourd is a member of the Cucurbitaceae family together with pumpkins and water melons (Decker-Walters, 2004). It is believed to have originated in Africa and it exhibits a great diversity in nature. This alone indicates wide environmental adaptation (Chimonyo and Modi, 2013). The leaves of the crop are consumed the same way as those of pumpkins, water melons and other

popular cucurbits. They are usually consumed as a relish with maize staple. The seeds of the crop, on the other hand, are popular snacks in Africa and are reported to contain high levels of proteins as is the case with the seeds of its closest relative pumpkins (van Wyk and Gericke, 2001). The mature fruit can be used as a container to store water, food and as a musical instrument (Milind and Satbir, 2011). In addition Asia, bottle gourd is used as a rootstock in winter production of water melons and squashes to prevent root-borne pathogens such as *Fusarium oxysporum* (Han *et al*., 2004). A lot has also been documented about its medicinal properties, especially in countries like India and Pakistan. Bottle gourd has been reported to contain high levels of choline which is a compound that is reported to heal mental disorders (Rahman, 2003). In India, it has been reported to cure stomach complications (Milind and Satbir, 2011).

Given all these benefits, it is important to note that the potential of bottle gourd landraces as a possible food security crop has been overlooked by many researchers. These landraces have been preserved by the communities who have been utilizing them for over 100 years and form a possible germplasm resource. They may have adapted to ecological niches from which they have been preserved. This makes them an important food security crop for cultivation in marginal areas of crop production. However, owing to the popularity of exotic members of the Cucurbitaceae family (pumpkins, water melon, butternut and squashes), the popularity and cultivation of bottle gourd landraces has faced neglect. There is a need to conduct research that will contribute to the documentation of the agronomy of bottle gourd landraces and their potential to contribute to food security in marginal areas. The aim of this study was to establish the agronomic potential of *Lagenaria siceraria* landraces and to evaluate their potential contribution to food security through assessing nutritional content in their leaves.

1.1 Specific objectives of the study:

- To determine the seed quality of bottle gourd landraces compared with two conventional cucurbits (*Cucurbita maxima* and *Cucurbita pepo*).
- To determine the effect of water stress on growth, development, physiology and yield of bottle gourd landraces compared with two conventional cucurbits (*Cucurbita maxima* and *Cucurbita pepo*) under controlled environment conditions.
- To determine changes in nutritional content of bottle gourd landraces compared with two species of *Cucurbita maxima* in response to sequential harvesting of leaves.
- To compare winter and summer planting of bottle gourd landraces in comparison with commercial hybrids of other cucurbits for yield determination.

CHAPTER 2

LITERATURE REVIEW

2.1 Crop History and Classification

Bottle gourd is one of man's first domesticated crops (Decker-Walters *et al*., 2004). It is believed to have originated in Africa with tropical and sub-tropical distribution (Yetisir *et al.,* 2008; Decker-Walters *et al*., 2004). However, the centre of origin of the crop has long been a point of academic debate with various scientists suggesting different centres of origins. Milind and Satbir (2011) argued that bottle gourd originated in India because its wild traces are still found in Dehradoon (high humid area) and Malabar coastal area. Molecular analysis by Decker-Walters *et al.* (2001) suggested that the crop dispersed from Africa to Asia and the Americas during pre-Colombian times followed by independent domestication in all these continents. It is believed that the crop reached Asia and the Americas about 9 000 years ago, probably as a wild species whose fruits had floated across the sea and ocean (Decker-Walters *et al*., 2004). This hypothesis was tested by Whitaker and Carter (1954) who demonstrated that bottle gourd fruit still contained viable seeds even after floating in the sea for more than seven months. Bottle gourd remains were also found in Egyptian tombs dating back to about 3 000 – 3 500 BC, Thailand 10 000 – 6 000 BC, in Mexico 7 000 – 5 000 BC, Peru 4 000 –3 000 BC and in China 500 AD (Yetisir *et al.,* 2008). Archaeological evidence suggests that people have been using the crop for at least 12 000 years in both old and new worlds (Yetisir *et al.,* 2008).

Lagenaria siceraria belongs to the *Cucubitaceae* family (Meeuse, 1962; Warrier *et al.,* 1995; Milind and Satbir, 2011) (Table 2.1). This family consists of about 118 genera and 825 species that are distributed along the warmer regions of the world (Milind and Satbir 2011). It is commonly referred to as calabash, bottle gourd, white flowered gourd plant and moli (English), iselwa (Zulu), moraka (Sesotho), iselwa (Xhosa) and segwana (Tswana). Bottle gourd belongs to the genus *Lagenaria*, which is derived from the word 'lagena' meaning 'the bottle' (Milind and Satbir, 2011). The genus *Lagenaria* also contains five wild species: *Lagenaria brevifilora* (Benth.) Roberty, *Lagenaria abyssinica* (Hook. f.) C. Jeffrey, *Lagenaria rufa* (Gilg) C. Jeffrey, *Lagenaria sphaerica* E. Mey and *Lagenaria guineensis* (G. Don) C. Jeffrey (Chimonyo and Modi, 2013).

Table 2.1: Botanical classification of bottle gourd based on Milind and Satbir (2011).

Kingdom	**Plantae**
Division	Magnoliophyta
Class	Magnoliospida
Order	Cucurbitales
Family	*Cucurbitaceae*
Genus	*Lagenaria*
Species	*siceraria*
Scientific name	*Lagenaria siceraria*

Bottle gourd fruit are found all over the world having different fruit size, shape and length. This variation in fruits indicates great genetic diversity within and between countries as well as across continents. Bottle gourd landraces also show great diversity compared to their wild relatives which are found in the same region.

2.2 Botany

The crop is an annual herbaceous, monoecious (male and female flower occur on the same plant) and it is insect pollinated. It exhibit tendril growth habit (Fig. 2.1). The leaves are kidney shaped (heart shape or bean shape), alternate and variable in size. Depending on the cultivars, the flowers open late in the afternoon to early hours of the night (Teppner, 2004). Bottle gourd can be pollinated by many insects. Morimoto *et al.* (2004), in Kenya, observed hawk moth, moths A-D and skipper butterflies to be active flower visitors during pollination. In America, hummingbirds were observed to be attracted by nectar which is only produced by male flowers during pollination (Morimoto *et al*., 2003). Heiser (1997) observed that bottle gourd flowers were pollinated mainly by hawk moths at night, but during the day cucumber beetles, mumble bees and other insects were active. Since *Lagenaria siceraria* is monoecius, cross pollination is highly

favourable to the plant. The ratio of male: female ratio is high like in the case of other members of the *Cucurbitaceae* family (Sivarai and Pandravada, 2005). This has serious implication on decreasing yields thus other methods to overcome the problem has to be used. Plant growth regulators and environmental manipulation for instance has been used to increase yields in the crop (Desai *et al*., 2011).

The seeds of bottle gourd are flat, more or less rectangular to narrow trapezial, whitish to dark brown at the distal end. They develop inside the fruit and show great diversity in seed shape and seed size. Bottle gourd fruit vary greatly in size and shape (Fig. 2.1). Elongate bottle gourds and related cultivars that are nearly cylindrical in shape can reach a length of up to 1-2 m in length, Teppner (2004) and round fruits on the other hand can have a diameter of up to 50 cm.

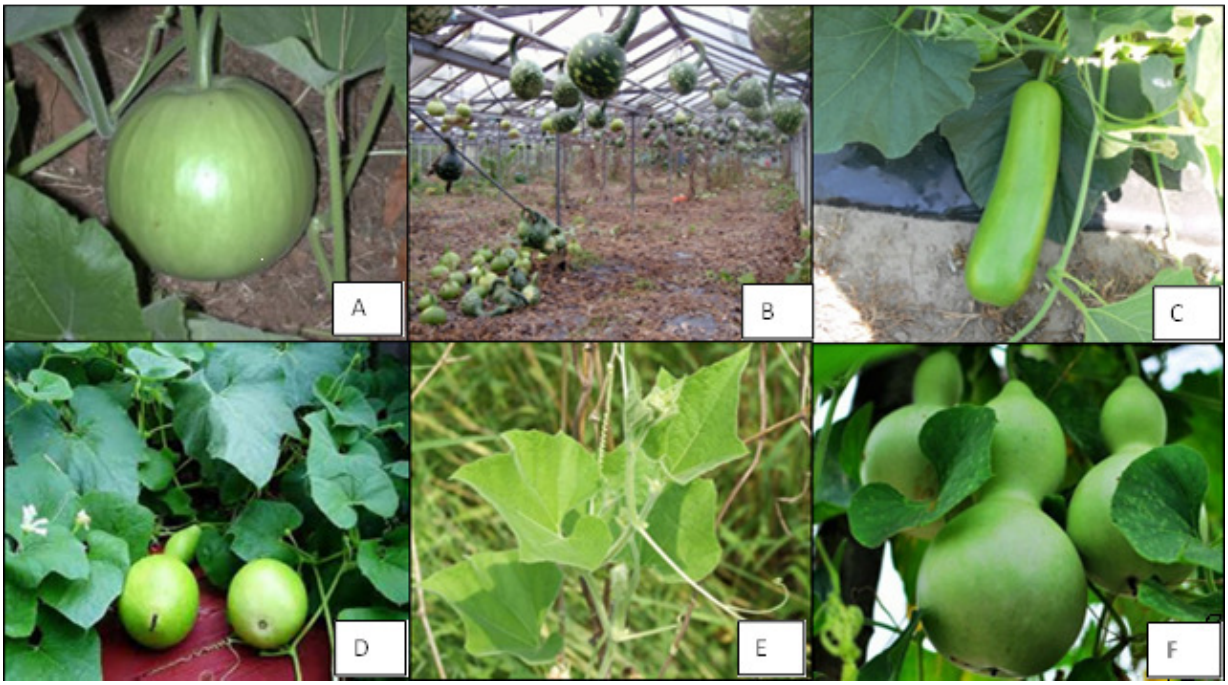

Figure 2.1: Morphology of different bottle gourd plants. A - Cylindrical fruit shape, B & E– calabash fruit shape, white flowers and lobed kidney shaped leaves, C – cucumber fruit shape, long vines and kidney shape leaves, D – tendrils and bean shape leaves and F – bottle shape calabash. Source: Teppner (2004).

2.3 Bottle gourd ecology

Bottle gourd is found distributed along the tropical regions of the African continent (Decker-Walters *et al.,* 2004). It has been observed to grow well in temperate, tropical and sub-tropical climates as well as low lying areas of arid to semi-arid climates (Grubben and Dento, 2004). Bottle gourd can grow well over a wide range of soils although sandy loamy soils with good drainage and a pH near 6.5 are desirable (Milind and Satbir, 2011). The crop has been observed to grow best in areas with annual rainfall ranging from 400 to 1 500 mm per year (Haque *et al.,* 2009). However, the crop is not tolerant to water logging. It grows well under warm temperatures of between 25°C - 35°C (Grubben and Denton, 2004). According to Chimonyo and Modi (2013) the optimum germination temperature is between 20°C and 25°C. Temperatures above 35°C and below 15°C have been observed to decrease germination rate (Chimonyo and Modi 2013). Bottle gourd flowering is highly sensitive to photoperiodism. High temperatures and long day lengths result in more female flowers than male flowers while short days coupled with low night

temperatures promote the production of more male than female flowers (Chimonyo and Modi, 2013).

2.4 Uses of the crop and its Potential as a Food Security Crop

2.4.1 Economic and medical uses

Commercially, about 30 years ago, the crop was planted for the pipe making industry in South Africa (Welman, 2005). Walman (2005) further reported that the neck of the fruits were bent in such a way that they grow to form bowls of pipes and when the fruits were dry and ripe, the necks sawn off, cleaned and exported to pipe markets overseas. In Malaysia rural farmers have been reported to increase their income due to planting and selling bottle gourd leaves and fruits (Awasthi, 2012). A lot has been reported on medical properties of the crop.

Bottle gourd has been shown to contain triterepenoide cucurbitanics B, D, G, and H, two sterols (fucosterol and campesterol), aerpene, byonolic acid, flavone-C glycosides and lagenin (Milind and Satbir, 2011). The extract from the seed was found to contain antibiotic properties and the fruit juice is helpful in constipation, premature greying hair, urinary disorder and insomnia. To date, the crop has been found to contain high levels of choline (Rahman, 2003 and Milind and Satbir, 2011). Choline serves as a precursor of the neurotransmitter acetylecholine which in turn is important for retaining and enhancing memory. Milind and Satbir, (2011) further reported that bottle gourd juice helped to regulate blood pressure in hypertensive patients because of its high potassium content, reducing weight quickly because of its high diet fibre and low fat and cholesterol content.

Furthermore, the crop has been reported to lower blood cholesterol. Ghule *et al.* (2006) explored the antihyperlipidermic effects (anti-lipids effect) of four different extracts: chloroform, petroleum ether, alcoholic and aqueous extract from bottle gourd. They found that both chloroform and alcoholic extract had a significant effect on lowering total cholesterol, triglycerides and low density lipoprotein along with increase in high density lipoprotein as compared to others. Their results also suggested a marked antihyperlipidemic and hypolipidemic of the extract.

2.4.2 Bottle gourd as a possible food security crop

In most parts of the world and in South Africa, bottle gourd is grown mainly as a vegetable for human consumption. Leaves of the crop are consumed the same way as those of popular cucurbits (water melon, pumpkin and squashes) and other popular leafy vegetables (spinach, *amaranthus spp*, spider flower, chines cabbage, etc.). The young fruit of the crop is a popular vegetable in many part of the world (Prasad and Prasad, 1979). According to Chimonyo and Modi (2013) the leaves of the crop can also be added fresh and mixed with maize porridge in southern Africa and they can also be dried and stored for later use in the off season. The seeds of the crop have been reported to contain high levels of oil that is comparable to those of sunflower and grape oil (Axtell and Fairman, 1992). Loukou *et al.* (2011) reported that bottle gourd was rich in protein, oil and energy. Apart from these nutritional uses, bottle gourd has been used for decades in Asia as a root stock for water melon to promote the root system under stressful conditions of water deficit and salinity (Park *et al.,* 2012), low temperature (Yetisir *et al*., 2008) as well as root borne pathogens (Han *et al.,* 2004). In South Africa and the neighbouring countries (Botswana and Zimbabwe), the oil is extracted from the seed and used as an alternative for vegetable oil (Grubben and Denton, 2004). According to Chimonyo and Modi (2013), the defatted cake can be used as a protein supplement in rural communities. *Lagenaria siceraria* seeds have been reported to contain about 45% oil and 35% proteins. Loukou *et al.* (2011) argued that the potential for bottle gourd as a food security crop lay on the use of its seed kernel in food and livestock industry. Given such benefits of the crop, it is a wonder that in South Africa the benefits of the crop have not yet been fully exploited.

The country is still faced with the problem of malnutrition. In recent years, obesity has increasingly become problematic in both rural and urban communities. High incident of stunted, underweight children, and increasing infant mortality due to marasmus and Kwashiorkor have been reported (Chimonyo and Modi, 2013). The use of bottle gourd seeds or defatted cake could help in mitigating protein deficiencies in rural communities. Oil extracted from bottle gourd is reported to be rich in sterolic compounds and fatty acids (Axtell and Fairman, 1992). Thus, the use of bottle gourd seeds could contribute significantly in providing much needed amino acids in the diets of vulnerable communities. Nutritionists have argued that inclusion of leafy vegetables in diets could increase dietary diversity, nutrient availability and absorption contributing to the reduction of malnutrition (Maunder and Meake, 2007). Milind and Satbir (2011) reported the

crop to form excellent diet that is rich in iron vitamins and minerals. In addition, the seeds and fruit of the crop can also be used to supplement livestock feeds in rural communities where grazing land is also becoming a problem. Observations in Zimbabwe have shown that leaves, seeds and fruits are being used to supplement livestock feeds (Chikwanha, 2006).

Chimonyo and Modi (2013) added that bottle gourd also contained sodium, potassium, essential elements and trace minerals. They concluded that the crop could be useful to hypertensive patients since it contained high levels of potassium and sodium. Global population and that of South Africa, continues to increase rapidly. Statistics South Africa (2013) recently reported that the South African population had increased from 45 million in 2001 to 52.98 million in 2013. This necessitates the production of more food to meet the growing demand of population. To achieve this, it is necessary for the country to look at the diversity that exists in traditional crops. Another problem that the country and the world face is that of climate change. It is important therefore to also look for diverse crops that can withstand high temperatures and possible outbreak of diseases in order to ensure food security, especially in the marginal areas of crop production. This makes bottle gourd attractive for a range of uses.

Table 2.2: Nutritional content of whole *Cucurbitaceae* seeds, total protein and phenolic compounds (Achu *et al*., 2005)

Sample	Moisture content (g/100 g FM)	Protein content (g/100 g DM)	Lipid content (g/100 g DM)	Ash content (g/100 g DM)	Crude fibre content (g/100 g DM)	Carbohydrate content (g/100 g DM)	Total protein (%)	True Protein (%)	Phenolic compound (%)
Lagenaria siceraria	6.09	34.19	50.08	3.68	4.08	8.01	68.52	25.89	0.34
Cucumis sativus	5.65	28.68	50.08	3.68	4.04	10.01	61.91	8.15	0.34
Cucumero mannii	6.49	40.49	44.85	3.74	3.81	7.11	73.59	35.95	0.39
Cucurbita Maxima	6.94	34.93	49.05	3.95	3.44	8.62	68.72	39.53	0.42
Cucurbita moschata	5.65	28.68	53.76	3.47	4.14	10.01	68.52	25.06	0.43

2.5 Agronomy of bottle gourd

2.5.1 Seed germination and establishment

Germination begins with water uptake by the seed (imbibition) and ends with the start of elongation by the embryonic axis, usually radicle (Bewley and Black, 1986). Knowledge of environmental requirements for germination is important because it enables us to know when exactly to plant. It also enables us to assess the suitability of climatic conditions for crop growth particularly with regards to suitable and optimum planting dates, which largely depends on local temperature regimes and the growing period of crop (Motsa *et al.,* 2012). It is also important for scheduling sequential planting in order to match supply with demand; this has implications on the monetary value of a crop (Wang, 2005). Germination in non-dormant seeds is controlled by a number of factors such as temperature, light, water, oxygen content and the type of seed (Ghaderi *et al.,* 2008). In the presence of adequate soil water and absence of water logged conditions, temperature, light and dormancy are the three factors that affect germination (Bewley and Black, 1986).

The rate at which germination occurs usually increases, linearly, within a well-defined temperature range and then declines sharply at higher temperatures above the optimum (Motsa *et al.,* 2012). The enzymes become denatured at high temperatures of about 40°C because of structural damage to the proteins. Different crops have well–defined differences in optimum germination temperatures. Seed dormancy is one of the major factors which inhibit germination in many seeds. Dormancy can be defined as the failure of seed to germinate under optimal conditions favouring germination (Bradford and Nonogaki, 2007). According to Thomson (2005), dormancy is an important survival mechanism for plants which allows time for dispersal and prevents germination of all seeds at the same time when conditions appear favourable. Dormancy can be caused by several factors that have been grouped into physical and chemical imposed dormancy. Physical dormancy occurs when hard seed with impermeable seed coat prevents water and gases from entering the seed. Furthermore, an impermeable hard seed coat acts as a barrier by preventing embryo expansion or radical growth (Materechera and Matterechera, 2001). In addition, immature embryos, light and temperature requirements as well as germination inhibitors are other factors causing dormancy (Finch-Savage and Leubner-Metzger, 2006). Physiological dormancy in seed may be related to the proportion between

inhibitors (especially abscisic acid) and growth regulators (gibberellins) (Fenner and Thomson, 2005).

Information describing germination requirements of bottle gourd landraces in South Africa has not been well–documented. Recently Chimonyo and Modi (2013) evaluated seed performance of selected bottle gourd landraces. They found that most of the traits evaluated were not indicators of good quality seeds and it was concluded that morphology could be a useful trait for selection of planting material in the context of seed germination as a trait. Although it is well–documented in other countries like India where its production is high, local conditions (South Africa) are not similar to Indian conditions. As such, it is important to generate local information describing the germination characteristics of locally cultivated bottle gourd landraces. However, *Cucurbita maxima* (pumpkin) landraces that have been recently studied can serve as a guide because they belong to the same family as bottle gourd. In an experiment conducted by Jansen van Rensburg *et al.* (2007), results showed that the minimum temperature for germination in *Cucurbita maxima* was 16°C. Time to 50% germination was shortest at 28°C to 36°C. Maximum temperature for germination was 40°C. Kurtar (2010) observed minimum and maximum temperature for germination of cucurbits to be 15°C and 45°C respectively, with large differences amongst cultivars, while the reported optimum germination temperatures ranged from 20°C to 32°C. In addition, seeds of *Cucurbita maxima* appeared not to possess any dormancy mechanism when subjected to pre-chilling, potassium nitrate KNO_3 treatments and scarification because there was no difference between them and the control.

2.5.2 Planting date

Based on the preceding argument on seed germination and ecology of bottle gourd, it is clear that it is a summer crop which requires relatively high temperatures (20 – 30°C) in order to germinate and establish well under field conditions. In the absence of specific information on bottle gourd, information on convention cucurbits that have been well–studied could be used as a starting point. According to Latifi *et al.* (2012), the planting of cucurbits can commence after the danger of frost occurrence because they are highly sensitive to frost. This can be early to late spring. This period coincides with the start of the rain in South Africa which is also crucial for crop growth and development.

2.5.3 Plant nutrient requirements

Plant nutrients and water are key growth factors. Crop growth and yield increase as the rate of fertilizer application increases until an optimum point at which growth and yield are maximum (van Averbeke *et al.,* 2012). Beyond this optimum point, typically the crop will not respond to any additional fertilizer application following which crop growth and yield may decline due to toxicity. In rural areas, low nutrient content in soils is one of the most limiting factors in crop production. The positive interaction effects between water and nutrients are well known as a result to obtain high yields; both nutrient and the water requirements must be known (Tisdale *et al.,* 1985). The most important nutrients in plants are nitrogen, phosphorus and potassium. These nutrients are required in larger amounts by the plants (macro nutrients) particularly by the species that are grown for their leaves. However, crops differ in the in the way they respond to the availability of different nutrients in soil because they vary in terms of their distribution and density of root system (van Averbeke *et al.,* 2012).

All species of *Cucurbitaceae* family respond well when organic and inorganic fertilizer is applied. The dose of fertilizer depends on soil type, climatic condition and systems of cultivation (Teppner, 2004). Due to these variation and the lack of literature on application rate of fertilizer on different soil types and climatic conditions it is difficult to give specific recommendations on the amount of fertilizer to apply per hectare in order to obtain maximize yield and reduce cost associated with production of bottle gourd landraces. However, the Institute of Vegetable Research of India Council of Agricultural Research recommended applying fertilizer at a rate of 50-100 kg N, 40-60 kg P_2O_5, 30-60 kg K_2O /ha in cucurbits. However this can vary from soil to soil and can be affected by climate conditions.

2.5.4 Production

Depending on weather conditions, seed can be sawn directly or it may be raised in the nursery and then transplanted. In the case of transplanting, seeds can be sown in poly pots filled with mixture of soil and compost manure and transplanted at four to five leaf stages. With some variation, seedlings are transplanted with inter-row spacing of 1.5 to 2 m, and intra-row spacing of 1 to 2 m (Millind and Satbir, 2011). Vines are allowed to trail on the ground or allowed stacking. In Asia local landraces were observed to produce less than 25 tones/ha while hybrid

varieties were on the other hand observed to produce yield of more than 40tones/ha under optimum conditions (Heque *et al.* 2009). Yield of 35 t/ha in subtropical and tropical areas of Bangladesh were recorded while in semi-arid to arid regions, yield of less than 20 t/ha was recorded.

2.5.5 Pest and diseases

Diseases in bottle gourds are very common. Despite that it has been used in many countries for years as a rootstock for watermelon because of its resistance to some root diseases and low soil temperature. Grafting of watermelon onto bottle gourd root stocks was first performed in Japan in the late 1920s (Ashita, 1927) and showed high compatibility rate with watermelon (Lee, 1994; Oda, 1995; Yetisir, 2003). In Japan for example, they used this technique to control Fusarium wilt in watermelon caused by *Fusarium oxysporum* because root stocks were immune to causal fungus (Kuniyasu, 1980). However, recently *Fusarium oxysporum* has been reported by Cumagun *et al.,* (2010) to be pathogenic and aggressive on 7-day and 1-month old seedlings raised in the nursery. The results of this study also showed that isolates from infested soil were non-pathogenic to bottle gourd as compared to sweet gourd. Furthermore, the crop has been reported to be very susceptible to different types of diseases when grown in the wrong season (Van Wyl and Gericke, 2000). Powdery mildew was reported by Van Wyl and Gericke (2000) to be prevalent under humid conditions and can quickly spread to all seedlings in the nursery when not prevented. The control of this disease can be achieved by spraying with copper oxychloride (Van Wyl and Gericke, 2000).

In India several viruses like Cucumber mosaic virus, Chlorotic curly stunt, Cucumber green mottle mosaic virus, Papaya ring sport virus, Water melon mosaic virus and Zuccchini yellow mosaic virus are known to affect bottle gourd cultivation according to Sohrab *et al.* (2010). In China recently the crop has been confirmed as a new host of *Ralstonia solanacearum* (Gao *et al.* 2007). The symptom for the later appears 1 week after transplanting. According to Gao *et al.* (2007), initially the upper leaves of affected plants become wilted then after 3-5 days later almost all leaves of the diseased plant become wilted. Other symptoms are yellowing of the leaves extending to stem and then death 7-14 days after the first appearance of wilt. For Chlorotic curly stunt disease the affected plants become severely stunted and bear very small chlorotic and

mildly curled leaves (Sohrab *et al.,* 2010). The disease for chlorotic curly stunt can be easily transmitted by whitefly, *Bemisiatabaci* but not by sap.

Furthermore, seeds of bottle gourd have been reported to be affected by diseases at germination. For example, *Lasiodiplodia theobromae* has been reported to reduce seed germination (up to 40%) in various cucurbites (Sohi and Maholay, 1974). Some research has been done to find control methods for reducing seed and seedling infection of the crop. For example, (Sultana and Ghaffar, 2010) investigated the effect of fungicides and microbial antagonists in the control of *lasiodiplodia theobromae,* the cause of seed rot, seedling and root infection of bottle gourd. The experiment was conducted in vitro and in vivo. The overall results showed that the most effective seed treatment was Benlate, Topsin-M, Carbendazin and Aliette at 3 g/kg seeds which enhanced seed germination and reduced seed infection in bottle gourd. On the other hand, *Trichoderma harzianum, T. viride, Gliocladium virens, Stachybotrysatra* and *Bacillus subtilis* showed better results in the control of pre- and post-emergence infection of *Lasiodiplodia theobromae* in seedling of bottle gourd in vitro and in vivo. *G. virens* has been found most effective to reduce seed and root infection in vivo whereas *B. subtilis* performed best to reduce seed and seedling infection of bottle gourd in vitro.

2.5.6 Yield potential

The fruits can be harvested when they are still young and mixed with leaves for food or they can also be harvested when they are mature after four months. Yield can vary greatly with season, variety, soil, water and nutrient management practices. Fruits are usually 40-45 cm long and picked in about two months. Yields are variable and usually range from 25-27.5 t/ha (Haque *et al.*, 2009). It can be highly influenced by the planting dates which are related to photoperiodism as discussed above that short days promote the development of more male flowers than female which in turn reduces fruits development in winter and more fruits in summer.

2.5.7 Genotype by environment interaction

Genotype × environment (G × E) interaction has been widely studied by many researches (Crossa *et al.,* 1990). It is defined as the failure of genotypes to achieve the relative performance in

different environments (Becker, 1988). Inconsistent genotypic responses to environmental factors such as temperature, soil type and rainfall and fertility level from location to location are as a result of Genotype × Environment interaction (Kang, 1988). Good estimation of G × E and Genotype × Year interaction are good in evaluating the efficiency of testing program and optimum allocations and year (Muir *et al*., 1992). Existence of G × E interactions and their effects on selection process are widely recognized (Delacy *et al.,* 1990).

Selection of right material for planting is important for improved seed yield and quality. The decision of selection is one of the important that farmers must make before the planting season. Variety selection is a foundation of effective and successful management plan. Although it is difficult to predict the weather during the growing season, selection of right material can minimize risk associated with environmental conditions (Patel and Hall, 1990).

There are different types of varieties of *Lagenaria siceraria* that are available for use in South Africa. Selection of right variety ensures that maximum production potential yield of the crop is achieved that is determined genetically. This maximum production yield potential is achieved when management and environmental conditions are complementary (Ehlers and Hall, 1996). The performance of a variety may vary from year to year even on the same field indicating that the environment as a factor has an effect on the yield of the crop. In addition, when the different varieties are tested in the fields of different climatic condition over years, it is possible to find varieties that are adapted to specific climatic conditions.

2.6 Crop responses to water stress

Most of the areas in the rural areas of South Africa or former homelands are arid or semi-arid. This necessitates studies of crop response to water stress if the objective is to promote the crops in these areas. Water stress or drought is one of the important environmental factors limiting plant growth and development. Drought can be defined as an extended period (months or years) where a region experiences a deficiency in water supply whether surface or underground water (Oval Myers *et al*., 1986). South Africa is a semi-arid country hence crop production is water limited. Water deficit in crops causes a reduction in crop productivity and in turn causing

economic losses (Fuglie, 2007; Hyman *et al.,* 2008). Unlike animals which they can move if the environmental conditions are not conducive, plants they cannot move if they are under stressful conditions instead they develop certain physiological response in reaction to stressful conditions. It has been reported that water stress affect plant growth by altering metabolism and gene expression (Ludlow, 1993). Drought inhibits cell elongation, reduces photosynthesis, reduce nutrient uptake and alters plant hormone level (Pennypacker *et al.* 1990), because water is a fundamental constituent in maintenance of normal physiological process and membrane transport activities in plants (Slabbert *et al.,* 2012). Furthermore, research studies done in many plants indicate that plants develop mechanisms to survive and grow under conditions of extreme low and frequently changing water supply and extreme heat. According to Monneveux and Belhassen (1996) plant response to abiotic stress includes various mechanism of escape, tolerance or avoidance.

2.7 Proline accumulation

The increase in proline level in the tissues during drought stress is unique compared to other free amino acids in plants although it is similar to other low molecular weight solutes such as sugars and organic acids (Ain-Lhout *et al.* 2001). Proline is the amino acid that is found in many proteins especially in collagen. This compound (proline) was firstly observed by Kemble and MacPherson in 1954 and since then it has been widely used as an indicator of plant response to environmental stress (especially water stress) (Yancey *et al*., 1982). This highly soluble amino acid molecule is accumulated in apical meristems and leaves (Boggess *et al.,* 1976 and Jones *et al.,* 1980) in root apical region growing at low water potential (Voetberg and Sharp, 1991) and in suspension cultured plant cells adapted to water stress (Handa *et al.,* 1986; Rhodes *et al.,* 1986). Studies done suggest that proline may act as osmotic solute and protect protein structure and membrane from damage and to reduce enzymes from denaturation (Ain-Lhout *et al.* 2001). Ain-Lhout *et al.* (2001) further suggested that proline accumulation might serve as a nitrogen storage mechanism in plants during stressful conditions.

During stressful conditions where water is a deficit, plants tend to close their stomata to avoid water loss by transpiration and thus carbon dioxide uptake is prevented. It has been proposed that under these conditions, proline act as an electron acceptor avoiding damage by proto inhibition

(Ain-Lhout *et al.*, 2001). There have been some long academic arguments on whether prolile is an indicator of water stress tolerance or just crop stress water injury sensor. The large body of data suggests that it is an indicator of water stress tolerance. Garcia *et al.* (1987) observed significantly high levels of proline in maize seedling subjected water stress conditions. Vendruscolo *et al.* (2007) and Naidu *et al.* (1990) observed stress imposed on wheat to increase proline on the leaves of the crop.

2.8 Conclusions

South Africa is faced with a problem of malnutrition, HIV and Aids, growing population and that of climate change. It is important therefore to look for a wide variety of crops in order to solve the problems of malnutrition, HIV and Aids, climate change and to meet the demands of the growing population. Bottle gourd is the one of the traditional under-utilized crop in South Africa which has been ignored by researchers. There is no research done describing its agronomy despite the fact that it has a potential to contribute to food security. Landraces are possible adapted to the ecological niches of which they grow. It has been described in literature that landraces can tolerate stressful conditions of water shortages. The seeds of the crops are reach in proteins that are fundamental important for human nutrition. The fruits contain medical properties that are important for human health and the leaves are also important source of nutrients. Given all these benefits and its potential as a food security crop in other countries, in South Africa it has been ignored by researchers for many years thus there is a need to establish nutritional information and agronomic requirements of the crop based on our local conditions and varieties that we have in order to promote the utilization of the crop.

CHAPTER 3

MATERIALS AND METHODS

3.1 Plant Materials

Mature fruit of bottle gourd landraces were collected from famers' fields in Richards Bay (28°19'S; 32°06'E; 30 m.a.s.l.), Nkandla (28^{0}37'21''S31^{0}5'22''E; altitude) in KwaZulu-Natal Province, South Africa and Chimbwanda East (18º19'S; 31º12'E; 1484 m a.s.l), in Mashonaland East Province, Zimbabwe. Seeds of two commercially grown, exotic species of pumpkin (the green and gold) and one cucumber variety were sourced from a local seed company (McDonalds Seeds, Pietermaritzburg) and used as check varieties. After removing the landrace seeds from the fruit, all the seeds were surface sterilized with 50% ethanol by immersion for five minutes. Seeds were then air dried. Table 3.1 provides a brief description of the characteristics of the landraces and the three exotic cucurbits used in the study. Figure 3.1 and 3.2 illustrate the morphology of fruit and seeds used.

Table 3.1: Description of *Cucurbitaceae* varieties and associated climate of where they were collected. Note: M01 – 3 = bottle gourd landraces; GRH = pumpkin; GOH = pumpkin; CA = cucumber.

Variety	Fruit shape	Fruit texture	Fruit colour	Fruit length (cm)	Single seed mass (g)	Ten seed mass (g)	Location	Climate
M01	Spherical	Warted	Brownish-green	26.9	0.18	1.79	Chimbwanda East	Semi-arid
M02	Calabash	Warted	Brown	28.3	0.24	2.71	Richards Bay	Sub-tropical
M03	Calabash	Smooth	Brown	32.1	0.16	1.72	Nkandla	Semi-arid
GRH	Cylindrical	Warted	Green	-	0.18	1.79	-	-
GOH	Cylindrical	Warted	Gold	-	0.17	1.70	-	-
CA	Cucumber	Warted	Green	-	0.14	1.59	-	-

Figure 3.1: Fruit and seed morphology of bottle gourd landraces (M01, M02 & M03).

Figure 3.2: Fruit and seed morphology of pumpkins (GRH and GOH) and cucumber (CA).

3.2 Seed Quality

3.2.1 Standard germination test

Seeds were germinated according to the guidelines set by AOSA (1992) with slight modifications. Four replications of twenty seeds per variety were placed on a double layered moistened germination paper towel. The germination paper was then rolled and tied at either end using rubber bands and put in zip-lock bags to seal off any moisture loss. The sealed zip lock bags were then placed in a germination chamber set at oscillating temperatures of 20/30°C (16/8 hours). Germination counts were taken daily for 22 days. Germination was defined as radicle protrusion of at least 2 mm. On day 22, final germination percentage was calculated based on normal seedlings according to AOSA (1992). Thereafter, measurements of root and shoot lengths, root: shoot ratio and seedling fresh and dry mass were taken. In addition, the following indices were calculated:

Germination velocity index (GVI) indicates the speed of germination and was calculated using the formulae by Maguire (1962):

$$GVI = G1/N1 + G2/N2 + \ldots + Gn/Nn \qquad \textbf{Equation 3.1}$$

where:

GVI = germination velocity index

G1, G2…Gn = number of germinated seeds in first, second… last count.

N1, N2…Nn = number of sowing days at the first, second… last count.

Mean time to germination (MGT) was calculated according to the formulae by Ellis and Roberts (1981):

$$MGT = \frac{\sum \boldsymbol{Dn}}{\sum \boldsymbol{n}} \qquad \textbf{Equation 3. 2}$$

where:

MGT = mean germination time,

n = the number of seed which were germinated on day D, and

D = number of days counted from the beginning of germination.

3.2.2 Seed electrical conductivity (EC)

Electrical conductivity was assessed using the CM 100-2 Model Single Cell analyser. Three replicates of 15 seeds per variety were used in this experiment. Seeds were initially weighed before being put into 2 ml wells filled with distilled water. Electrical conductivity of the seeds was then read over a period of 24 hours.

3.2.3 Tetrazolium (TZ) test

A 1% TZ solution was prepared by adding 1 g of 2, 3, 5 triphenyl tetrazolium chloride powder to 100 ml of distilled water in a glass beaker. Four seeds of each variety were replicated four times and soaked in distilled water for 24 hours. Following this, seeds were dissected longitudinally using a surgical blade to expose their cotyledons (AOSA, 2001) and placed them in petri dishes. The TZ solution was then poured into the petri dishes and incubated at 35°C for 8 hours and then scored for viability (AOSA, 2001).

3.2.4 Seedling emergence

The seedling establishment experiment was carried out under controlled environmental conditions (27/15°C day/night; 65% Relative Humidity; natural day length) at the University of KwaZulu-Natal's Controlled Environment Research Unit. Four replicates of 16 seeds from each variety were planted in seedling trays using a seedling mix as a media. The field capacity (FC) of the seedling mix was determined (by gravimetric method) prior to planting. Based on the pre-determined FC of the seedling mix, seedling trays were weighed and watered at a two-day interval to maintained FC. Seedling emergence measurements were collected daily for a period of 21 days. On termination of the experiment, the data collected included leaf number, leaf area, root length and shoot length. Leaf area was measured using Potable Area Meter: LI – 3000C.

Mean time to emergence was determined according to Bewley and Black (1994):

$$MET = \frac{\sum fx}{fx} \qquad \textbf{Equation 3. 3}$$

where:

MET= mean emergence time

f = number of newly germinating seeds at a given time (day)

X = number of days from the date of sowing

3.3 Controlled Environmental Experiment: Crop Responses to Water Stress

The experiment was conducted at the University of KwaZulu-Natal, Pietermaritzburg, South Africa under semi-controlled environment conditions (27°/15°C day/night; 60% RH and natural day length). Temperature and relative humidity were monitored electronically using a HOBO 2K logger (Onset Computer Corporation, Bourne, USA).

3.3.3 Experimental design and trial management

The pot trial experiment was laid out as a randomized complete block design with two treatment factors: variety [landraces M01 & M02 and commercial cucurbits hybrids GRH & CA] and water regimes [30%, 60% and 100% crop water requirement (ETc)], replicated three times. Soil used in the experiment was collected from the University of KwaZulu-Natal Research Farm (Ukulinga). Soon after collection it was to fill thirty six 10 ℓ pots. A brief description of the soil's physical and chemical properties is given below (Table 3.2). Three seeds were planted in each pot and later thinned to one seedling per pot after emergence. All pots were initially watered to field capacity and thereafter all pots were irrigated at 100% ETc in order to allow for maximum possible crop stand. Thereafter, water treatments were imposed. Pots were routinely hand weeded to ensure there was no competition for water and solar radiation. Plants were sprayed with Cypermethrin® (15: 10L) for control of aphids. Fertilizer was not applied in order to simulate the predominant conditions under which subsistence farmers cultivate the crop; in most cases people in rural areas do not apply fertilizer.

Table 3.2: Brief description of physical and chemical characteristics of the soil used.

Soil Texture	**Soil colour**	**Organic carbon (%)**	**N (%)**	**P mg/L**	**K mg/L**	**PH (KCL)**	**FC (v%)**	**PWP (v%)**
Clay loamy	Brown	1.70	0.23	20	227	5.16	36.1	22.0

3.3.4 Data collection

Plants were allowed to establish until 40 days after planting (DAP). Thereafter, the following measurements were taken; vine length, leaf number, stomatal conductance (SC), chlorophyll content index (CCI) and soil water content. Vine length was measured from the base of the plant to the apical meristem of the plant and leaf number was counted as a fully expanded green leaf. Stomatal conductance was measured using a steady state leaf porometer (Model SC-1, Decagon Devices USA) on the abaxial surface. Chlorophyll content index was measured using a chlorophyll content meter (CCM-200 *Plus*, Opti-Science, USA) on the adaxial surface. These measurements were taken on fully exposed, fully expanded leaves between 11 and 2 pm during the day before irrigation. Soil water content was measured using an ML2x Theta probe connected to an HH2 handheld moisture meter (Delta-T, UK). These parameters were taken weekly until the termination of the experiment. On termination of the experiment, measurements of leaf fresh and dry mass, root fresh and dry mass were taken using a sensitive balance (METTLER SM 3000). In addition, proline and protein content were also determined.

3.3.4.1 Proline content determination

At harvest, proline accumulation in leaves was determined according to the method of Bates *et al*. (1973) with slight modifications. Samples weighing 0.1 g of freeze-dried leaf tissue were homogenised in 10 ml of 3% sulfosalycic acid (w/v) and ultraturaxed for 60 seconds. The homogenate were then vortexed at room temperature for 2 minutes. Supernatant were added to 2 ml of acid ninhydrin and 2 ml of acetic acid. The mixture was then incubated in a hot water bath (100°C) for 1 hour with constant shaking and the reaction terminated in ice. The reaction mixture was then extracted with 4 ml toluene, and vortexed for 15-20 sec. The toluene phase was used to

measure the absorbance at 520 nm (Beckman Coulter DU® 800). Toluene was used as a blank. A standard curve was used to determine the concentration of proline by using the equation:

[(µg proline/ml x ml toluene)/ (115 µg/µmole)]/ [(g sample)/5] = µmoles proline/g of dry mass material.

3.3.4.2 Amino acid (protein) determination

Total soluble protein extraction

Total soluble proteins were extracted according to Kanellis and Kalaitzis (1992), with slight modifications. Freeze-dried leaf sample (0.1 g DM) was put into 5mL 50mM Tris–HCl buffer (pH 7.4) containing 0.2 M NaCl, 20 mM MgSO4, 1 mM EDTA, 5 mM -mercaptoethanol, 0.5 mM PMSF, 10 mM leupeptin, and 10% (v/v) glycerol. The samples were then homogenised using the ultrasonic cell disrupter to extract free and membrane-bound proteins. Subsequently, the mixture was allowed to stand on ice for 15 min and centrifuged at 20,000 × g for 20 min. The supernatant was used for enzyme assays after being filtered through Miracloth®.

Total protein quantification

The protein concentration of the samples was quantified by the Bradford micro assay (Bradford, 1976). Bradford dye reagent was prepared by diluting the dye concentrate with distilled water at a ratio of 1:4. The diluted dye (1 mL) was added to test tubes containing 2 L sample extract; thereafter samples were mixed by three times inversion. Subsequently, samples were incubated at room temperature for 5 min and read spectrophotometrically at 595 nm. The protein concentration was determined by comparing results with a standard curve constructed using bovine serum albumin (BSA) as a standard.

3.4 Controlled Environmental Experiment: Nutritional Value

3.4.1 Experimental design

The experiment was conducted as in section 3.3 above. It was laid out as a randomized complete block design with two treatment factors, varieties and harvesting replicated three times (however, plants that were harvested every two weeks until termination were replicated 18 times to make enough material for analysis). The harvesting treatments included; 1) harvesting once, two weeks after crop establishment, 2) harvesting every two weeks until termination of the experiment and, 3) no harvest (control). A brief description of the layout of the experiment is available in Appendix 3.

A total of 96, 10 ℓ drained pots were filled with soil collected from the University of KwaZulu-Natal Research Farm (see section 3.3.3 for detailed soil characteristics). Seedlings were established in seedling trays for 23 days. Thereafter, seedlings were transplanted into pots. All pots were connected to an online drip irrigation system and irrigation was scheduled to meet full crop water requirement (100% ETc). Other agronomic practices such as weeding, fertilisation and pest control were similar to those described in section 3.3.3.

3.4.2 Data collection

Measurements of leaf number, vine length, chlorophyll content index, stomatal conductance and soil water content were taken weekly until termination of the experiment. Refer to section 3.3.4 for detailed descriptions on data collection. Leaves were initially harvested at the 4^{th} leaf stage, when all four leaves were fully expanded and exposed. For each harvest, healthy edible leaves were targeted and removed from the stem with petiole. Sequential harvesting was done based on the preferences of rural households who still consume the leaves. The number of leaves harvested at each harvest was two. Immediately after harvesting, plant leaf material were freeze dried at -53°C for three days. Soon after removal from the freeze–drier, leaf material were ground under liquid nitrogen using mortar and pestle and stored at -12°C. On termination of the experiment plant leaf material were sent to Cedara laboratory for nutrient analysis.

3.4.2.1 Nutrient content assessment in relation to nutrient requirements

The means for five individually analysed samples were used to calculate and process the data used in calculating recommended daily allowance (RDA). Nutrient retention factors for "veg, greens, boiled, little water drain" (USDA Table of nutrient retention factors, 2007) were used to account for nutrient losses during cooking. The following nutrient retention factors were used: phosphorus and potassium = 0.90; calcium, magnesium, sodium, copper, iron and zinc (USDA Table of retention factors, 2007). For protein and manganese, the USDA Table of nutrient retention factors (2007) does not provide nutrient retention factors for "veg, greens, boiled, little water for these nutrients. It was assumed therefore for this calculation that there is no loss during cooking and a nutrient retention factor of 1 was used.

Average portion size (Faber *et al.,* 2007) was set at 130 g boiled leaves for adult females and 90 g for young children. A raw to cooked yield factor of 1.3 was applied based on the method by van Jaarsveld *et al.* (2014). The nutrient composition of average portion cooked leaves to nutrient intake of individuals was calculated and expressed as a percentage of Dietary Reference Intake (DR) (RDA, adequate intakes (AI)) for 4-8 years old children and 19-30 years old non–pregnant and non–breast feeding females (Ross *et al*., 2011).

3.5 Field Trials

Two field trials were carried out at the University of KwaZulu-Natal's Research Farm in Pietermaritzburg (29°37'S; 30°16'E; 805 m a.s.l) from April to June (winter trial) and August to February (summer trial) under rain–fed conditions. The farm has a subtropical climate with 694 mm annual precipitation received mainly during the summer season (October–March). The farm represent semi-arid environment characterized by clay loamy soil. Weather parameters were monitored by automatic weather station situated with 50 m radius from the trial.

3.5.1 Experimental design

The experimental design was a randomized complete block design (RCBD) with three replicates. There were 5 varieties, two landraces (M01 & M02) and three hybrid varieties (GOH, GRH & CA). The trial was planted on an area of 242.2 m^2. The sub plot size was 7.2 m^2 with inter-plot spacing of 1.5 m and plant spacing of 1.5 × 0.8 m.

3.5.2 Agronomic practices

Before the start of trials, soil samples were taken for soil fertility and textural analysis. The details of the results and soil characteristics were presented in section 3.3.3. Land preparation involved disking and rotovating to achieve fine seed beds. Weeding was done by hand hoeing.

3.5.3 Data collection

Data collected included emergence until more than 70% of the plants had emerged. Canopy characteristics (plant vine length and leaf number) and physiological parameters (stomatal conductance and chlorophyll content index) were only measured for winter while in summer trial only emergence and yield were measured. Refer to section 3.3.4 for detailed descriptions on data collection.

3.5.3.1 Weather data

Weather (rainfall, Tmin and Tmax) data for the duration of the study was obtained from an automatic weather station (AWS) situated 75 m from the study site. The AWS is managed by the Agricultural Research Council – Institute for Climate, Soil and Water (ARC–ISCW).

3.5.3.2 Seed quality test after harvesting

After harvesting, mature fruits from plants free of diseases were selected and cut open to remove seeds from fruit. Thereafter, seeds were sun dried for 10 hours per day to remove dormancy for the period of 10 days without any other treatment. Following air drying, seeds were subjected to seed quality tests for viability and vigour in the laboratory. See section 3.2 for details.

3.6 Statistical Analyses

All data were subjected to analysis of variance (ANOVA) using GenStat® (Version 14, VSN International, UK). Means of significant different variables were separated using least significant differences (LSD) at a probability level of 5% (Appendix 3). Nutrient data obtained from the analysis were entered and analysed in a spreadsheet using Microsoft Office® Excel 2010. However, as the data was limited to a few samples, statistical data are not presented in this paper.

CHAPTER 4

SEED QUALITY OF SELECTED BOTTLE GOURD LANDRACES (*LAGENARIA SICERARIA* (MOLINA) STANDL.) COMPARED WITH POPULAR CUCURBITS

4.1 Introduction

Seed quality has been described by Hampton (2002) as a standard of excellence that will determine the performance of seeds when sown. According to Basu (1994), it is a multiple concept which includes the genetic, physical, physiological, pathological and entomological attributes of seed lots. In addition to the above mentioned traits, Thomson (1976) included in his definition of seed quality, aspects of genetic purity, analytical purity (absence of contaminants from bacteria and fungi), pure seed and healthy seed, correct moisture content and uniform mixing and blending of size. The two most used indicators of seed quality are viability and vigour.

Seeds that are viable are those that are alive and have the potential to germinate when exposed to favourable conditions (Basu, 1994). According to Perry (1973) and McDonald (1980), seed vigour comprises those seed properties that determine the potential for rapid, uniform emergence and development of seedlings under a wide range of environmental conditions. Hence, the success in sustainable crop production lies in the use of high quality seeds as it is essential for a good (even and rapid) crop stand. Good crop stands have been correlated to better yields due to their ability to compete better for resources (Zelitch, 1982). Furthermore, in the event of stress, a good stand can escape the risk of total crop loss better than a poorly established stand (Dornbos, 1995). Therefore, seeds of high quality are a cornerstone to high agricultural output, especially if NUS (neglected underutilized species) such as bottle gourd are to be promoted for food security. However, not much information on seed quality and factors affecting it is available for bottle gourd.

Bottle gourd is an open-pollinated crop with different fruit morphology and seed forms, size, shape and colour. This suggests that may be a wide genetic variation in its seeds. According to Chimonyo and Modi (2013) this affects seed quality. In comparison to hybrids, seeds of

landraces have been observed by several authors (Mabhaudhi and Modi, 2010; Bidinger *et al*., 2008; Shimelis and Laing, 2012) as inferior since they are heterogenous. According to Shimelis and Laing (2012), hybrid seeds are genetically uniform and possess hybrid vigour. On the other hand, large variation in genetic constitution has been identified as an added bonus in improving crop resilience towards risk (Bewley, 1997). Differences in times to germination and emergence can be used as an avoidance mechanism in the event of risk.

Genetic characteristic can also affect phenotypic characteristics and thus affect seed quality. While holding all other traits constant and varying seed coat colour, Odindo (2007) and Rolston (1978) observed differences in seed quality in cowpea and *Trifolium alexandrinum*, respectively. In bambara groundnut, Sinefu (2011) observed better germination of brown than white seeds. Seeds of bottle gourd vary from white to dark brown. This implies that seed coat colour can have an effect on seed quality. In addition to seed coat colour, other paramenters such as seed size, seed coat thickness, and seed mass have been shown to have an effect on seed quality.

A number of tests can be conducted to determine seed quality and these vary in degree of complexity and duration. According to Mabhaudhi and Modi (2010), determining seed quality of landraces from a single test is often misleading due to the large variation. Chimonyo and Modi (2013) concluded that a combination of tests should therefore be done. For the purpose of this study it was necessary to assess seed quality of bottle gourd landraces for the establishment of field and pot trials since they were collected from different locations. Furthermore, farmers need information on seed quality before planting. It is imperative for farmers to plant high quality seed because they cannot recover the cost and time lost after planting has commenced. Information on seed quality is also important for implementing effective breeding programs where higher performing seeds are the starting point of selection. Therefore, the objective of this study was to evaluate the seed quality of local bottle gourd landraces compared to exotic cucurbits. The landraces differed with respect to fruit morphology. The popular cucurbits were used as benchmark crops for the purposes of future studies.

4.2 Results

4.2.1 Standard germination test

There were highly significant differences ($P < 0.001$) observed across all varieties in terms of germination time and final germination (Fig. 4.1). Landrace M01 showed rapid germination; reaching 50% four days after incubation (also see Fig. 4.2). On the other hand, M02 and GRH were very slow to germinate compared with the rest of the other varieties. Based on the LSD (15.01), there were no significant differences ($P > 0.05$) in terms of final germination between M01, M03, GRH and CA.

Significant differences ($P < 0.05$) were observed across the varieties with respect to T_{50} (Fig. 4.2). Landrace M01 was observed to take the less time to germinate (6.5 days) than all other varieties while landrace M03 was observed to take more days (10 days) than all other varieties to reach T_{50}. Landrace M02 and GRH did not reach 50% germination in the period of 22 days.

Highly significant differences ($P < 0.001$) were observed across the varieties (Table 4.1) for GVI, EC, root and shoot length, and root: shoot ratio. Landraces had, on average, a 4% higher GVI, 4% higher MGT and 6% higher root: shoot ratio than hybrid varieties. The results showed no significant differences ($P > 0.05$) in EC across all varieties except for landrace M02, which had a 71% greater EC than all other varieties (Table 4.1). The highest root lengths were observed for landrace M01, hybrid GRH and CA while the lowest was observed for the hybrid GRH. Hybrid CA had the highest shoot length than all varieties while the lowest was observed in hybrid GRH.

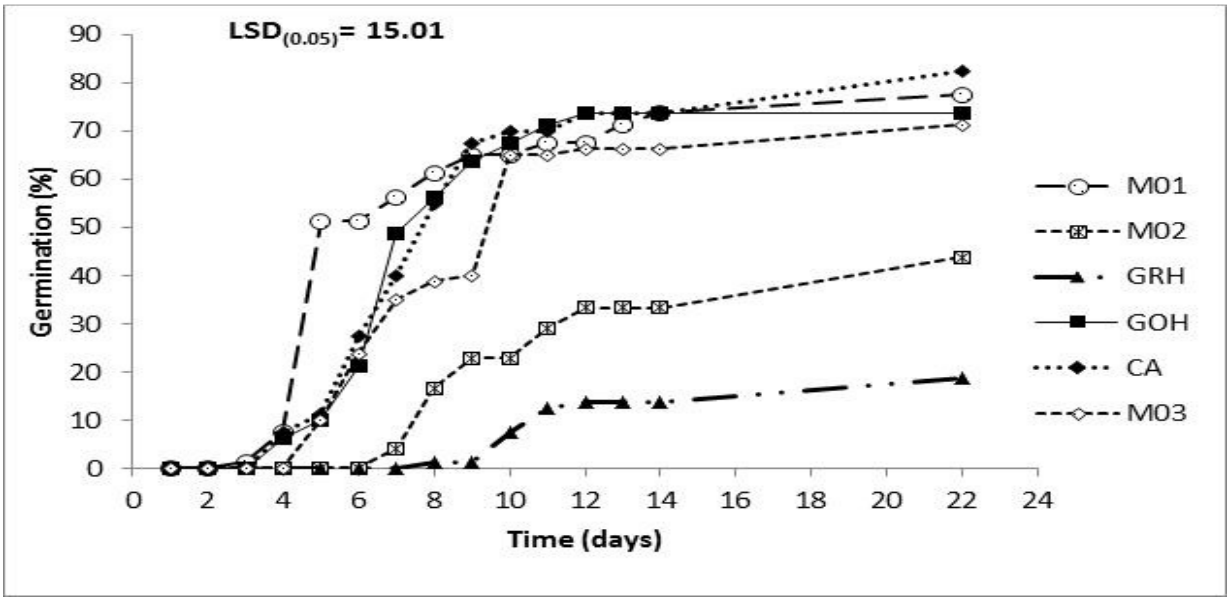

Figure 4.1: Daily germination of bottle gourd landraces (M01, M02 and M03) compared with selected hybrid cucurbits (GRH, GOH and CA).

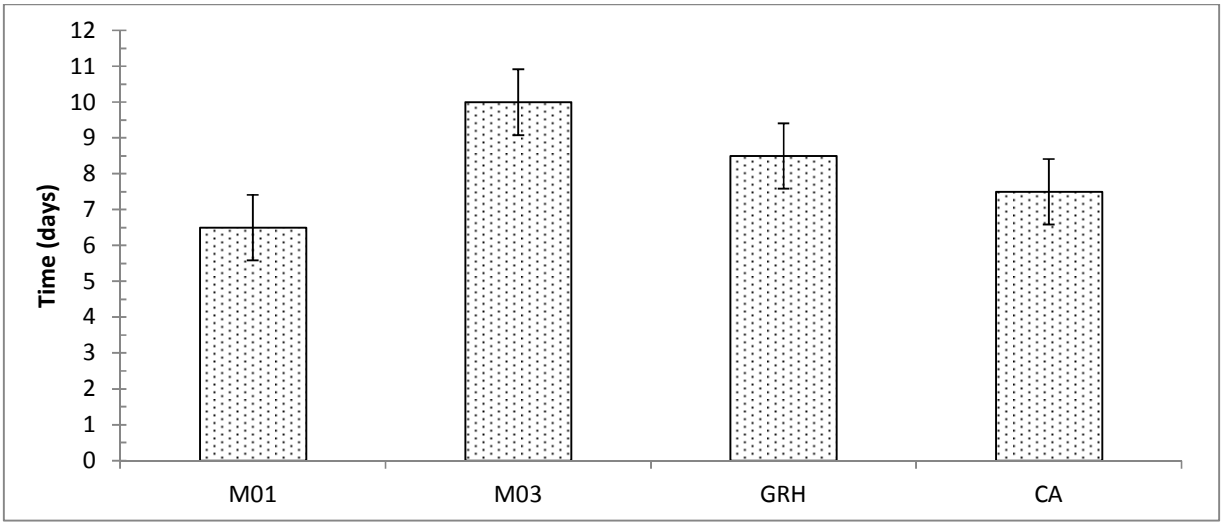

Figure 4.2: Time (days) taken by bottle gourd landraces (M01, M02, and M03) and conventional cucurbits (GRH, GOH and CA) to 50% germination (T_{50}).

Table 4.1: Seed performance of bottle gourd landraces (M01, M02 and M03) compared with selected hybrid cucurbits (GRH, GOH and CA) during the standard germination test. [1]Values represented by the same letter are not significantly different from each other at $P < 0.05$.

Variety	Final germination (%)	Germination velocity index (GVI)	Mean germination time (days)	Electrical conductivity (µS/g)	Root length (cm)	Shoot length (cm)	Root:shoot ratio
M01	77.50^{c1}	13.37^{b}	7.17^{ab}	92.8^{a}	15.17^{d}	7.70^{c}	1.72^{c}
M02	43.75^{b}	3.94^{a}	12.22^{c}	1021^{b}	7.06^{b}	4.20^{b}	1.04^{b}
M03	71.25^{c}	10.01^{b}	5.22^{a}	149^{a}	10.50^{c}	7.28^{c}	1.08^{b}
GRH	73.75^{c}	10.77^{b}	7.35^{ab}	64.9^{a}	15.65^{d}	7.23^{c}	1.70^{c}
GOH	18.75^{a}	3.21^{a}	10.37^{bc}	53.4^{a}	0.27^{a}	0.11^{a}	0.098^{a}
CA	82.50^{c}	11.34^{b}	8.77^{b}	60.0^{a}	16.01^{d}	10.00^{d}	1.64^{c}
LSD	18.44	3.84	3.35	102.9	2.97	1.71	0.23
P value	*< 0.001*	*< 0.001*	*=0.006*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*

4.2.2 Electrical conductivity (EC)

There were highly significant differences (*P < 0.001*) in electrolyte leakage (Table 4.1) across the varieties tested. Landraces M01, M03 and hybrids GRH, GOH, CA were not statistically different from each other while landrace M02 were statistically different from all other varieties.

4.2.3 Tetrazolium (TZ) test

All seeds were found to be viable to expect both good germination and seedling establishment (Fig. 4.3).

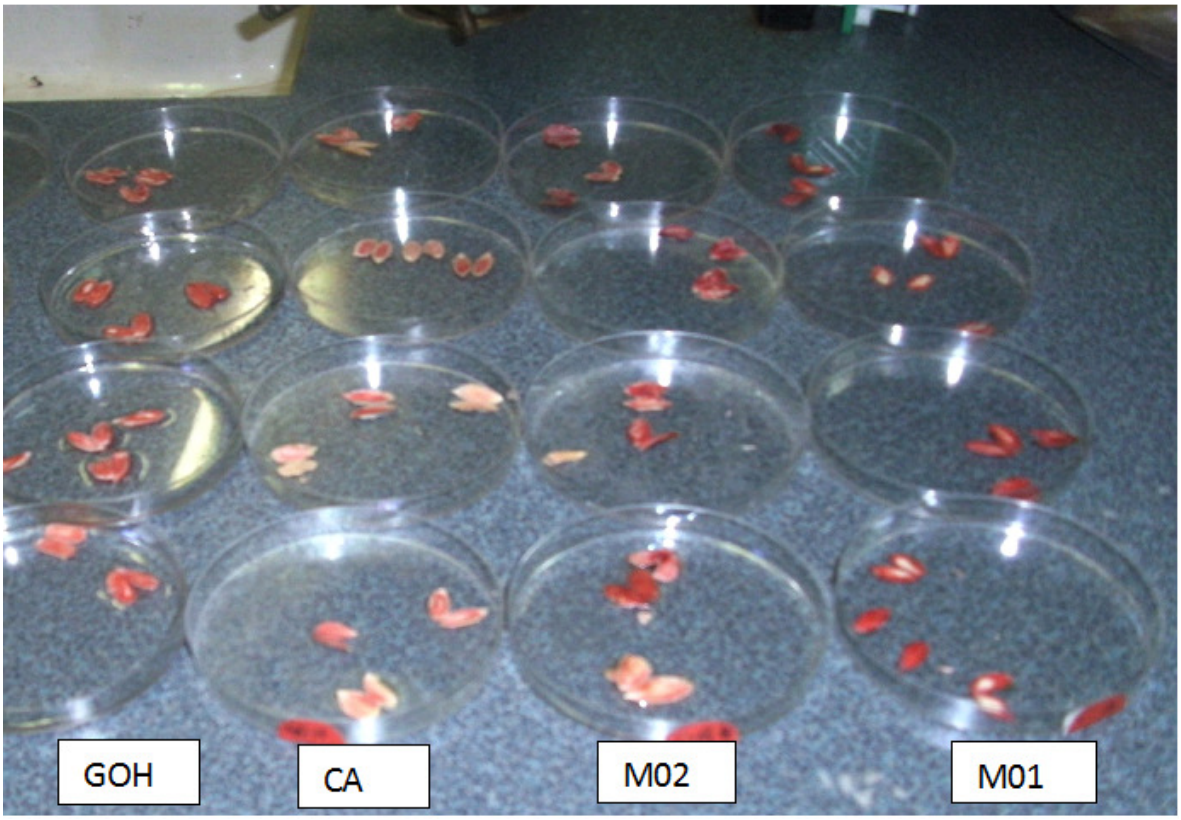

Figure 4.3: Comparison of intensity of staining of the seeds during TZ test. Red colour is an indication of viability.

4.2.4 Correlation of germination traits

Strong positive correlations of traits among the following variables were observed during the standard germination test: GVI and root: shoot ratio (r = 0.84, P < *0.01*), GVI and shoot length (r = 0.90, P < *0 .05*), GVI and root length (r = 0.91, *P < 0.05*) (Table 4.2). A negative non-significant correlation was observed between EC and the following variables: GVI (r = -0.5, *P > 0.05*), root length (r = -0.21, *P > 0.05*, shoot length (r = -0.14, *P > 0.05*, and root: shoot ratio (r = -0.05, *P > 0.05*) (Table 3). However, a highly significant and strong correlation (r = 0.99, P < *0.01*) was observed between EC and seed mass. Final germination was significantly and strongly correlated to root: shoot ratio (r = 0.92, P = 0.025), shoot length (r = 0.99, P < *0.001*), root length (r = 0.96, P = 0.008) and GVI (r = 0.94, P = 0.002) (Table 4.2). However, it was weakly and negatively correlated to EC (r = -0.25, P = 0.69). Seed mass was observed to be negatively correlated to final germination (r = -0.22, P = 0.72).

Table 4.2: Association of seed quality traits during the standard germination test. Note: GVI = germination velocity index; EC= electrical conductivity; MGT = mean germination time; RL = root length; R: S = root to shoot ratio; SL = seedling length.

Final germination	**1**	-							
GVI	**2**	0.9381	-						
EC	**3**	-0.248	-0.507	-					
MGT	**4**	-0.760	-0.821	0.6980	-				
RL	**5**	0.9647	0.9100	-0.209	-0.600	-			
R:S	**6**	0.9238	0.8439	-0.054	-0.480	0.9837	-		
SL	**7**	0.9932	0.8959	-0.142	-0.714	0.9516	0.9236	-	
Seed mass	**8**	-0.224	-0.473	0.9905	0.7287	-0.151	0.0135	-0.123	-
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**

4.2.5 Emergence

Highly significant differences ($P < 0.001$) were observed across varieties in terms of their emergence time (Fig. 4.4). Hybrid CA emerged on day 6 and reached 40% emergence and by day 7 it was standing above 75%. Significant differences ($P < 0.05$) were observed also in the final emergence. Landrace M01 had the slowest emergence (76.6%) compared to all other varieties while hybrid CA had the highest emergence of 98.44%. There were no significant differences ($P > 0.05$) observed across the varieties in terms of their final emergence (Table 4.3). Significant differences ($P < 0.05$) were, however, observed for MET, leaf number, leaf area, root and shoot length (Table 4.3). Hybrid CA had the shortest emergence time of about 7 days followed by GRH with 9 days. Landraces M01 and M03 had MET of 10 days. Leaf area and leaf number were significantly ($P < 0.001$) higher for hybrids than landraces. There were no significant differences ($P > 0.05$) observed for root: shoot ratio.

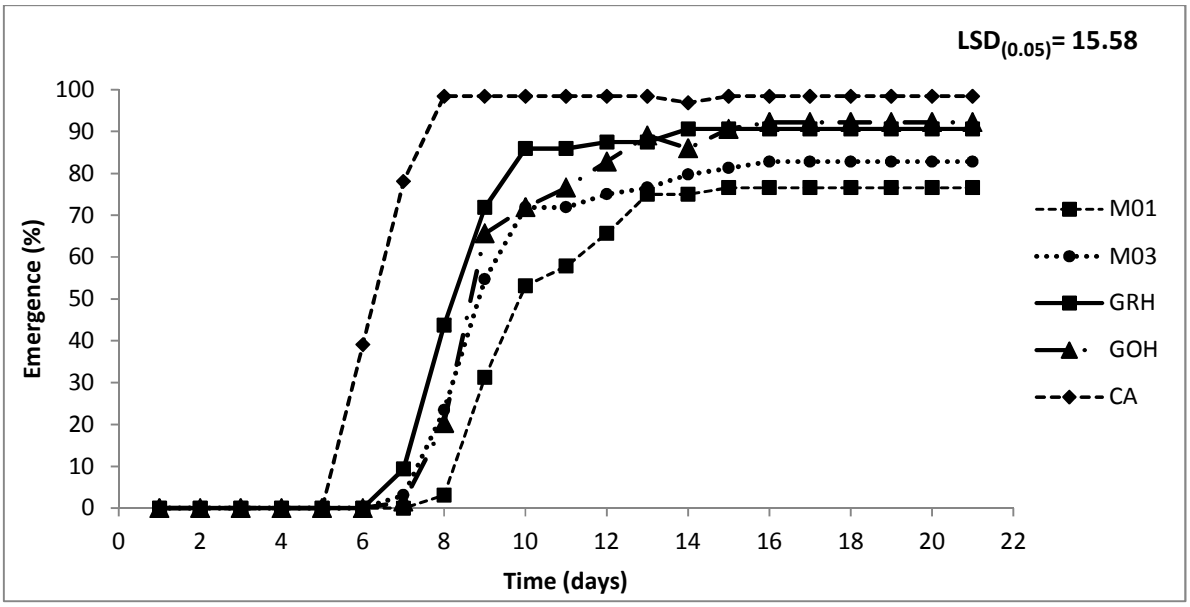

Figure 4.4: Seedling emergence of landraces (M01, M03 and M03) and conventional cucurbit hybrids (GRH, GOH and CA) over the period of 21 days.

Table 4.3: Seedling emergence of landraces (M01, M02 & M03) and hybrids GRH, GOH and CA) and parameters associated with growth. Note: Values not sharing the same letter within the same column differ significantly at P = 0.05.

Variety	Emergence (%)	MET (days)	Leaf No.	Leaf area (cm^2)	Root length (cm)	Shoot length (cm)	Root: shoot
M01	76.6	10.1^{c}	1.0^{a}	5.00^{a}	5.70^{b}	4.64^{b}	1.27^{a}
M03	82.8	9.55^{bc}	1.0^{a}	4.09^{a}	3.91^{a}	3.49^{a}	1.16^{a}
GRH	90.6	8.82^{b}	1.7^{b}	13.13^{c}	5.87^{b}	5.33^{b}	1.12^{a}
GOH	92.2	9.60^{c}	1.7^{b}	9.10^{b}	5.96^{b}	5.34^{b}	1.16^{a}
CA	98.4	6.65^{a}	2.0^{c}	17.5^{d}	10.32^{c}	9.03^{c}	1.16^{a}
$\mathbf{P_{value}}$	NS	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	NS

4.2.6 Correlation of emergence traits

Seed mass was observed to be weakly correlated (r = 0.27, $P > 0.05$) to root: shoot ratio (Table 4.4), negatively correlated (r = -0.73, $P > 0.05$) to final emergence and strongly correlated to MET (r = 0.80, $P > 0.05$). A strong negative correlation (r = -0.71, $P > 0.05$) was observed between final emergence and root: shoot ratio (Table 4.4).

Table 4.4: Association of seed quality traits and some growth parameters during the seedling emergence. Note: MET = mean emergence time; R: S = root to shoot ratio; RL = root length; SL = shoot length.

Emergence	1	-						
Leaf No.	2	0.9606	-					
MET	3	-0.815	-0.776	-				
R:S	4	-0.712	-0.600	0.4303	-			
RL	5	0.7245	0.7772	-0.893	-0.098	-		
SL	6	0.7893	0.8304	-0.919	-0.197	0.9948	-	
Leaf area	7	0.9995	0.9407	-0.900	-0.519	0.8674	0.9058	-
Seed size	8	-0.733	-0.602	0.7976	0.2716	-0.752	-0.771	-0.590
		1	2	3	4	5	6	7	8

4.3 Discussion

Results obtained in this study showed that seeds obtained from morphologically different bottle gourd landraces portrayed different seed quality and emergence traits. Similar to landrace selections, varietal differences in seed quality and emergence were also observed between the three hybrid varieties. The observed results of final germination pulled together were contrary to the expectation that hybrid seeds should perform better than landraces in terms of seed quality. They are, however, similar to results observed by Mabhaudhi and Modi (2010) when they compared hybrid and landrace maize. On the other hand, it could be argued that, the most important factor affecting seed germination is not always in the genetic makeup, rather post and pre-environmental conditions as alluded to in the earlier part of the discussion.

Results obtained in this study also showed that final germination, GVI, MGT, and root: shoot ratios were strongly linked. High number of seeds that germinate per day results in high GVI because GVI represents the relative speed of the germinating seeds. On the other hand, high negative correlation between final germination and MGT may be explained by the fewer number of days taken for seeds to germinate; the lower their mean and the higher the final germination. High GVI resulted also in high root: shoot ratio because of quick use of the seed reserves by the germinating seeds. Similar observations were made by Sinefu (2011).

Low germination of M02 could have been due to poor seed coat integrity due to the observed higher EC. Higher electrolyte conductivity is associated with leakage of soluble solutes, which may compromise seed quality. Borji *et al.* (2007) observed a positive correlation between EC and the thickness of the seed coat. Thus, poor germination percentage observed in landrace M02 could be due to the thickness of the seed coat. To substantiate this, although the average seed mass of this landrace was significantly higher than that of all the other varieties, it failed to perform better, given all conditions required for germination. Similar results were obtained by Akita *et al* (1986) that heavy seeds did not always mean high plant growth rate. Thus, this reemphasizes the need to understand pre and post-harvest activities which often affect seed quality.

The results obtained for viability test indicated that most seeds for all varieties were alive. However, these results were not in direct agreement with those obtained in germination test. This indicates that viability test was a poor indicator of seed quality in this case and suggests that this test should be used with other tests to determine the combined seed quality.

Seedling emergence is one of the critical stages in crop establishment. Establishment of the crop depends on the interaction between seed quality and seed bed environment (Khajeh-Hosseini *et al.,* 2003). Water availability during this stage has been found to have a profound impact on crop establishment (Bayoumi *et al*., 2008). In this study the results obtained for seedling emergence were in agreement with what was found in seed germination and seed vigour tests, with a few exceptions. In standard germination test, the final germination for hybrid GOH was very low (18.75%), while in emergence it was high (92.2%). In addition, it was observed that hybrid varieties produced a significantly higher leaf number and leaf area than the landraces in the period of 21 days.

The results of the study indicated that seed quality of bottle gourd landraces with different fruit morphologies collected from different localities varies in terms of seed different measures including viability, germination, germination rate and stand seedling establishment. Bottle gourd landraces compared favourably with cultivars in terms of seed performance. Landraces can perform better than cultivars and vice versa.

CHAPTER 5

RESPONSES OF SELECTED BOTTLE GOURD LANDRACES TO WATER STRESS UNDER CONTROLLED ENVIRONMENT

5.1 Introduction

South Africa is water scarce and most agricultural activities depend on irrigation (Republic of South Africa National Water Act, 1998; DWAF, 2004).The country receives less than 500 mm of rainfall per year which is far below the world average of 836 mm (The World Bank, 2013). Rains occur mostly in summer, between October and March. Within this time frame, rainfall fluctuates greatly across space and time often resulting in sporadic and at times severe episodes of water stress (DEAT, 2004; Laker, 2007). The observed trend is expected to worsen due to the effects of climate change (Schulze, 2011). Due to these challenges, it is not clear whether the continued production of conventional crop species will provide food security for present and future generations, especially subsistence farmers often located in the drier regions of South Africa (Mabhaudhi *et al*., 2013). The introduction of a more home based solution was proposed, such as neglected underutilized species (NUS), into current cropping systems as an assistance in buffering the negative effect of predicted climate change (FAO, 2012; Padulosi, 2011; Mabhaudhi *et al.,* 2013). Neglected underutilized species are those species whose potential to improve people's livelihoods as well as food security and sovereignty are not fully recognized because of limited competitiveness with commercial crops in main stream agriculture (Dawson *et al*., 2007).

Bottle gourd [*Lagenaria siceraria (Molina)* Standly] is a cucurbit commonly cultivated in sub–Saharan Africa using landraces. These landraces may have evolved to become drought tolerant through farmer and natural selection in environments characterized by numerous abiotic stresses, mainly water stress (Mabhaudhi *et al.,* 2013). Chimonyo and Modi (2013) identified bottle gourd as an excellent model crop for improving food security and helping economic prosperity of rural communities in Africa. In spite of this potential, in-depth investigations on the crop are scant and it is difficult to use reports about other cucurbit species due to variations in morphology and phenology, as well as to describe its tolerance to water stress.

Plant responses to water stress are complex (Blum, 2009) and it causes a reduction or a total failure in plant production. The reduction of soil water content results in accumulation of abscicic acid and this is the major mechanism leading to stomatal closure (Chaves *et al*., 2002), which leads to reduced stomatal conductance and affecting growth processes in plants. Cell division and expansion are severely affected by drought stress (Prasal *et al.,* 2008); consequently, leaf expansion will also be inhibited (Seyed *et al*., 2012). Continued water stress during crop growth and development leads to reduced chlorophyll content; as a result photosynthetic efficiency is also reduced (Forooq *et al.,* 2009; Anjum *et al.,* 2003; Kiani *et al.*, 2008). Arunyanark *et al.* (2008) observed significantly reduced chlorophyll content in water stressed leaves causing a reduction in photosynthetic activity and membrane bound chloroplast antioxidants.

Water stress affects plants at any stage of development (Liu *et al.,* 2003) and its effects vary depending on the stage of crop development (Abo-El-Kheir and Mekki, 2007). Severe water stress at early establishment, vegetative growth, flowering and yield formation, decreases yields significantly (Dhillon *et al*., 1995) with early crop establishment stage being particularly susceptible (Liu *et al.*, 2003). In many crops water stress decreases plant height, leaf number, fruit number and quality, harvest index as well as to delay and / or interfere with time to flowering (Grimes, 1970; Prasad and Staggenborg, 2008). Zhi-min *et al*. (2000) observed significantly lower plant height, leaf area ratio and relative growth rate when cucumber plants were exposed to water stress. In severely stressed wild water melon plants there was a significant reduced plant height and dry matter production, whereas in mild and optimum water stress there were no differences; therefore, wild water melon showed moderate tolerance to water stress (Zulu and Modi, 2010).

Tough progress has been made in South Africa to define some of the NUS responses to drought stress (WRC, 2013) in order to select and develop drought tolerant cultivars, little or none has been reported about bottle gourd landraces; thus, it is necessary to characterize drought tolerance of this crop. Besides, since bottle gourd is relatively unknown, owing to its status as a NUS, its performance *viz a viz* that of popular and elite commercial varieties must be carried out.

Therefore, the aim of the study was to evaluate responses of bottle gourd landraces to water stress in comparison with two commercially produced cultivars of pumpkins.

5.2 Results

5.2.1 Soil water content

There were significant interactions between variety, water regime and days after planting over time (Fig. 5.1). Highly significant differences ($P < 0.001$) were observed in soil water content with respect to different water regimes. Although there were large fluctuations in soil water content, 100% ETc was significantly higher (9%) than 30% ETc and 5% higher than 60% ETc.

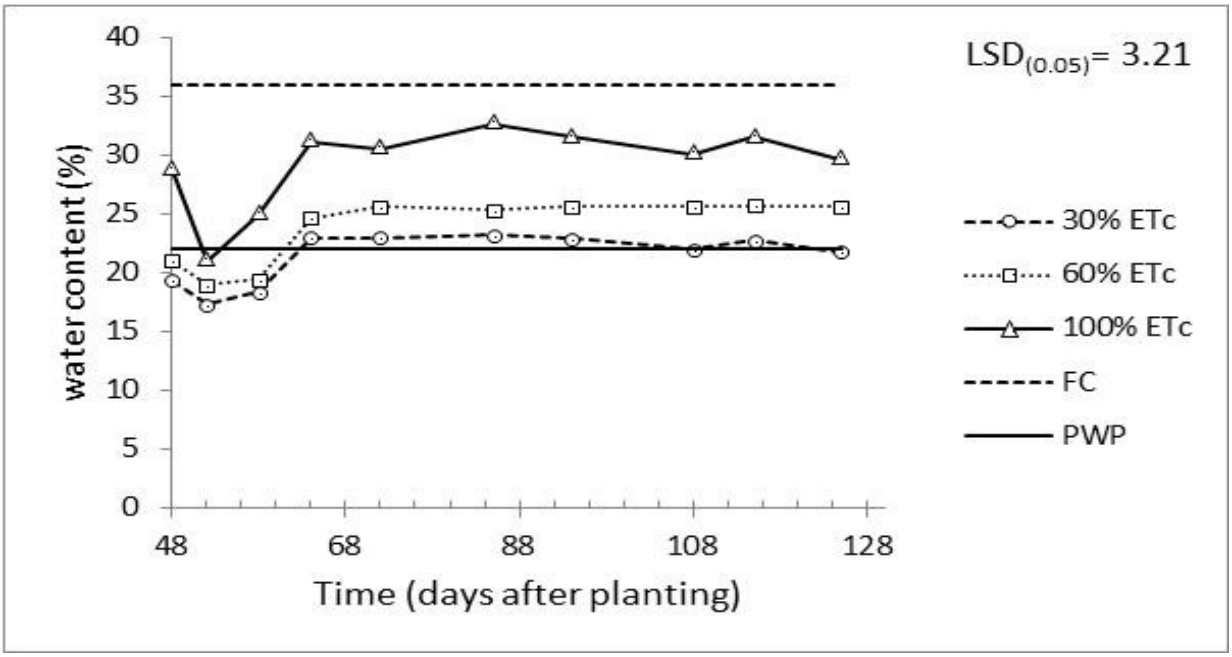

Figure 5.1: Volumetric soil water content of varieties at different water regimes over a period of 125 days.

5.2.2 Crop physiology

5.2.2.1 Stomatal Conductance (SC) and Chlorophyll Content Index (CCI)

The results showed no significant interaction ($P > 0.05$) among varieties, water regimes and time with respect to SC (Fig. 5.2). Although not significant, clear differences were apparent in varieties with respect to ETc. Varieties in 30% ETc were observed to close their stomata with increase in water stress while in 100% ETc varieties were shown to have higher values of stomatal conductance. Significant differences ($P < 0.01$) were also observed between landraces and hybrid varieties in stomatal SC in 30 and 60% ETc with hybrid varieties showed higher SC while in 100% ETc there were no significant differences observed.

There was no significant ($P > 0.05$) interaction among varieties, water regimes and time with respect to CCI (Fig. 5.3). However, the interaction between varieties and water regimes was shown to be highly significantly ($P < 0.001$). It was shown to increase with increase in ETc. Chlorophyll content index, for all varieties, was higher during the early growth stages and declined with time. Hybrid GRH had a higher chlorophyll content index than other varieties; the other varieties did not show any clear trend in chlorophyll content index. When comparing landraces and hybrid varieties, hybrid varieties on average had the highest CCI in 30 and 60% ETc while on 100% ETc landraces had the highest CCI but however this was not significant.

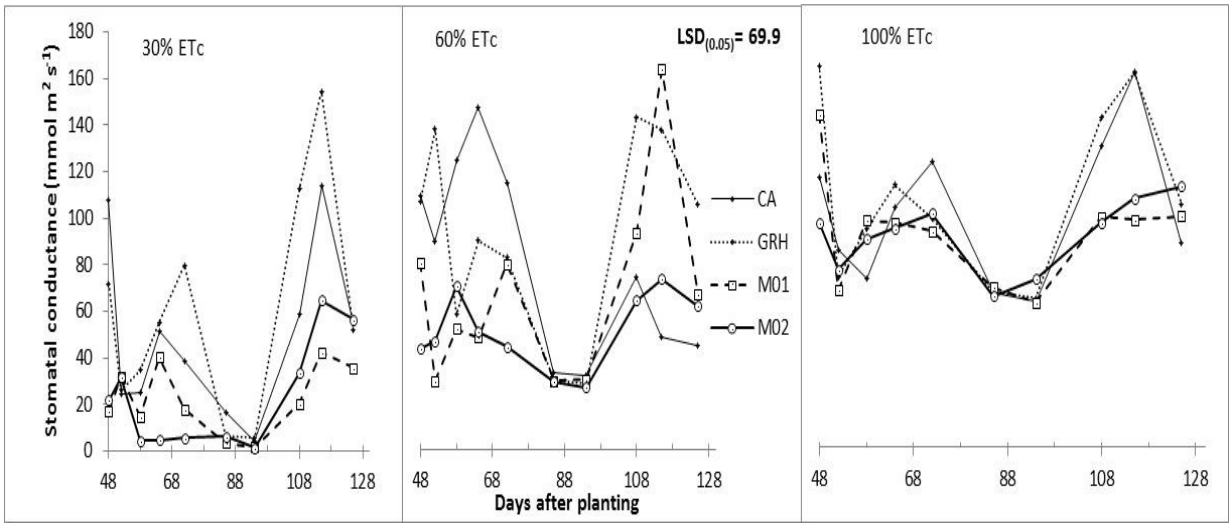

Figure 5.2: Changes in chlorophyll contet index of different varieties in response to varying water regimes.

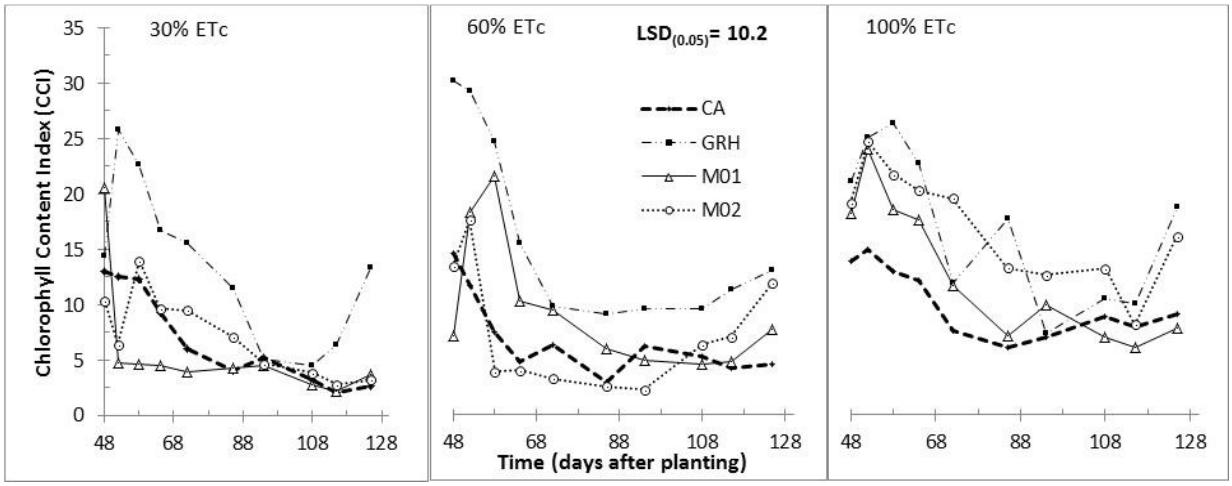

Figure 5.3: Changes in stomatal conductance of varieties in response to varying water regimes.

5.2.3 Crop growth

5.2.3.1 Vine length and leaf number

Results showed that the interaction of variety, water regime and time was significant ($P < 0.001$) (Fig. 5.4). Overall, vine length of landraces was significantly longer than for hybrid varieties. Hybrid CA was observed to have the shortest vine length than all varieties while landrace M01 had the longest vine length compared with all other varieties. The vine lengths were observed to be longer under 100 % ETc followed by 60 % and 30 % ETc, respectively for all varieties with the exception of CA where vine length was observed to be high at 60 % ETc. However, leaf number was higher in hybrids than landraces (Fig. 5.5). They were also observed to decrease with increase in water stress.

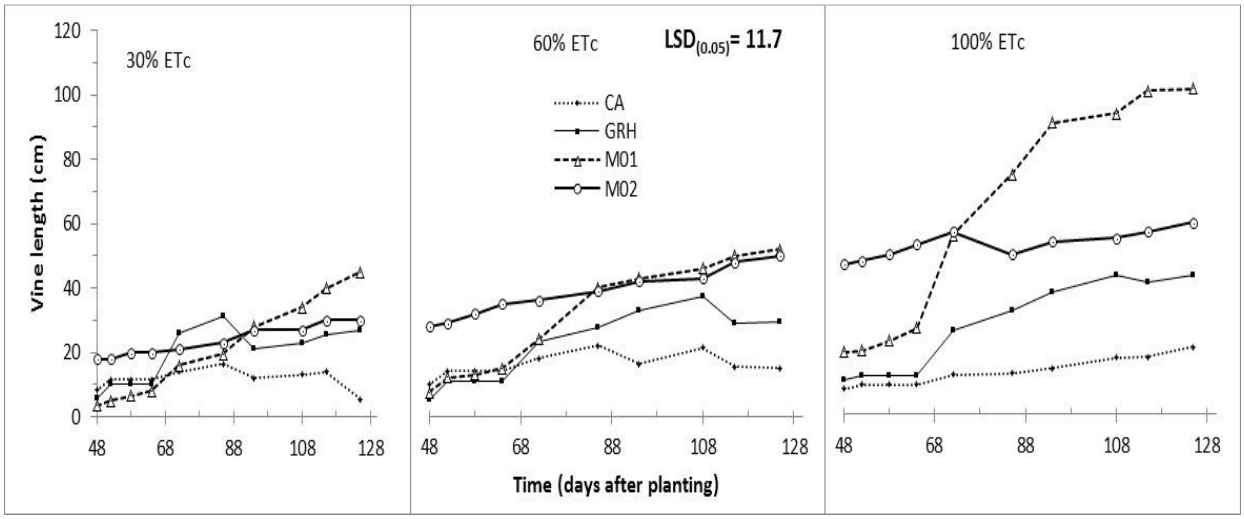

Figure 5.4: Vine length of bottle gourd landraces (M01 and M02) and commercial hybrids members of cucurbits (GRH & CA) at 30%, 60% and 75% ETc.

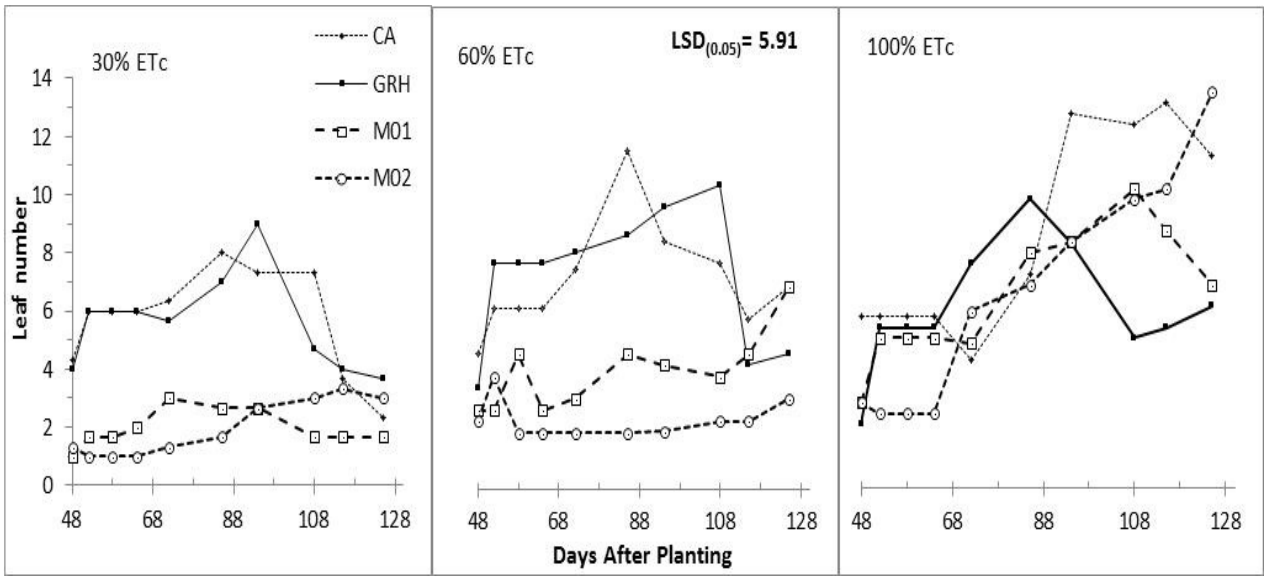

Figure 5.5: Leaf number of bottle gourd landraces (M01 & M02) and commercial hybrids (CA & GRH) of cucurbits at 30%, 60% and 75% ETc.

5.2.4 Proline and protein content

High significant differences (*P < 0.001*) were observed in proline accumulation with respect to ETc (Fig. 5.6). Varieties were observed to increase in proline content with increase water stress. Hybrid varieties were observed to accumulate more proline than landraces.

Highly significant differences (*P < 0.001*) were observed among varieties in protein content (Table 5.1). Hybrid varieties had higher protein content than landraces. Protein content was observed to be higher in hybrid GRH followed by hybrid CA. Landraces, on the other hand, had small amounts of proteins when compared to hybrid varieties. The interaction between varieties and water regimes was shown to be highly significant (*P < 0.001*). At 100% ETc protein content was observed to be higher than all other water regimes. However, there was no clear trend observed in protein content between 30 and 60% ETc.

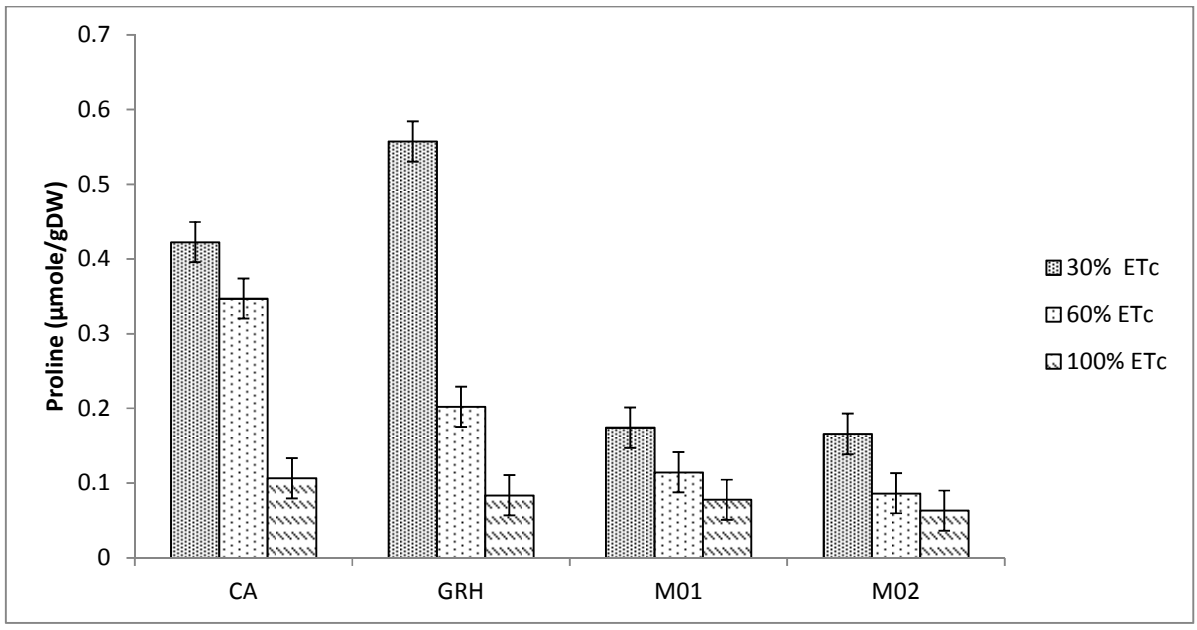

Figure 5.6: Proline accumulation in leaves of bottle gourd landraces (M01 & M02) and hybrid varieties of cucurbits (Ca & GRH).

Table 5.1: Protein content in leaves of the bottle gourd landraces (M01 & M02) and cucurbits hybrids (GRH & CA) under simulated water stress.

Variety	Protein content (mg/g)		
	30% ETc	**60% ETc**	**100% ETc**
Landrace M01	0.720[b]	3.069[e]	4.277[g]
Landrace M02	2.275[c]	0.467[a]	2.643[d]
Mean	**1.50[a]**	**1.77[b]**	**3.46[c]**
Hybrid GRH	5.985[i]	8.633[j]	17.416[k]
Hybrid CA	4.837[h]	4.165[f]	8.604[j]
Mean	**5.411[a]**	**6.40[b]**	**13.01[c]**

LSD(P= 0.05) variety * water regime = 0.055

CV (%) variety * water regime = 0.60

[1]Values represented by the same letter are not significantly different from each other at $P < 0.05$.

5.2.5 Yield and yield component

The results showed no significant interaction (*P > 0.05*) among varieties and water regimes with respect to time to flowering (Table 5.2). However, it was observed that hybrid varieties flowered earlier than landraces. On average, landraces took 73 days after planting (DAP) to flower while hybrid varieties only took 51 DAP. Although not statistically significant, water stressed plants were observed to take a longer time to flower compared with unstressed plants. For all varieties, results showed that there were more male than female flowers produced. This may have resulted in low number of fruits produced for all varieties. A significant interaction (*P < 0.05*) between varieties and water regimes was observed with respect to leaf fresh mass. It was observed to increase with increasing water availability. Other yield parameters measured (branch number, stem fresh mass and dry and root fresh and dry mass) did not show a significant interaction between varieties and water regimes.

Table 5.2: Parameters associated with yield of different cucurbit varieties varieties in 30, 60 and 75% ETc.

Water regime	Variety	Brench no.	Leaf fresh mass (g)	Stem fresh mass (g)	Stem dry mass (g)	Root fresh mass (g)	Root dry mass (g)	Fruit number	Fruit mass	Time to flowering (days)	No. of male flowers	No. of female flowers
30% ETc	**M01**	3.30^{a}	0.76^{a}	1.3^{a}	0.16^{a}	0.51^{a}	0.145^{a}	0.33^{a}	0.58^{a}	83.3^{a}	1.83^{ab}	0.0^{a}
	M02	3.7^{a}	2.39^{a}	2.9^{a}	0.36^{a}	0.41^{a}	0.071^{a}	0.33^{a}	0.00^{a}	91.0^{a}	0.50^{a}	0.0^{a}
	GRH	11.7^{a}	2.49^{a}	9.1^{a}	1.36^{a}	1.66^{ab}	0.143^{a}	0.33^{a}	2.28^{a}	60.3^{a}	2.67^{ab}	0.25^{ab}
	CA	6.30^{a}	2.17^{a}	6.0^{a}	0.94^{a}	0.65^{ab}	0.106^{a}	0.00^{a}	0.00^{a}	51.3^{a}	1.50^{ab}	0.33^{ab}
	Mean	6.25^{a}	1.95^{a}	4.82^{a}	0.70^{a}	0.86^{a}	0.12^{a}	0.25^{a}	0.71^{a}	71.5^{a}	1.62^{a}	0.14^{a}
60% ETc	**M01**	9.3^{a}	4.64^{ab}	8.9^{a}	0.74^{a}	0.71^{ab}	0.084^{a}	0.00^{a}	0.00^{a}	57.3^{a}	1.58^{ab}	0.0^{a}
	M02	5.7^{a}	2.92^{ab}	3.5^{a}	0.44^{a}	0.41^{a}	0.086^{a}	0.00^{a}	0.00^{a}	91.7^{a}	0.50^{a}	0.0^{a}
	GRH	13.3^{a}	4.24^{ab}	12.5^{a}	1.87^{a}	1.71^{ab}	0.141^{a}	0.33^{a}	0.33^{a}	58.3^{a}	2.50^{ab}	0.17^{ab}
	CA	11.3^{a}	2.33^{a}	11.2^{a}	1.81^{a}	0.41^{a}	0.081^{a}	0.00^{a}	0.00^{a}	49.7^{a}	1.83^{ab}	0.83^{ab}
	Mean	9.90^{a}	3.53^{ab}	9.02^{a}	1.21^{a}	0.76^{a}	0.098^{a}	0.082^{a}	0.082^{a}	64.2^{a}	1.60^{a}	0.25^{a}
100% ETc	**M01**	18.3^{a}	6.00^{ab}	13.2^{a}	1.94^{a}	2.49^{b}	0.345^{a}	0.00^{a}	0.00^{a}	56.3^{a}	3.08^{b}	0.0^{a}
	M02	8.0^{a}	12.6^{b}	12.8^{a}	2.08^{a}	1.68^{ab}	0.296^{a}	0.00^{a}	0.00^{a}	61.7^{a}	1.42^{ab}	0.0^{a}
	GRH	15.7^{a}	4.74^{ab}	17.1^{a}	2.81^{a}	0.97^{ab}	0.585^{a}	0.67^{a}	1.10^{a}	39.7^{a}	2.75^{ab}	0.25^{ab}
	CA	18.7^{a}	8.37^{ab}	15.6^{a}	2.55^{a}	0.81^{ab}	0.232^{a}	1.00^{a}	4.96^{a}	48.0^{a}	2.00^{ab}	0.92^{b}
	Mean	15.2^{a}	7.93^{b}	14.7^{a}	2.34^{a}	1.49^{a}	0.36^{a}	0.42^{a}	1.51^{a}	51.4^{a}	2.31^{a}	0.29^{a}
$LSD_{(0.05)}$ FC*variety		**11.24**	**5.56**	**10.63**	**1.62**	**1.05**	**0.39**	**0.75**	**3.11**	**45.9**	**1.38**	**0.70**

Note: Numbers not sharing the same letter in the column differ at LSD (P= 0.05).

5.3 Discussion

The objective of this study was to evaluate the responses of bottle gourd landraces to water stress compared with commercial cucurbit hybrids. It has been reported that under sub-optimum conditions landraces may out-perform hybrid varieties because of their adaptability and continued selection under such conditions (Zeven, 1998). On the other hand, hybrid varieties have been shown to perform better under optimum conditions (Stoskopf, 1981).

Commercial cucurbit hybrid varieties had higher stomatal conductance than landraces. On average, stomatal conductance was observed to decrease with increase in water stress. According to Chaves (1991), stomatal control in stressed plants is an avoidance measure and forms part of initial defence response to water stress. The closing of stomata reduces transpirational losses and this in turn minimizes water losses through transpiration. The closing of stomata has been suggested to be an initial response to decreasing soil water content. This has been characterized by Forooq *et al* (2009) as a drought avoidance mechanism as well as being a characteristic of increased water use efficiency under drought stress (Blum, 2009). Thus a more reduced SC in landraces may symbolise sensitivity and / or avoidance mechanism of these landraces to water stress. In Bambara groundnut Collinson *et al*. (1997) argued that drought avoidance was achieved in part due to increase in stomatal control and regulation of transpirational losses.

Chlorophyll content is usually an indicator of plant nutritional status, photosynthetic capacity and developmental stage (senescence) (Filella and Penuelus, 1994). In this regard, a reduced carbon dioxide uptake as a result of stomatal closure during photosynthesis over time results to reduced chlorophyll content in stressed plants (Makakheri *et al*., 2010). Similar observations were made in this study where optimal conditions of water were shown to have high concentration of chlorophyll compared with stressed plants. Furthermore, chlorophyll content during development in all varieties was observed to drop significantly. This drop in chlorophyll concentration was attributed to the decreased in temperatures at the start and during winter season. Low winter temperature results in a decreased rate of stomatal opening accompanied by lowered rates of net photosynthesis (Drew and Bazzaz, 1982). With regard to the landraces and hybrid varieties, it was observed that CCI was on average higher in hybrid varieties than landraces.

Landraces, on average, had longer vines than hybrid varieties. However, leaf number was higher in hybrids than landraces. Simulated drought stress may have significantly reduced plant vine length and leaf number in a number of varieties. This may be also due to genetic and morphological difference of these varieties. Similar results in maize showing a reduction in height due to water stress were reported (Porro and Cassel, 1986). In cucumber plants, Doss *et al.* (1977) and Elkner (1985) showed also a significant decrease in vine length and leaf number which in turn greatly reduced fruit yield and quality. A decrease in plant vine length is due to a physiological decrease in cell enlargement Hsiao (1973) and the decrease in leaf number on the other hand is associated with the decrease in the leaf appearance rate (Sharp *et al*., 1979). With optimum and sub-optimum condition, varieties were shown to flower around the same time. However, differences in flowering period were observed between landraces and hybrid varieties with hybrid varieties being observed to take less time to flowering. The study showed the contrasting results with many findings in literature which have reported a delay in flowering in water stressed plant. Mwanamwenge *et al.* (1998) in faba bean observed a significant delayed in time to 50% flowering in most cultivars of faba bean while in few others; it was observed that stress had no effect on flowering. Furthermore, the male is to female flower ratio was observed to be high in all varieties. This resulted in a significant reduced and lower number of fruits that were produced. This is a common problem has been reported in cucurbits production. Lower number of fruits produced may also be due to controlled environmental condition which restricted the availability of pollinators. The morphological differences observed when plants are subject to water stress conditions were the results of physiological changes that take place within the plants.

Proline was shown to accumulate more in hybrid varieties than in landraces and its accumulation was observed to be more in stressed plants compared to unstressed. Similar observations were made de Ronde *et al.* (2000) in cotton plants where he observed accumulation of proline in stressed plants. Accumulation of this compatible solute has been well reported as plant response to environmental stress like water stress (Hasegawa *et al*., 1994; Van Rensburg and Kruger, 1994). Its accumulation however varies depending on the plant and variety. For instance in certain plant species it has been reported to play a major role in osmotic adjustment while in others like tomatoes it accounts for small concentration of total active solutes (Claussen, 2005).

Hybrid varieties were also shown to contain more proteins than landraces. With respect to ETc, proteins were observed to increase with the decrease in water stress. Many studies have shown that in high rainfall areas or in high irrigated areas nitrogen is usually a deficiency in plants because it leaches down the profile during the rain which can limit the synthesis of the proteins. On the other hand, it has been observed that drought stress activates certain proteins in plants. Many studies (Hsiao, 1973) have pointed the reduction of protein synthesis in the vegetative tissue with increase water stress. However, some studies (Hsiao, 1973) have reported increase in proteins.

CHAPTER 6

A PRELIMINARY ASSESSMENT OF NUTRITIONAL VALUE OF BOTTLE GOURD LANDRACES AS A POTENTIAL FOOD SECURITY CROP

6.1 Introduction

Africa is the only developing continent in the world with increasing numbers of underweight, stunted and hungry people (Schonfeldt and Gibson, 2009). Sub-Saharan Africa has the highest prevalence of malnutrition with one in three people being chronically hungry (Uusiku *et al*., 2010). In South Africa, malnutrition is attributed to food insecurity with one in two households experiencing hunger (Hart, 2011; Schonfeldt and Pretorius, 2011). According to Labadarios *et al*. (2011), the diet of South Africans often lacks food variety and micronutrient deficiencies especially vitamin A, iron and zinc are wide spread (Labadarios, 2007). Issues surrounding food insecurity have been associated more with a lack of access to nutritious foods (Hart, 2011) rather than availability of food at a country level. In order to have a healthy nation that can promote development, the relationship between nutrition and health should be reinforced (Achu *et. al*., 2005). One way that has been proposed is to re-look at the previously neglected African leafy vegetables (Modi *et al.*, 2006; Schonfeldt and Pretorius, 2011; Odhav *et al*., 2007).

South Africa has a large number of underutilized crop species of which knowledge of nutritional value is relatively under-researched (Odhav *et al*., 2007). Urgent attention should be given to these crops if they are to be promoted for utilization by rural households. Reportedly, these crops may grow on soils with low fertility, are relatively drought tolerant, provide good ground cover and can be harvested within a short period of time after planting (Shiundu, 2002). Improved availability of knowledge on nutritional status has been proposed to encourage the cultivation and consumption of these crops, more especially those with high nutrient content (Maunder and Meake, 2007; van Jaarsveld *et al.*, 2014). This is a serious challenge that needs to be addressed in order to promote the cultivation of these crops and alleviate poverty, health problems and malnutrition in the country. Consuming leafy vegetables like bottle gourd can offer a solution to this problem.

Several studies conducted in Sub-Saharan Africa (Odhav *et al.,* 2007; Schonfeldt and Pretorius, 2011; Uusiku, 2010) have evaluated the nutritional content of several traditional underutilized crops and their potential contribution to household nutrition and food security. Some progress has been made for other leafy vegetables such as: *Amaranthus dubius, Amaranthus hybridus, Amaranthus spinosus, Asystasia gangetica, Bidens pilosa, Centella asiatica, Ceratotheca triloba, Chenopodium album, Cleome monophylla, Cucumis metuliferus, Emex australis, Galinsoga parviflora, Justicia flavaandv*. However, bottle gourd has not benefited from these efforts and remains under-researched. This is despite knowledge that bottle gourd is well–known for its nutritional composition and medicinal properties in the other countries. The edible portion of bottle gourd contains carbohydrates, proteins, fats and minerals including phosphorous and calcium (Ahmad *at al.,* 2011). It is a good source of vitamin B complex, vitamin C (ascorbic acid), β-carotene, amino acids and pectin dietary soluble fibres (Habibur-Rahman, 2003; Duke, 1999; Modgil *et. al*., 2004). This information is derived done in other countries; South African bottle gourd landraces remain underutilized in favour of introduced species whose nutritional information is well defined. It is also difficult to superimpose information from other places to our local conditions because of landrace variability. This highlights the need for research on local bottle gourd landraces in order to successfully promote them as viable alternatives to introduced elite cultivars.

It was hypothesized that local bottle gourd landraces may have similar nutritional value as popular commercial cucurbit varieties. The primary aim of the study was to determine the leaf nutritional value of bottle gourd landraces at different stages of crop growth, and in doing so, determine optimum harvest times. Secondary to this, the study evaluated nutritional potential of bottle gourd leaves by estimating their potential contribution to dietary requirements. Such information could be a starting point in developing valuable knowledge about the crop, allowing better food selection and improvement of nutrient status of the diet of local people in South Africa.

6.2 Results

6.2.1 Stomatal conductance and chlorophyll content

Stomatal conductance was shown to vary significantly ($P < 0.001$) with time (Fig. 6.1). For all varieties, SC decreased over time. Plants subjected to sequential harvesting generally had high levels of stomatal conductance during the later stages of crop growth.

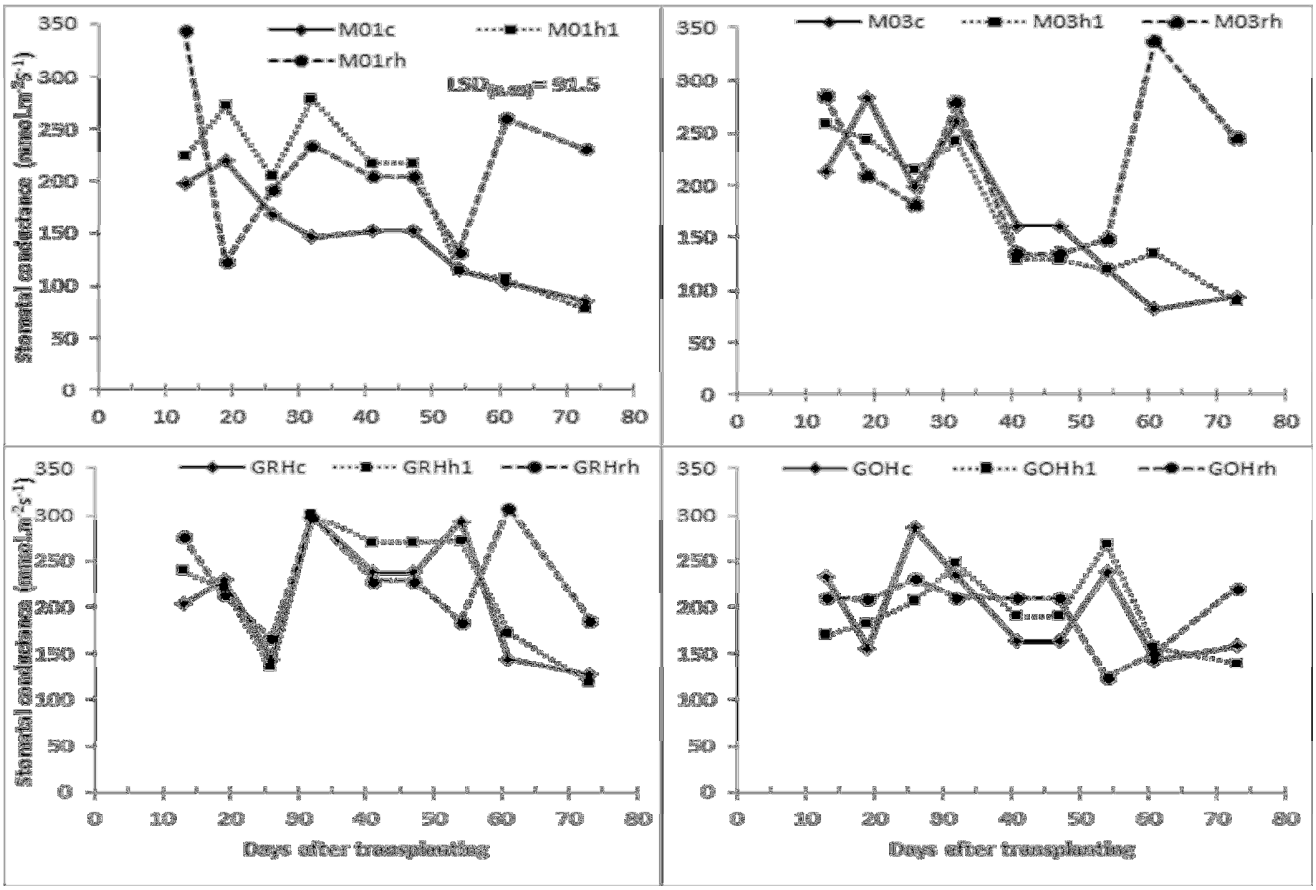

Figure 6.1: Stomatal conductance (mmol m^{-2} s^{-1}) of bottle gourd landraces (M01 & M03) and pumpkin hybrids (GRH & GOH). C denotes control plants; h1 – plants harvested once and rh – plants harvested repeatedly.

There was a significant ($P < 0.05$) interaction among varieties over time with respect to chlorophyll content index (CCI) (Fig. 6.2). For all varieties, CCI was shown to be low during the early crop growth stages and it gradually increased with time, reaching a peak and then declining steadily over time.

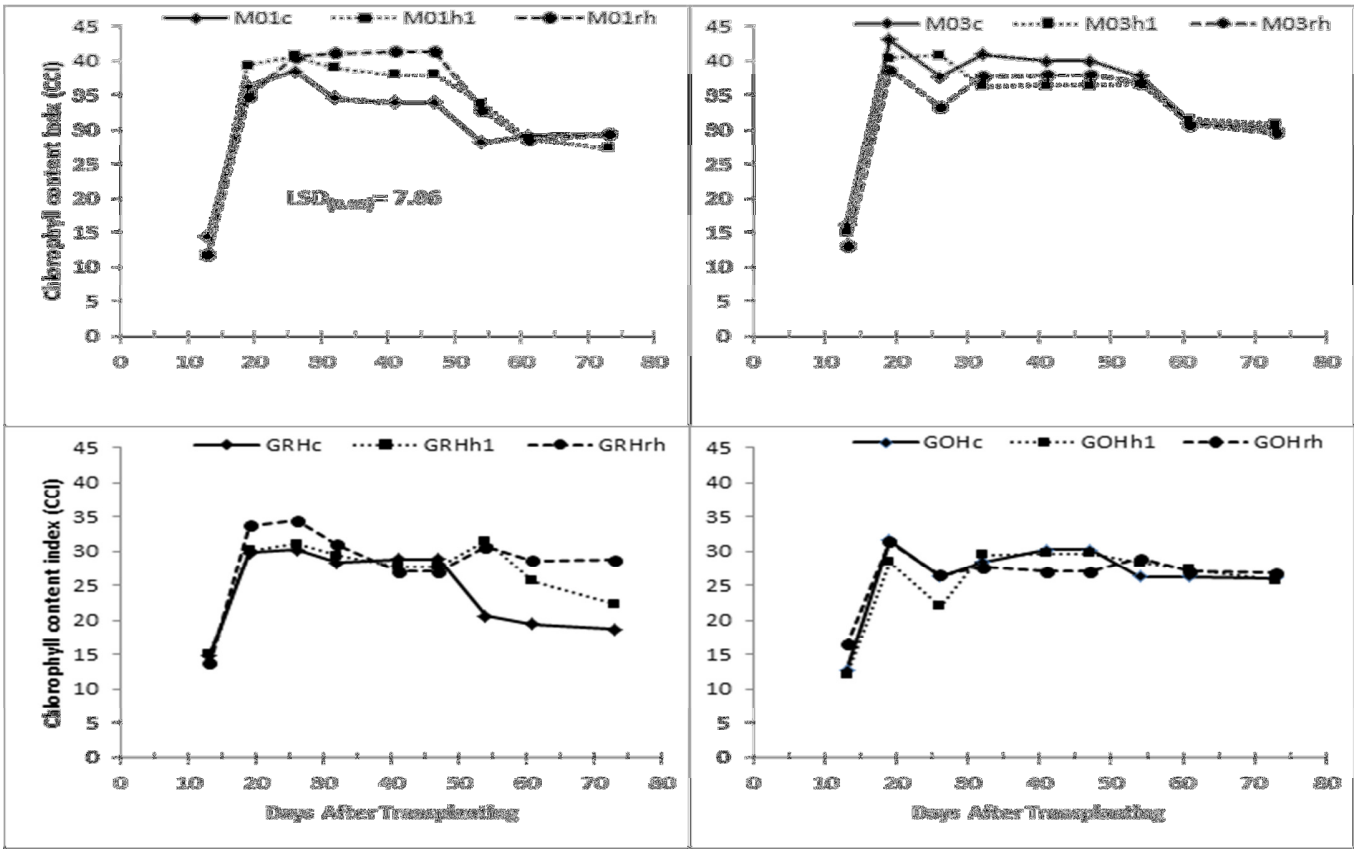

Figure 6.2: Comparison of bottle gourd landraces (M01 & M03) and pumpkin hybrids (GRH & GOH) CCI of controlled plants (c), harvested once plants (h1) and repeated harvested plants (rh) over time.

6.2.2 Crop growth

Vine length varied significantly ($P < 0.001$) over time (Fig. 6.3). On average, the landraces had longer vines than hybrid varieties with landrace M01 having the longest vines. Differences in vine length were not clear during early establishment; however, as growth proceeded clear differences were observed among landraces. Sequential harvesting appeared to inhibit vine growth of landraces while in hybrid varieties the opposite was true; hybrid GOH for instance, sequentially harvested plants had the longest vines.

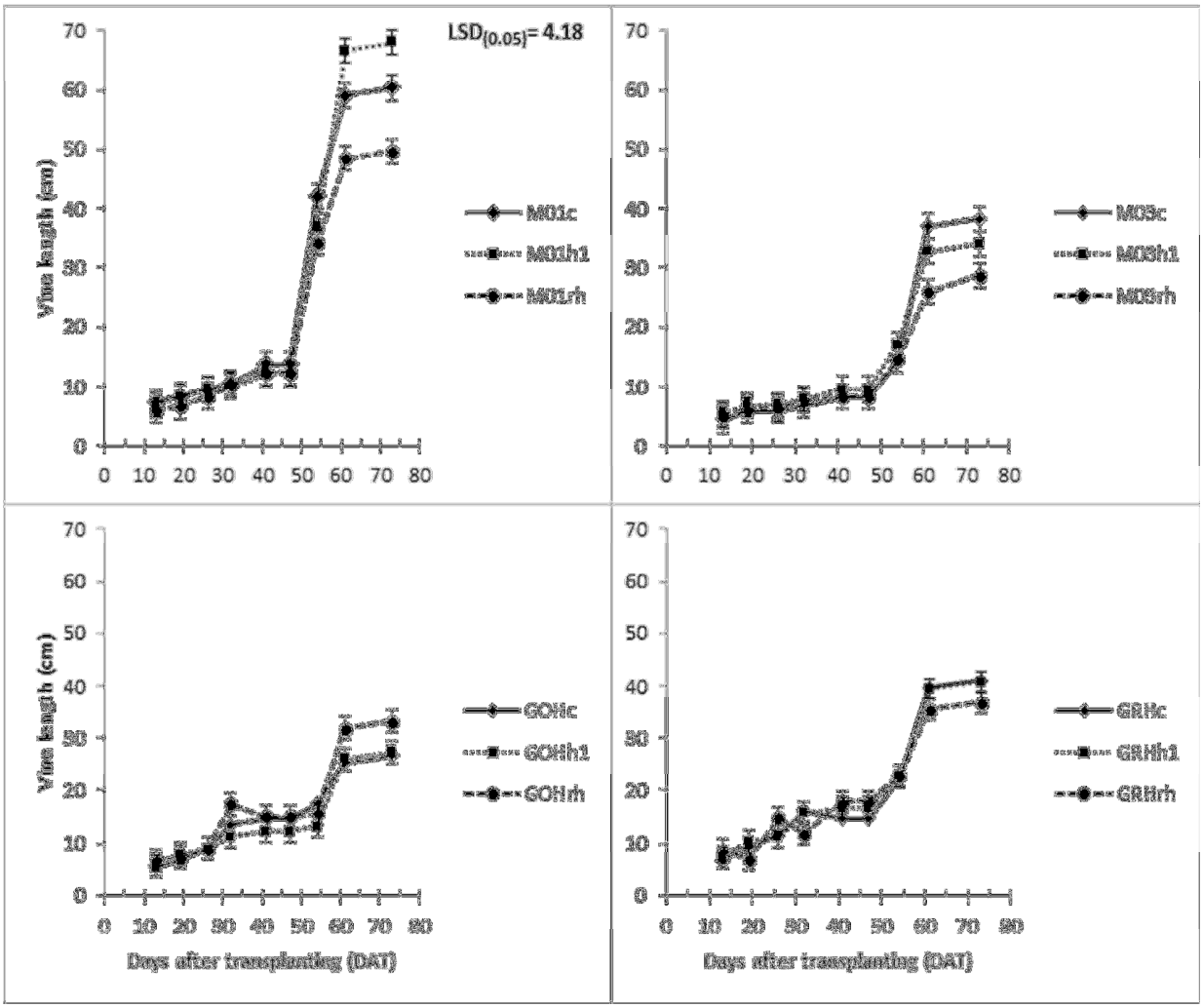

Figure 6.3: Comparison of bottle gourd landraces (M01 & M03) and pumpkin hybrids (GRH & GOH) vine length of controlled plants (c), harvested once plants (h1) and repeated harvested plants (rh) over time.

Highly significant differences ($P < 0.001$) were also observed among varieties with respect to leaf number, where hybrid varieties had more leaf number than landraces (Fig 6.4). Sequential harvesting significantly lowered final leaf number in landraces than hybrid varieties. In hybrid varieties, pronounced lowering in leaf number was observed for hybrid GRH in response to sequential harvesting. Hybrid GOH showed no significant differences between plants that were harvested once and those harvested repeatedly (Fig 6.4). Overall, harvesting the cucurbits once

resulted in a positive response to leaf production. This was clearer in hybrid varieties than landraces.

Hybrid varieties (GRH & GOH) started to flower from day 45 after transplanting while landraces on the other hand were observed flower from 55 days on wards. Flowering corresponded with the decrease of chlorophyll content in both landraces and hybrids also from 50 days (Fig 6.2). Leaf and vine growth at this period was also observed to be reduced (Fig 6.3 & 6.4).

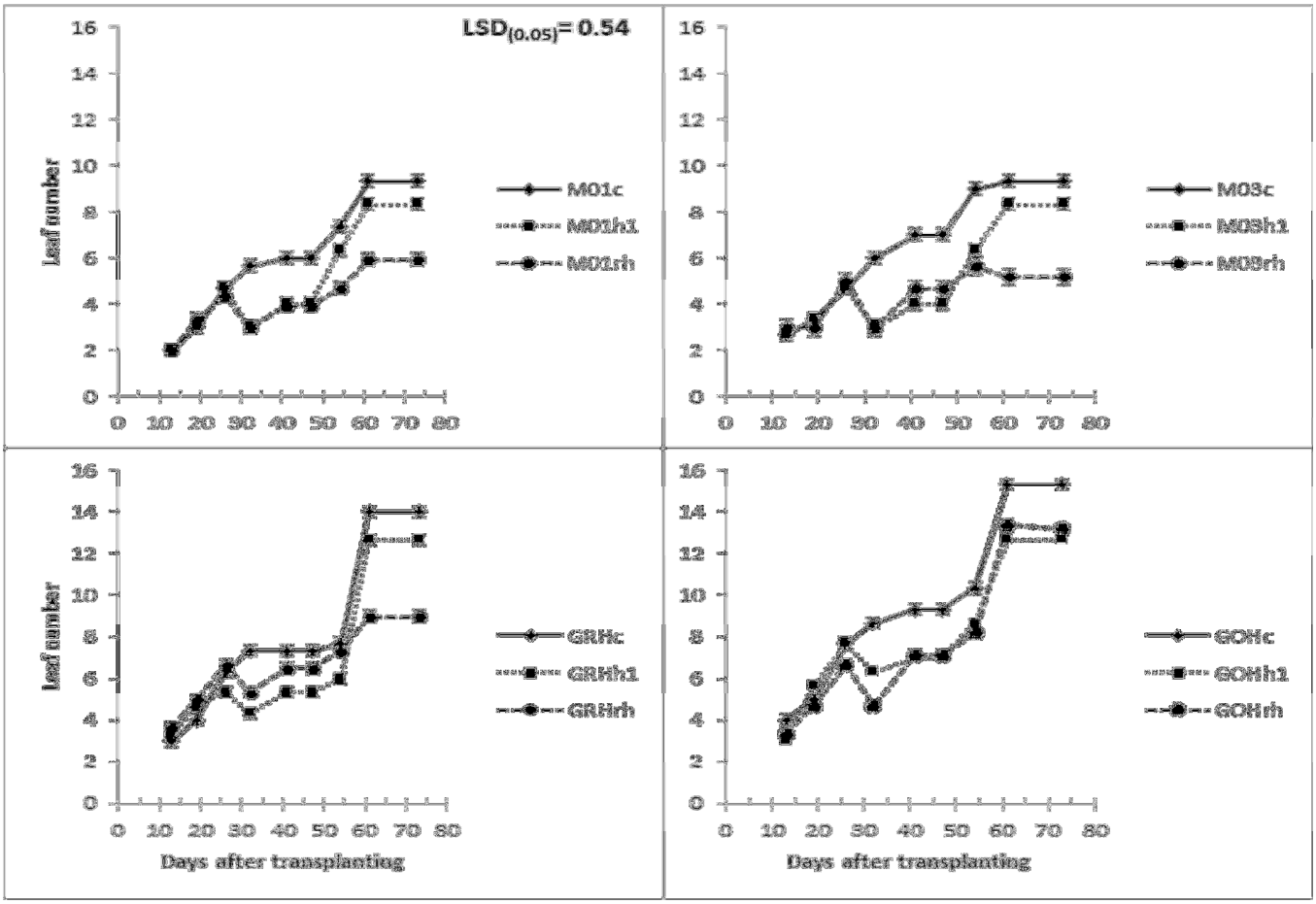

Figure 6.4 : Comparison of bottle gourd landraces (M01 & M03) and pumpkin hybrids (GRH & GOH) leaf number of controlled plants (c), harvested once plants (h1) and repeated harvested plants (rh) over time.

Highly significant differences (*P < 0.001*) were observed across varieties with respect to leaf area for plants that were sequentially harvested at 54 days after transplanting (Fig. 6.5). Landraces had the highest leaf area compared to hybrid varieties; leaf area was highest for landrace M01 and lowest for hybrid GOH. Hybrid varieties had the more leaves but however, their leaf area was small compared that of landraces. The opposite was true for landraces.

Highly significant differences (*P < 0.001*) were observed among varieties for fresh mass (Fig. 6.6). Landraces were shown to have higher fresh mass than hybrid varieties. Landrace M03 had the highest fresh mass (16.21 g) and the lowest (4.40 g) was observed in hybrid GOH. Differences were also observed between control, plants that were harvested once and sequential harvested plants. Control plants were shown to have more fresh mass than plants that were sequentially harvested once and repeatedly, respectively. For dry mass, there were no significant differences (*P > 0.05*) observed among varieties. Although there were no significant differences, dry mass was observed to be higher for plants that were sequentially harvested relative to control plants.

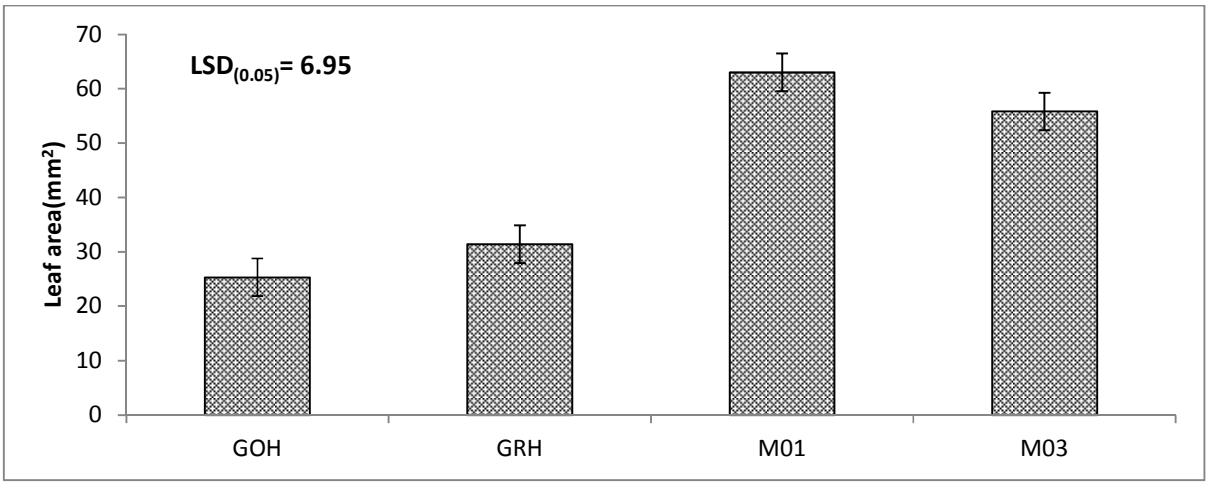

Figure 6.5: Leaf area of landraces (M01 & M03) and hybrid varieties (GRH & GOH) observed 54 days after transplanting.

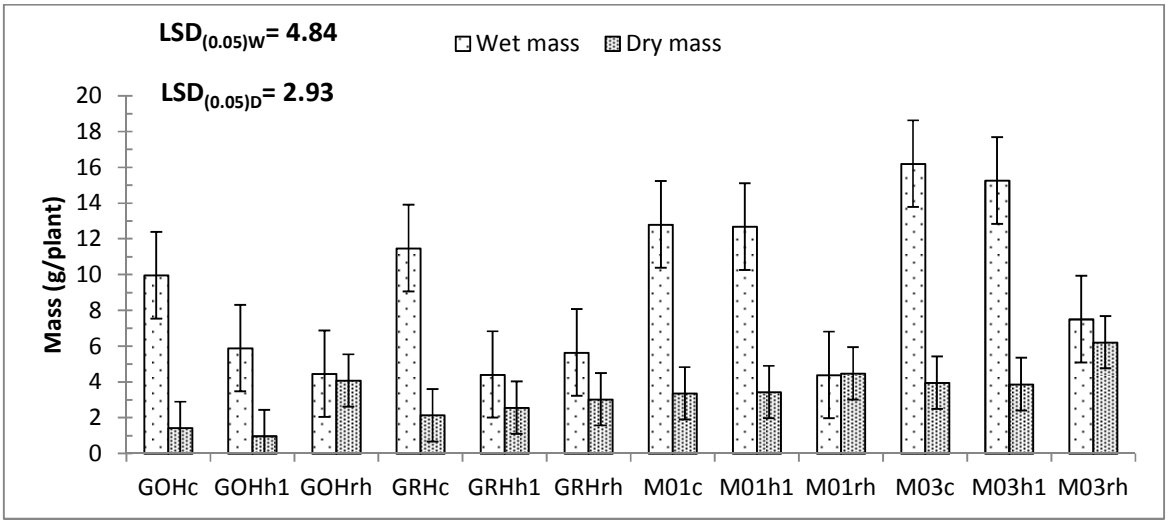

Figure 6.6: Comparison of bottle gourd landraces (M01 & M03) and pumpkin hybrids (GRH & GOH) wet and dry weight of controlled plants (c), harvested once plants (h1) and repeated harvested plants (rh) measured 54 days after transplanting.

6.2.3 Mineral levels

Mean values for mineral content of nutritional importance are presented in Tables 6.1 and 6.2. Hybrid varieties were shown to contain higher levels of minerals than landraces (Table 6.1). In response to sequential harvesting, hybrid varieties also had higher nutrient content (Table 6.2). Both landraces and hybrids contained remarkably high amounts of calcium (> 1 000 mg 100 g^{-1}). With respect to sequential harvesting, Ca levels were also observed to be high; Ca levels were even higher in hybrid varieties where it averaged around 5 000 mg 100 g^{-1} per harvest. The range in calcium was 1 826 between hybrid varieties and landraces (Table 6.1). The phosphorus content in leaves did not vary greatly between landraces and hybrid varieties; it ranged between 306 mg 100 g^{-1} (Landrace M03) and 599 mg 100 g^{-1} (hybrid GOH) (Table 6.2).

With response to sequential harvesting phosphorus content was observed to relatively increase with time in all varieties (Table 6.2). High nitrogen content (> 1 000 mg 100 g^{-1}) was observed in all varieties, with hybrid varieties having higher N content than landraces (Table 6.1). However, there was a huge range of 523 mg 100 g^{-1} between hybrid varieties and landraces in nitrogen concentration. Within landraces there was a very narrow range of 60 mg 100 g^{-1} while in hybrid

varieties the range was 271 mg 100 g^{-1}. With respect to sequential harvesting, N concentration was shown to increase in landraces while in hybrid varieties it fluctuated within narrow ranges. Both landraces were shown to be excellent sources of potassium (> 1 000 mg 100 g^{-1}) (Table 6.1). A huge range of 739 mg 100 g^{-1} was observed between the landraces and hybrids, with hybrid varieties having higher K concentration (Table 6.1). Within landraces, a narrow range of 288 mg 100 g^{-1} was observed while in hybrid varieties there was a huge range of 669 mg 100 g^{-1}. With respect to sequential harvesting, K was observed to be lower with small fluctuations (Table 6.2). Magnesium ranged from 451 mg 100 g^{-1} in landraces to 1 902 mg 100 g^{-1} in hybrids varieties. Magnesium was shown to be relatively constant when leaves were sequentially harvested (Table 6.2). All varieties were shown to contain low levels (< 0.6 mg 100 g^{-1}) of copper and in both hybrid varieties and landraces the level was, on average, equal. Iron concentration between landraces and hybrids ranged between 13 mg 100 g^{-1} and 17 mg 100 g^{-1} in sequentially harvested plants while in control plants it ranged between 8 mg 100 g^{-1} and 11 mg 100 g^{-1} with hybrid varieties having relatively higher Fe levels. For both landraces and hybrids, leaf Fe content was observed to fluctuate in response to sequential harvesting.

Table 6.1: Concentration of selected minerals (mg/100g) in raw leaves of bottle gourd landraces and commercial cultivars of pumpkins in control plants.

Variety	N	P	K	Ca	Mg	Na	Zn	Cu	Mn	Fe	Al
	(mg)										
M01c	1087	322	1111	2481	519	6.1	3.6	0.4	7.5	9.5	13.6
M03c	1027	306	1399	2021	451	4.0	3.6	0.5	7.5	6.5	9.5
Mean	**1057**	**314**	**1255**	**2251**	**485**	**5**	**3.6**	**0.4**	**7.5**	**8**	**12**
GRHc	1167	328	1660	5456	1902	17.0	8.1	0.3	17.9	13.2	22.6
GOHc	1993	599	2329	2699	975	8.4	6.3	0.6	7.6	8.2	11.2
Mean	**1580**	**464**	**1994**	**4077**	**1439**	**13**	**7**	**0.4**	**12.7**	**11**	**17**

*100 g of leaf sample equals about 3 cups

Table 6.2: Concentration of selected minerals (mg/100g) in raw leaves of bottle gourd landraces and commercial cultivars of pumpkin in response to sequential harvesting**.**

Variety	DAT	N	P	K	Ca	Mg	Na	Zn	Cu	Mn	Fe	Al
		(mg)										
Landrace M01	26	2033	226	2010	2666	688	10.5	4.9	0.2	8.0	21.5	17.9
	39	1460	193	1450	2713	782	6.2	5.6	0.3	9.3	9.1	9.1
	54	1022	244	871	2922	721	8.2	4.5	0.3	10.3	9.7	22.2
	65	1628	486	1339	3140	743	9.2	5.3	0.5	10.8	13.1	17.2
	73	1904	533	1692	2622	578	6.1	4.3	0.7	8.8	7.6	7.8
Landrace M03	26	1905	218	2144	3467	736	10.6	5.7	0.3	8.9	26.1	25.4
	39	1460	222	1592	2077	577	6.2	5.5	0.4	8.2	9.2	8.6
	54	1097	289	1118	2051	483	4.1	4.1	0.3	7.8	16.9	21.9
	65	1444	496	1655	2566	678	5.7	5.1	0.6	9.7	10.8	15.4
	73	1635	494	1690	2300	515	6.3	4.0	0.7	8.7	6.5	5.1
Landrace mean		1559	340	1556	2652	650	7.0	5.0	0.4	9.1	13	15
Hybrid GRH	26	1532	381	2495	5976	1722	30.0	10.1	0.3	12.6	29.3	29.1
	39	1605	425	2411	5713	1926	18.6	8.9	0.4	13	10.7	11.4
	54	1534	354	1838	5769	1958	22.7	8.7	0.3	15	25.1	41.6
	65	1490	375	2046	6619	2500	23.3	6.1	0.4	26	12.6	30.5
	73	1718	493	1766	4505	1840	10.5	6.5	0.5	14.4	13.0	9.6
Hybrid GOH	26	1584	322	2325	5598	1577	26.8	9.3	0.3	10.1	28.0	29.9
	39	1601	370	2560	5912	2127	25.0	6.9	0.4	18.7	9.8	10.4
	54	1660	387	2093	6504	2341	33.1	6.6	0.4	20.9	17.2	41.6
	65	1592	475	1839	6305	2449	20.3	9.7	0.4	19.1	22.1	52.2
	73	1814	546	2015	4698	1784	10.5	5.7	0.5	18.3	6.9	10.9
Hybrids mean		1613	413	2139	5760	2022	22	8.0	0.4	16.8	17	27

*100 g of leaf sample equals about 3 cups.

Table 6.3: Nutrient content per 100g edible portion of bottle gourd landraces leaves and hybrids of pumpkin.

Variety	N	P	K	Ca	Mg	Na	Zn	Cu	Mn	Fe	Al
	(mg)										
Landrace M01	1609	336	1472	2813	702	8	4.9	0.401	9.4	12.17	14.8
	(1011)	(340)	(1139)	(518)	(204)	(4.4)	(0.6)	(0.5)	(2.8)	(13.9)	(14)
Landrace M03	1508	344	1640	2492	598	6.6	4.91	0.46	8.7	13.92	15.3
	(808)	(278)	(1026)	(1416)	(253)	(6.5)	(1.7)	(0.4)	(1.9)	(10.4)	(20)
Hybrid GRH	1576	406	2111	5716	1989	21	8.03	0.389	16.2	18.15	24.4
	(106)	(139)	(729)	(2114)	(778)	(19.5)	(4.0)	(0.2)	(1.8)	(18.6)	(32)
Hybrid GOH	1650	420	2166	5803	2056	23.1	7.62	0.389	17.4	16.8	29
	(230)	(224)	(721)	(1806)	(872)	(22.6)	(4.0)	(0.2)	(10.8)	(21)	(42)

Values are mean and (range) of five samples analysed individually.

Table 6.4: Estimated[1] amount of nutrient retained after cooking 100g leaves of bottle gourd landraces and hybrids of pumpkin.

Variety	Proteins	P	K	Ca	Mg	Na	Zn	Cu	Mn	Fe	Al
	(mg)										
Landrace M01	0.9	302	1325	2672	667	8	5	0.4	9.4	12	14
Landrace M03	0.9	310	1476	2367	568	6	5	0.4	8.7	13	15
Hybrid GRH	0.9	365	1900	5430	1890	20	8	0.4	16.2	17	23
Hybrid GOH	0.8	378	1949	5513	1953	22	7	0.4	17.4	16	28

[1]The nutrient content of cooked leaves and was calculated from the mean nutrient value in raw leaves using the following retention factors: calcium, sodium, magnesium, copper, iron zinc, copper and manganese = 0.95; phosphorus and potassium = 0.90 (USDA Table of nutrient (USDA Table of nutrient retention factors, 2007).

Table 6.5: Estimated nutrient contribution of an average portion size[1] of leaves of bottle gourd landraces and two commercial cultivars of pumpkin to the RDA for children aged 4-8 years and woman 19-30 years.

	Unit	Landrace M01	Landrace M02	Hybrid GRH	Hybrid GOH
Proteins	% RDA 4-8y[2]	0.85	0.85	0.85	0.76
	% RDA 19-30y	1.5	1.5	1.5	1.3
Phosphorus	% RDA 4-8y	42	43	50	52
	% RDA 19-30y	43	44	52	54
Potassium	% Al 4-8y[3]	31	35	45	46
	% Al 19-30y	37	41	52	54
Calcium	% RDA 4-8y	301	266	470	477
	% RDA 19-30y	347	308	406	716
Magnesium	% RDA 4-8y	355	302	1006	1040
	% RDA 19-30y	217	184	614	635
Sodium	% Al 4-8y	0.6	0.45	1.5	1.6
	% Al 19-30y	0.7	0.52	1.7	1.9
Zinc	% RDA 4-8y	69	69	111	97
	% RDA 19-30y	89	89	95	82
Copper	% RDA 4-8y	67	67	67	67
	% RDA 19-30y	58	58	58	58
Manganese	% Al 4-8y	546	520	973	1047
	% Al 19-30y	679	628	1172	1255
Iron	% RDA 4-8y	85	92	115	108
	% RDA 19-30y	200	212	275	262
Aluminium	% RDA 4-8y	ND[4]	ND	ND	ND
	% RDA 19-30y	ND	ND	ND	ND

[1] 90g cooked leaves of bottle gourd and pumpkin for young children and 130g cooked bottle gourd leaves and pumpkin using yield factor of 1.3 from raw to cooked.

[2] RDA= recommended daily allowance – average daily dietary intake level that is sufficient to meet the nutrient requirement of nearly all (97-98%) health individuals in a particular life stage and gender group.

[3] Al= adequate intake as there is no RDA. Al is a recommended intake value that is assumed to be adequate (Otten *et al*., 2006 and Ross *et al*., 2011).

[4] ND= not determined

Nutrients retention factors were used to calculate the nutrients retained for cooking 100g raw leaves (Table 6.4). The retention factors used to account for amount of nutrients retained after cooking are estimates as cooking method (temperature and time) all effect cooking method (Greenfield and Southgate, 2003). To estimate dietary reference intakes (DRI), two age groups were selected in this study (Table 6.5). Namely, children of 4-8 year old and 19-30 year old non-pregnant, non-breast feeding females because young children and woman of child-bearing age are nutritional most vulnerable (van Jaarsveld *et al*., 2014). The results from the estimated nutrient contribution of an average cooked portion size of both landrace and pumpkin cultivar leaves (90 g) of 4-8-year-old child showed that they provide less than 2% of the recommended daily allowance (RDA) of proteins. However when comparing other nutrients, both landraces and pumpkin cultivars were shown to be good sources of calcium, iron, magnesium, zinc, copper and manganese for this age group. On these, calcium, magnesium and manganese provided more that 100% of the RDA. Phosphorus and potassium were found to contribute a reasonable amount (> 30 and < 50%) to the RDA. Similar trends were observed for calculated estimate of an average cooked portion size of both landraces and pumpkin cultivar leaves (130 g) in nutrient content and their percentage contribution to RDA of 19-30 year old woman. In both age groups, sodium was found to be less than 1% and 2% of the RDA in both landraces and hybrid varieties of pumpkin respectively.

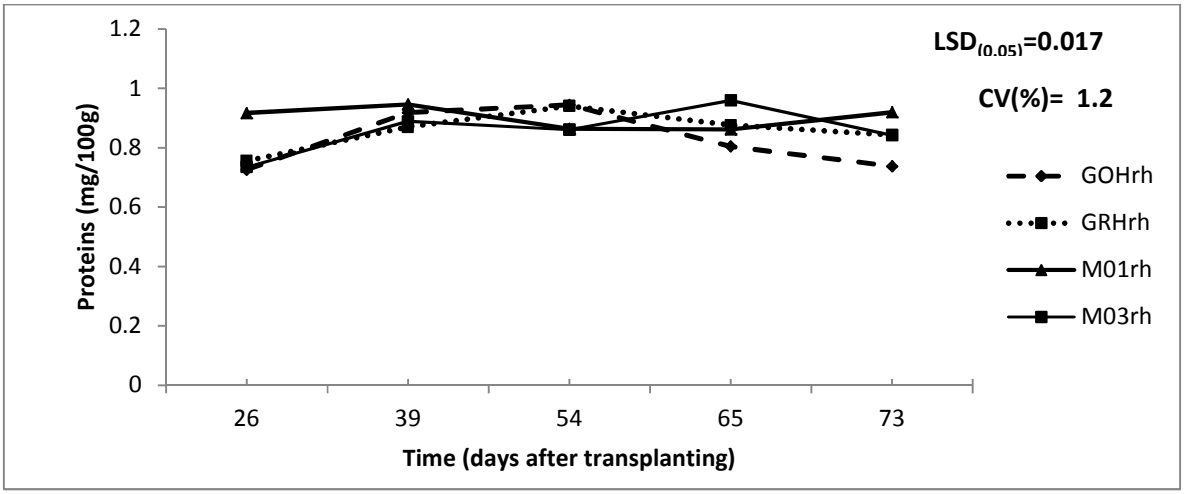

Figure 6.7: Protein content in leaves of bottle gourd landraces (M01 & M03) and hybrid varieties of pumpkin (GOH & GR).

Highly significant differences ($P < 0.001$) were observed among varieties over time with respect to protein content (Fig. 6.7). Protein content, for all varieties, was observed to fluctuate over time. Landrace M01 had higher protein content at 26 and 39 days after transplanting, respectively, than all other varieties. Protein content of hybrid varieties was observed to drop significantly at 54 days after transplanting while landraces in that period were observed to increase in amount of protein.

6.3 Discussion

Bottle gourd, as a leafy vegetable, it can be harvested at any time during the stage of crop growth and development; however, data on nutritional value with plant age is limited for landraces. The objective of this study was to determine the nutritional changes of the crop at different time intervals and in doing so, determine the optimum harvest time.

The results found significant higher levels of nutrients in commercially produced varieties when compared with landraces. These results were similar to the observation made by Modi (2009) that introduced species generally contain more nutrients compared to their native counterparts. This may also be the due to the fact that hybrid varieties have been breed to produce higher amount of nutrients and differences also may also be attributed to species differences. With respect to sequential harvesting of the leaves, the analysis of the results found variations in nutrient content over time depending on the type of nutrient in question. Although notable inconsistences were observed for different nutrients over time, it was evident that high nutrient content can be attained 39 days after transplanting and before flowering because the crop flowers around 50 DAP. These results were similar to those obtained by Modi (2007) in amaranths species where early stage of crop development was observed to contain appreciable amount of plant nutrients. In the early stages of plant growth and development, leaves are more of sinks than sources of nutrients. However, other phenological stages like flowering and fruit development may affect source sink relationship. Older leaves may act as a source of nutrients to the developing floral structures and fruits (Venkateswarlu and Visperas, 1987). From human and animal nutrition perspective these results could mean that bottle gourd should be harvested during early stage of crop development

before the onset of flowering period to attain highest nutrients in the leaves. However, since the leaves of the crop are mixed with young immature fruits this stage is usually compromised and leaves are consumed after flowering and fruit development.

Lower proteins observed in this study were contrary to those observed by Schonfeldt and Pretorius (2011) in *Cucurbita maxima*. This was also in disagreement to the findings by Kruger *et al*. (1998) and Langenhoven *et al.* (1991) where they observed higher amounts of proteins in locally grown leafy vegetables compared with their commercial produced counterparts. Differences observed in proteins and other nutrients could be attributed to difference in growth factors, growth development stages and redistribution between new developing and developed leaves. Variation of nutrients observed in different studies may also be associated with handling and processing after harvesting and different methods used in nutrient quantification (Gupta *et al*., 2004). As such, van Jaarsveld *et al*. (2014) advised that nutrients comparison should be interpreted with great caution. In addition, higher amounts of certain nutrients may not necessarily mean that they are bio-available, especially in older leaves because they may be associated with anti-nutrients (oxalates and phytates) which reduce their bio-availability (Fincham *et al.,* 1986).

Furthermore, the crop (bottle gourd) was shown to contribute a significant appreciable amount of nutrients to RDA of most nutrients. This suggests the potential of the crop to contribute nutritionally and meet RDA of the rural communities. Contrary to this study, van Jaarsvel *et al*. (2014) reported a significant lower amount of RDA of iron in both age groups 4-8 year- old children and 19-30 year-old woman in pumpkin leaves. Large variation and high iron content in low lying leaves such as that of pumpkin could be due to soil contamination and van Jaarsveld *et al*. (2014) advised that it should be interpreted with great caution. In their study, the leaves were meticulously socked and washed with several changes of water before the samples were homogenised while in the current study leaves after harvest were taken into freeze drier without washing.

The results of crop physiology and morphological responses of crop to sequential harvesting indicated that the CCI was not affected by sequential harvesting of leaves but was observed to be affected by phenological stage of development. The observed reduction in CCI corresponded with flowering of plant species and was also associated with the decline in nitrogen content. On the other hand, leaf number was more reduced in landraces than in hybrid varieties which responded by producing more leaf number but of significant lower surface area when compared to landraces in response to leaf harvesting. From these observations, higher SC observed in the later stage of crop development can be ascribed plants acquiring more energy for the production of new leaves due to sequential pressure of leaf harvesting.

CHAPTER 7

FIELD PERFORMANCE OF BOTTLE GOURD LANDRACES IN WINTER AND EARLY SUMMER PLANTING UNDER RAIN FED CONDITION

7.1 Introduction

Planting time is one of the cultural practices that results in greatest differences in growth, development and yield without involving any additional cost such as fertiliser and/ irrigation systems. Optimum planting date varies according to the type of cultivar planted. There is no information in scientific literature describing optimum planting times of bottle gourd owing to its status as neglected underutilised species (NUS). In South Africa, the crop is usually planted by subsistence farmers during the summer season as an intercrop with maize, which is the staple crop. This practise is also the same for other *Cucurbitaceae* family members such as pumpkin and water melons which are seldom planted as sole crops but rather intercropped with maize. Climate change and variation have resulted in shifts in planting dates, and this has affected farmers who have been unable to respond to these changes (Mabhaudhi *et al*., 2013). In addition, predicted effects of climate change and concerns around food security have recently shifted interest to NUS (Mabhaudhi, *et al*., 2013). As such, there is now a need to generate agronomic information on the agronomy of NUS like bottle gourd. As previously stated, planting date selection is as an important yield determining factor and currently there is no information describing optimum planting dates of NUS like bottle gourd.

Environmental factors have a profound influence on growth, development and yield of any crop (Agele *et al.,* 1999, 2002), with temperature and soil water content considered to be major factors driving these developments (Tingle and Chandler, 2003). Most plants are exposed to extreme water deficit in semi– and arid environments in South Africa due to erratic and uneven rainfall distribution. Drought stress can occur at any time during the growing season and the sensitivity to this effect in crops varies depending on stage of crop development (Laker, 2007). Under these conditions, planting date selection has been suggested as a useful tool for managing water stress (Mabhaudhi and Modi, 2010; Sinefu, 2011). Early planting, before the onset of rainy season (August), resulted in significantly higher emergence in maize landraces compared with commercial hybrid counterpart (Mabhaudhi and Modi, 2010). However, highest leaf number and

height was attained for the optimum planting date with late and early planting dates having the least heights and leaf numbers (Mabhaudhi and Modi, 2010). Zulu (2010) observed slow emergence in wild water melon planted early due to colder temperatures. Delayed and/or slow emergence due to cool conditions has been observed to also encourage pathogen development; this may be of particular relevance to bottle gourd since its germination is epigeal. In this study it was hypothesized that different planting seasons, due to their great variability in weather, will provide different growing conditions. The objective of the study was to compare winter and summer planting of bottle gourd landraces and commercial varieties of other cucurbits were used as check varietis.

7.2 Results

7.2.1 Meteorological data

Monthly average temperatures and rainfall measured from April to June showed a significant decrease (Fig. 7.1). This period (April to June) coincided with the first planting date, planted on the 9th of April 2013. The lowest mean temperature measured between June and August was less than 10°C. Rainfall and temperature started to increase from August. This coincided with planting of the summer trial.

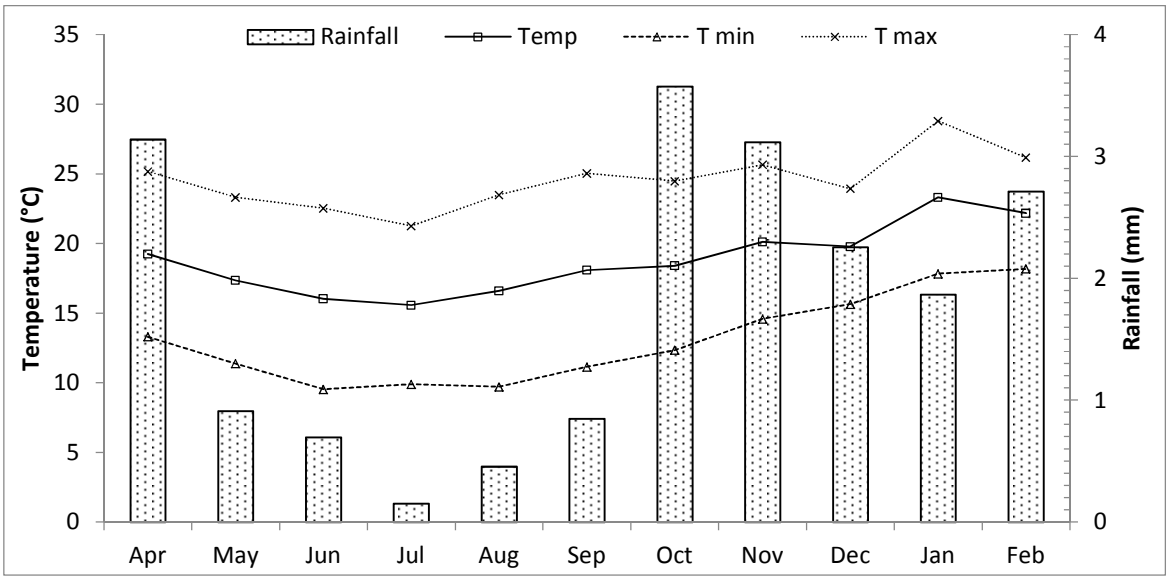

Figure 7.1: Monthly average temperatures (maximum and minimum) and rainfall recorded at Ukulinga Farm from April 2013 to February 2014.

7.2.2 Crop establishment

Differences were observed in time taken to emergence between winter and summer trial (Fig. 7.2). For the winter trial, plants took 2 WAP to emerge while for the spring/summer trial, plants took 4-5 WAP to emerge. On average, hybrid varieties emerged faster than landraces. Hybrid GRH had the poorest emergence during winter trial while for the spring/summer trial it improved by 50%.

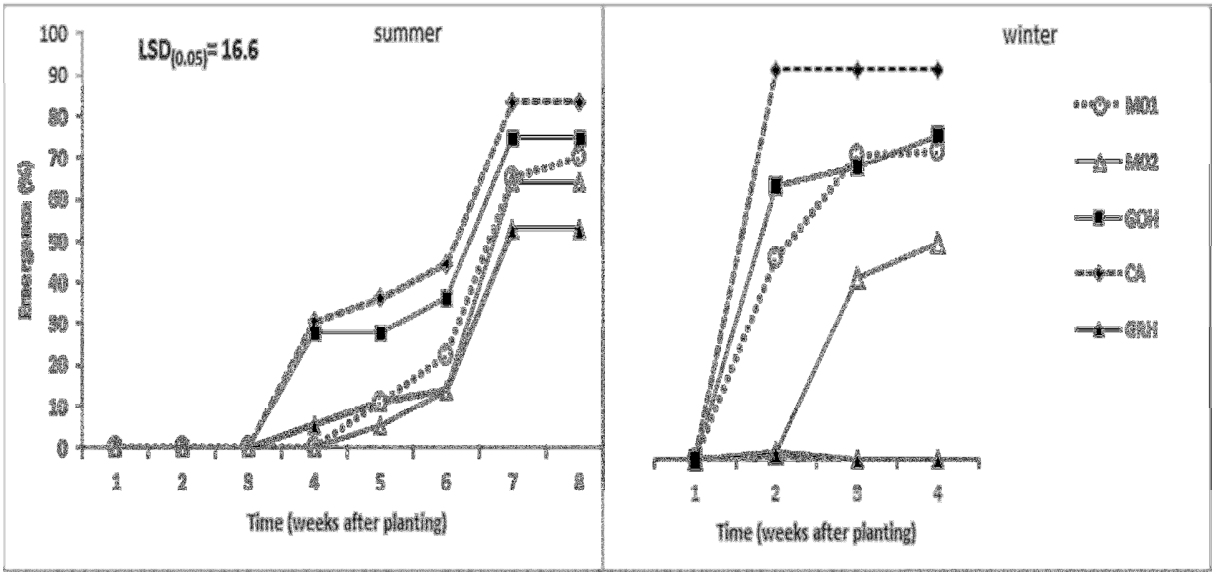

Figure 7.2: Emergence percentage of landraces (M01 & M02) and hybrid varieties (GOH, GRH & CA) recorded overtime for summer and winter season.

7.2.3 Physiological and growth associated parameters

Stomatal conductance (SC) and CCI of all varieties increased with time until 8 weeks after planting when a sharp decline was observed for both SC and CCI (Fig. 7.3). This period also coincided with frost occurrence (Fig. 7.3 and 7.4). There were no significant differences ($P > 0.05$) among varieties with respect to SC. However, CCI was observed to be significant ($P < 0.05$). Landraces, in general, had higher CCI than hybrid varieties (Fig. 7.3). Similar trends as in SC and CCI were observed for plant growth parameters (Fig. 7.4). At week 8 after planting, leaf number and vine length were observed to decline drastically as a result of frost occurrence. Leaf number and vine length were higher in hybrid varieties than landraces. Landrace M02 had the lowest leaf number and vine length and the highest was observed in hybrid GRH.

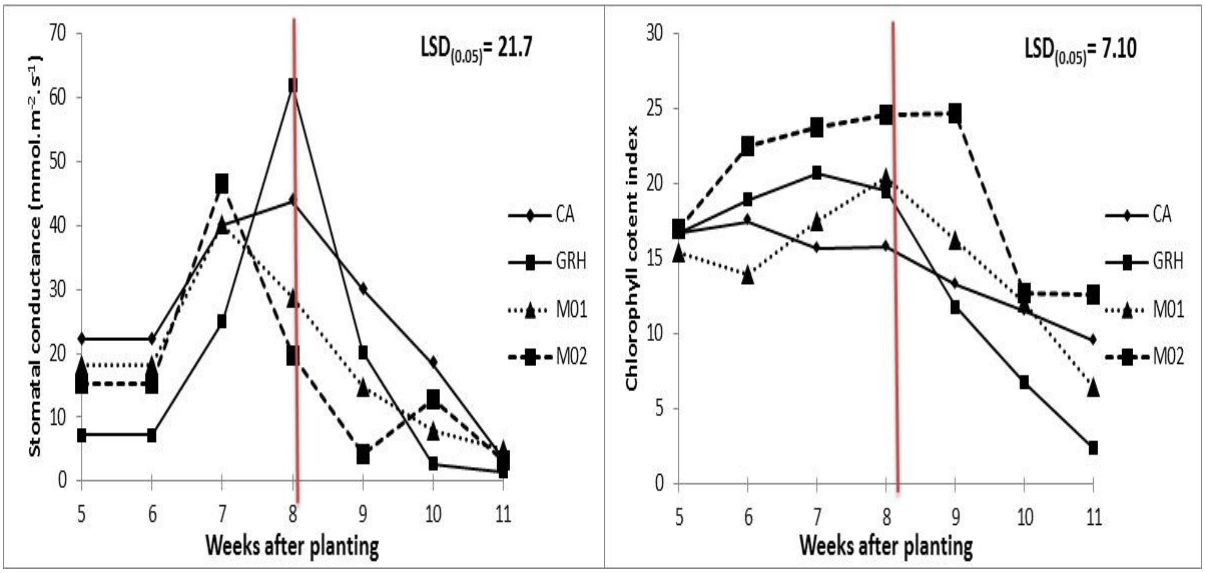

Figure 7.3: Stomatal conductance and CCI of landraces (M01 & M02) and hybrid varieties of cucurbits (GRH & CA) recorded overtime. The red line denotes frost occurrence.

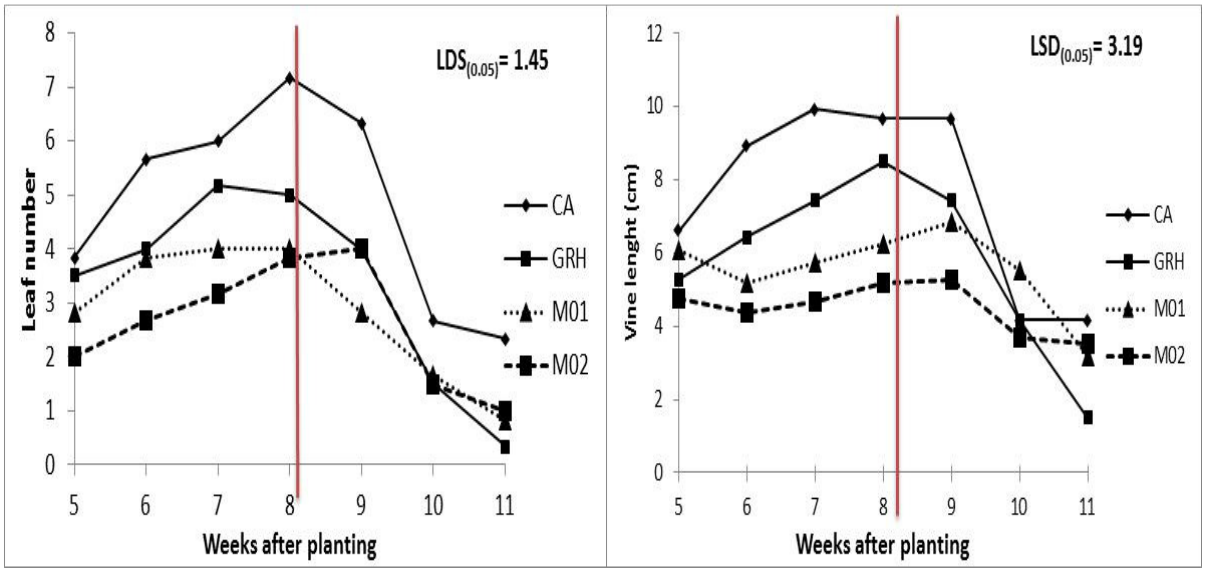

Figure 7.4 : Leaf number and vine length of landraces (M01 & M02) and hybrid varieties (GRH & CA) recorded over time. The red line denotes frost occurrence.

7.2.4 Yield

Significant differences (*P < 0.05*) were observed among varieties with respect to fruit number, fruit mass plot^{-1} and fruit mass plant^{-1} (Table 7.1). High variation within and between the varieties with respect to fruit number plot^{-1} (CV = 36.8), fruit mass plot^{-1} (CV=60.4) and fruit mass plant^{-1} (CV= 59.2) were observed. This variation was observed mostly on landraces. Landraces were observed to produce the same fruit shape as the initial plant.

On average, the yield was higher for landraces than hybrid varieties (Fig. 7.6). The estimated fruit yield in landraces varied between 14.7 to 21.9 t ha^{-1} while in hybrid varieties it ranged between 3.05 to 11.9 t ha^{-1}.

Figure 7.5: Fruits of bottle gourd landraces, pumpkin cultivars and a cucumber species. 1= bottle gourd leaves, 2= M01 fruits, 3= M02 fruits, 4= GRH fruit, 5= CA fruit and 6= GOH fruit.

Table 7. 1: Comparison of fruit yield of bottle gourd landraces and commercial commonly produced hybrid of cucurbits.

Variety	Fruit number plot^{-1}	Fruit mass plot^{-1} (kg)	Fruit mass plant^{-1} (kg)	Fruit shape
Landrace M01	11.67^{a}	10.6^{ab}	1.07^{ab}	Cylindrical
Landrace M02	13.36^{a}	15.8^{a}	1.74^{a}	Calabash
Hybrid GOH	11.33^{a}	7.6^{ab}	0.94^{b}	Spherical
Hybrid CA	12.00^{a}	8.6^{ab}	0.98^{b}	Oval
LSD	6.97	10.2	0.75	-
P_{value}	*< 0.05*	>0.05	<0.05	-
CV (%)	36.8	60.4	59.2	-

[1]Values sharing the same letter are not significantly different at P < 0.05.

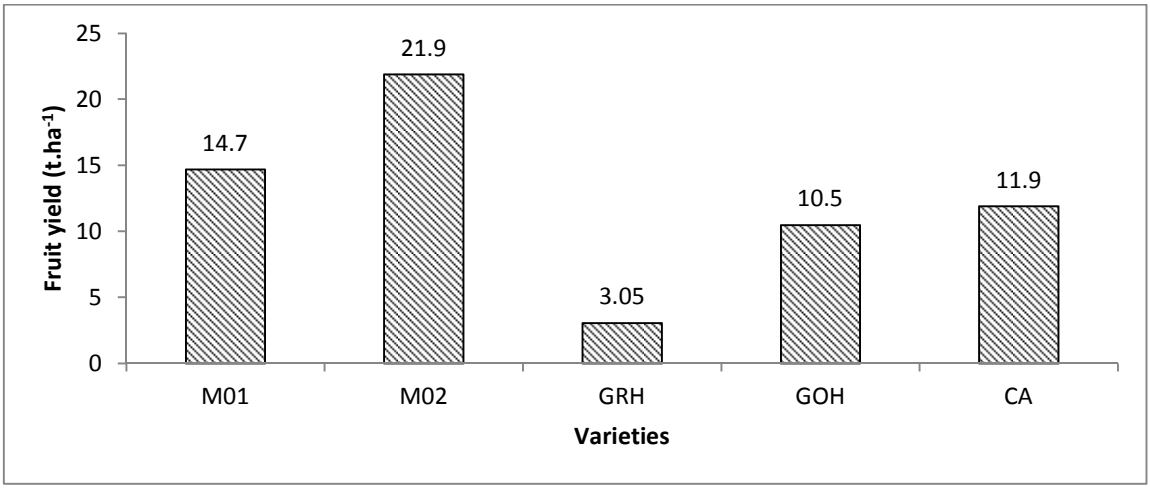

Figure 7.6: Estimated fruit yield of bottle gourd landraces (M01 & M02) and hybrid varieties of cucurbits (GRH, GOH & CA) obtained in early summer planting.

7.2.5 Seed quality test

Highly significant differences ($P < 0.001$) were observed with respect to germination time (Fig. 7.7). Hybrid varieties (CA and GOH) germinated faster than landraces. Differences ($P < 0.05$) were also observed for final germination with landrace M01 having very low germination (< 10%). Germination velocity index was similar for all varieties except for landrace M01 where it was observed also to be significant lower (Table 7.2).

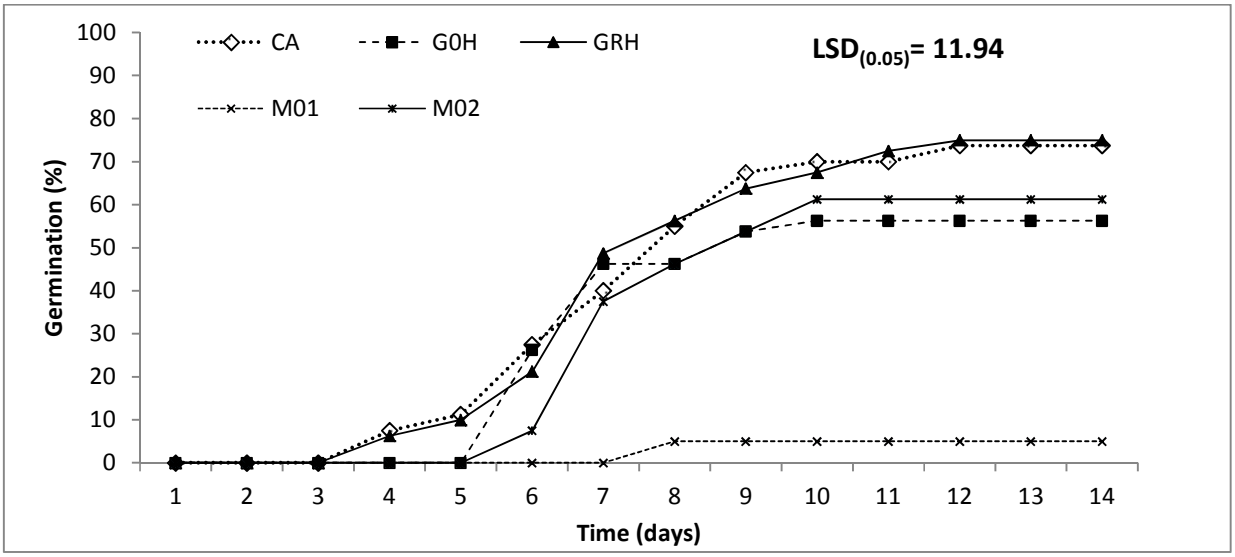

Figure 7.7: Daily germination of bottle gourd landraces (M01, M02 & M03) compared with selected hybrid cucurbits (GRH, GOH & CA) during the standard germination test.

Table 7.2: Seed perfomance of bottle gourd landraces (M01, M02 & M03) as compared with selected hybrid cucurbits (GRH, GOH & CA) during the standard germination test.

Variety	Final germination (%)	Germination velocity index (GVI)	Mean germination time (MGT)	Electrical conductivity (µS/g)	Root length (cm)	Shoot length (cm)	Root: shoot ratio
M01	5.0[a1]	0.62[a]	4.00[a]	89.7[ab]	1.09[a]	0.64[a]	0.12[a]
M02	61.2[b]	7.86[b]	7.70[a]	109[b]	10.5[b]	7.28[b]	1.08[b]
GRH	75.0[b]	10.8[b]	7.37[a]	62.9[a]	15.6[c]	7.23[b]	1.70[c]
GOH	56.2[b]	8.31[b]	6.91[a]	68.9[a]	15.2[c]	7.70[b]	1.72[c]
CA	73.8[b]	10.4[b]	7.00[a]	60.3[a]	15.1[c]	10.0[c]	1.62[c]
LSD	14.7	2.35	3.22	26.1	2.59	1.46	0.35
P value	*< 0.001*	< .001	=0.157	*< 0.05*	*< 0.001*	*< 0.001*	*< 0.001*

[1]Values represented by the same letter are not significantly different from each other at P < 0.05.

7.3 Discussion

Planting times, influenced by different biotic and abiotic environmental factors had a significant effect on performance of the crops during its development which subsequent impact on final yield. Availability of water in the soil and optimal temperatures are the two most profound influencing factors during crop development. In this study availability of water and low temperatures during summer season were shown to delay emergence in a number of varieties. This led to a poor crop establishment compare to a winter season trial where soil water content was relatively available during early stages of crop development. Similar trends were observed by Sesay *et al.* (2008) in Bambara ground nut, where delayed and prolonged seedling emergence was observed in trials established before the onset of rainy season in Swaziland. Poor emergence due to drought stress exposes plants to various factors such as diseases which can attack plants at the early stage of development and subsequent leads to poor yield. Thus small holder farmers need this information in order to optimize their yield and combat food security issues associated with poor yields. These results were in agreement with what was observed in seed quality test, where it was observed that hybrid GRH and landrace M02 had poor germination. This was apparent in winter field trial where emergence was significantly lower (< 10%) while in summer trial it improved by more than 40%.

Stomatal conductance of all varieties was observed to be reduced significantly when compared with plants that were planted in the controlled environment under full irrigation (chapter 4). Stomatal conductance was low in landraces than in hybrid varieties. Closure of stomata is the initial response of plants to decrease in water content and According to Chaves (1991), stomatal control in stressed plants is an avoidance measure and forms part of initial defence response to water stress. Similar observations were made in CCI, where winter trial had a low amount of CCI compared with the plants with full irrigation in controlled environment.

Although the crops that were planted in winter trial were quick to emerge due to the prevailing soil water content, their grown and development was significantly reduced due to low temperature which was associated with drought at the onset and during winter season. Low temperatures and water stress decrease metabolic activity of the plants which in turn is translated to growth and development inhibition. As a result, leaf number and vine length was significantly reduced. Similar trends were observed in in five cucumber varieties (Eifediyi and

Remison, 2009). Occurrence of frost in winter season damaged the plants significantly and led to growth retardation and the death of the plants. This effect limited the possibility of fruit production during this season. Nu (1998) stated that cucurbits are warm season crops which can be cultivated anytime but with little or no tolerance to frost that growth and development are favoured by temperatures more than 20°C.

Seed quality characteristics with respect to viability and vigour of the varieties of the same age were not much different to one obtained in seed quality test in chapter 2 except some few changes. Landrace M01 which performed well and hybrid GRH which had a poor germination during standard germination test had a contradicting results compared with the current study. It was observed that the seeds of this landrace were not fully matured at the time of harvest compared to other varieties. While on the other hand hybrid GRH might have gained vigour strength due to age influence.

CHAPTER 8

GENERAL DISCUSSION

8.1 Introduction

The prevalence of malnutrition due to food insecurity continues to increase in developing countries particularly in Africa and sub-Saharan Africa. In South Africa, issues surrounding food security have been associated more with lack of access to nutritious food rather than availability of food because the country is deemed to be food secure at a national level. At the same time, the population of South Africa and that of the world continues to increase. The population of South Africa has just reached 52 million (STAT SA, 2013) and that of the world is currently sitting at 7 billion 2012 and is expected to reach 9.1 billion in 2050. This necessitates the production of more food on the limited amount of land that we have. The challenge of food security is expected to worsen due to the predicted effects of climate change especially in developing countries which are characterized by semi- or arid climate condition which limits crop production due to water scarcity. Climate change is expected to increase the frequency and severity of drought. In order to meet these demands (food security, malnutrition, increasing population and climate change); traditional underutilized species (NUS) have been proposed because of their likely adaptability to the marginal areas of crop production. These crops may have evolved through natural and farmer selection and thus may have acquired drought tolerance (Mabhaudhi, 2012).

Bottle gourd [*Lagenaria siceraria (Molina) Standl.*] is one such traditional indigenous and underutilized crop species in South Africa and sub-Saharan Africa. The crop is only available as a landrace with no commercially produced seeds. Underutilised traditional crops can be defined as those crops that originate in South Africa or those that have become indigenised over many years of farmers' cultivation and natural selection (Schippers, 2006). Azam-Ali (2010) added that these crops have never been classified as major crops, are under-researched and occupy low levels of use which is usually confined to small-scale rural farmers. As an underutilised crop, there is no South African literature describing the agronomy of the crop and/ or its potential as a food security crop; its nutritional and medical properties have been reported elsewhere. The review of literature relied on generalisations on information from other cucurbits and species of bottle gourd found in other countries. That information is not enough because of the different climatic conditions and different genetic diversity found in

different regions of the world. Hence, there was a need to research local species of the crop because of their likely suitability to local climates if they are to be promoted for utilisation.

8.2 Aims and Objectives

The overall aim of the study was to assess the potential contribution of bottle gourd landraces to food security, looking at the agronomic perspective. To meet the overall objective of the study, the specific objectives were set as follows:

i. the study determined the seed quality of selected bottle gourd landraces and these were compared with commercially produced members of pumpkin and one cucumber species and this was the case for all the studies conducted. Pumpkin and cucumber were used for comparison because there were no commercial produced bottle gourd hybrid seeds in South Africa. This test was conducted in order to identify "viable" seed lots as a starting point for establishment of field and pot trial experiments,
ii. the study evaluated the responses of landraces to water stress. Since the country is water scarce and most people who utilise the crop reside in rural areas characterised by semi-arid climate. This information was also important for characterisation of crops for drought tolerance,
iii. the study evaluated the nutritional content of the crop at different growth stages. Secondary to this, it estimated potential contribution of the crop to RDAs. In South Africa there was no information on nutritional value of the crop despite its potential contribution to food security. So this information was important if the crop is to be promoted, and
iv. lastly, crop growth and development in different seasons was compared.

8.3 Challenges

- Sourcing seed was an arduous task as the seed was not commercially produced and the farmers who grow it generally do not have established seed systems. Seed used in this study was sourced from farmers' fields during the growing season.
- Once field trials were established, wild animals were a persistent threat to field trials.

8.4 Future Teaching, Learning and Research Possibilities

The following recommendations may be made, based on observations made during the study;

- The huge diversity demonstrated by landraces used in this study suggests that DNA fingerprinting should be done to determine the extent to which these landraces differ.
- Proper seed systems need to be developed to support smallholder farmers in rural communities who still rely on traditional and underutilised crops
- Strategies to enhance seed quality should be explored. Low cost strategies such as hydropriming could be evaluated.
- Since water stress hardly acts alone under field conditions, the effect of other abiotic factors such as temperature and fertilisers should be evaluated in order to develop best management recommendations for farmers.
- Multidisciplinary studies that focus on the entire value chain of traditional crops should be emphasised if there is going to be progress in promoting these crops as well as influencing policy formulation.

8.5 Final Comments and Summary Conclusions

The results of seed quality from fruits of different morphologies collected from different places indicated the variability in seed quality and emergence traits. The differences observed were attributed to seed coat thickness and high EC of the landrace collected from Richards Bay. Other factors that could account for the differences observed are pre- and post-harvest handling. Contrary to expectations that hybrids would have superior seed quality than landraces, this study showed that some landraces may have similar seed quality as hybrids; however, the uniformity and consistency of hybrids makes them superior to landraces which often showed huge variability within and between landraces.

Crop responses to water stress are multifold (Blum, 2009). In this study, physiological responses (stomatal conductance and chlorophyll content) and plant growth responses (vine length and leaf number) were evaluated. With respect to physiological responses; stomatal conductance was observed to decrease with increase in water stress. Similar observations were made for CCI. Closure of stomata in response to declining soil water content is an initial response of plants to water stress. Stomatal regulation has been associated with drought avoidance mechanisms (Chaves, 1991). Hybrids generally had higher levels of SC than landraces under optimum and sub-optimum conditions. This suggests that under optimum

conditions would out-perform landraces while the opposite would be true under water limited conditions. Plant growth (vine length and leaf number) was inhibited by water stress. Landraces had higher vine length than hybrids; however, hybrid varieties were observed to produce more leaves than landraces. This suggests that hybrids are bred to transpire more and produce more biomass under optimum conditions. The smaller canopy size of landraces implies that they are adapted to transpiring less and hence may be suitable for areas with limited water availability. Proline accumulation in stressed plants has been reported as a widespread plant response. In this study it was used as an index for assessing the severity of water stress. Similar observations were made also in this study. Hybrid varieties were observed to accumulate more proline than landraces. This further confirmed the hypothesis that hybrids may be more sensitive to water stress than landraces based on Mabhaudhi's (2009) argument that high proline accumulation could be an indication of stress severity and not tolerance. This study also measured the amount of proteins in leaves under varying water regimes.

The results of leaf nutrient analysis showed that bottle gourd contains most of the nutrients required for good health. It was shown to be an excellent source of calcium, magnesium, iron, zinc, nitrogen, manganese and copper. On average, hybrids had higher nutritional content than landraces. However, landraces met the DRAs of all nutrients for the two chosen age groups (4-8 year olds and 19-30 years old women), with the exception of sodium and proteins which were found to be very low (< 2% of the DRA). The results of leaf harvest at different stages of growth indicated that most nutrients analysed were found during early stages of crop development, before flowering. Based on these preliminary results it is recommended to harvest the leaves before the onset of flowering period.

Under field conditions, bottle gourd landraces responded well to summer than winter planting. The winter trial failed before flowering due to frost occurrence. Therefore, bottle gourd is sensitive to frost; as such even early planting should be avoided in areas with high incidence of frost occurrence. Overall, the study confirmed the potential of bottle gourd to contribute to food security in marginal areas.

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APPENDICES

Appendix 1: Analysis of variance tables for chapter 4

Variate: Germination (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	2827.4	942.5	8.13	
Rep.*Units* stratum					
Variety	4	86938.6	21734.7	187.39	<.001
Day	14	141357.6	10097.0	87.05	<.001
Variety.Day	56	40023.3	714.7	6.16	<.001
Residual	222	25748.9	116.0		
Total	299	296896.0			

Variate: Final Germ (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	3	364.9	121.6	0.78	
rep.*Units* stratum					
variety	4	11857.5	2964.4	18.92	<.001
Residual	12	1880.3	156.7		
Total	19	14102.6			

Variate: GVI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.127	0.042	0.01	
Rep.*Units* stratum					
Variety	4	342.884	85.721	11.43	<.001
Residual	12	89.964	7.497		
Total	19	432.976			

Variate: MGT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	8.016	2.672	0.46	
Rep.*Units* stratum					
Variety	4	72.932	18.233	3.11	0.057
Residual	12	70.424	5.869		
Total	19	151.372			

Variate: EC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	14	292082.	20863.	0.86	
Rep.*Units* stratum					
Variety	4	10913209.	2728302.	112.47	<.001
Residual	56	1358423.	24258.		
Total	74	12563714.			

Variate: Root length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	46.85	15.62	0.22	
Rep.*Units* stratum					
Variety	4	15081.43	3770.36	52.34	<.001
Residual	360	25933.51	72.04		
Total	367	41061.79			

Variate: Shoot length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	111.03	37.01	1.61	
Rep.*Units* stratum					
Variety	4	4560.03	1140.01	49.58	<.001
Residual	360	8278.23	23.00		
Total	367	12949.29			

Variate: Root: shoot lenght

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	2.595	0.865	0.50	
Rep.*Units* stratum					
Variety	4	154.242	38.561	22.09	<.001
Residual	360	628.309	1.745		
Total	367	785.146			

Correlation Germination

Final_G	1	-						
GVI	2	0.9381	-					
EC	3	-0.2481	-0.5070	-				
MGT	4	-0.7604	-0.8215	0.6980	-			
RL	5	0.9647	0.9100	-0.2094	-0.6221	-		
R:S	6	0.9238	0.8439	-0.0537	-0.4796	0.9837	-	
SL	7	0.9932	0.8959	-0.1416	-0.7141	0.9516	0.9236	-
seed_mass	8	-0.2241	-0.4732	0.9905	0.7287	-0.1512	0.0135	-0.1235
		1	2	3	4	5	6	7

seed_mass	8	-
		8

Number of observations: 5

Two-sided test of correlations different from zero

Final_G	1	-				
GVI	2	0.0183	-			
EC	3	0.6874	0.3833	-		
MGT	4	0.1356	0.0881	0.1899	-	
RL	5	0.0079	0.0320	0.7353	0.2625	-
R:S	6	0.0250	0.0723	0.9316	0.4136	0.0025
SL	7	<0.001	0.0397	0.8204	0.1754	0.0127
seed_mass	8	0.7171	0.4208	0.0011	0.1625	0.8082
		1	2	3	4	5

R_L	6	-		
SL	7	0.0251	-	
seed_mass	8	0.9828	0.8432	-
		6	7	8

Variate: Emerg_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	10584.1	5292.0	19.21	
Rep.*Units* stratum					
Variety	4	164648.3	41162.1	149.43	<.001
Day	2	16074.0	8037.0	29.18	<.001
water	2	66.0	33.0	0.12	0.887
Variety.Day	8	13736.0	1717.0	6.23	<.001
Variety.water	8	17883.2	2235.4	8.11	<.001
Day.water	4	2505.1	626.3	2.27	0.068
Variety.Day.water	16	11154.9	697.2	2.53	0.003
Residual	88	24241.1	275.5		
Total	134	260892.7			

Correlations emergence

Emergence	1	-						
Leaf_no	2	0.9606	-					
MET	3	-0.8151	-0.7757	-				
R_L	4	-0.7121	-0.6002	0.4303	-			
R_length	5	0.7245	0.7772	-0.8927	-0.0980	-		
S_length	6	0.7893	0.8304	-0.9186	-0.1972	0.9948	-	
leaf_area	7	0.8895	0.9407	-0.8996	-0.5192	0.8674	0.9058	-
seed_size	8	-0.7325	-0.6024	0.7976	0.2716	-0.7522	-0.7708	-0.5890
		1	2	3	4	5	6	7

seed_size	8	-
		8

Number of observations: 5

Two-sided test of correlations different from zero

Emergence	1	-				
Leaf_no	2	0.0093	-			
MET	3	0.0928	0.1231	-		
R_L	4	0.1772	0.2845	0.4695	-	
R_length	5	0.1662	0.1219	0.0415	0.8754	-
S_length	6	0.1124	0.0816	0.0275	0.7506	<0.001
leaf_area	7	0.0433	0.0172	0.0376	0.3699	0.0568
seed_size	8	0.1593	0.2823	0.1059	0.6585	0.1425
		1	2	3	4	5

S_length	6	-		
leaf_area	7	0.0342	-	
seed_size	8	0.1271	0.2960	-
		6	7	8

Appendix 2: Analysis of variance tables for chapter 5

Variate: Moisture content (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	56.91	28.46	1.35	
Rep.*Units* stratum					
Variety	3	25.74	8.58	0.41	0.748
FC	2	1143.95	571.97	27.14	<.001
DAP	9	1240.86	137.87	6.54	<.001
Variety.FC	6	494.83	82.47	3.91	<.001
Variety.DAP	27	2402.44	88.98	4.22	<.001
FC.DAP	18	1330.51	73.92	3.51	<.001
Variety.FC.DAP	54	1880.11	34.82	1.65	0.006
Residual	238	5016.76	21.08		
Total	359	13592.11			

Variate: Chlorophyll content index (CCI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2056.42	1028.21	25.71	
Rep.*Units* stratum					
Variety	3	3343.65	1114.55	27.87	<.001
FC	2	2869.12	1434.56	35.87	<.001
DAP	9	6855.60	761.73	19.05	<.001
Variety.FC	6	958.30	159.72	3.99	<.001
Variety.DAP	27	1092.86	40.48	1.01	0.453
FC.DAP	18	727.24	40.40	1.01	0.449
Variety.FC.DAP	54	2503.85	46.37	1.16	0.227
Residual	238	9518.59	39.99		
Total	359	29925.62			

Variate: Stomatal conductance (SC)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	61195.	30597.	16.20	
Rep.*Units* stratum					
Variety	3	72386.	24129.	12.77	<.001
FC	2	39543.	19771.	10.47	<.001
DAP	9	279732.	31081.	16.45	<.001
Variety.FC	6	4232.	705.	0.37	0.895
Variety.DAP	27	66911.	2478.	1.31	0.146
FC.DAP	18	39395.	2189.	1.16	0.297
Variety.FC.DAP	54	116664.	2160.	1.14	0.247
Residual	238	449556.	1889.		
Total	359	1129613.			

Variate: Height (cm)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	994.12	497.06	9.34	
Rep.*Units* stratum					
Variety	3	38066.18	12688.73	238.47	<.001
FC	2	24191.00	12095.50	227.32	<.001
DAP	9	33777.84	3753.09	70.53	<.001
Variety.FC	6	16898.99	2816.50	52.93	<.001
Variety.DAP	27	20950.30	775.94	14.58	<.001
FC.DAP	18	3422.90	190.16	3.57	<.001
Variety.FC.DAP	54	5882.49	108.94	2.05	<.001
Residual	238	12663.84	53.21		
Total	359	156847.65			

Variate: Leaf_number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	564.76	282.38	20.94	
Rep.*Units* stratum					
Variety	3	876.09	292.03	21.66	<.001
FC	2	739.71	369.85	27.43	<.001
DAP	9	595.90	66.21	4.91	<.001
Variety.FC	6	100.04	16.67	1.24	0.288
Variety.DAP	27	272.12	10.08	0.75	0.815
FC.DAP	18	949.58	52.75	3.91	<.001
Variety.FC.DAP	54	770.06	14.26	1.06	0.379
Residual	238	3209.41	13.48		
Total	359	8077.67			

Variate: Proline

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.000422	0.000211	0.21	
Rep.*Units* stratum					
Variety	3	0.270818	0.090273	88.98	<.001
FC	2	0.369426	0.184713	182.07	<.001
Variety.FC	6	0.190075	0.031679	31.23	<.001
Residual	22	0.022319	0.001015		
Total	35	0.853060			

Variate: Proteins

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.001787	0.000894	0.84	
Rep.*Units* stratum					
Variety	3	435.124924	145.041641	1.362E+05	<.001
FC	2	161.953217	80.976609	76028.42	<.001
Variety.FC	6	114.994417	19.165736	17994.59	<.001
Residual	22	0.023432	0.001065		
Total	35	712.097778			

Variate: Day to flowering

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	1592.4	796.2	1.08	
rep.*Units* stratum					
variety	3	5652.8	1884.3	2.56	0.081
FC	2	1227.1	613.5	0.83	0.448
variety.FC	6	2736.1	456.0	0.62	0.713
Residual	22	16194.3	736.1		
Total	35	27402.6			

Variate: Brench number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	695.72	347.86	7.89	
Rep.*Units* stratum					
Variety	3	308.22	102.74	2.33	0.102
FC	2	401.56	200.78	4.56	0.022
Variety.FC	6	223.78	37.30	0.85	0.548
Residual	22	969.61	44.07		
Total	35	2598.89			

Variate: Leaf fresh mass (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	94.81	47.40	4.39	
Rep.*Units* stratum					
Variety	3	28.43	9.48	0.88	0.467
FC	2	157.20	78.60	7.29	0.004
Variety.FC	6	168.72	28.12	2.61	0.046
Residual	22	237.33	10.79		
Total	35	686.48			

Variate: Male flower

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	16.431	8.215	2.82	
Rep.*Units* stratum					
Variety	3	65.472	21.824	7.50	<.001
FC	2	15.597	7.799	2.68	0.074
Days_after_planting	3	175.806	58.602	20.14	<.001
Variety.FC	6	8.569	1.428	0.49	0.814
Variety.Days_after_planting	9	181.583	20.176	6.93	<.001
FC.Days_after_planting	6	11.236	1.873	0.64	0.695
Variety.FC.Days_after_planting	18	28.375	1.576	0.54	0.930
Residual	94	273.569	2.910		
Total	143	776.639			

Variate: Female Flower

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.5417	0.2708	0.72	
Rep.*Units* stratum					
Variety	3	11.5764	3.8588	10.23	<.001
FC	2	0.5417	0.2708	0.72	0.490
Days_after_planting	3	1.9097	0.6366	1.69	0.175
Variety.FC	6	1.9028	0.3171	0.84	0.542
Variety.Days_after_planting	9	5.2847	0.5872	1.56	0.140
FC.Days_after_planting	6	3.0694	0.5116	1.36	0.240
Variety.FC.Days_after_planting	18	5.1528	0.2863	0.76	0.741
Residual	94	35.4583	0.3772		
Total	143	65.4375			

Variate: Brench number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	695.72	347.86	7.89	
Rep.*Units* stratum					
Variety	3	308.22	102.74	2.33	0.102
FC	2	401.56	200.78	4.56	0.022
Variety.FC	6	223.78	37.30	0.85	0.548
Residual	22	969.61	44.07		
Total	35	2598.89			

Variate: Root dry mass (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.01263	0.00631	0.12	
Rep.*Units* stratum					
Variety	3	0.12598	0.04199	0.77	0.526
FC	2	0.11750	0.05875	1.07	0.360
Variety.FC	6	0.52166	0.08694	1.58	0.199
Residual	22	1.20686	0.05486		
Total	35	1.98462			

Variate: Root fresh mass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	4.6825	2.3413	6.08	
Rep.*Units* stratum					
Variety	3	3.7813	1.2604	3.27	0.040
FC	2	7.2915	3.6458	9.47	0.001
Variety.FC	6	4.2864	0.7144	1.86	0.134
Residual	22	8.4674	0.3849		
Total	35	28.5091			

Variate: Stem fresh mass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	597.29	298.64	7.58	
Rep.*Units* stratum					
Variety	3	237.69	79.23	2.01	0.142
FC	2	301.63	150.82	3.83	0.037
Variety.FC	6	333.75	55.62	1.41	0.255
Residual	22	866.77	39.40		
Total	35	2337.12			

Variate: Stem dry mass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	12.4024	6.2012	6.76	
Rep.*Units* stratum					
Variety	3	8.1381	2.7127	2.96	0.055
FC	2	7.9798	3.9899	4.35	0.026
Variety.FC	6	9.7567	1.6261	1.77	0.151
Residual	22	20.1763	0.9171		
Total	35	58.4533			

Variate: Fruit number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.3889	0.1944	1.00	
Rep.*Units* stratum					
Variety	3	1.1111	0.3704	1.90	0.158
FC	2	0.7222	0.3611	1.86	0.180
Variety.FC	6	1.7222	0.2870	1.48	0.232
Residual	22	4.2778	0.1944		
Total	35	8.2222			

Variate: Fruit mass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	7.762	3.881	1.15	
Rep.*Units* stratum					
Variety	3	17.359	5.786	1.72	0.192
FC	2	12.370	6.185	1.84	0.183
Variety.FC	6	43.355	7.226	2.15	0.088
Residual	22	74.061	3.366		
Total	35	154.907			

Appendix 3: Analysis of variance tables for chapter 6

Variate: Water content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	103.64	51.82	3.22	
REP.*Units* stratum					
VARIETY	11	230.91	20.99	1.30	0.223
DAT	8	160.52	20.06	1.25	0.273
VARIETY.DAT	88	1839.09	20.90	1.30	0.066
Residual	214	3444.25	16.09		
Total	323	5778.41			

Variate: CCI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	4.35	2.18	0.11	
REP.*Units* stratum					
VARIETY	11	4675.53	425.05	22.07	<.001
DAT	8	12633.26	1579.16	82.01	<.001
VARIETY.DAT	88	2378.62	27.03	1.40	0.025
Residual	214	4120.66	19.26		
Total	323	23812.42			

Variate: SC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	37151.	18575.	5.74	
REP.*Units* stratum					
VARIETY	11	167200.	15200.	4.70	<.001
DAT	8	301005.	37626.	11.63	<.001
VARIETY.DAT	88	725321.	8242.	2.55	<.001
Residual	214	692521.	3236.		
Total	323	1923197.			

Variate: proteins

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0004872	0.0002436	2.37	
Rep.*Units* stratum					
variety	3	0.0434546	0.0144849	140.64	<.001
DAT	4	0.1267785	0.0316946	307.74	<.001
variety.DAT	12	0.1463684	0.0121974	118.43	<.001
Residual	38	0.0039137	0.0001030		
Total	59	0.3210025			

Variate: Hight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	406.12	203.06	3.34	
REP.*Units* stratum					
VARIETY	11	5639.00	512.64	8.42	<.001
DAT	8	49677.08	6209.63	102.03	<.001
VARIETY.DAT	88	10067.19	114.40	1.88	<.001
Residual	214	13024.35	60.86		
Total	323	78813.73			

Variate: Leaf number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	3.865	1.932	1.89	
REP.*Units* stratum					
VARIETY	11	784.656	71.332	69.92	<.001
DAT	8	1862.600	232.825	228.20	<.001
VARIETY.DAT	88	388.248	4.412	4.32	<.001
Residual	214	218.335	1.020		
Total	323	3257.704			

Variate: Leaf wet mass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	21.994	10.997	1.34	
Rep.*Units* stratum					
Variety	11	634.548	57.686	7.06	<.001
Residual	22	179.882	8.176		
Total 35	836.425				

Variate: Leaf dry mass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	87.336	43.668	14.55	
Rep.*Units* stratum					
Variety	11	66.571	6.052	2.02	0.078
Residual	22	66.019	3.001		
Total 35	219.926				

Variate: Leaf_Area

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	5	463.4	92.7	0.63	
rep.*Units* stratum					
variety	3	24203.8	8067.9	54.98	<.001
Residual	87	12767.8	146.8		
Total 95	37435.0				

Experimental lay out

rh	c	rh	h1
rh	rh	rh	rh
rh	h1	rh	rh
rh	rh	c	rh
c	rh	h1	rh
rh	rh	rh	rh

Appendix 4: Analysis of variance tables for chapter 7

Variate: Fruit weight. Plant^{-1}

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	5	2.4182	0.4836	1.26	
rep.*Units* stratum					
variery	4	4.7061	1.1765	3.06	0.041
Residual	20	7.6912	0.3846		
Total 29 14.8155					

Variate: Fruit number

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	188.93	94.47	6.89	
rep.*Units* stratum					
variety	4	274.27	68.57	5.00	0.026
Residual	8	109.73	13.72		
Total 14	572.93				

Variate: Fruit weight. $Plot^{-1}$

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	54.98	27.49	0.94	
rep.*Units* stratum					
variety	4	294.73	73.68	2.51	0.125
Residual	8	234.76	29.35		
Total 14	584.47				

Variate: Germination percentage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	1622.41	540.80	7.33	
Rep.*Units* stratum					
Variety	4	55899.11	13974.78	189.43	<.001
Day	13	151478.66	11652.20	157.94	<.001
Variety.Day	52	33983.39	653.53	8.86	<.001
Residual	207	15271.34	73.77		
Total 279	258254.91				

Variate: Final germination (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	213.75	71.25	0.78	
Rep.*Units* stratum					
Variety	4	13157.50	3289.38	36.13	<.001
Residual	12	1092.50	91.04		
Total	19	14463.75			

Variate: Germiation velocity index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	7.661	2.554	1.10	
Rep.*Units* stratum					
Variety	4	270.284	67.571	29.02	<.001
Residual	12	27.937	2.328		
Total	19	305.883			

Variate: Mean germination time

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	14.152	4.717	1.08	
Rep.*Units* stratum					
Variety	4	35.243	8.811	2.01	0.157
Residual	12	52.593	4.383		
Total	19	101.988			

Variate: Electrolyte conductivity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	14	28821.	2059.	1.61	
Rep.*Units* stratum					
Variety	4	25578.	6395.	5.01	0.002
Residual	56	71459.	1276.		
Total 74	125859.				

Variate: Root length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	349.94	116.65	1.68	
Rep.*Units* stratum					
Variety	4	12735.04	3183.76	45.94	<.001
Residual	392	27166.25	69.30		
Total 399	40251.23				

Variate: Shoot length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	233.90	77.97	3.51	
Rep.*Units* stratum					
Variety	4	3957.10	989.28	44.52	<.001
Residual	392	8710.90	22.22		
Total 399	12901.90				

Variate: Root: Shoot ratio

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	9.235	3.078	2.43	
Rep.*Units* stratum					
Variety	4	152.010	38.002	29.97	<.001
Residual	392	497.140	1.268		
Total	399	658.385			

Variate: Emerge percent

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1188.7	594.3	5.68	
Rep.*Units* stratum					
Variety	4	5964.1	1491.0	14.25	<.001
WAP	7	85851.3	12264.5	117.19	<.001
Variety.WAP	28	4360.0	155.7	1.49	0.088
Residual	78	8163.2	104.7		
Total	119	105527.2			