Pre-Breeding of Bottle Gourd [*Lagenaria siceraria* (Molina) Standl.]

By

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Thesis Abstract

Bottle gourd [*Lagenaria siceraria* (Molina.) Standl.] is potentially a useful food crop that could contribute to crop diversification among smallholder farmers and the alleviation of malnutrition in South Africa. However, the crop can be considered as being among one of the most neglected, underutilized and under-researched. The crop is widely grown by smallholder farmers in the drier regions of South Africa who use predominantly landrace varieties. There is no genetic improvement programme in the country which impacts on breeding and strategic conservation of the crop. Therefore, the genetic diversity of this crop needs to be studied and documented to enhance its production and productivity. This knowledge will be essential for effective bottle gourd pre-breeding and breeding programs in South Africa. Evaluation of the available genetic resources of the crop using agro-morphological and horticultural attributes and molecular markers will be useful in the selection of promising genotypes for the development of unique varieties with improved yield, yield components and quality and for resistance breeding against abiotic and biotic stresses. Therefore, the objectives of this study were: 1) to determine genetic diversity present among bottle gourd landraces widely grown in South Africa using qualitative and quantitative morphological traits, 2) to determine genetic diversity among bottle gourd landraces using single sequence repeat (SSR) markers; 3) to apply correlation and path coefficient analyses to determine level of association among qualitative and quantitative traits and subsequently to select unique genotypes with suitable traits for breeding and strategic conservation; 4) to determine drought tolerance of a diverse set of bottle gourd landraces using drought tolerance indices and to identify promising genotypes for direct production or breeding and 5) to determine the relationship between cucurbitacin accumulation with leaf gas exchange and chlorophyll fluorescence in bottle gourd under water stress conditions.

In the first study, the genetic diversity present among 36 diverse landrace selections of bottle gourd was investigated using morphological qualitative and quantitative traits. Phenotypic differences were observed among bottle gourd landraces. Principal component analysis (PCA) of qualitative and quantitative traits identified five and seven principal components (PCs) which accounted for 78 and 87% of the total variation, respectively. Among qualitative traits, presence or absence of

fruit neck, fruit shape, degree of neck bending and fruit neck length positively correlated with PC1, which accounted for 31.9% of the total variation. Presence or absence of seed lines and seed texture was highly correlated with PC2 which accounted for 14.9% of the total variation. For quantitative traits, plant height, number of nodes on the main stem, total number of nodes, number of male flowers, number of branches, fruit neck diameter positively correlated with PC1, which accounted for 39.6% of the total variation. Fruit mass, fruit width, fruit size, shell thickness, seed length, seed width, seed size and hundred seed weight highly correlated with PC2 which accounted for 13.7% of the total variation. Unique genotypes such as BG-16, BG-25, BG-09, BG-37 and BG-10 showing suitable qualitative traits and BG-07, BG-13, BG-67, BG-12, BG-09 and BG-06 showing suitable quantitative traits were identified for genetic improvement.

In the second study, the genetic diversity of 67 bottle gourd landraces was determined using 14 selected polymorphic simple sequence repeat (SSR) markers**.** The markers amplified a total of 86 alleles with allele sizes ranging from 145 to 330 base pairs (bp). Number of effective alleles (N_e) ranged from 1.58 to 6.14 with a mean of 3.10. Allelic richness varied from 3.00 to 8.90 with a mean of 5.23. Expected heterozygosity (H_e) values ranged from 0.37 to 0.84 with a mean of 0.65. The mean polymorphic information content (PIC) was 0.57. Jaccard's coefficient of similarity values ranged from 0.00 to 1.00, with a mean of 0.63. Analysis of molecular variance revealed that 79%, 17% and 4% of the variation was attributable to among landraces, within landraces and between populations, respectively. Cluster analysis classified the bottle gourd landraces into three major clusters namely: cluster I, cluster II and cluster III. Overall, landraces such as BG-04, BG-06, BG-08, BG-09, BG-15, BG-55, BG-42, BG-57, BG-58, BG-28, BG-23, BG-29 and BG-34 selected for breeding and systematic conservation.

In the third study, simple correlation and path coefficient analysis were used to determine the level of association between qualitative and quantitative traits among 36 bottle gourd landraces. Simple correlation analysis showed that fruit width, number of seeds per fruit, seed width, seed size and hundred seed weight significantly and positively correlated with fruit yield and seed yield, respectively. Highly significant and negative correlations ($P < 0.001$) were observed between primary fruit colour, secondary fruit colour, fruit texture, fruit shape, degree of corrugation, neck bending degree and fruit neck length with either fruit and seed yield. Path coefficient analysis

revealed high direct positive path coefficient values between number of female flowers, seed size, and number of seeds per fruit and fruit width with fruit yield, respectively. High direct negative path coefficient values were observed between primary fruit colour and fruit shape with seed yield and presence or absence of fruit neck, fruit texture and neck bending degree with fruit yield, respectively. The identified quantitative and qualitative traits are useful for improving the yield potential of bottle gourd.

The focus in the fourth study was to evaluate selected bottle gourd landraces for drought tolerance and select genotypes for breeding using yield-based selection indices. Significant differences ($P < 0.05$) were observed amongst bottle gourd landraces with respect to edible fruit number and fruit yield under drought stress (DS) and non-stress (NS) conditions. The mean fruit number under DS and NS conditions were 15 457 and 31 088 ha^{-1}, respectively. The mean fruit yield under DS and NS were 8.75 t ha^{-1} and 22.4 t ha^{-1}, respectively. Drought stress reduced fruit number and yield by 49% and 62%, respectively. Landraces such as BG-79, BG 31, BG-48, BG-67, BG-52, BG-78 and GC were promising for large scale production or as parents for drought tolerance breeding. Correlation and principal component analyses revealed the significance of drought tolerant indices such as geometric mean productivity, stress tolerance, mean productivity, yield index and harmonic mean which allowed discrimination of drought tolerant bottle gourd landraces.

Lastly, twelve selected bottle gourd landraces were grown under non-stressed and water-stressed conditions under glasshouse conditions to determine whether the accumulation of cucurbitacins could be indicative of drought tolerance in bottle gourd. Leaf gaseous exchange and chlorophyll fluorescence parameters were measured and correlated to cucurbitacin content. Stomatal conductance, transpiration rate, net CO_2 assimilation rate, CO_2 assimilation rate/intercellular CO_2, intrinsic water-use efficiency and instantaneous water-use efficiency declined under water stress conditions. Intercellular CO_2 concentrations, ratio of atmospheric and intercellular CO_2 were significantly increased by water stress. The maximum photosystem II activity, quantum yield of photosystem II, photochemical quenching and non-photochemical quenching were not affected by water stress; whereas, electron transport rate, electron transport to oxygen molecules and alternative electron sink declined. Cucurbitacin E and I were detected under water stress condition in several bottle gourd landraces. Significant and positive correlations were observed between

cucurbitacin I content with electron transportation to O_2 molecules and alternative electron sinks suggesting their possible role in the regulation of photorespiration and photoprotection against oxidative stress. Accumulation of cucurbitacin I could be considered as a tool for selection of bottle gourd genotypes for drought tolerance.

Overall, the present study identified valuable bottle gourd genetic resources useful in the development of improved cultivars for large-scale production in South Africa or related environments.

Declaration

I, Jacob Mashilo, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain any other persons' data, pictures graphs, or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain any other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
    a) Their words have been re-written but the general information attributed to them has been referenced;
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5. This thesis does not contain text, graphics and tables copied and pasted from the internet unless specifically acknowledged, and the source being detailed in the dissertation and in the reference sections.

Signed

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Jacob Mashilo

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

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Dr. Alfred Odindo (Supervisor)

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Prof. Hussien Shimelis (Co-Supervisor)

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Dedication

This work is dedicated to my son, Onalenna.

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Publications Pertaining to This Thesis

Chapter two and three

Jacob Mashilo., Hussein Shimelis & Alfred Odindo. 2015. Genetic diversity of bottle gourd [*Lagenaria siceraria* (Molina) Standl.] landraces of South Africa assessed by morphological traits and simple sequence repeat markers. South African Journal of Plant and Soil. 33: 113-124.

Chapter three

Jacob Mashilo., Hussein Shimelis., Alfred Odindo., & Beyene Amelework. 2016. Genetic diversity of South African bottle gourd [*Lagenaria siceraria* (Molina) Standl.] landraces revealed simple sequence repeat markers. HortScience 51: 120-126.

Chapter four

Jacob Mashilo., Hussein Shimelis & Alfred Odindo. 2016. Correlation and path coefficient analysis of qualitative and quantitative traits in selected bottle gourd landraces. Acta Agriculturae Scandinavica, Section B – Soil & Plant Science. 66: 558-569.

Chapter five

Jacob Mashilo., Hussein Shimelis & Alfred Odindo. 2017. Yield based selection indices for drought tolerance evaluation in selected bottle gourd [*Lagenaria siceraria* (Molina) Standl.] landraces. Acta Agriculturae Scandinavica, Section B – Soil & Plant Science. 67: 43-50.

Thesis Introduction

Background

Bottle gourd [*Lagenaria siceraria* (Molina.) Standl.] is an important underutilized crop (van Rensburg *et al.*, 2007; van Wyk, 2011). The crop is grown for its fresh or dry fruits, seed and succulent leaf vegetable (Hart, 2011). It is mainly cultivated by smallholder farmers using unimproved landraces. There is no formal bottle gourd breeding program in South Africa impacting on breeding and strategic conservation of the crop and its commercial value. The commercial potential of this crop is currently known in South Africa being widely marketed in some supermarkets and informal open market systems. Landrace varieties of bottle gourd are widely grown in Limpopo Province of South Africa. However, limited studies are conducted that determine the presence and magnitude of bottle gourd genetic diversity in South Africa. Landraces of crop species are considered to be storehouses of valuable genetic diversity (Bashir *et al.*, 2015). Therefore, a better understanding of the extent of genetic diversity within and amongst the landraces of South African bottle gourd is essential for conservation and strategic breeding. For plant genetic resources to remain a foundation for future sustainable agricultural development, complementary conservation and breeding strategies are needed (Hawtin *et al.*, 1996).

Systematic study of landrace genotypes including bottle gourd is important to identify genetically unrelated genetic stocks for conservation and as possible source of novel genes for breeding programmes (Lai *et al.*, 2015). According to Afuape *et al.* (2011), proper management and effective utilization of plant genetic resources depends on detailed understanding of their genetic variability. The failure to identify suitable and potential parents affects the success and genetic gains of the breeding programme, which is dependent on the extent of genetic variability present in the source genetic pool (Tseng *et al.*, 2002). Therefore, there is need to initiate a dedicated pre-breeding of bottle gourd to fully exploit the genetic diversity and to identify candidate genotypes for breeding or direct production in South Africa.

Drought is currently the major production constraint affecting global crop production especially under dry-land farming systems. The frequency and intensity of droughts have increased over the last 30 years in many parts of the world due to climate change (Hall *et al.*, 2003). In southern Africa, drought intensity is forecasted to be steadily increasing (Fauchereau *et al.*, 2003).

Therefore, there is a continuous need to increase crop production and productivity by developing drought resilient varieties that are more adapted to grow under the changing environmental conditions and water-limited conditions.

There is a growing interest to introduce neglected and underutilised crops as possible drought tolerant crops (Mabhaudhi, 2012). Neglected underutilized crop species like bottle gourd are believed to be drought tolerant (Zeven, 1998). This is probably through many years of selection by famers living in marginal and drought prone areas. This might have led in the development of drought resilient landrace varieties that are unique and adapted to their local conditions. According to Blum and Sullivan (1986), farmers' local varieties or landraces may possess some unique genetic and physiological attributes that may not be present in modern varieties. Landraces are believed to be tolerant to abiotic stress (e.g. heat and drought stresses) making these materials a potential key genetic resource for crop improvement. There is no detailed record on the genetic basis of drought tolerance in the South African bottle gourd landraces despite the importance of the crop and occurrence of frequent droughts in the country.

Breeding for drought tolerance requires knowledge of genetic and physiological mechanisms associated with adaptation under drought stress conditions. Therefore, it is important to identify agronomic attributes and specific physiological mechanisms that improve adaptation to water-limited environments to develop drought tolerant cultivars (Subbarao *et al.*, 1995). Cucurbits accumulate more bitter-tasting secondary metabolites referred to as cucurbitacins in response to drought and heat stress (Haynes and Jones, 1975; Balkema- Boomstra *et al.*, 2003; Shang *et al.*, 2014). The role of this compounds and whether they could be linked to drought adaptation or tolerance has not been well-established. The literature indicated that secondary metabolites play essential roles in mediating interactions between the plant and its environment (e.g. UV-radiation, nutrient deficiencies, temperature and drought stress). Secondary metabolites have been regarded as metabolic waste or substances with no role in plant functions. However, evidence of their role in many plant process including plant growth and development, protection from UV-radiation and their role as antioxidants has been reported (Munne-Bosch and Alegre, 2000; Hura *et al.*, 2007; Hura *et al.*, 2008, 2009). Most secondary metabolites are linked to functions associated with coping mechanism against unfavorable environmental stresses. Therefore, the accumulation of

cucurbitacins may be associated with drought adaptation. However, very limited information is available linking cucurbitacins accumulation and drought tolerance. Understanding the physiological role of cucurbitacins may underpin the underlying regulatory processes at genotypic level and the identification of genes associated with their biosynthesis. This information could be applicable for drought tolerance breeding.

Rationale for pre-breeding and genetic improvement of bottle gourd

Bottle gourd is an important crop in the drier regions of sub-Saharan Africa including South Africa. The crop is mostly cultivated using unimproved landrace varieties mainly by smallholder farmers. Bottle gourd landraces are phenotypically and genetically diverse attributed to long-term selection by growers (Zeven, 1998), the diverse farming systems, variable environmental conditions and the predominant cross-fertilisation mating system of the crop (Morimoto *et al.,* 2005; Koffi *et al.,* 2009). Nass and Parterniani (2000) defined a landrace as 'a local variety of a plant species that evolved largely through selection by farmers in an unstructured way and which has becomes adapted to ecologies where it grows and survives'. Assessing the extent of genetic variation among landraces is useful for strategic conservation for effective breeding. Bottle gourd landraces can be systematically exploited in breeding programs through identification of promising genotypes via a dedicated pre-breeding program.

Pre-breeding is defined as the 'the art of identifying desired traits, and subsequent incorporation of these into modern breeding materials' (Nass and Parterniani, 2000). It is routinely applied in commercial breeding programs where desired traits are constantly sought and identified from source genotypes for use in cultivar development. Pre-breeding includes all activities directed at identification of desirable crop traits and/or genes, and their subsequent transfer into a suitable set of parents for cultivar development. Understanding the genetic variability and genetic interrelationship present among germplasm collections is valuable to avoid redundancy and to select potential parents with desirable traits to be used in development of new cultivars (Chaudhary and Singh, 1982; Yoshida, 2004).

Genetically diverse landrace varieties of bottle gourd are widely grown in the Limpopo Province of South Africa. The landraces show significant variations in fruit shape, neck shape, fruit colour and texture. However, this germplasm has not been adequately characterized and conserved. Detailed phenotypic and genotypic characterization of the bottle gourd germplasm is an important step to identify suitable genotypes as starting point for long term improvement of the crop. Farmers mostly use fruit traits for identification of bottle gourd genotypes making these traits important for strategic breeding according to the farmer's needs or for commercial purposes.

Bottle gourd landraces have been shown to be potential sources of disease resistance. Some landrace collections from Africa were reportedly used in disease resistance breeding programs in the USA (Ling and Levi, 2007; Kousik *et al.*, 2008; Ling *et al.*, 2013). Kousik *et al*. (2008) tested various bottle gourd landraces collected from Israel, Syria, South Africa, Ethiopia, Israel Zimbabwe and Zambia and reported resistance to powdery mildew. Resistance to zucchini yellow mosaic virus was described in bottle gourd landrace collections from Africa (i.e. Zimbabwe and South Africa) (Ling and Levi, 2007). Bottle gourd also shows a wide adaptability, growing from arid to humid environments and from temperate to tropical conditions with annual rainfall ranging between 400-1500 mm (Haque *et al.*, 2009). The capacity to grow under diverse climates could perhaps be explained by a broad genetic diversity which could be inherent in the species. This genetic variability may to a large extent account for the wide adaptation of the species to distinct environments and specifically to water limited conditions. In South Africa, bottle gourd landraces are normally planted under rain fed conditions in the marginal dry areas such as the Limpopo Province often characterized by prolonged dry spells. Farmers in these regions do not have access to irrigation facilities and bottle gourd grows under dry-land farming systems with low production inputs. Farmers reported that their landraces are tolerant to harsh conditions and produce reasonable fruit yield. The potential value of bottle gourd for biotic and abiotic stresses has not been well-documented.

Bottle gourd is under-utilized and under-researched crop and cultivated mostly using landraces in South Africa. There has been no strategic breeding of the crop and consequently there are no improved varieties released in Africa. Therefore, there is need to initiate a dedicated breeding

program to develop new cultivars with improved yield, enhanced horticultural attributes with tolerance to biotic and abiotic stresses integrating farmers and consumers preferences.

Research objectives

The main aim of this study is to initiate a pre-breeding programme of bottle gourd in South Africa for subsequent development and deployment of improved cultivars with high yield, enhanced horticultural quality and tolerance to abiotic and biotic stresses.

The specific objectives of the study were:

1. To determine the genetic diversity of bottle gourd landrace collections from the Limpopo Province, South Africa using qualitative and quantitative morphological traits.
2. To determine genetic diversity of bottle gourd landraces from the Limpopo Province, South Africa, using single sequence repeats (SSR) markers.
3. To use correlation and path coefficient analysis to determine level of association between qualitative and quantitative traits and subsequently to select suitable parents for breeding.
4. To determine drought tolerance of a diverse set of bottle gourd landraces using drought tolerance indices and to identify promising genotypes for direct production or breeding.
5. To determine the relationship between accumulation of cucurbitacins with leaf gas exchange and chlorophyll fluorescence in bottle gourd under water stress conditions.

Thesis outline

This thesis consisted of six chapters, which are outlined below. The referencing system used in this thesis is based on the referencing style of the Journal of Crop Science. The thesis is in the form of discrete research chapters, each following the format of a stand-alone research paper (whether or not the chapter has already been published). This is the dominant thesis format adopted by the University of KwaZulu-Natal. As such, there is some unavoidable repetition of references and some introductory information between chapters. The research outcomes covered in Chapter Two and Three have been published in HortScience (Volume 51, No. 2, 2016) and South African

Journal of Plant and Soil (Volume 33, No. 2, 2015). Chapter Four has been published in Acta Agriculturae Scandinavica, Section B - Soil & Plant Science (Volume 66. No. 7, 2016). Chapter Five has been published in Acta Agriculturae Scandinavica, Section B - Soil & Plant Science (Volume 67. No. 1, 2017).

The structure of the thesis is outlined below:

Chapter	Title
-	Thesis Introduction
1.	Review of Literature
2.	Assessment of genetic diversity among bottle gourd [*Lagenaria siceraria* (Molina) standl.] landraces using qualitative and quantitative traits
3.	Genetic diversity of bottle gourd [*Lagenaria siceraria* (Molina) standl.] landraces revealed by simple sequence repeat markers
4.	Correlation and path coefficient analyses of qualitative and quantitative traits in selected bottle gourd landraces
5.	Yield-based selection indices for drought tolerance evaluation in selected bottle gourd [*Lagenaria siceraria* (Molina) standl.] landraces
6.	Bottle gourd [*Lagenaria siceraria* (Molina) standl.] response to water stress: relationship between cucurbitacin accumulation with leaf gas exchange and chlorophyll fluorescence
-	An overview of the research findings

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Chapter 1: **Review of literature**

Abstract

Bottle gourd is under-utilized and under-researched crop widely grown for its fruits, seeds and succulent leaves. It is commonly grown by smallholder farmers in Limpopo Province of South Africa predominantly using unimproved landrace varieties. Thus far little effort has been directed towards genetic improvement of the crop in South Africa or sub-Saharan Africa. Landraces possess valuable genetic variability and are often well adapted to harsh environments characterized by poor soils, prolonged dry spells, low and erratic rainfall and high temperatures. Assessing the extent of genetic variation present among landraces is useful for strategic conservation and efficient use in bottle gourd improvement programmes. This review discusses flowering and fruit biology, economic importance, characterization, genetic diversity and population structure in bottle gourd germplasm and implications for breeding and conservation.

Keywords*:* Bottle gourd, breeding, conservation, crop improvement, hybrids, genetic, landrace

1.1 Introduction

Bottle gourd (*Lagenaria siceraria* (Molina) Standl.) also known as white-flowered gourd or calabash, is a diploid species (2n = 2x = 22) belonging to the genus *Lagenaria* of the *Cucurbitaceae* family (Beevy and Kuriachan, 1996; Morimoto *et al.*, 2005). The *Cucurbitaceae* family contains many other economically important species including, watermelon (*Citrullus lanatus*), cucumber (*Cucumis sativus*), melon (*Cucumis melo*) and pumpkin (*Cucurbita pepo*) (Xu *et al.*, 2011). The genus *Lagenaria* contains five other wild species, namely: *L. breviflora* (Benth.) Roberty, *L. abyssinica* (Hook f.) Jeffrey, *L. rufa* (Gilg.) Jeffrey, *L. sphaerica* (Sonder) Naudin and *L. guineensis* (G. Don) Jeffrey. All the six species are found in Africa, which is believed to be the centre of genetic diversity for *L. siceraria* (Whitaker, 1971). *Lagenaria siceraria* (Molina) Standley, is the only cultivated species. Within the species *L. siceraria*, two morphologically distinct sub-species of bottle gourd have been recognized, namely: *L. siceraria* ssp. *siceraria* and *L. siceraria* ssp. *asiatica*. The northern half of the African continent is the centre of diversity of five *Lagenaria* species, with the distribution of *Lagenaria sphaerica* (Sond.) Naud. extending to South Africa (Jeffrey, 1976).

1.2 Bottle gourd: origin, genetic diversity and distribution

Bottle gourd is one of the most ancient crops cultivated by humans in the tropics (Erickson *et al.*, 2005; Clarke *et al.*, 2006). Archaeological evidence suggested human utilization of bottle gourd at least 15,000 years ago in the New World and 12,000 years in the Old World (Richardson, 1972). The same evidence suggested that the independent use and possible cultivation of the crop started from around 9 000 to 10 000 BP (before present) in the Americas (New World), 6 000 to 10 000 BP in East Asia and 4 000 to 5 000 BP in Africa. Whitaker (1971) suggested that bottle gourd was indigenous to tropical Africa (south of Equator) and diffused to the New World by trans-oceanic drift or human transport. Heiser (1980) supporting the view of Whitaker concluded Africa as place of origin of bottle gourd. This author further conceded that there lacks a decisive evidence to distinguish between Africa and America as the original home of this species. However, recently, based on seed morphology and analysis of DNA, Erickson *et al*. (2005) reported that American

bottle gourd germplasm showed genetic variation and lineage aligned to bottle gourds derived from Eastern Asia. This suggested that domesticated bottle gourd was introduced to Americas from Asia by Paleoindians (Erickson *et al.*, 2005). Kistler *et al*. (2014) suggested that bottle gourds of America are most closely related to African gourds. The discovery of a wild indigenous bottle gourd species in Zimbabwe by Decker-Walters *et al*. (2004) reinforced Africa as the centre of origin. However, the evolutionary history of bottle remains unresolved.

1.3 Description of bottle gourd

1.3.1 Crop biology

The cultivated bottle gourd is an annual, viny, strong-growing climber or trailer (Figure 1.1 A & B) (Morimoto *et al.*, 2004). The stem has many branches. Prostrate or climbing branches of a single plant can cover varying space depending on weather conditions, soil fertility, moisture availability, and plant spacing. The plants have well developed branched root system. The nodes also develop out roots when they come in contact with moist soil. The leaves are commonly simple, reniform and wavy with entire margin. The leaves may have 3-5 shallow to deep lobes called segments. Segmented leaf shape has been reported to be dominant over the normal type. A single dominant gene 'S' is thought to be responsible for segmented leaf shape character in bottle gourd (Tiwari and Ram, 2009). The apex of the leaves may be pointed or blunt. Tendrils are borne in the axils of leaves (Singh, 2008).

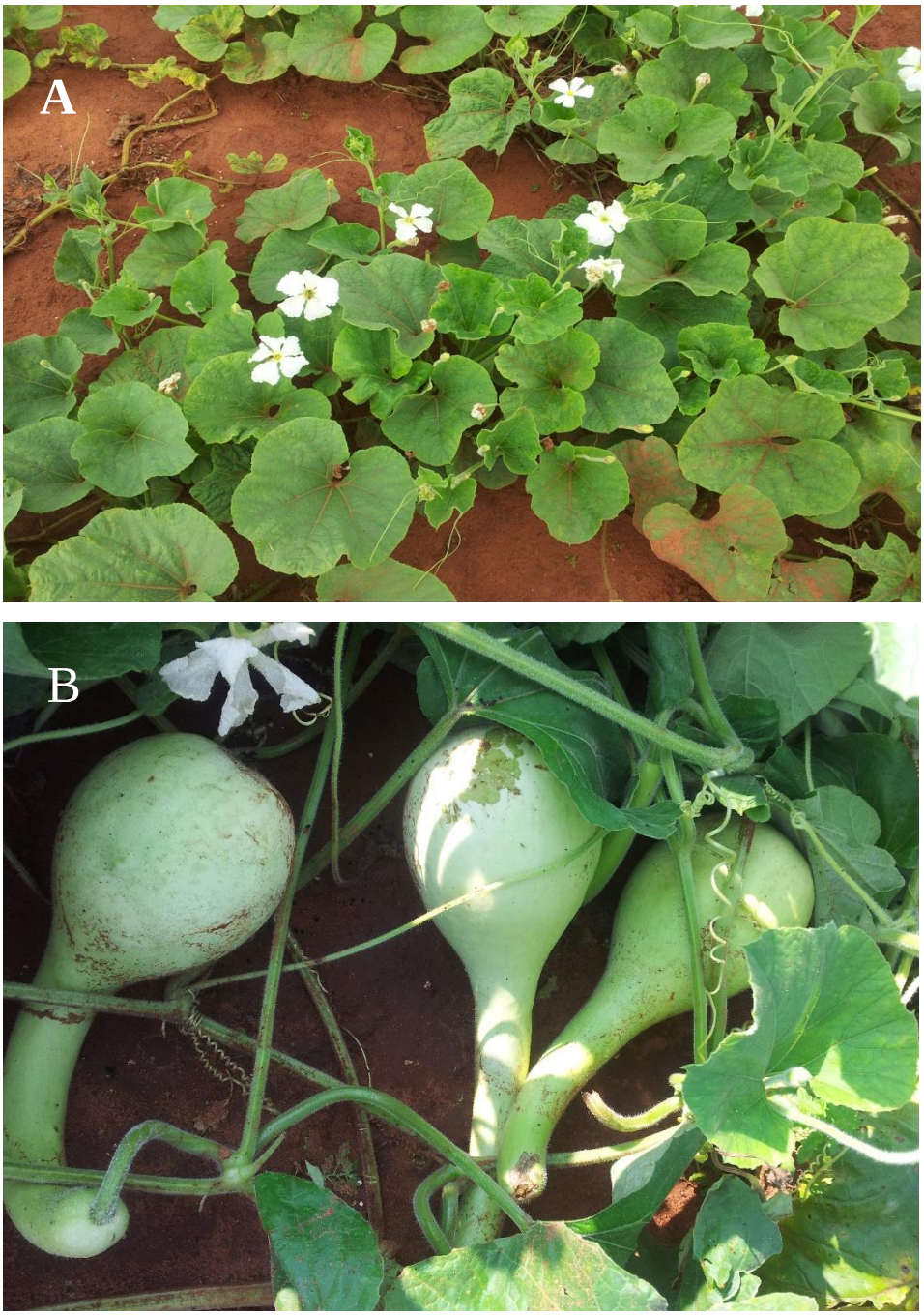


Figure 1.1. Bottle gourd plant growing in the field showing open male flowers, tendrils (A) and developed fruits (B) (photo by author).

1.3.2 Sex phenotypes

Bottle gourd is a monoecious species with male and female flowers found separately on the leaf axils of the same plant (Morimoto *et al.*, 2004; Achigan-Dako *et al.*, 2008; Singh, 2008). Though monoecious, bottle gourd is a highly cross pollinating crop (Tiwari and Ram, 2009). Dioecious (e.g. male and female flowers found on different plants) and andromonoecious sex forms (e.g. sex forms bearing male and perfect flowers) also exist in wild, non-cultivated species (Singh *et al.*, 1996). An andromonoecious sex form bearing hermaphrodite flower and male flower in the same plant have been isolated and named Andromon-6 by Singh *et al*. (1996). The male flowers of Andromon-6 are similar to normal monoecious, however; the hermaphrodite flowers exhibit a few distinguishing characteristics compared to normal female flowers. The distinguishing characteristics are: long corolla, 3.5 to 4.5 cm compared to 2.5 to 3.5 cm present in normal flowers; well-developed anthers encircling the fully developed stigma and oval/round ovary that develops into a drum-shaped fruit with 12 light grooves. Furthermore, the fruit bears scars and contain about 400 to 700 white/brown small empty non-viable seeds with under-developed seed coats. Only a few fruits bears about 1 to 25 normal viable seeds and fruit length and diameter can be about 25 and 45 cm, respectively (Singh *et al.*, 1996). The expression of monoecious and andromonoecious sex form in bottle gourd is genetically controlled (Singh *et al.*, 1996). The expression of sex form, flower, fruit morphology and seed characteristics in the F_1 generation demonstrated that monoecious sex form is completely dominant over andromonoecious sex form, normal size corolla dominant over large size corolla, long fruit shape dominant over drum-shaped oval fruit, small blossom scar dominant over large blossom scar and normal seed development dominant over abnormal seed development. Even though more than one sex form has been reported in bottle gourd, there is generally little sex phenotypes reported in this crop compared to other cucurbits. For example, in cucumber sex phenotypes includes monoecious or gynoecious (female flowers only), androecious (male flowers only), hermaphroditic (perfect flowers), andromonoecious (male and perfect flowers), and trimonoecious (male, perfect, and female flowers) (Behera *et al.*, 2009).

Male flowers in bottle gourd are borne on longer pedicels than female and hermaphrodite flowers. Both male and female flowers generally have large and white corolla with five petals. However, male flowers have larger petals than female flowers. In male flowers, stamens are apparently 3,

two are 2–celled and one is 1–celled (Singh, 2008). Female flowers have small prominent ovary, which may be round, ovate, long or cylindrical. Female flowers also have three stigmatic lobes with many ovules, generally between 400 to 700 (Morimoto *et al.*, 2004; Singh, 2008). Petals and sepals are fused. The spiny, sticky pollen is not windborne and the plants therefore require pollinators to move pollen from male to female flowers (Morimoto *et al.*, 2004).

1.3.3 Flowering and fruit set

Bottle gourd flowers are produced at leaf nodes and are solitary. Flowers open late during the day and only last for a day (Delesalle and Mooreside, 1995). Flowering starts from about 40 days after planting during summer, however; this may be influenced by cultivar differences (Morimoto *et al.*, 2004). Female flowers are normally formed on the 1st to 3rd leaf axils of the auxiliary vines surrounded by male flowers. They may also occur towards the tip of the main and auxiliary vines (Delesalle and Mooreside, 1995; Morimoto *et al.*, 2004). Generally male flowers appear before female flowers (Hossain *et al.*, 1990; Delesalle and Mooreside, 1995; Morimoto *et al.*, 2004). Thereafter, a flush of male and female flowers occurs continuesly. Male flowers remain open only for a few hours and eventually wither and die off; thus, the flowers have a short life span (Shah *et al.*, 2010; Sugiyama *et al.*, 2014). Therefore, the flowering period is normally shorter in male flowers than in female flowers (Morimoto *et al.*, 2004). Flower opening occurs late in the afternoon and sometimes during the night (Sugiyama *et al.*, 2014). Hossain *et al*. (1990) reported that both male and female flowers opened between 16h30 and 18h00 and closed 14 hours later. Morimoto *et al*. (2005) also reported that flowering began between 17h30 and 23h00 taking almost 60 to 90 minutes to open fully and closed between 07h00 and 13h00 the following day. Pollen of male flowers is only viable for two days (Sugiyama *et al*. 2014). The sex ratio in bottle gourd is a male-biased floral sex ratio (Delesalle and Mooreside, 1995). Floral sex ratio in bottle gourd is reported to be much more male-biased than is typical for monoecious species (Sutherland, 1986). The ratio of male: female flowers may vary from 5:1 to 15:1 in the common types. Higher male: female sex ratio of 20: 1 and 26:1 have also been reported (Delesalle and Mooreside, 1995; Morimoto *et al.*, 2004). Even though sex determination genes controls sex expression, several authors have indicated that environmental conditions (e.g. precipitation, temperature and light intensity) and plant growth regulators (e.g. auxins, ethylene, gibberellic acid) can alter sex ratio (Arora *et al.*,

1982; Quan Yu, 1999; Ingle *et al.*, 2000; Desai *et al.*, 2011; Mishra *et al.*, 2013). Higher temperature, long day length, and high light intensity and high nitrogen levels are reported to result in more male flowers (Singh, 2008; Tan *et al.*, 2009) while the opposite results in more female flowers.

Fruit formation can occur only after 24 hours following pollination (Singh, 2008). Fruit shape differences has been reported to have an effect on flowering in bottle gourd. Morimoto *et al*. (2004) reported that small round edible, often warted types produced more female flowers compared to the dipper, bilobal and club–shaped types. In general higher proportions of female flowers are borne in a plant even during summer but only a few turns into fruits. Morimoto *et al*. (2004) observed that female flowers appearing on the early leaf axils (early nodes) have a higher chance of developing into fruits than those appearing later. Female flowers developing towards the end of the creeping branches usually develop into smaller fruits which often die (Delesalle and Mooreside, 1995). The mechanisms responsible for premature death of fruits in bottle gourd are not well-understood. First edible fruits can be available between 55 to 65 days after sowing in early varieties (Singh, 2008).

1.3.4 Fruit and seed characteristics

Bottle gourd exhibits great diversity with respect to fruit shapes (Morimoto *et al.*, 2005; Singh, 2008; Yetişir *et al.*, 2008; Xu *et al.*, 2013). Figure 1.2 shows some common fruit shapes found from bottle gourd collections of South Africa (Figure 1.2A) and Turkey (Figure 1.2B) (Yetişir *et al.*, 2008). The specific shape of the fruits depends on the variety while the size of the fruits depends on amount of rainfall, and its position along the vine (Morimoto *et al.*, 2004). The fruit is often globular, bottle-or club-shaped, cylindrical, necked, oblate, flat, flat-round, conical, pear-shaped, white-yellow to dark green when young, with a hard and durable rind (Achigan-Dako *et al.*, 2008).

Fruit size varies considerably from 5 to 40 cm in diameter, and 20 to 90 cm in length. There also occurs a great range of variation in weight of the fruits (Singh, 2008). The fruit weight at maturity varies between 1 to 10 kg. However, at edible green stage commonly the fruit weight varies from

0.5 kg to 2.0 kg because at this stage the fruit size is smaller. At edible stage the fruits are pale green, green or dark green. At maturity the fruit turn into cream or creamish-brown in colour (Singh, 2008). Fruit characters in bottle gourd are not generally indicative of subspecies; however, plants producing large round fruits are typically native to tropical West Africa, whereas the long, thin, snake-like fruits are considered to be of Asian origin. Warted gourds occur only in Africa and the New World, and the 'maranka-type' gourds, which have 10 lengthwise, knobby, contorted ridges along the spherical region of the dipper-shaped fruit, occur in Africa (Heiser Jr, 1973).

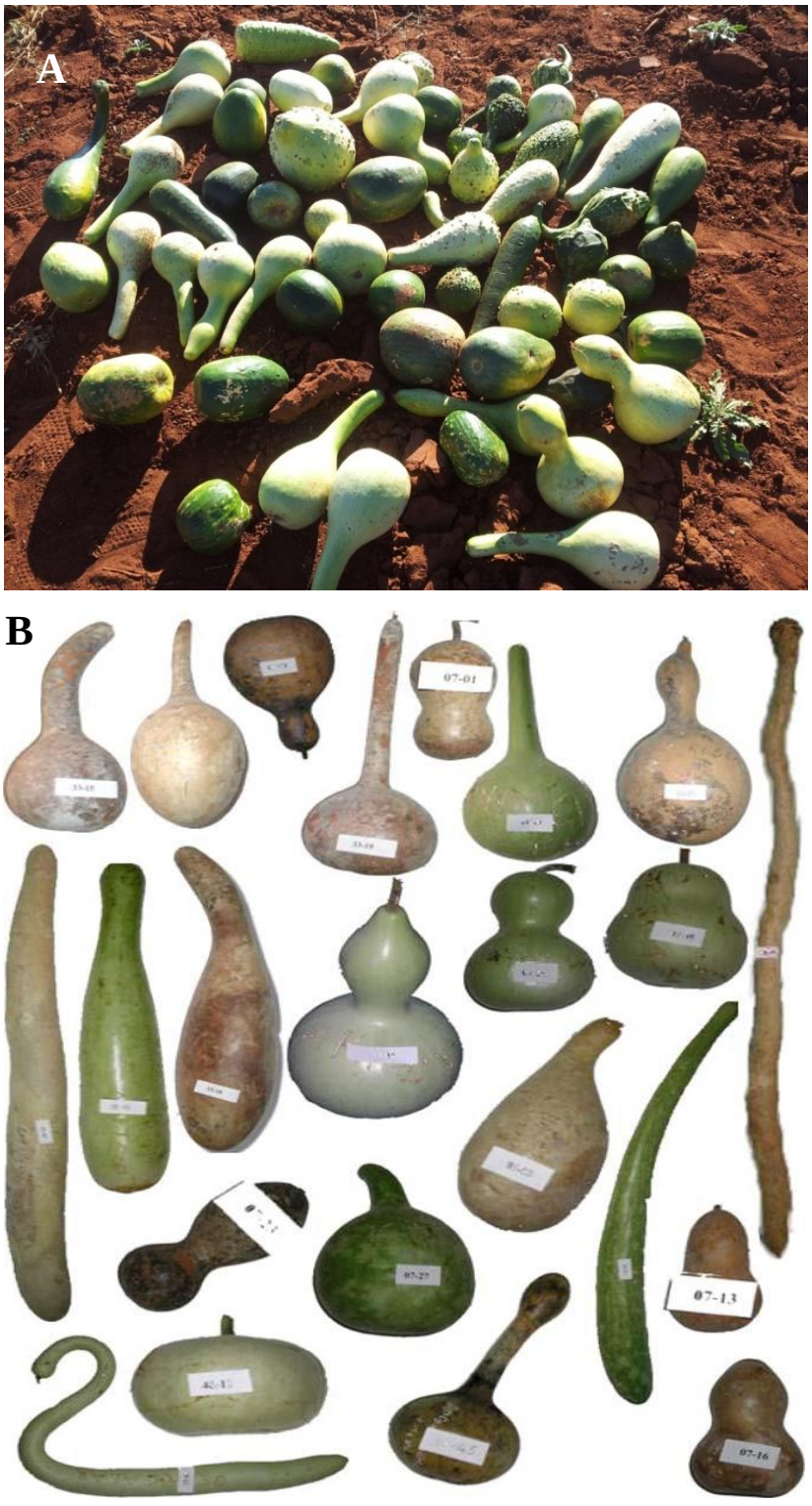


Figure 1.2. Common fruit shapes of bottle gourd genotypes grown in South Africa (A, Photo by author) and Turkey (B, Yetişir *et al*., 2008).

Edibility of bottle gourd fruit varies with size and shape. Edible fruits are relatively small and either oblate, spherical ovoid or pyriform in shape while non-edible types are characterized by relatively thin shells and had dipper, club or elongated cylindrical shapes, with handles (Morimoto *et al.*, 2005). The fruits may be sweet or bitter (Milind and Satbir, 2011). Bitter types are not edible, although they have medicinal importance. Bitter fruit taste has been reported to be dominant over the sweet taste and they are differentiated by single gene difference (Tyagi, 1976). The number of seeds per fruit varies from 400 to 700 with seed weight varying from 10 to 15g per 100 seeds (Singh, 2008). Furrows and ridges are also present on seeds. The seeds are oblong, generally up to 2 cm long, emarginated at the base, with two facial ridges, mostly smooth, whitish to brownish (Achigan-Dako *et al.*, 2008). Brown seed colour has been reported to be dominant over light brown and the mode of segregation is controlled by a pair of genes (Tyagi, 1976).

1.4 Economic importance of bottle gourd

Bottle gourd is mostly grown for its fruit either being harvested young and used as a vegetable or harvested mature and used as a bottle, utensil, or pipe (Hart, 2011). The fresh fruit, which usually has a light green smooth skin and a white flesh, is frequently used in many regions of Asia and Africa as either a stir-fry or vegetable soup (Morimoto and Mvere, 2004). The tender edible fruits may also be used to prepare sweets, pickles and other delicious dishes (Singh, 2008). It is a preferred vegetable for treating ailments because of its cooling effect to the stomach and easy digestibility (Singh, 2008). The young edible fruits are rich in dietary fibre, essential minerals (e.g. iron, phosphorus, potassium, zinc, magnesium etc.), amino acids and vitamins (e.g. vitamins B and E) (Milind and Satbir, 2011). The juice from the fruit helps control blood pressure due to its high potassium content. It also help in weight loss due to its high dietary fibre and low fat and cholesterol levels (Milind and Satbir, 2011; Sukhlecha, 2012). Fresh bottle gourd juice is used as medicine to cure diseases like flatulence, diabetes mellitus, hypertension, liver diseases and as a diuretic (Ghule *et al.*, 2007). The crop is regarded as a useful vegetable for the management of many diseases like cardiac disorders, hepatic diseases and ulcer. The seeds are rich in essential amino acids and are also used for oil extraction. The seeds are rich in protein, minerals, lipids and fatty acids (Achu *et al.*, 2005; Fokou *et al.*, 2009; Essien *et al.*, 2013) ideal for human food or for incorporation into livestock feed (Achu *et al.*, 2005; Ojiako and Igwe, 2007; Ogunbusola *et al.*,

2010). The fatty acid profile shows linoleic acid as the most abundant (62%) as compared to oleic (16.2%), palmitic (14.4%) and stearic (5.8%) acids. High linoleic and low linolenic acid levels of these oils suggest that they could be sources of good edible oils for cooking. The abundance of linoleic followed by oleic acid in bottle gourd seed makes them good oils for reducing serum cholesterol and low density lipoprotein (LDL) and increasing high density lipoprotein (HDL) levels, hence, they could be good oils to fight against cardiovascular illnesses (Fokou *et al.*, 2009). Furthermore, bottle gourd seed are also a good source of amino acids. A study by Ogunbusola *et al*. (2010) showed that glutamic acid (139–168 mg/g protein) was the most abundant amino acid followed by aspartic acid (89.0 to 116 mg/g protein). These authors further reported that the total essential amino acid ranged from 45.8 to 51.5%.

Further, bottle gourd is used as rootstocks for watermelon (*Citrullus lanatus var. lanatus*) owing to its tolerance to various biotic and abiotic stress factors. It is reported to be highly tolerant to fusarium wilt disease (Yetisir and Sari, 2003) and tolerant to salt (Colla *et al.*, 2005; Yetisir and Uygur, 2010). Production of seedless watermelon by pollinating with bottle gourd pollen has recently been demonstrated (Sugiyama *et al.*, 2014). As a result, plant breeders are increasingly interested in exploring the bottle gourd germplasm for use in watermelon breeding. However, to preserve the genetic richness of this crop for such purposes, efforts must be directed towards the achievement of reliable collection and conservation strategies (Bhawna *et al.*, 2015). Conservation of genetic resources is critical to plant breeding because economically important traits are constantly sought after to increase yield and quality and improve human health (Gaikward *et al.*, 2008).

1.5 Genetic diversity and crop improvement

Neglected and underutilized crops such as bottle gourd play a prominent role in sustaining the livelihood of poor rural people by increasing food availability (Padulosi *et al.*, 2002). The significance of ‘underutilized’ and ‘neglected’ crops such as bottle gourd for global food security has received attention by the researchers and food security policy organizations (Massawe *et al.*, 2007). In most cases, underutilized species exist only as landraces and wild collections due to many years of neglect by researchers (Massawe *et al.*, 2007). Recently, the importance of these

crop species has been realized and research has been re-directed towards these crops because of the important role they play in house-hold food security.

Genetic diversity within a crop gene pool is important for breeding and germplasm conservation (Smith *et al.*, 1991; Pagnotta *et al.*, 2009; Bhawna *et al.*, 2015). Genetic variation for crop improvement exists in modern varieties, exotic germplasm, genetically modified plants or mutants. The success of a breeding programme depends on careful selection of individuals that have superior or specific attributes needed by growers and consumers (Zamir, 2001). Knowledge of the genetic diversity among breeding materials is important to avoid the risk of increasing uniformity in elite germplasm, and in order to ensure long term selection gain. This is because crossing of a limited number of elite lines creates the danger of losing their genetic diversity.

Genetic diversity should be maintained at two different stages: (i) during formation of core collections where genetic diversity is maximized with minimum repetition (Pessoa-Filho *et al.*, 2010) and (ii) in the intermediate generations of a breeding programme to conserve genetic variability for selection in later generations (El-Basyoni *et al.*, 2013). Genetic diversity can be determined using different methods such as pedigree analysis, morphological, biochemical or DNA based markers. The first step towards identifying genetic diversity patterns in a given population is to estimate the similarities or differences among genotypes.

Despite its economic and nutritional values, bottle gourd is relatively under studied and under-utilized genetic resource in Africa. Further, there has been no targeted breeding of the crop and consequently there are no improved varieties of bottle gourd in South Africa where it is mostly grown using unimproved landraces maintained by farmers. Therefore, detailed genetic diversity analyses of the crop need to be undertaken to understand population structure and to maximize the genetic potential of the crop. Characterization of genetic diversity among cultivated bottle gourd varieties may be important for the selection of traits for breeding.

1.6 Germplasm characterization

Characterization of genotypes is fundamental for breeding and strategic conservation. To serve the diverse human needs the genetic diversity of 'underutilized' and 'neglected' crops like bottle gourd needs to be harnessed through an effective pre-breeding program. The standard descriptors for bottle gourd have been used as guidelines in phenotypic characterization (Morimoto *et al.*, 2005; Yetişir *et al.*, 2008). Table 1.1 summarizes some qualitative traits that have been used for bottle gourd phenotyping. The diversity represented especially by fruit qualitative provides an important basis for the breeding of new bottle gourd cultivars. Qualitative traits form discrete phenotypic classes that can be assessed visually and are therefore useful for breeding and genetic analysis of the crop (Manivannan *et al.*, 2016). Several genes controlling different qualitative traits have been described in bottle gourd (Table 1.2). Inheritance studies of several qualitative traits are yet to be studied in bottle gourd including blossom and stem-end fruit shape, presence or absence of fruit neck, fruit neck length and other fruit shapes (e.g. necked vs non-necked, pyriform vs elongated and snake-like vs. curvilinear etc.).

Table 1.1. Summary of qualitative traits used in genetic diversity analysis in bottle gourd.

Trait	Class	Reference
Seed size	Small	Yetisir *et al*. (2008)
	Medium	
	Large	
Seed margin	Absent	Yetisir *et al*. (2008)
	Thin and uniform	
	Thin and irregular	
	Thick uniform	
	Thick irregular	
Seed margin color	Absent	Yetisir *et al*. (2008)
	White	
	Tan	
	Yellow	
	Orange	
	Brown	
	Grey	
	Black	
	Light brown	
	Dark brown	
Cotyledon size	Small	Yetisir *et al*. (2008)
	Intermediate	
	Large	
Cotyledon color	Light green	Yetisir *et al*. (2008)
	Intermediate	
	Dark green	
Leaf shape	Ovate	Yetisir *et al*. (2008)
	Orbicular	
	Reniform	
	Retuse	
	Heart	
Leaf size	Small	Yetisir *et al*. (2008)
	Intermediate	
	Large	
Leaf edge	Smooth	Yetisir *et al*. (2008)
	Toothed	
Leaf pubescence	Small	Yetisir *et al*. (2008)
	Intermediate	
	Large	
Pubescence of upper surface of leaf	Small	Yetisir *et al*. (2008)
	Intermediate	
	Large	
Branching pattern	Central	Mashilo *et al*. (2015)
	Basal	
	All over	

Table 1.1. (Continued)

Trait	Class	Reference
Peduncle transactional Shape	Round Slightly angled Angled	Yetisir *et al*. (2008)
Peduncle attachment	Easy Intermediate Difficult	Yetisir *et al*. (2008)
Stem end fruit shape	Depressed Flattened Rounded Pointed	Yetisir *et al*. (2008) and Sivaraj and Pandravada (2005)
Blossom end shape	Depressed Flattened Rounded Pointed	Yetisir *et al*. (2008) and Sivaraj and Pandravada (2005)
Fruit shape	Oblate Circular Pyriform Elongated pyriform Cavate Cylindrical	Sivaraj and Pandravada (2005)
Variation in fruit shape	Low Intermediate High	Yetisir *et al*. (2008)

Table 1.2. Genes controlling some qualitative traits in bottle gourd.

Gene	Description	Reference
Bb	Bottle-shaped fruit	Tyagi (1976)
BB	Disk-shaped fruit	Tyagi (1976)
Rr	Produces round-shaped fruit	Tyagi (1976)
GG	Dark green fruit colour	Tyagi (1976)
Gg	Light green fruit colour	Tyagi (1976)
Lblb	Light brown seed coat colour	Tyagi (1976)
LbLb	Brown seed coat colour	Tyagi (1976)
WtWt	Warty fruit texture	Mladenovic *et al*. (2013)
Wtwt	Smooth fruit texture	Mladenovic *et al*. (2013)
YY	Yellow fruit colour in ornametal gourd	Paris *et al*. (2003)
Yy	Green fruit colour in ornamental gourd	Paris *et al*. (2003)
SS	Segmented leaf shape	Tiwari and Ram (2009)
Ss	Normal leaf shape	Tiwari and Ram (2009)

Genetic diversity in bottle gourd has also been described based on quantitative phenotypic traits (Morimoto *et al.*, 2005; Yetişir *et al.*, 2008; Koffi *et al.*, 2009; Mladenovic *et al.*, 2012; Xu *et al.*, 2014). Quantitative traits widely reported in genetic diversity studies are summarized in Table 1.3. Coefficient of variation (CV's) in these studies tended to be larger for fruit traits than other quantitative traits indicating that bottle gourd is more diversified in fruit size and fruit shape (Morimoto *et al.*, 2005; Sivaraj and Pandravada, 2005).

Table 1.3. Quantitative phenotypic traits and corresponding mean values reported in bottle gourd.

Quantitative trait	Mean	Reference
Days to 50% female flowering	54.2 (7.38)	Harika *et al*. (2012)
Number of nodes on main-stem	5.21 (5.9)	Harika *et al*. (2012)
Sex ratio	17.4 (9.2)	Harika *et al*. (2012)
Number of branches	8.89 (5.1)	Harika *et al*. (2012)
Number of fruits per plant	7.62 (7.5)	Harika *et al*. (2012)
Fruit mass (kg)	1.1 (8.3)	Harika *et al*. (2012)
Fruit length (cm)	35.6 (7.2)	Harika *et al*. (2012)
	17.8 (60)	Morimoto *et al*. (2005)
	38.7 (51.7)	Sivaraj and Pandravad (2005)
Fruit width (cm)	8.77 (17.7)	Harika *et al*. (2012)
	10.8 (33)	Morimoto *et al*. (2005)
	19.3(38.3)	Sivaraj and Pandravad (2005)
Fruit circumference (cm)	42.2(39.1)	Morimoto *et al*. (2005)
Volume of fruit (cm^3)	1016 (93)	Morimoto *et al*. (2005)
Length between apical point and wide fruit part (cm)	5.6 (40)	Morimoto *et al*. (2005)
Relative length of fruit width	0.76 (45)	Morimoto *et al*. (2005)
Relative length of the apical point	0.36 (32)	Morimoto *et al*. (2005)
Shell thickness (cm)	2.87 (10.6)	Harika *et al*. (2012)
	0.42 (39)	Morimoto *et al*. (2005)
	0.31 (9.7)	Sivaraj and Pandravad (2005)
Number of seeds per fruit	304 (50)	Morimoto *et al*. (2005)
Seed length (cm)	1.32 (16)	Morimoto *et al*. (2005)
	1.67(10.7)	Sivaraj and Pandravad (2005)
Seed width (cm)	0.68 (16)	Morimoto *et al*. (2005)
	0.74 (9.6)	Sivaraj and Pandravad (2005)
Seed size (cm^2)	0.92 (32)	Morimoto *et al*. (2005)
Hundred seed weight (g)	14.05 (8.1)	Harika *et al*. (2012)
	17.9 (26.8)	Sivaraj and Pandravad (2005)
Days to maturity	63.61 (6.9)	Harika *et al*. (2012)

Values in parenthesis are coefficient of variation (CV) in percentages.

Germplasm characterization using morphological markers is an important first step in the description and classification of crop genetic resources because a successful breeding programme

mainly depends upon the magnitude of morphological variability (Smith *et al.*, 1991; Belaj *et al.*, 2011). Morphological characterization facilitates efficient utilization of germplasm collections in a breeding program, providing direct useful information about the genetic relationships and specific traits of agronomic importance (Laurie *et al.*, 2012). However, assessment of variability based on agro-morphological characteristics has limitations, since most of the characters are influenced by environmental factors and plant developmental stage (Morimoto *et al.*, 2005; Dey *et al.*, 2006; Behera *et al.*, 2012). Therefore, genetic diversity analysis using morphological characterization can be complemented by other tools such as DNA based molecular markers for accuracy or repeatability.

1.7 Molecular marker based characterization

Molecular markers are powerful complementary tool to phenotyping. Molecular marker-based characterisation measures genetic distances independent to genotype × environment interaction and show higher levels of polymorphism (Decker-Walters *et al.*, 2004; Pagnotta *et al.*, 2009). DNA markers relate variability directly at the genetic level and provide reliable data that permit a reproducible estimate of genetic diversity within the germplasm (Pandey *et al.*, 2008).

Several molecular markers have been used to assess genetic variability in bottle gourd namely: chloroplast markers, random amplified polymorphic DNA (RAPD), sequence related amplified polymorphism (SRAP), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSRs) or microsatellite markers, inter-simple sequence repeats (ISSR), single nucleotide polymorphism (SNP's) and allozyme markers (Table 1.4) (Decker-Walters *et al.*, 2001; Koffi *et al.*, 2009; Xu *et al.*, 2011; Saxena *et al.*, 2015). Simple sequence repeat markers are currently the marker of choice for genetic diversity analysis studies because of their high degree of polymorphism and random distribution across the genome (Varshney *et al.*, 2005; Gong *et al.*, 2012; Ji *et al.*, 2012). Xu *et al*. (2011) designed 400 SSR loci and selected a subset of 14 SSR markers for bottle gourd genotyping. Bhawna *et al*. (2015b) also developed 40 SSRs useful for cultivar identification and breeding in bottle gourd. The SSR markers developed for bottle gourd can be employed to screen segregating population to identify breeding lines possessing desirable traits in a marker-assisted breeding of the crop. This will facilitate speedy release of improved

bottle gourd varieties (Xu *et al.*, 2011; Bhawna *et al.*, 2015b). Further research is needed to develop SSR markers linked to important qualitative and quantitative traits. This can be applied for marker-assisted selection to improve traits of interest and to facilitate efficient breeding and variety development.

Table 1.4. Marker systems used in genetic diversity analysis of bottle gourd.

Country	Marker type	No. of landraces evaluated	No. of cultivars evaluated	Bottle gourd species	References
USA	RAPD	31	-	*L. siceraria & L. sphaerica*	Decker-Walters *et al*. (2001)
Côte d'Ivoire.	Allozyme	30	-	*L. siceraria*	Koffi *et al*. (2009)
Kenya	RAPD	95	-	*L. siceraria, L. sphaerica L. abyssinica, L. breviflora*	Morimoto *et al*. (2006)
India	SSR		40	*L. siceraria*	Bhawna *et al*. (2015b)
India	SSR	-	20	*L. siceraria*	Sarao *et al*. (2014)
India	SSR	-	44	*L. siceraria*	Bhawna *et al*. (2015a)
India	ISSR	-	42	*L. siceraria*	Bhawna *et al*. (2014)
China	SSR	39	5	*L. siceraria*	Xu *et al*. (2011)
Turkey	SSR and SRAP	-	30	*L. siceraria*	Yildiz *et al*. (2015)
Turkey	Chloroplast and SSR	60	31		Gurcan *et al*. (2015)

1.8 Intra-and-inter population structure among *Lagenaria* species

Intra-population diversity among *Lagenaria* species namely: *L. siceraria*, *L. sphaerica*, *L. abyssinica*, and *L. breviflora*, revealed great genetic differentiation (Morimoto *et al.*, 2005). Yildiz *et al*. (2015) using SSR and SRAP markers discriminated *L. siceraria* and *L. cylindrica*. Further, successful hybrids have been developed between *L. sphaerica and L. siceraria* (Meeuse, 1962) indicating that the level of gene flow and compatibility among these species is relatively high. However, there is little information documented on discriminant morphological traits among *Lagenaria* species. There is also limited information on gene flow among *Lagenaria* species and the extent of introgression among the different species (Decker-Walters *et al.*, 2004). Whether outcrossing between the *Lagenaria* species will enhance or reduce genetic diversity is yet to be determined. The current gap in species delimitation suggests the need for further research to

broaden our understanding of the associations between phenotypic and genotypic variation among *Lagenaria* species for efficient germplasm utilization, breeding or strategic conservation.

Some bottle gourd types called "egusi" are exclusively grown for their seeds (Achigan-Dako *et al*., 2008). Intra-species analysis between "egusi" and "gourd type" indicated that the gourd type has a smaller genome size in comparison to the egusi type (Achigan-Dako *et al*., 2008). Differences in genome size between the egusi and gourd type may suggest genetic differences between the two forms. In watermelon, Achigan-Dako *et al*. (2015) reported that egusi watermelon (*C. mucosospermus*) was genetically differentiated from other *Citrullus* species. Further, domesticated forms of bottle gourd are non-bitter whereas wild forms are very bitter (Sivaraj and Pandravada, 2005). Bitterness is caused by cucurbitacins. The low levels of cucurbitacins in domesticated bottle gourd suggest that growers could have selected for non-bitterness through domestication and unconscious selection (Qi *et al.*, 2013). There is little information that documented genetic differences between bitter and non-bitter bottle gourd types and whether these two forms could be genetically similar or distinct.

1.9 Pre-breeding

Nass and Paterniani (2000) defined pre-breeding activities to include the following: (1) the production of new base populations for a structured breeding program; (2) identify heterotic group for either hybrid production or further selection procedures; and (3) the establishment of a core collection when working with wild species and landraces. The Global Partnership Initiative for Plant Breeding Capacity Building (GIPB)/FAO and Biodiversity International use the term 'pre-breeding' to describe the various activities of plant breeding research that have to precede the stages involved in cultivar development, testing and release (Biodiversity International and GIPB/FAO, 2008). Further, the Global Crop Diversity Trust defined pre-breeding as 'the art of identifying desired traits, and incorporation of these into modern breeding materials. Pre-breeding is routinely applied in commercial breeding programs where desired traits are constantly sought and identified from source genotypes for use in cultivar development. Overall, pre-breeding includes all activities directed at identification of desirable crop traits and/or genes, and their

subsequent transfer into a suitable set of parents for further selection. Identification of suitable genotypes with complementary economic traits is an important step for crop improvement and sustainable crop production (Tseng *et al.*, 2002; Elameen *et al.*, 2011). Understanding the genetic variability and genetic interrelationship present among germplasm collections is valuable to avoid redundancy, and allows plant breeders to select potential parents with desirable traits to be used in development of new cultivars (Chaudhary and Singh, 1982; Yoshida, 2004).

A pre-breeding program may help to identify suitable parent and agronomic traits for crop improvement. Pre-breeding refers to all concerted activities and/or procedures designed to identify desirable characteristics and/or heritable genes from otherwise un-adapted and unimproved plant genetic resources and their subsequent manipulation in the actual breeding of improved cultivars (Nass and Parterniani, 2000). It is a vital step that links conservation and the use of plant genetic resources especially for breeding. Pre-breeding enables precise and fast selection of suitable genetic sources and forms the initial steps of breeding. Pre-breeding is the route for genetic enhancement whose valuable agronomic characteristics can be used by plant breeders. How such activities are conducted, varies among breeders and crop species. Plant genetic resources in pre-breeding program include wild species and landraces because they harbour desirable genes necessary for improving yield, pest and disease resistance, food quality and adaptation to heat and drought stress.

1.10 Landraces as source of genetic diversity for breeding

Camacho *et al.* (2005) defined a landrace as ‘‘a dynamic population of cultivated plants that has historical origin and distinct identity, and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems’’. Landraces are crop genetic resources that have evolved under natural and farmer selection rather than plant breeding (Harlan, 1975; Zeven, 1998) and are a valuable genetic resource of potentially useful traits. Landraces or traditional varieties have played an important role in the introduction of improved adaptive characteristics (Hawtin *et al.*, 1996). For example, in wheat breeding programmes, the first improved varieties consisted of selections of local landraces. One such bread wheat variety, ‘Aragon 03’, selected from the indigenous landrace population ‘Catalan de Monte’,

was the leading variety in Spain during the period 1960–1976 due to its capacity for drought tolerance (Royo and Briceño-Félix, 2011). Pigeonpea and chickpea traditional landraces or selections from them were released directly as varieties (Asthana *et al.*, 1996; Remanandan, 1996). A large number of varieties evolved from local cowpea landraces has also been commercialized in India (Sharma, 1996). Hedge and Mishra (2009) also reported that cowpea landraces were found as an important source of genetic variability for grain yield, drought and heat tolerance. Therefore, landraces are potential sources of genes for characters that can be used in breeding programmes. However, for genetic resources to remain a foundation for future sustainable agricultural development, their conservation and breeding strategies are needed (Hawtin *et al.*, 1996).

Despite their importance, the cultivation or use of landraces is challenging because in many cases they have already disappeared or cannot be properly identified, which in turn, prevent the possibility for their conservation and utilization (de Carvalho *et al.*, 2013; Bashir *et al.*, 2015). Worldwide initiatives for the conservation and utilization of landraces are currently active (e.g. Biodiversity International and more recently the initiatives of the Global Crop Diversity Trust, The Global Seed Vault, etc.) (Bashir *et al.*, 2015). Various landraces and wild type accessions of bottle gourd are held in various organizations around the globe (Table 1.5). Ensuring that the inbred lines or landraces that have been sequenced are generally available to researchers through germplasm banks will be an important step to widening the genetic composition of bottle gourd in cultivar improvement programmes. In some parts of South Africa particularly the Limpopo Province, farmers cultivate unimproved landrace varieties of bottle gourd which exhibits tremendous genetic diversity useful for genetic improvement of the crop (Mashilo *et al.*, 2016).

Landraces provide a rich source of genes, but at the same time plant breeders, who want to create new high-yielding varieties, tend to make crosses among elite lines where they have the highest likelihood of developing new varieties (Baenziger and DePauw, 2009). As a result, landraces have rarely been utilized in hybridization programme to understand their breeding value to improve grain yield and other economically important traits (Hawtin *et al.*, 1996). Landraces, which have evolved and mixed through natural and artificial selection processes makes them the most genetically diverse of the cultivated lines (Zeven, 1998). Landraces can be considered as likely sources of putatively lost variability and may provide new genes or alleles, which could be

incorporated into modern varieties by hybridization. Modern crop varieties have evolved from either genetically homogeneous (e.g. clones) or heterogeneous parents (e.g., seeds resulted from self- or cross fertilization) through careful selection and hybridization (Bonnin *et al.*, 1996). These genetic resources are the basis for present and future food security. However, the importance of widening genetic diversity requires several actions in addition to hybridization in breeding programmes. These include monitoring genetic diversity and increasing the frequency of rare alleles using landraces in breeding programmes; finding 'new' allelic variation for known functional genes among landraces; and promoting phenotypic characterization of landraces for adaptation to climate change.

Table 1.5. Bottle gourd landrace or accession collections maintained by various institutions worldwide.

Organization/Institution	Number of bottle gourd collections	Country	Reference
Agricultural Research Institute (AARI)	-	Turkey	Yetisir *et al.* (2008)
U.S. National Plant Germplasm System (NPGS)	-	USA	Decker-Walters *et al.* (2001)
USDA-ARS Plant Genetic Resources Conservation Unit (Griffin, GA)	234	USA	Kousik *et al.* (2008)
International Plant Genetic Resource Institute (IPGRI)	425	Kenya	Morimoto *et al.* (2005)
Kenya Resource Center for Indigenous Knowledge	425	Kenya	Morimoto *et al.* (2005)
Genbank of the Leibniz-Institute of Plant Genetics and Crop Research	-	Germany	Achigan-Dako *et al.* (2008)
Department of Vegetable Science	-	India	Srivastava *et al.* (2014)
University of Abobo-Adjamé	-	Côte d'Ivoire	Koffi *et al.* (2009)
Zhejiang Academy of Agricultural Sciences	-	China	Xu *et al.* (2014)
Department of Agriculture and Fisheries	-	South Africa	-

- Unknown number of collections held

1.11 Breeding neglected and underutilized crops for drought tolerance

Neglected and underutilized crops like bottle gourd are reported to be adapted to dry areas with limited rainfall (Backeberg and Sanewe, 2010; Mabhaudhi, 2012) probably attributed to many

years of directed selection by famers living in arid and semi-arid areas. These suggest that breeding these crops for drought tolerance is possible. The development of drought tolerant cultivars largely depends on the existence of genetic diversity and the comprehensive exploration of potential genetic resources (Rampino *et al.*, 2006). The initial population for evaluation must be large and diverse to maximize strong selection response. Selected cultivars, landraces or wild species present some of the genetic resources that can be exploited for drought tolerance breeding. Crosses made between locally adapted varieties with improved cultivars are recommended for drought tolerance improvement (Fischer *et al.*, 2003). Landraces are more phenotypically and genotypically diverse than pure lines, and are excellent sources of genetic variation for breeding (Zeven, 1998). According to Blum and Sullivan (1986), farmers' local varieties may possess some unique physiological attributes that may not be present in germplasm not exposed to abiotic stress (e.g. heat and drought stress); therefore, making them a potential key genetic resource for crop improvement. Bottle gourd exhibits abundant genetic and morphological variation signifying wide ecological adaptation. It has been reported that the adaptation and distribution of bottle gourd is bi-hemisphere and it grows well in the tropical and temperate areas in Africa. The species is also adapted to high altitude sub-tropical, tropical and temperate climates as well as semi-arid to arid climates; and grows well in areas with rainfall between 400-1500 mm per annum (Haque *et al.*, 2009). The capacity of bottle gourd to grow under diverse climates could perhaps be explained by a broad genetic diversity which could be inherent in the species. This genetic variability may to a large extent account for the wide adaptation of the species to distinct environments and specifically to water limited conditions.

1.11.1 Physiological response to drought stress

Water stress leads to a decrease in plant water content, turgor reduction and consequently causing a decrease in cellular expansion and alteration of various vital physiological, biochemical and molecular processes that affect growth and productivity (Reddy *et al.*, 2004; Costa *et al.*, 2008; Lobato *et al.*, 2008). However, the negative effects due to drought stress can be reduced by triggering various physiological drought adaptive mechanisms (Hura *et al.*, 2007; Hura *et al.*, 2009a). One important physiological mechanism that enhances drought tolerance is osmotic

adjustment. Osmotic adjustment is defined as the accumulation of solutes within the plant tissue (either in roots or shoot) in response to a lowering of soil water potential leading to the lowering of the water potential, which provides the driving force for water extraction at low water potential (Morgan, 1984; Ramanjulu and Sudhakar, 2000). Osmotic adjustment is thought to enable the maintenance of turgor which might help in sustaining physiological processes such as stomatal opening, photosynthesis, cell enlargement, delay leaf senescence and death, improve plant growth and increase water extraction under drought stress conditions (Morgan, 1984; Munns, 1988; Ludlow and Muchow, 1990; Kusaka *et al.*, 2005). Plants that use tolerance mechanism maintain turgor through osmotic adjustment by accumulation of compatible solutes in the cell like sugars, proteins, proline, which increase cell elasticity, decrease cell volume and resistance to desiccation (Turner, 1986; Agbicodo *et al.*, 2009). These, compatible solutes can act as free-radical scavengers/antioxidants and directly stabilize proteins and membranes (Wang *et al.*, 2003). Therefore, breeding for drought tolerance requires understanding of the physiological mechanisms involved in drought tolerance (Subbarao *et al.*, 1995) and studying these responses may help in breeding cultivars adapted to drought conditions (Yordanov *et al.*, 2000).

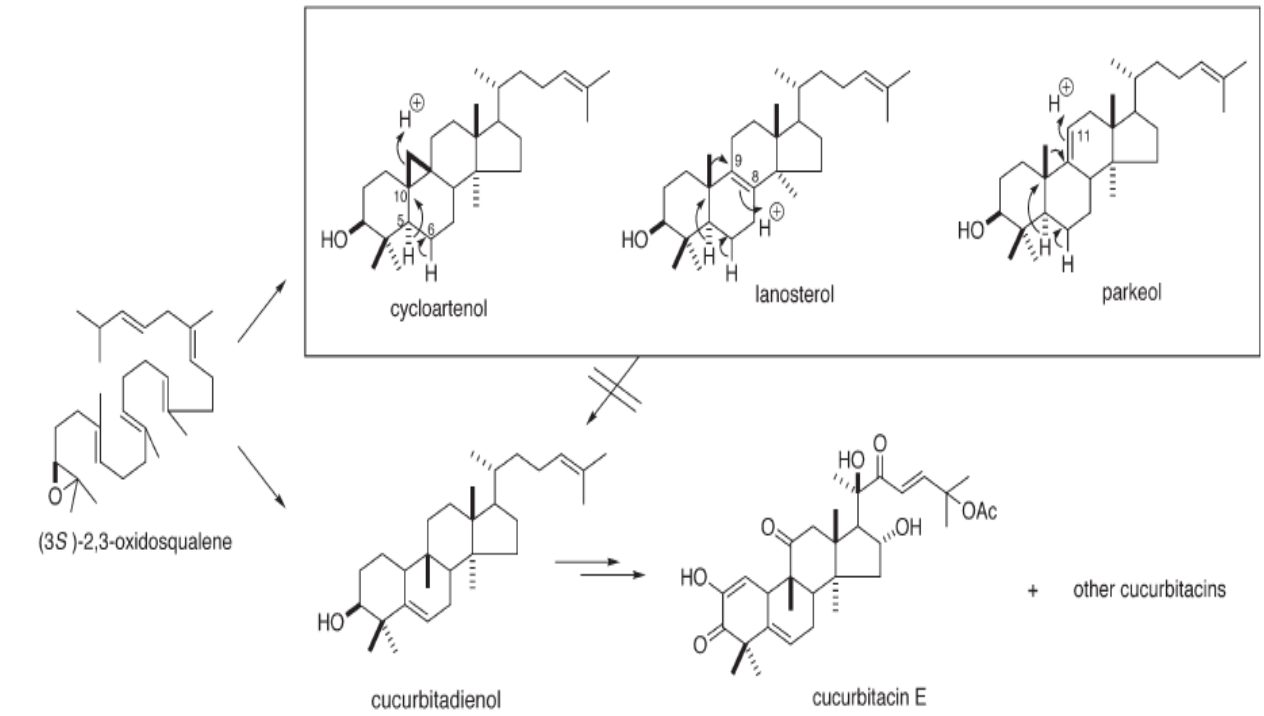
1.11.2 Cucurbitacins and drought adaptation

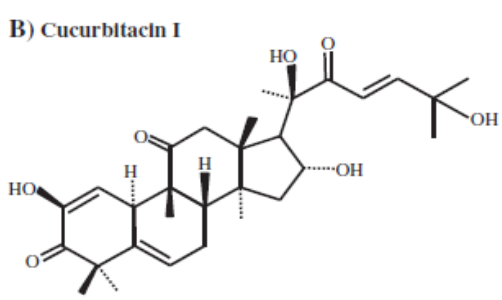
Plants in the *Cucurbitaceae* family produce the toxic tetracyclic triterpenoid (C_{32}-H_{48}-O_8) called cucurbitacins, a secondary metabolite which are responsible for the bitter taste (Sharma *et al.*, 2006; Sharma *et al.*, 2012; Sukhlecha, 2012). Cucurbitacins were originally isolated from *Cucurbitaceae* plants including: *Ecballium elaterium, Cayaponia tayuya*, *Trichosanthes kirilowii*, *Citrillus colocynthis* and *Cucurbita pepo* (Tannin-Spitz *et al.*, 2007; Wakimoto *et al.*, 2008). Also they have been found in other plant families such as *Scrophulariaceae, Brassicaceae* and *Polemoniaceae* (Sturm and Stuppner, 2000; Greige-Gerges *et al.*, 2007). They are also produced primarily in the family *Cucurbitaceae*, including cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), bitter watermelon (*Citrullus lanatus* var. citroides) and pumpkin (*Cucurbita pepo* L. and *Cucurbita maxima* L.). The isolation of 10-alpha-cucurbita-5, 24- dien-3beta-ol (9a), the simplest tetracyclic triterpene with a cucurbitane skeleton from germinating seeds of *Bryonia dioica* (*Cucurbitaceae*) validated the view that cucurbitacins are biosynthesized by plants and that 24-

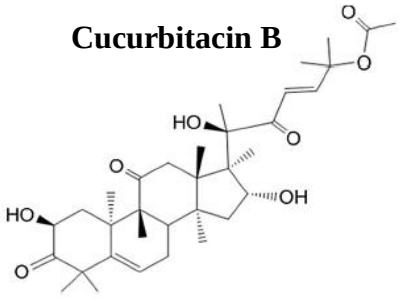
dien-3beta-ol is the general precursor of cucurbitacins (Cattel *et al.*, 1981). Naturally occurring cucurbitacins are classified into 12 cucurbitacins namely: A, B, C, D, E, F, I, L, 23, 24-dihydrocucurbitacin F, and hexanorcucurbitacin F. They differ from each other by hydroxylation at C-2, -3,-19, -24, the presence of ketone function at C-3, double bond between C-23 and C-24, and by the acetylation of the C-26 hydroxy group (Che *et al.*, 1985).

1.11.3 Biosynthesis of cucurbitacins

Cucurbitacins are a group of highly substituted triterpenoids characterized by a common tetracyclic cucurbitane backbone (Chen *et al.*, 2005). Triterpenoids are synthesized from mevalonic acid via the isoprenoid pathway, resulting in over 20 000 different plant metabolites built up by condensation of isopentenyl pyrophosphate (IPP) oligomer (Munnisse *et al.*, 2013). Triterpenoids include the membranal sterols, steroids and triterpenoid saponins, synthesized from IPP via the 30-carbon intermediates squalene and 2, 3-oxidosqualene. The different triterpenoid backbones are primarily determined by the particular cyclization of 2, 3-oxidosqualene by different oxidosqualene cyclases (OSCs) (Phillips *et al.*, 2006). Biosynthesis of cucurbitacins begins with the cyclization of 2,3-oxidosqualene to cucurbitadienol (Figure 1.3). Cucurbitadienol is further metabolized to a variety of different cucurbitacins by subsequent hydroxylation, acetylation and glucosylation steps (Chen *et al.*, 2005). Cucurbitadienol, a triterpene synthesized from oxidosqualene, is the first committed precursor for cucurbitacins produced by a specialized oxidosqualene cyclase termed cucurbitadienol synthase (Figure 1.3) (Davidovich-Rikanati *et al.*, 2015).







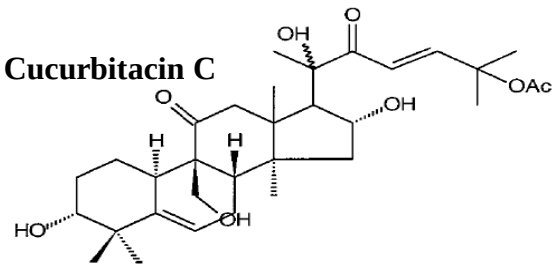
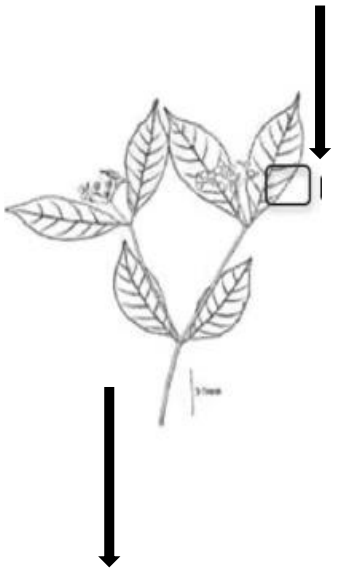


Figure 1.3. Biosynthesis of cucurbitacins in higher plants (Shibuya *et al*., 2004) and chemical structure of cucurbitacin E, I, B and C (Balkema- Boomstra *et al*., 2003; Sadzuka *et al*., 2012; Hassan *et al*., 2017).

It has been reported that cucurbitacin accumulation is triggered by environmental stress including heat and water stress (Shang *et al.*, 2014). Cucumber has bitter foliage and non-bitter fruits, but the fruits may become bitter as a result of water stress (Balkema- Boomstra *et al.*, 2003). Also, wilted cucumber seedlings contained twice as much cucurbitacins than non-wilted seedlings (Haynes and Jones, 1975) suggesting that they are drought-induced. However, limited information is available concerning the relationship between accumulation of cucurbitacins and to drought tolerance. Whether the accumulation of cucurbitacins may be a unique drought adaptation mechanism in plants within the *Cucurbitaceae* family is not known. Secondary metabolites play essential roles in mediating interactions between the plant and its environment (e.g. UV-radiation, temperature and drought stress (Shang *et al.*, 2014). Their role in many plant process including plant growth and development, protection from UV-radiation and their role as antioxidants has been reported (Munne-Bosch and Alegre, 2000; Hura *et al.*, 2007; Hura *et al.*, 2008, 2009b). Most secondary metabolites are linked to functions associated with survival by coping with unfavourable environmental stresses. It is possible that the accumulation of cucurbitacins plants may be linked to drought adaptation (Figure 1.4). However, several questions need to be addressed, for example, if cucurbitacins accumulate in response to drought stress, what types of cucurbitacins accumulate? What effect does drought stress have on the enzyme/s that may be related to the biosynthesis of cucurbitacins? Can resistance/susceptibility to drought stress be attributed to the level of cucurbitacins accumulation? Does cucurbitacins accumulation influence leaf gas exchange and photosynthetic performance under water limited conditions? Therefore, a more detailed understanding of the role of cucurbitacins under drought stress conditions is needed.

Abiotic stress [heat and drought stress]



Cucurbitadienol synthase

Cucurbitacins [e.g. Cucurbitacin B, C, D, E, F, I, L, 23, 24-dihydrocucurbitacin F, and hexanorcucurbitacin F]

Possible roles?

Photo-protection of photosynthetic apparatus?
Confer protection against oxidative stress caused by reactive oxygen species (ROS)?
Regulation of photorespiration?
Maintenance of plant water status and yield performance/productivity?

Figure 1.4. A hypothetical model depicting the effect of drought stress on cucurbitacin biosynthesis and possible roles.

1.12 Conclusions

Genetic resources are critical to plant breeding programs because economically important traits are constantly sought after to increase yield and quality and improve human nutrition. Such genetic resources must be evaluated to allow for their effective and efficient use in plant improvement programs (Gaikward *et al.*, 2008). Bottle gourd is an important underutilized crop in South Africa. Due to non-availability of improved cultivars, its cultivation has been largely dependent on local unimproved landraces which are not systematically selected and bred. For efficient utilization of this crop in breeding programs, it is important to initiate a pre-breeding program to harness the genetic diversity and identify potential parents.

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Chapter 2: **Assessment of Genetic Diversity among Bottle Gourd [*Lagenaria siceraria* (Molina) Standl.] Landraces Using Qualitative and Quantitative Traits**

Abstract

Bottle gourd [*Lagenaria siceraria* (Molina) Standl.] is an important crop in the rural communities in South Africa but it remains under-researched. The objective of this study was to assess the genetic variability present among bottle gourd landraces grown by smallholder farmers in the Limpopo Province, South Africa using qualitative and quantitative traits. Thirty six bottle gourd landraces were phenotyped in the field at Towoomba Research Station, Bela-Bela, South Africa using a 6 x 6 α-lattice design with two replications. Significant differences were observed among qualitative and quantitative traits suggesting considerable genetic variability. Principal component analysis (PCA) on qualitative and quantitative traits identified five and seven principal components (PCs) which accounted for 78 and 87% of the total variation, respectively. Among qualitative traits, presence or absence of fruit neck, fruit shape, degree of neck bending and fruit neck length positively correlated with PC1, which accounted for 31.9% of the total variation. Presence or absence of seed lines and seed texture was highly correlated with PC2 which accounted for 14.9% of the total variation. For quantitative traits, plant height, number of nodes on the main stem, total number of nodes, number of male flowers, number of branches, fruit neck diameter positively correlated with PC1, which accounted for 39.6% of the total variation. Fruit mass, fruit width, fruit size, shell thickness, seed length, seed width, seed size and hundred seed weight highly correlated with PC2 which accounted for 13.7% of the total variation. The present study demonstrated considerable genetic variability among bottle gourd landrace collections from South Africa useful for strategic improvement, direct production or conservation. Unique genotypes such as BG-16, BG-25, BG-09, BG-37 and BG-10 showing suitable qualitative traits were identified. BG-07, BG-13, BG-67, BG-12, BG-09 and BG-06 showing suitable quantitative traits were also identified. These are recommended for breeding and strategic conservation.

Keywords*:* bottle gourd, genetic diversity, landrace, principal component analysis, qualitative traits, quantitative traits

2.1 Introduction

Bottle gourd [*Lagenaria siceraria* (Molina) Standl.] belonging to the *Cucurbitaceae* family is a diploid (2n = 2x = 22) vine crop mainly grown for its fruits (Beevy and Kuriachan, 1996; Achigan-Dako *et al.*, 2008). The genus *Lagenaria* consists of five wild species: *L. breviflora* (Benth.) Roberty, *L. abyssinica* (Hook F.) Jeffrey, *L. rufa* (Gilg.) Jeffrey, *L. sphaerica* (Sonder) Naudin and *L. guineensis* (G. Don) Jeffrey (Whitaker, 1971). *L. siceraria* is the only economically cultivated species worldwide for diverse uses such as for food, medicine, decoration, household utensils and musical instruments (Jeffrey 1976). Fresh bottle gourd fruit juice is used as medicine to cure various diseases including flatulence, diabetes mellitus, hypertension, liver diseases and as a diuretic (Ghule *et al.*, 2007). The seeds of this crop are rich in essential amino acids and oil. Some bottle gourd types are exclusively grown for their seeds (Achigan-Dako *et al.*, 2008). Bottle gourd serves as a rootstock in watermelon breeding to control soil-borne diseases and to manage low soil temperature stress (Lee, 1994; Yetisir and Sari, 2003).

Bottle gourd exhibits significant genetic variation for fruit sizes and shapes (Morimoto *et al.*, 2005; Yetişir *et al.*, 2008; Xu *et al.*, 2014), fruit shell thickness, fruit length and fruit width (Morimoto *et al.*, 2005; Koffi *et al.*, 2009; Harika *et al.*, 2012), seed morpho types (Morimoto *et al.*, 2005; Yetişir *et al.*, 2008) and other agro-morphological characteristics (Morimoto *et al.*, 2005; Sivaraj and Pandravada, 2005; Morimoto *et al.*, 2006; Achigan-Dako *et al.*, 2008; Xu *et al.*, 2014). This variation is attributed to farmers' long-term selection of the crop which is often driven by specific socio-cultural preferences and use, cultural practices and the environment (Mlandenovic *et al.*, 2012).

Bottle gourd is an important but neglected and under-researched crop in South Africa (van Rensburg *et al.*, 2007; van Wyk, 2011). In the country, smallholder farmers grow unimproved landraces which exhibit great morphological diversity. Despite the possible genetic variability and potential use of bottle gourd in South Africa, there is no recent and detailed information regarding its systematic characterization using morphological markers.

The bottle gourd landraces maintained by smallholder farmers in South Africa could be useful genetic resources for breeding (Blum and Sullivan, 1986; Sharma, 1996; Hedge and Mishra, 2009). Genes that could contribute to bottle gourd improvement may exist in the landraces which are yet cultivated and maintained by smallholder farmers under diverse marginal growing environments. Phenotypic characterization of the variability present among the bottle gourd landraces is helpful to identify useful genotypes for breeding, direct production or systematic conservation. Therefore, the objective of this study was to assess the genetic variability present amongst bottle gourd landraces grown by smallholder farmers in the Limpopo Province of South Africa using qualitative and quantitative morpho-agronomical traits.

2.2 Materials and methods

2.2.1 Plant materials

Thirty-six bottle gourd landraces collected from various geographic locations of the Limpopo Province of South Africa were used for the study (Table 2.1). Entries were collected from the following localities: Kgohloane (23°47’39.76” S; 29°22’13.45” E), Ga-Kgoroshi (23°40’57.89” S; 29°15’03.47” E), Phokwane (24°52’36.15” S; 29°44’36.15” E), Tshikonelo (22°52’49.82” S; 30°44’16.33” E), Ga-Rapitsi (23°35’48.37” S; 29°06’25.08” E) and Ga-Phasa (23°40’57.30” S; 29°15’57.30” E). Table 2.1 summarizes information related to the collection sites of landraces.

Table 2.1. List of bottle gourd landraces used in the study and description of geographic location of collection sites.

Sr.No.	Entry	Location	District	Sr.No.	Entry	Location	District
1	BG-08	Tshikonelo	Vhembe	19	BG-44	Kgohloane	Capricorn
2	BG-27	Kgohloane	Capricorn	20	BG-55	Kgohloane	Capricorn
3	BG-61	Kgohloane	Capricorn	21	BG-24	Ga-Phasa	Capricorn
4	BG-09	Tshikonelo	Vhembe	22	BG-60	Kgohloane	Capricorn
5	BG-28	Kgohloane	Capricorn	23	BG-41	Kgohloane	Capricorn
6	BG-25	Phokwane	Sekhukhune	24	BG-57	Kgohloane	Capricorn
7	BG-18	Kgohloane	Capricorn	25	BG-10	Tshikonelo	Vhembe
8	BG-52	Kgohloane	Capricorn	26	BG-32	Kgohloane	Capricorn
9	BG-12	Tshikonelo	Vhembe	27	BG-17	Ga-Kgoroshi	Capricorn
10	BG-37	Kgohloane	Capricorn	28	BG-04	Kgohloane	Capricorn
11	BG-43	Kgohloane	Capricorn	29	BG-11	Tshikonelo	Capricorn
12	BG-16	Ga-Kgoroshi	Capricorn	30	BG-30	Kgohloane	Capricorn
13	BG-13	Tshikonelo	Vhembe	31	BG-23	Kgohloane	Capricorn
14	BG-07	Tshikonelo	Vhembe	32	BG-19	Kgohloane	Capricorn
15	BG-36	Kgohloane	Capricorn	33	BG-06	Tshikonelo	Vhembe
16	BG-62	Kgohloane	Capricorn	34	BG-63	Kgohloane	Capricorn
17	BG-31	Kgohloane	Capricorn	35	BG-42	Kgohloane	Capricorn
18	BG-47	Kgohloane	Capricorn	36	BG-67	Ga-Rapitsi	Capricorn

Sr. No. = Serial number

2.2.2 Study site

A field study was conducted during the 2014 growing season, under dry-land conditions at Towoomba Research Station, Bela-Bela, South Africa (28º21'E, 24º25'S; 1 184 m above sea level). The soils are of the Hutton and Arcadia form. The area usually receives mean annual rainfall of 627 mm with erratic distribution. Daily average maximum and minimum temperatures range between 29.7°C and 16.5°C during the growing season.

2.2.3 Experimental design and field establishment

The 36 bottle gourd landraces were evaluated using a 6 x 6 α-lattice design with two replications and six incomplete blocks. Landraces were planted with an intra-and inter-row spacing of 2 m. In each replication 20 plants per landrace were established. A soil analysis was conducted before planting that resulted a pH (water) value of 6.95, N content of 0.05 mg/kg, exchangeable K of 129

mg/kg and phosphorus of 4.25mg/kg. Fertilizers were applied at the following rates: 100 kg N/ha of LAN (lime ammonium nitrate) and 165 kg P/ha SSP (single superphosphate) based on the results of a soil fertility analysis to achieve the establishment of healthy and vigorous plants. Weed control was done manually using hand-hoes and the chemical pesticides Malasol was used to control aphids infestation. A supplementary irrigation of about 27 mm was applied three times a week until maturity.

2.3 Data collection

Both qualitative and quantitative data were collected during the study. Descriptors of other related cucurbits were used as a reference (Maggs-Kölling *et al*., 2000; Marr *et al*., 2005; Morimoto *et al*., 2005; Yetişir *et al*., 2008; Koffi *et al*., 2009; Aruah *et al*., 2010). The qualitative data collected with their descriptors are summarised in Table 2.2. Quantitative data measured included: days to 50% emergence, days to 50% flowering; cotyledon length (cm), cotyledon width (cm), leaf length (cm), leaf width (cm), leaf size (cm^2), plant height (m); number of nodes of main-stem, total number of nodes, number of male flowers, number of female flowers, proportion of male to female flowers representing sex ratio, number of branches, number of fruits per plant; fruit mass (kg), fruit length (cm), fruit width (cm), fruit size (cm^2), fruit neck length (cm), fruit neck diameter (cm), fruit shell thickness (cm), number of seeds/fruit, seed length (cm), seed width (cm), seed size (cm^2) and hundred seed weight (g). Individual measurements were collected from 10 randomly selected and tagged plants.

Table 2.2. List of qualitative traits with descriptions used for genetic diversity assessment of 36 bottle gourd landraces.

Characters	Description	Code
Presence of tendrils	0=absent, 1=present	T
Growth habit	3= bushy, 5= intermediate, 7= spreading	GH
Stem colour	1-light green, 2-dark green	SC
Flower colour	1=white, 2=yellow	FC
Plant vigour	1= vigorous, 2= non-vigorous	PV
Foliage cover	1=very poor,3 = poor, 5 = moderate, 7= good , 9 = very good	FCV
Male flower size	1=small, 2=medium, 3= large	MFS
Female flower size	1=small, 2=medium, 3= large	FFS
Leaf colour	1=light green, 2=medium green, 3=dark green	LC
Leaf size	1=small, 2=medium, 3= large	LSZ
Leaf shape	1= heart,2=ovate	LS
Overlapping of petals of male flowers	1=free, 2=touching to slightly overlapping ,3= strongly overlapping	OPMF
Overlapping of petals of female flowers	1=free, 2=touching to slightly overlapping ,3= strongly overlapping	OPFF
Leaf edge	1=pointed, 2=blunt	LE
Branching pattern	1-central, 2-basal, 3-all over	BP
Place of male flowers	1=main stem, 2=lateral stem, 3=both	PMF
Place of female flowers	1=main stem, 2=lateral stem, 3=both	PFF
Primary fruit colour	0= No colour, 1=light green, 2=medium green, 3=dark green	PFC
Secondary fruit colour	0= No colour ,1=light green, 2=medium green, 3=dark green	SFC
Presence of fruit neck	0=no, 1=yes	PFN
Fruit neck shape	1=fusiform shaped, 2=cylindrical	FNS
Fruit texture	1=smooth,2=verrucose, 3=corrugated, 4=verrucose+corrugated, 5=smooth+verrucose 6=smooth+corrugated	FT
Degree of warts	0=none, 1=few, 2=medium, 3=many	DW
Fruit shape	1=oblate,2=circular, 3=pyriform, 4=elongated pyriform, 5=cavate, 6=cylindrical	FS
Degree of corrugation	0= None, 1=slightly, 2=moderate, 3=high	DC
Degree of neck bending	0= No neck, 1=straight, 2=slightly curved, 3=curved	DNB
Stem end fruit shape	1=flat, 2=rounded, 3=pointed	SEFS
Flesh colour of fruit	1=white, 2=yellow	FCF
Fruit neck length	0= No neck, 1=short ≤ 5cm, 2=medium 6-12cm, 3=long,13-20cm 4=very long>20cm	FNL
Seed coat colour	1=light brown, 2=dark brown	SCC
Presence of seed lines	0=absent, 1=present	PSL
Seed texture	1=smooth, 2=slightly rough	ST

2.4 Data analysis

Qualitative data were subjected to analyses using the Kruskal-Wallis non-parametric test procedure and descriptive statistics. Quantitative data were subjected to analysis of variance (ANOVA) of a lattice procedure using the SAS statistical program and Agrobase (2006). Mean comparisons among genotypes were performed using the LSD test procedure at 5% level of significance. Data for qualitative and quantitative traits was further subjected to principal component analysis (PCA) using SPSS16.0. For qualitative traits, PCA was only performed for traits that were significantly different. Principal components (PC's) with eigenvalues > 1.0 were selected and those characters with load coefficient values > 0.6 were considered highly relevant for that PC (Guttieri *et al.,* 2001; Morimoto *et al.,* 2005).

2.5 Results

2.5.1 Qualitative traits

Results of observations on qualitative traits amongst bottle gourd landraces are summarized in Table 2.3. All landraces produced tendrils and exhibited a spreading growth habit. There were no differences observed between bottle gourd landraces with respect to stem colour, flower colour and plant vigour. Stem colour was generally medium to green, while male and female flowers were white in colour. All landraces grew vigorous showing rapid ground cover. There were no differences observed amongst landraces with respect to male and female flower size. Male flowers were generally medium to large in size, while female flowers were generally smaller in size. No differences were also observed for leaf colour and leaf shape. Leaf colour was generally green and all landraces exhibited a hearty leaf shape. No differences were observed amongst landraces with respect to overlapping of petals of male and female flowers. No differences were observed for leaf edge, branching pattern and location of male and female flowers. Leaf edge was blunt, while branches were spread all over the main vine. Male flowers were borne on the main vine and lateral branches while female flowers were only borne on the lateral branches. Significant differences were observed amongst landraces with regards to leaf size.

Table 2.3. Distribution of qualitative traits of 36 bottle gourd landraces collected from the Limpopo Province, South Africa.

Entry	PFC	SFC	PFN	FT	DW	FS	DC	NBD	SEFS	FNL	FNL	SL	ST
BG08	1.0	0.0	0.0	1.0	0.0	4.0	0.0	0.0	1.0	0.0	0.0	1.0	2.0
BG27	3.0	0.0	1.0	4.0	1.0	5.0	2.0	2.0	2.0	3.0	3.0	1.0	2.0
BG61	2.0	1.0	1.0	3.0	0.0	5.0	2.0	2.0	2.0	2.0	2.0	1.0	2.0
BG09	1.0	0.0	1.0	1.0	0.0	5.0	0.0	3.0	1.0	3.0	3.0	1.0	2.0
BG28	3.0	1.0	1.0	3.0	0.0	5.0	2.0	3.0	3.0	3.0	3.0	1.0	2.0
BG25	1.0	0.0	1.0	2.0	2.0	3.0	2.0	1.0	1.0	1.0	1.0	1.0	2.0
BG18	3.0	1.0	1.0	3.0	0.0	5.0	1.0	3.0	3.0	3.0	3.0	1.0	2.0
BG52	3.0	1.0	1.0	3.0	0.0	5.0	2.0	3.0	3.0	3.0	3.0	1.0	2.0
BG12	2.0	0.0	1.0	1.0	0.0	4.0	0.0	2.0	1.0	1.0	1.0	1.0	2.0
BG37	3.0	1.0	1.0	3.0	0.0	5.0	1.0	2.0	3.0	3.0	3.0	1.0	2.0
BG43	3.0	0.0	1.0	3.0	0.0	5.0	2.0	3.0	3.0	4.0	4.0	1.0	2.0
BG16	3.0	1.0	1.0	3.0	0.0	5.0	0.0	2.0	3.0	2.0	2.0	1.0	2.0
BG13	2.0	0.0	1.0	1.0	0.0	4.0	2.0	1.0	1.0	2.0	2.0	1.0	2.0
BG07	3.0	0.0	1.0	1.0	0.0	3.0	0.0	1.0	1.0	1.0	1.0	1.0	2.0
BG36	3.0	1.0	1.0	5.0	0.0	5.0	1.0	2.0	2.0	2.0	2.0	1.0	2.0
BG62	3.0	1.0	1.0	3.0	0.0	5.0	2.0	2.0	2.0	3.0	3.0	0.0	2.0
BG31	3.0	0.0	1.0	5.0	1.0	5.0	1.0	2.0	2.0	3.0	3.0	1.0	2.0
BG47	3.0	1.0	1.0	3.0	0.0	5.0	3.0	3.0	2.0	2.0	2.0	1.0	2.0
BG44	3.0	1.0	1.0	3.0	0.0	5.0	1.0	3.0	3.0	2.0	2.0	1.0	2.0
BG55	3.0	1.0	1.0	3.0	0.0	5.0	2.0	2.0	2.0	2.0	2.0	1.0	2.0
BG24	3.0	1.0	1.0	3.0	0.0	5.0	0.0	1.0	2.0	2.0	2.0	0.0	1.0
BG60	3.0	1.0	1.0	3.0	0.0	5.0	2.0	2.0	2.0	2.0	2.0	0.0	2.0
BG41	1.0	2.0	1.0	3.0	0.0	5.0	1.0	2.0	2.0	2.0	2.0	1.0	2.0
BG57	2.0	1.0	1.0	3.0	0.0	5.0	2.0	2.0	2.0	2.0	2.0	1.0	2.0
BG10	1.0	0.0	1.0	1.0	0.0	5.0	0.0	2.0	1.0	3.0	3.0	1.0	2.0
BG32	3.0	1.0	1.0	3.0	0.0	5.0	1.0	2.0	1.0	2.0	2.0	1.0	2.0
BG17	1.0	2.0	1.0	2.0	2.0	7.0	2.0	1.0	2.0	1.0	1.0	1.0	2.0
BG04	3.0	1.0	1.0	3.0	0.0	5.0	2.0	2.1	1.9	2.0	2.0	1.0	2.0
BG11	1.0	0.0	1.0	1.0	0.0	5.0	0.0	2.0	1.0	2.0	2.0	1.0	2.0
BG30	3.0	1.0	1.0	3.0	0.0	5.0	1.0	2.0	1.0	2.0	2.0	1.0	2.0
BG23	1.0	0.0	0.0	1.0	0.0	4.0	3.0	0.0	1.0	0.0	0.0	1.0	2.0
BG19	3.0	1.0	0.0	6.0	1.0	1.0	0.0	0.0	1.0	0.0	0.0	1.0	2.0
BG06	1.0	0.0	1.0	6.0	0.0	4.0	1.0	1.0	1.0	2.0	2.0	1.0	2.0
BG63	3.0	0.0	1.0	3.0	0.0	5.0	2.0	2.0	3.0	3.0	3.0	0.0	1.0
BG42	3.0	1.0	1.0	3.0	0.0	5.0	2.0	2.0	1.0	2.0	2.0	1.0	2.0
BG67	3.0	1.0	1.0	2.0	3.0	4.0	0.0	2.0	2.0	2.0	2.0	1.0	2.0
KWSL	0.002	0.001	0.00	0.001	0.005	0.00	0.005	0.001	0.007	0.002	0.002	0.003	0.014

PFC= Primary fruit colour; SFC= Secondary fruit colour; PFN = Presence of fruit neck; FT= Fruit texture; DW= Degree of warts; FS = Fruit shape; DC= Degree of corrugation; DNB = Degree of neck bending; SEFS = Stem-end fruit shape; FNL = Fruit neck length; SL = Prescence or absence of seed lines; ST = Seed texture. KWSL = Kruskal-Wallis significance level.

Qualitative fruit and seed traits amongst bottle gourd landraces and corresponding number and percentage of landraces possessing the respective trait are presented in Table 2.4. Significant differences were observed for primary and secondary fruit colour. The dominant primary fruit colour amongst landraces was dark green exhibited by 64% of entries (23 landraces), while 9 landraces (25%) had light green primary fruit colour. Only four landraces (11%) showed a medium green primary fruit colour. It was also noted that 14 landraces (39%) had no secondary fruit colour while light green secondary fruit colour was observed amongst 20 landraces. Differences were observed with regards to intensity of warts. Thirty landraces (83%) had no warts and only 6 exhibited warted fruit skins. The dominant fruit shapes of the 36 landraces are displayed in Figure 2.1. Fruit shape differed amongst bottle gourd landraces (Figure 2.1). Twenty six landraces (72%) had a cavate fruit shape (Figure 2.1, entries: 2, 3, 4 and 5). Most landraces (56%) exhibited a corrugated fruit texture with few displaying a smooth and verrucose/rough fruit texture. A combination of smooth, corrugated or verrucose fruit texture was observed for some landraces. Thirty three landraces (92%) possessed fruit necks while only 3 had no fruit necks (Figure 2.1, entries: 1, 31 and 32). Degree of neck bending differed amongst bottle gourd landraces. Twenty landraces had a slightly curved fruit neck while seven had a curved fruit neck. Twenty landraces exhibited medium fruit necks while eight displayed long fruit necks. Fourteen landraces exhibited a smooth and pointed stem end fruit shape, whereas six landraces had a pointed stem end fruit shape. Differences were observed for presence of seed lines and seed texture (Figure 2.1). Thirty four landraces had seed lines while only two (Figure 2.2, entries: 16 and 21) had no seed lines. Thirty four landraces had slightly rough seed coats and only two (Figure 2.2, entries: 16 and 21) had smooth seed coats.

Table 2.4. Distribution of qualitative traits amongst 36 bottle gourd landraces and corresponding number and percentage.

Traits	Score & description	Number of landraces bearing the trait	Landraces bearing the respective traits (%)
Primary fruit colour	1. Light green	9	25
	2.Medium green	4	11.1
	3.Dark green	23	63.9
Secondary fruit colour	0. No colour	14	38.9
	1. Light green	20	55.6
	2.Medium green	2	5.6
	3.Dark green	0	0
Intensity of warts	0. No warts	30	83.3
	1. Few	3	8.3
	2. Medium	2	5.6
	3. Many	1	2.8
Fruit shape	1.Oblate	1	2.8
	3. Pyriform	2	5.6
	4.Elongatedpyriform	6	16.7
	5. Cavate	26	72.2
	6. Cylindrical	1	2.8
Intensity of corrugation	0. None	10	27.8
	1. Slightly	9	25
	2. Moderate	15	38.9
	3. Severe	2	5.6
Presence of fruit neck	0. Neck absent	3	8.3
	1. Neck present	33	91.7
Neck bending degree	1. Straight	6	16.7
	2. Slight curved	20	55.6
	3. Curved	7	19.4
Fruit neck length	1. Short	4	11.1
	2. Medium	20	55.6
	3. Long	8	22.2
	4. Very long	1	2.8
Fruit texture	1. Smooth	8	22.2
	2. Verrucose	3	8.3
	3.Corrugated	20	55.6
	4. Verrucose+corrugated	1	2.8
	5. Smooth+verrucose	2	5.6
	6. Smooth+corrugated	2	5.6
Stem end fruit shape	1. Smooth	14	38.9
	2. Rounded	14	38.9
	3. Pointed	8	22.2
Seed lines	0. Absent	2	5.6
	1. Present	34	94.4
Seed texture	1. Smooth	2	6
	2.Slightly rough	34	94

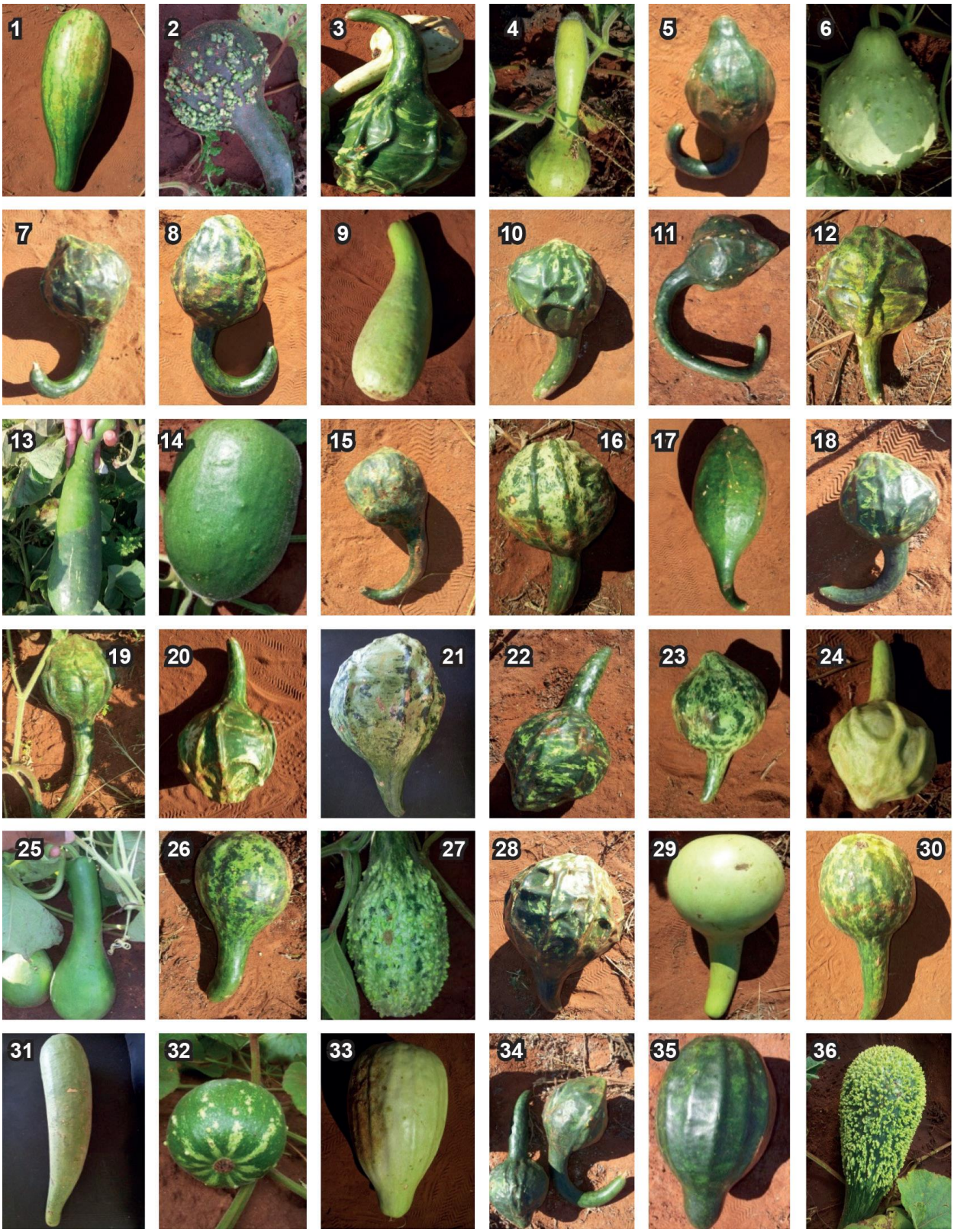

Figure 2.1. Variation in fruit traits among 36 bottle gourd landrace collections from the Limpopo Province of South Africa. Note: 1 to 36 designates entry numbers (Table 2.1) and fruit traits are described in Table 2.4.

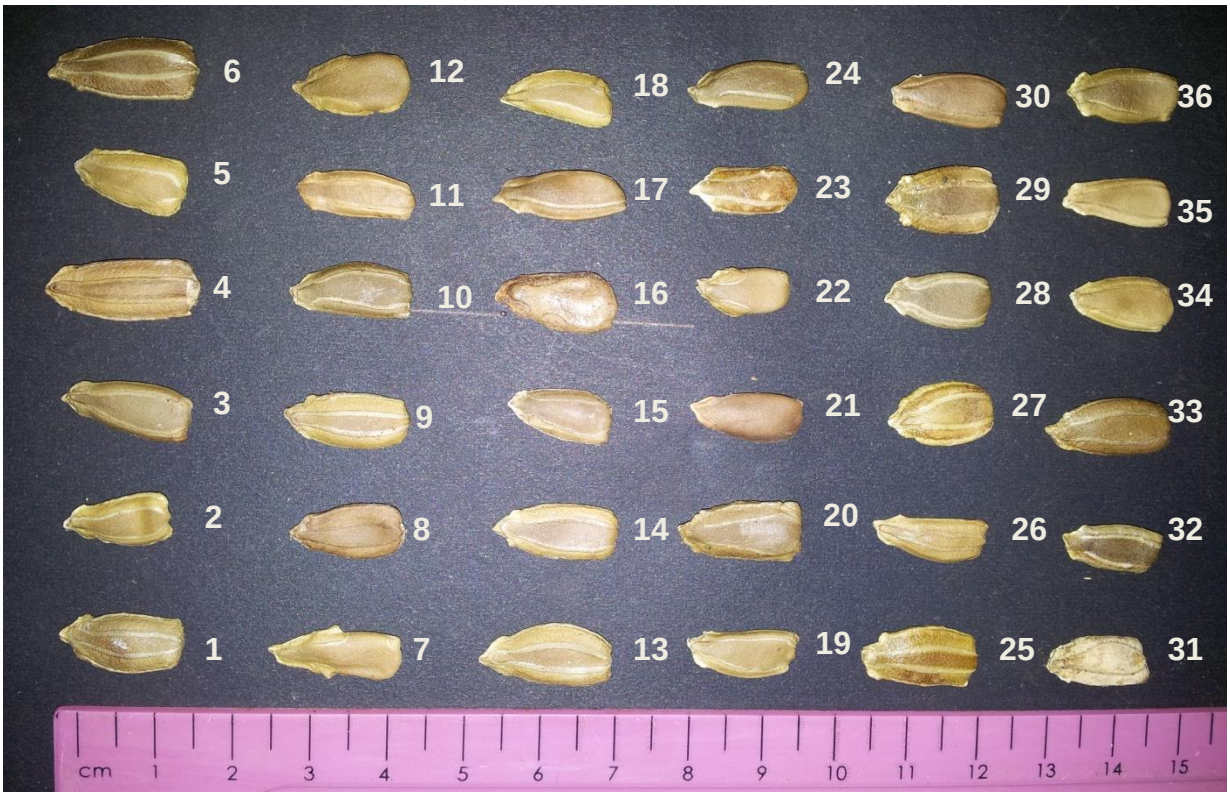

Figure 2.2. Variation in seed morphology among 36 bottle gourd landrace collections from the Limpopo Province of South Africa. Note: 1 to 36 denotes entry numbers (Table 2.1) and seed morpho-types are described in Table 2.4.

2.5.2 Quantitative traits

Mean values and standard error of quantitative phenotypic parameters observed in the study are presented in Table 2.5. Significant differences ($P < 0.05$) were observed amongst the landraces for quantitative traits measured except for days to 50% emergence. Days to 50% flowering ranged from 38 to 74 days. Cotyledon length varied from 2.56 to 4.98 cm. Cotyledon width and size ranged from 1.58 to 2.98 cm, and 4.29 to 14.82 cm^2, respectively. Leaf length varied from 12.81 to 22.5 cm, while leaf width varied from 16.53 to 31.1 cm. Leaf size varied from 258.1 to 700 cm^2. Plant height varied from 3.25 to 8.36 m (Table 2.5). Number of nodes on the main-stem varied from 9.4 to 34.1, while total number of nodes ranged from 87 to 392. Number of male and female flowers varied from 85 to 380 and 2 to 16, respectively. Sex ratio ranged from 14 to 115 among landraces. Number of branches varied from 9 to 34, whereas number of aborted fruits ranged from

0 to 7. Number of fruits per plant varied from 2 to 16. Fruit mass varied from 0.23 to 1.81 kg per fruit. Fruit length and width varied from 9.51 to 37.8 cm and 6.59 to 13.2 cm, respectively. Fruit size varied from 94.96 to 456.82 cm^2. Fruit neck length and diameter varied from 0.53 to 24.1 cm and 0.84 and 5.78 cm, respectively. Shell thickness varied from 0.12 to 0.43 cm. Number of seeds per fruit varied from 90 to 381. Seed length, width and size varied from 1.13 to 1.88 and, 0.5 to 0.87 cm and, 0.59 to 1.64 cm^2, respectively. Hundred seed weight varied from 9 to 23.91 g. Also, the CV's tended to be larger in fruit traits (e.g. number of fruits/plant, fruit mass, fruit neck length and shell thickness) than other traits indicating that bottle gourd landraces were more variable in size and shape.

Table 2.5. Means and coefficients of variation for quantitative traits of 36 bottle gourd landraces collections from the Limpopo Province of South Africa.

Qualitative traits	Mean ± SE	P-value	CV (%)
Days to 50% emergence	10.39 ± 1.8	NS	16.77
Days to 50% flowering	48.8 ± 5.4	< 0.012	10.53
Cotyledon length (cm)	4.038 ± 0.51	< 0.001	8.87
Cotyledon width (cm)	2.15 ± 0.29	< 0.013	13.30
Leaf length (cm)	18.12 ± 0.88	< 0.003	4.63
Leaf width (cm)	23.86 ± 0.97	< 0.001	4.04
Leaf size (cm^2)	436.49 ± 25.9	< 0.001	5.63
Plant height (m)	5.25 ± 0.35	< 0.001	6.56
No. of nodes on main-stem	19.19 ± 1.68	< 0.001	8.31
Total no. of nodes	205.7 ± 14.9	< 0.001	7.25
No. of male flowers	199.12 ± 14.9	< 0.001	7.00
No. of female flowers	6.56 ± 0.78	< 0.001	11.92
Sex ratio	37.9 ± 7.5	< 0.001	19.64
No. of branches	19.19 ± 1.68	< 0.001	8.31
No. of aborted fruits	1.79 ± 0.62	< 0.001	11.89
No. of fruits/plant	6.56 ± 0.8	< 0.001	34.03
Fruit mass (kg)	0.68 ± 0.18	< 0.001	25.99
Fruit length (cm)	24.8 ± 2.65	< 0.001	10.15
Fruit width (cm)	9.48 ± 0.66	< 0.001	7.01
Fruit size (cm^2)	236.15 ± 34.9	< 0.001	14.38
Fruit neck length (cm)	9.96 ± 2.53	< 0.001	23.71
Fruit neck diameter (cm)	2.95 ± 0.42	< 0.001	14.00
Shell thickness (cm)	0.25 ± 0.06	< 0.010	24.10
Number of seeds/fruit	235.2 ± 34.5	< 0.001	14.65
Seed length (cm)	1.44 ± 0.13	< 0.001	8.94
Seed width (cm)	0.67 ± 0.05	< 0.001	7.97
Seed size (cm^2)	0.97 ± 0.14	< 0.001	14.90
Hundred seed weight (g)	13.24 ± 1.42	< 0.001	10.02

SE = Standard error; CV = Coefficient of variation; NS = Non-significant

2.6 Principal component analysis

2.6.1 Quantitative traits

Results of the principal component analysis for 28 quantitative traits among bottle gourd landraces are presented in Table 2.6. All 28 traits were allocated under the seven principal components (eigenvalues ≥ 1) which accounted for 87% of the variation. The seven principal components (PCs) and corresponding correlation coefficients (eigenvectors) for quantitative traits are presented in Table 6. Plant height, number of nodes on the main stem, total number of nodes, number of male flowers, number of branches, fruit neck diameter positively correlated with PC1, which accounted for 39.6% of the total variation. Fruit mass, fruit width, fruit size, shell thickness, seed length, seed width, seed size and hundred seed weight were highly correlated with PC2 which accounted for 13.7% of the total variation. Leaf length, leaf width and leaf size highly correlated with PC3, which accounted for 9.6% of the total variation. Number of female flowers, number of aborted fruits and number of fruits per plant were highly correlated with PC 4, while sex ratio negatively correlated with PC4. The total variation accounted by PC 4 was 8.6%. Fruit length and fruit neck length positively correlated with PC5 which accounted for 6.5% of the total variation. Cotyledon length and width positively correlated with PC6 which accounted for 5.2% of the total variation. Days to emergence positively correlated with PC7 while days to 50% flowering also negatively correlated with PC7 which accounted for 3.6% of the total variation.

Table 2.6. Principal component analysis showing eigenvectors, eigenvalues, and percent variance explained by seven principal components (PC's) on quantitative traits of 36 bottle gourd landraces.

Quantitative traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Days to 50% emergence	-0.13	0.12	0.24	-0.09	-0.16	0.28	**0.71**
Days to 50% flowering	-0.21	0.29	0.08	-0.09	-0.22	-0.14	**-0.72**
Cotyledon length	0.08	-0.01	0.12	-0.17	0.28	**0.81**	0.18
Cotyledon width	-0.04	0.28	0.12	0.13	-0.01	**0.78**	0.30
Leaf length	0.27	0.31	**0.80**	0.11	-0.01	-0.14	0.01
Leaf width	0.10	0.14	**0.82**	-0.03	-0.16	0.41	0.07
Leaf size	0.19	0.26	**0.90**	0.06	-0.12	0.21	0.03
Plant height	**0.80**	0.17	0.27	0.16	-0.17	0.04	-0.05
No. of nodes on main-stem	**0.92**	0.17	0.13	0.22	0.04	-0.01	0.11
Total no. of nodes	**0.90**	0.34	0.10	0.07	0.04	0.05	0.01
No. of male flowers	**0.90**	0.33	0.10	0.04	0.04	0.05	0.00
No. of female flowers	0.62	0.20	0.12	**0.72**	-0.14	0.06	0.02
Sex ratio	0.06	0.08	-0.05	**-0.93**	-0.02	0.09	0.02
No. of branches	**0.92**	0.18	0.13	0.22	0.04	-0.01	0.11
No. of aborted fruits	0.62	0.20	0.12	**0.72**	-0.14	0.06	0.02
No. of fruits/plant	0.51	0.09	-0.09	**0.71**	-0.33	0.01	0.05
Fruit mass	0.51	**0.76**	0.09	-0.12	-0.21	-0.16	0.10
Fruit length	0.04	0.49	0.01	-0.04	**0.69**	0.20	0.39
Fruit width	0.54	**0.71**	-0.03	-0.12	-0.17	0.06	-0.21
Fruit size	0.31	**0.74**	-0.03	-0.08	0.48	0.19	0.21
Fruit neck length	-0.07	-0.01	-0.22	-0.20	**0.87**	0.11	-0.07
Fruit neck diameter	**0.6**	0.43	-0.03	-0.18	0.05	-0.09	-0.27
Shell thickness	0.33	**0.63**	-0.05	0.01	-0.50	0.17	0.08
Number of seeds/fruit	0.54	0.55	0.24	-0.01	-0.32	-0.20	0.23
Seed length	0.15	**0.84**	0.13	0.12	0.23	0.06	-0.09
Seed width	0.18	**0.89**	0.23	0.15	-0.02	0.13	0.03
Seed size	0.17	**0.91**	0.19	0.14	0.13	0.11	-0.03
Hundred seed weight	0.32	**0.80**	0.15	-0.01	-0.08	0.15	-0.17
Explained variance (Eigenvalue)	11.49	3.98	2.79	2.50	1.88	1.51	1.03
Proportion of total variance (%)	39.61	13.73	9.60	8.62	6.48	5.20	3.55
Cumulative variance (%)	39.61	53.34	62.95	71.57	78.05	83.25	86.79

Loadings greater than ≥ 0.6 are shown in bold faced fonts

The wide variation observed for selected quantitative traits among the bottle gourd landraces used in this study were expressed by the PCA biplot (Figure 2.3) using the loading scores of the two principal components (PC1 and PC2) which accounted 53.34% of the variation. The landraces were scattered within the four quadrants produced by the PC1 and PC2 biplot. In terms of their genetic variability, the landraces displayed a pairing orientation suggesting that they shared most of the quantitative traits that were studied. Conversely, some landraces scattered far apart within the axes and this suggests they were distantly related to other landraces within the same quadrant. Accordingly, genotypes such as BG-25, BG-67, BG-12, BG-13, BG-07, BG-09 were positioned in the same similarity group. The associations between PC1 and PC2 loading scores of quantitative traits used to classify bottle gourd landraces is shown in Figure 2-4. The landraces in the top right quadrant were closely associated by fruit size, fruit width, fruit mass, number of seeds per fruit and fruit neck diameter.

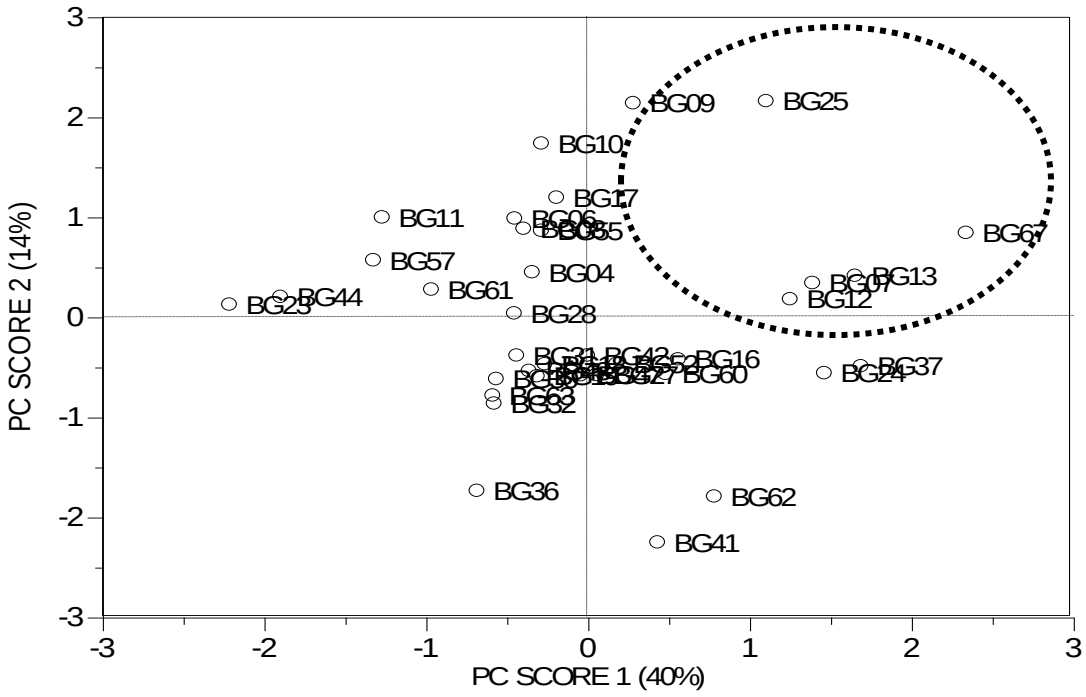

Figure 2.3. Rotated principal component scores of quantitative traits and percent explained variance of PC1 and PC2 showing similarities among 36 bottle gourd landraces.

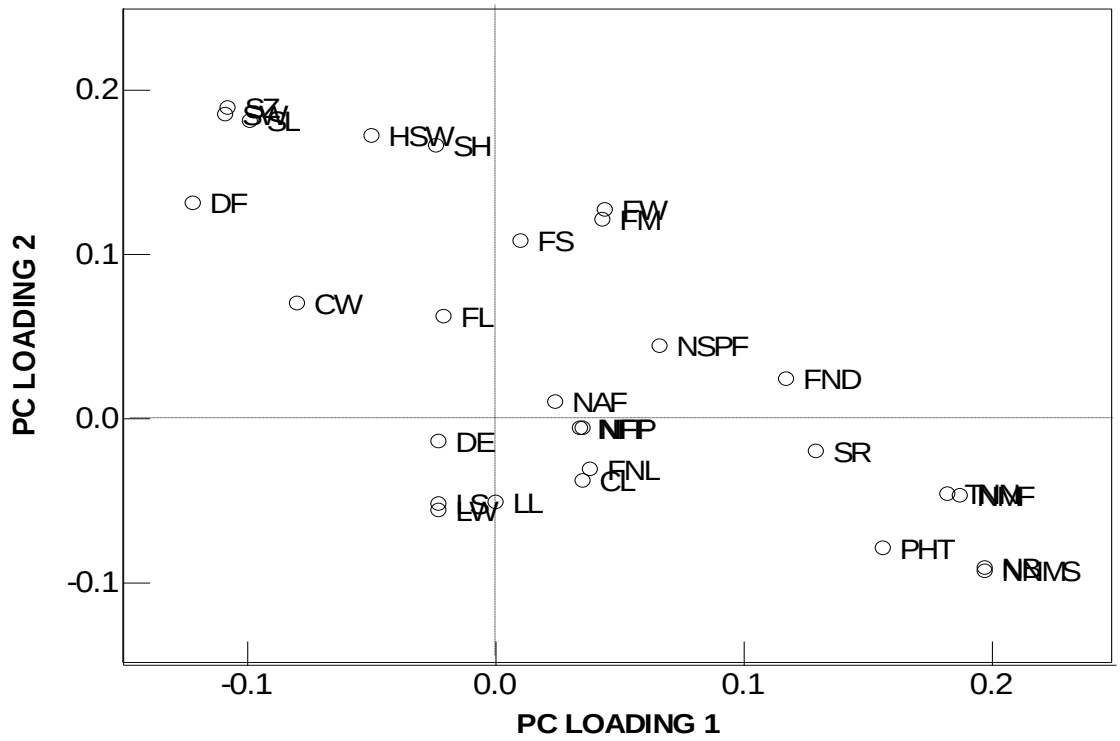

Figure 2.4. Varimax rotated principal component loadings showing similarities among quantitative traits of 36 bottle gourd landraces. CL = Cotyledon length; CW = Cotyledon width; LL = Leaf length; LW = Leaf width; LS = Leaf size; PHT = Plant height; NNMS = Number of nodes on the main-stem; TNN = total number of nodes; NMF = Number of male flowers; NFF = Number of female flowers; SR = Sex ratio; NB = Number of branches; NAF = Number of aborted fruits; NFPP = Number of fruits per plant; FM = Fruit mass; FL = Fruit length; FW = Fruit width; FS = Fruit size; FNL = Fruit neck length; FND = Fruit neck diameter; SH = Shell thickness; NSPF = Number of seeds per fruit; SL = Seed length; SW = Seed width, SZ = Seed size; HSW = Hundred seed weight.

2.6.2 Qualitative traits

Principal component analyses showing corresponding correlation coefficients (eigenvectors) for qualitative traits among bottle gourd landraces are presented in Table 2.7. The qualitative traits were grouped under the five principal components (eigenvalues ≥ 1) which accounted for 78% of total variation. Presence or absence of fruit neck, fruit shape, degree of neck bending and fruit neck length positively correlated with PC1, which accounted for 31.9% of the total variation. The presence or absence of seed lines and seed texture was highly correlated with PC2 which accounted for 14.9% of the total variation. The associations between PC1 and PC2 loading scores of

qualitative traits used to classify bottle gourd landraces is shown in Figure 2.6. The landraces in the bottom right quadrant were closely associated by primary fruit colour, degree of warts, stem-end fruit shape, fruit shape and presence or absence of fruit neck.

Table 2.7. Principal component analysis showing eigenvectors, eigenvalues, and percent variance explained by five principal components (PC's) on qualitative traits of 36 bottle gourd landraces.

Qualitative traits	PC1	PC2	PC3	PC4	PC5
Primary fruit colour	0.29	-0.17	**0.79**	0.03	-0.05
Secondary fruit colour	0.14	0.01	0.29	**0.61**	0.50
Presence of fruit neck	**0.86**	-0.06	-0.01	0.00	0.13
Fruit texture	-0.06	0.02	**0.83**	0.08	0.07
Degree of warts	-0.12	0.09	-0.01	-0.14	**0.85**
Fruit shape	**0.68**	-0.13	-0.26	0.53	0.06
Degree of corrugation	0.03	0.03	0.08	**0.79**	-0.24
Neck bending degree	**0.89**	0.16	0.19	0.10	-0.15
Stem-end fruit shape	**0.60**	-0.19	0.47	0.33	0.04
Fruit neck length	**0.87**	-0.09	0.21	-0.02	-0.22
Seed length	-0.04	**0.90**	-0.09	-0.10	0.08
Seed texture	-0.03	**0.92**	-0.05	0.09	0.02
Explained variance (Eigenvalue)	3.83	1.79	1.54	1.15	1.01
Proportion of total variance (%)	31.90	14.93	12.83	9.57	8.43
Cumulative variance (%)	31.90	46.83	59.66	69.23	77.66

Loadings greater than ≥ 0.6 are shown in bold faced fonts

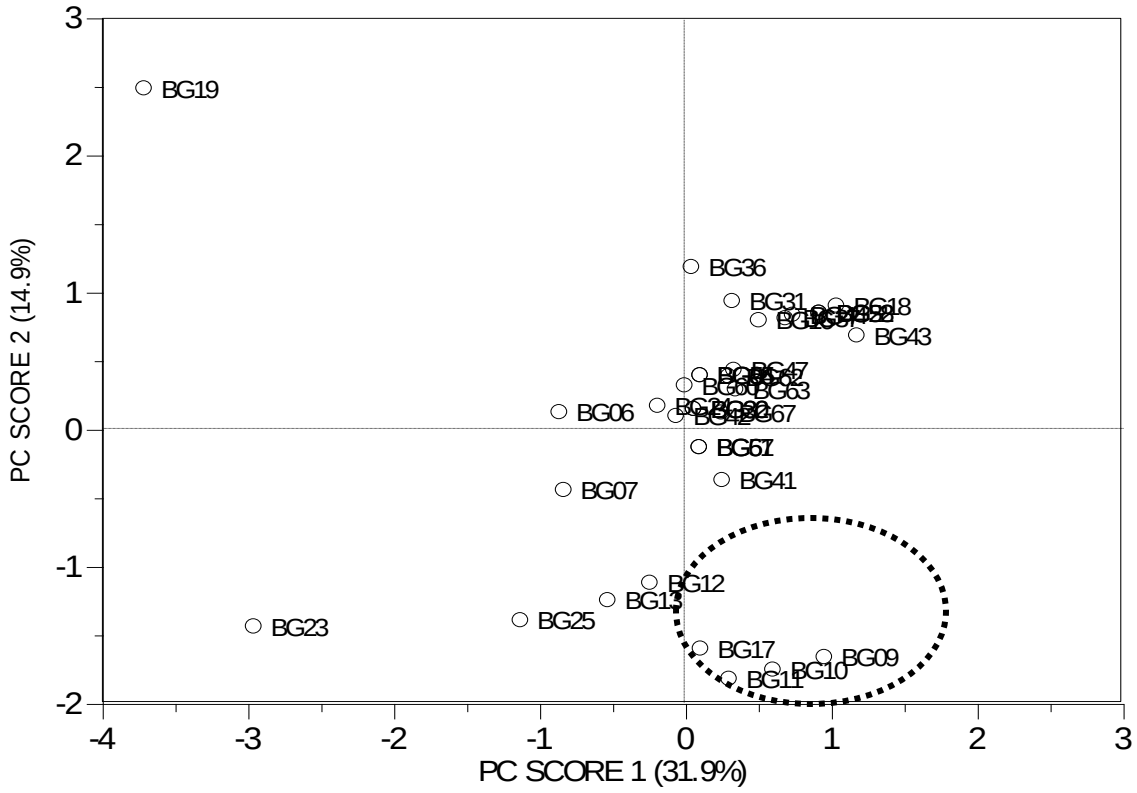

Figure 2.5. Rotated principal component scores of qualitative traits and percent explained variance of PC1 and PC2 showing similarities among 36 bottle gourd landraces.

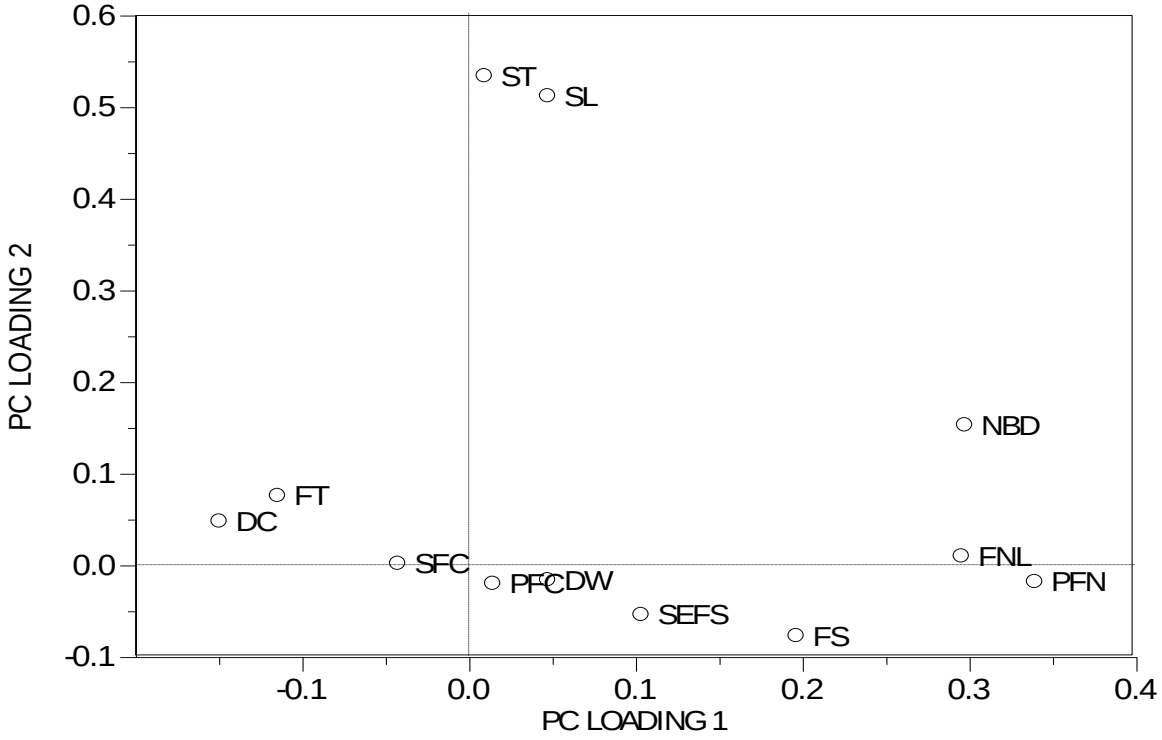

Figure 2.6. Varimax rotated principal component loadings showing similarities among qualitative traits of 36 bottle gourd landraces. PFC= Primary fruit colour, SFC= Secondary fruit colour, PFN = Presence of fruit neck, FT= Fruit texture, DW= Degree of warts, FS = Fruit shape, DC= Degree of corrugation, DNB = Degree of neck bending, SEFS = Stem-end fruit shape, FNL = Fruit neck length, SL = Prescence or absence of seed lines, ST = Seed texture.

2.7 Discussion

This study analyzed genetic diversity of bottle gourd landraces collected from the Limpopo Province of South Africa on the basis of qualitative and quantitative morphological traits. Many qualitative traits observed on bottle gourd landraces showed high genetic diversity. The extent of genetic diversity observed in this study agrees with this proposition. Most of the genetic diversity was observed with respect to fruit qualitative traits. Important fruit traits that represented the diversity of the landraces were: primary fruit colour, secondary fruit colour, fruit shape, and fruit skin texture, presence of fruit neck and fruit neck shape, fruit neck length and stem end fruit shape. Results observed in this study agree with work by several authors that bottle gourd exhibits significant variation with respect to fruit characteristics (Morimoto *et al.*, 2005; Morimoto *et al.*,

2006; Achigan-Dako *et al.*, 2008; Yetişir *et al.*, 2008; Xu *et al.*, 2013). The observed variation in fruit qualitative traits among the bottle gourd landraces in this study could be partly due to the result of long-term selection by growers, the farming system and environmental effects and the mating system of the crop (Morimoto *et al.*, 2005; Koffi *et al.*, 2009). Probably farmers may have selected various unique fruit shapes for various uses (Morimoto *et al.*, 2005). Bottle gourd is a highly monoceious plant species and has high rates of cross pollination and result in considerable variation which may change the genetic identity of populations. This may explain the higher variation in open pollinated populations (Robinson and Decker-Walters 1997). Variation in fruit characteristics observed amongst bottle gourd landraces in this study could also be explained by the fact that these landraces were the result of cross pollination. In South Africa, small-scale farmers typically plant more than one landrace in the field and the possibility of out-crossing is high. Morimoto *et al*. (2004) observed that honeybees (*Apis mellifera*) are the main agents of cross pollination among different species. The authors suggested that bottle gourd growing in farmers' fields may be pollinated not just by pollen of its own species, but also by pollen from adjacent species, resulting in a large gene pool within the species.

Principal component analysis (PCA) on quantitative traits indicated that PC1 was composed of a number of traits that contributed for the greatest variation, followed by PC2. Characters with high coefficients in the first and second PCs were considered more important since these axes explaining nearly half of the total variation. Plant height, number of nodes on main-stem, total number of nodes, number of male flowers, number of branches, fruit neck length, number of seeds/fruit, fruit mass, fruit width, fruit size, shell thickness, seed length, seed width, seed size and hundred seed weight constituted most the variability amongst bottle gourd landraces. The PCA in this study agree with Morimoto *et al*. (2005) and Koffi et *al*. (2009) who also reported that fruit and seed traits contributed most of the variation in bottle gourd. Unique genotypes including: BG-25, BG-67, BG-12, BG-13, BG-07, BG-09 showing suitable quantitative traits (e.g. tall plant height, maximum nodes on the mainstem, total number of nodes, male flowers, number of branches, fruit neck diameter, fruit mass, fruit width, fruit size, and shell thickness) were identified for breeding. Among qualitative traits, presence or absence of fruit neck, fruit shape, degree of neck bending and fruit neck length positively correlated with PC1. Presence or absence of seed lines and seed texture was highly correlated with PC2. Results in this study suggest that differences

among bottle gourd landraces are determined by qualitative fruit and seed characteristics. Identification of influential qualitative traits in bottle gourd using PCA has not been reported previously in bottle gourd. Farmer preferred qualitative traits include fruit shape, smooth, warted and corrugated skin texture (Mapitsi Kobe and Raisibe Mahlong, personal communication). The following landraces such as BG-09, BG-10 and BG-11 are useful to make containers for drinking water and traditional beer (Mashilo *et al.*, 2015). The bottle gourd landraces with warted and corrugated fruit texture are selected for direct production and included BG-16, BG-25, BG-09, BG-37 and BG-10. The bottle gourd landraces evaluated in this study represent an opportunity to initiate a breeding programme aimed at the development of new varieties in South Africa.

2.8 Conclusions

This study assessed genetic diversity of bottle gourd landraces grown by smallholder farmers in the Limpopo Province of South Africa. An analysis of qualitative and quantitative traits in this study showed the presence of genetic variation among bottle gourd landraces useful for bottle gourd genetic improvement.

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Chapter 3: **Genetic Diversity of Bottle Gourd [*Lagenaria Siceraria* (Molina) Standl.] Landraces Revealed by Simple Sequence Repeat Markers**

Abstract

Bottle gourd [*Lagenaria siceraria* (Molina) Standl.] landraces are widely grown in South Africa and genetic diversity analysis is necessary to identify promising genotypes for breeding or systematic conservation. Sixty seven diverse bottle gourd landraces were genotyped using 14 selected SSR markers. The number of alleles detected per marker ranged from 4 to 11, with a total of 86 putative alleles being amplified. Allele sizes ranged from 145 to 330 bp. Number of effective alleles (N_e) ranged from 1.58 to 6.14 with a mean of 3.10. Allelic richness varied from 3.00 to 8.90 with a mean of 5.23. Expected heterozygosity (H_e) values ranged from 0.37 to 0.84 with a mean of 0.65. The mean polymorphic information content (PIC) was 0.57. Jaccard's coefficient of similarity values ranged from 0.00 to 1.00, with a mean of 0.63. Analysis of molecular variance (AMOVA) revealed that 79%, 17% and 4% of the variation in bottle gourd landraces was attributable to among landraces, within landraces and between populations, respectively. The study established the existence of considerable genetic diversity among South African bottle gourd landraces. Unique landraces such as BG-4, BG-6, BG-8, BG-9, BG-15 from cluster I, BG-55, BG-42, BG-57 and BG-58 from cluster II, BG-28, BG-23, BG-29 and BG-34 from cluster III were selected based on their highest dissimilarity index. These could be useful for bottle gourd breeding and systematic conservation.

Keywords: Breeding, genotypes, simple sequence repeats, conservation

3.1 Introduction

Bottle gourd [*Lagenaria siceraria* (Molina) Standl.] belongs to the *Cucurbitaceae* family. It is a diploid (2n = 2x = 22) vine crop widely grown in rural communities in South Africa (Beevy and Kuriachan, 1996; Achigan-Dako *et al*., 2008). The genus *Lagenaria* consists of five wild species: *L. breviflora* (Benth.) Roberty, *L. abyssinica* (Hook F.) Jeffrey, *L. rufa* (Gilg.) Jeffrey, *L. sphaerica* (Sonder) Naudin and *L. guineensis* (G. Don) Jeffrey (Whitaker, 1971). *L. siceraria* is the only cultivated species with economic value grown worldwide for diverse uses such as for food, medicine, decoration, to make household utensils and musical instruments (Jeffrey, 1976). Fresh bottle gourd fruit juice is used as medicine to cure various diseases including flatulence, diabetes mellitus, hypertension, liver diseases and as a diuretic (Ghule *et al*., 2007). The seeds of this crop are rich in essential amino acids and oil. Some bottle gourd types are exclusively grown for their seeds (Achigan-Dako *et al.*, 2008). Bottle gourd also serves as a rootstock in watermelon breeding to control soil-borne diseases and to manage low soil temperature stress (Lee, 1994; Yetisir and Sari, 2003).

Bottle gourd exhibits significant genetic variation with respect to fruit size and shape (Morimoto *et al*., 2005; Yetişir *et al*., 2008; Xu *et al*., 2014; Gurcan *et al*., 2015), fruit shell thickness, fruit length and fruit width (Morimoto *et al*., 2005; Koffi *et al*., 2009; Harika *et al*., 2012) and seed morpho types (Decker-Walters *et al*., 2004; Morimoto *et al*., 2005; Yetişir *et al*., 2008; Schlumbaum and Vandorpe, 2012). This variation is attributed to farmers' long-term selection of the crop which is often driven by specific socio-cultural preferences and use, cultural practices and the environment (Mladenovic *et al*., 2012). Genetic advancement during selection depends on the availability of genotypes possessing favourable alleles for desired traits, which relies on the available genetic diversity (Smith *et al*., 1991).

Phenotypic markers have been used to characterize and evaluate bottle gourd genetic resources which employed various descriptor lists of morpho-agronomic traits (Morimoto *et al*., 2005; Yetişir *et al*., 2008; Koffi *et al*., 2009). Molecular marker-based characterization is a powerful and complementary tool to phenotyping. Molecular markers are independent of environmental effects

and provide more robust data on genetic distance estimates (Lefebvre *et al*., 2001; Decker-Walters *et al*., 2004; Pagnotta *et al*., 2009).

Various molecular markers have been used to assess genetic variability in bottle gourd namely: Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSRs) or microsatellite markers, Inter-Simple Sequence Repeats (ISSR), Single Nucleotide Polymorphism (SNP's) and allozyme markers (Decker-Walters *et al*., 2001; Saxena *et al*., 2015; Xu *et al*., 2014; Koffi *et al*., 2009). SSRs are the marker of choice for genetic diversity analysis studies because of their high degree of polymorphism and random distribution across the genome (Varshney *et al*., 2005; Gong *et al*., 2012; Ji *et al*., 2012). SSR markers have been successfully used to determine the level of genetic diversity in bottle gourd (Gonzalo *et al*., 2005; Watcharawongpaiboon and Chunwongse, 2008; Bhawna *et al*., 2015; Saxena *et al*., 2015). Sarao *et al*. (2013) fingerprinted 20 accessions of bottle gourd in India using 20 SSR primers and reported the discriminatory power of these markers. Xu *et al*. (2011) also used SSR markers to determine the genetic diversity of bottle gourd genotypes from China.

Bottle gourd is an under-researched genetic resource in South Africa (van Rensburg *et al*., 2007; van Wyk, 2011). In the country, small-holder farmers grow unimproved landraces which exhibit great morphological diversity with respect to fruit and seed morphology. Despite the possible genetic variability and potential use of bottle gourd in South Africa, there is no recent and detailed information regarding its systematic characterization using molecular markers. Genetic diversity analysis using molecular markers may effectively characterise the South African bottle gourd landraces for systematic selection for breeding or for strategic conservation. Therefore, the objective of this study was to assess the genetic diversity present among 67 bottle gourd landraces in South Africa using selected polymorphic simple sequence repeats markers.

3.2 Materials and methods

3.2.1 Plant materials

Sixty seven bottle gourd landraces collected from Capricorn (23°36’44.38”S; 29°13’55.48” E) and Vhembe (22°03’53” S; 28°50’00.03” E) districts of the Limpopo Province of South Africa were used in the study. Table 3.1 summarises information related to the collection sites of landraces.

Table 3.1. List of 67 bottle gourd landraces used in the study with collection districts in Limpopo Province of South Africa.

Sr. No	Entry	District	Sr. No	Entry	District
1	BG-03	Vhembe	35	BG-43	Capricorn
2	BG-04	Capricorn	36	BG-44	Capricorn
3	BG-05	Capricorn	37	BG-45	Capricorn
4	BG-06	Vhembe	38	BG-46	Capricorn
5	BG-07	Vhembe	39	BG-47	Capricorn
6	BG-08	Vhembe	40	BG-48	Capricorn
7	BG-09	Vhembe	41	BG-51	Capricorn
8	BG-10	Vhembe	42	BG-52	Capricorn
9	BG-11	Vhembe	43	BG-53	Capricorn
10	BG-12	Vhembe	44	BG-55	Capricorn
11	BG-13	Vhembe	45	BG-56	Capricorn
12	BG-15	Vhembe	46	BG-57	Capricorn
13	BG-16	Capricorn	47	BG-58	Capricorn
14	BG-17	Capricorn	48	BG-59	Capricorn
15	BG-18	Capricorn	49	BG-60	Capricorn
16	BG-19	Capricorn	50	BG-61	Capricorn
17	BG-22	Capricorn	51	BG-62	Capricorn
18	BG-23	Capricorn	52	BG-64	Capricorn
19	BG-24	Capricorn	53	BG-65	Capricorn
20	BG-25	Vhembe	54	BG-66	Capricorn
21	BG-26	Capricorn	55	BG-67	Capricorn
22	BG-27	Capricorn	56	BG-68	Capricorn
23	BG-28	Capricorn	57	BG-70	Capricorn
24	BG-29	Capricorn	58	BG-71	Capricorn
25	BG-30	Capricorn	59	BG-72	Capricorn
26	BG-31	Capricorn	60	BG-73	Vhembe
27	BG-32	Capricorn	61	BG-74	Vhembe
28	BG-33	Capricorn	62	BG-75	Vhembe
29	BG-34	Capricorn	63	BG-76	Vhembe
30	BG-35	Capricorn	64	BG-77	Capricorn
31	BG-36	Capricorn	65	BG-79	Capricorn
32	BG-40	Capricorn	66	BG-80	Capricorn
33	BG-41	Capricorn	67	BG-81	Capricorn
34	BG-42	Capricorn			

Sr. No = Serial Number

3.2.2 DNA extraction, purification and quantification

Seed of diverse bottle gourd landraces were planted in seedling trays at the Controlled Research Facility (CEF), University of KwaZulu-Natal, Pietermaritzburg (29°37'51.75" S; 30°23'59.10" E), South Africa. Young fresh leaves were harvested from 20 plants per landrace four weeks after planting. The leaf samples were sent to INCOTEC PROTEIOS laboratory (Incotech, SA Pty Ltd, Mkondeni, Pietermaritzburg, South Africa) for SSR analysis. The DNA was extracted following the CTAB (mixed alkyltrimethyl-ammonium bromide protocol (DNA extraction buffer) as described by CIMMYT (2005). The concentration of the extracted DNA was determined using 0.7% Tris-Borate-EDTA (TBE) agarose gel. A working concentration of 10 ng μl^{-1} was standardized for all extracted DNA (Erasmus, 2008). The samples were bulked and used in SSR amplification.

3.2.3 PCR and SSR analyses

All samples were used in bulked amplification, using DNA extracted from the leaf material. SSR sequences were amplified through polymerase chain reaction (PCR) using SSR primers specific for bottle gourd. Fourteen SSR markers were used for the analysis (Table 3.2). The markers were selected based on their high polymorphic information content and that they were developed being specific for bottle gourd (Xu *et al*., 2011). High polymorphic information content values suggest markers may have high discriminatory power to distinguish differences between the genotypes. PCR were performed using 12 µl of reaction mixture containing 1 x PCR buffer, 2.5 mM Mg^{++}, 0.2 µl each of dNTPs (Bioline), 1 unit of Taq polymerase (Bioline) and 5-10 ng of genomic DNA. Primers were labeled with a 104 fluorescent dye. Two primers were provided for the amplification of each SSR locus: one tailed forward primer (0.25 µmol) and one normal reverse primer (0.25 µmol). The initial denaturation step was performed at 94ºC for 2 minutes, followed by 33 cycles at 94ºC for 30 seconds. Annealing of primer at primer specific 3ºC for 30 seconds and 72ºC for 45 seconds with a final extension for 20 m minutes (Erasmus, 2008). PCR products were fluorescently labelled and separated by capillary electrophoresis on a ABI 3130 automatic sequencer (Applied Biosystems, Johannesburg, South Africa).

Table 3.2. Description of simple sequence repeats (SSR) primers used for bottle gourd genetic diversity analysis.

SSR primer	Forward primer (**5'→3'**)	Reverse primer (**3'→5'**)	Repeat type
LSR011	TTCGCCTCAGTCCATCTAGTTT	ATGTCGTACCTTTTCCCCTTTT	ATT
LSR015	CTTACCTTCACAAAACCCCATC	ACTCTGTTTCGACTCTGCTTCC	CTT
LSR020	AACTGAAACCATTAACGAAGGC	AATAAGCAGCAACCATGTCAAC	G
LSR030	GGAGACAAAACCAACAACGAA	GAAAATGCAGACAAAGAAAGCC	AT
LSR040	TTCCATCCAGACCAAACCTATC	CAAAGGCCATAGACAAACACAA	TTC
LSR047	CAATAGAGTAGGGTGGGGCATA	TAAAATAGTGGGAGAGCAAGGG	TC
LSR056	TAATAATGCCACTGCACATGGT	AGATGAATCCCAATATCCCAGA	CTT
LSR063	AAGAGAGGGGCAGGAAGTAAAT	AGAAAACACACAGTACGCCTCC	ATT
LSR074	TATCAACTCCAGAAGACGGGTT	TGGTAAACGTAGGGATACAAAGAAG	TA
LSR077	GACAGATCCTTCTGGGACTTTT	TTCTGCAATAGAGTACGTTGGC	TC
LSR088	CCAACTATCACCCCTACAATCA	GGACAGAACCTAAAAGAAAGAAGAG	ATA
LSR108	AGCTCTGGGAAGAGGAAGAGTA	GCAGACAGAAGAAGAAAGTTAGAGA	AG
LSR109	TGGGGTAGAAATTGAAGAGGAG	TTGGATCAGCTTGGGTTTTACT	GA
LSR112	CTCTCTATATGTCTAATTCCTCGCC	CAAATTCACAGTTGTTGTCACG	TTCT

3.3 Data analysis

3.3.1 Genetic parameters

Genetic diversity parameters, such as number of alleles per locus (N_a), number of effective alleles per locus (N_e), allelic richness (A_r) and expected heterozygosity (H_e) were calculated using GenAlex version 6.5 (Peakell and Smouse, 2007). Polymorphic information content (PIC) was calculated using the formula: $PIC = 1-\Sigma P_{ij}^2$, where P_{ij} is the frequency of j^{th} allele of the i^{th} locus (Nagy *et al*., 2012). The number of polymorphic loci was estimated for each predetermined group, based on the districts of collection. Allelic richness was estimated by using the rarefaction method implemented in HP-Rare 1.0 (Kalinowski, 2005). Further, an indirect estimate of the level of gene flow (N_m) between the genotypes was calculated using the formula: $Nm = 0.25\ (1 - F_{ST}/F_{ST})$ using GenAlex. Nei's unbiased genetic distance was also estimated using GenAlex. The F-statistics such as genetic differentiation (F_{ST}), fixation index or inbreeding coefficient (F_{IS}), and overall fixation index (F_{IT}) were calculated according to Wright's original derivation (Wright, 1951).

3.3.2 Analysis of molecular variance

The partitioning of total genetic variation using Analysis of molecular variance (AMOVA) was performed to estimate population genetic structure and differentiation among and within sweet and wild watermelon landrace collections. AMOVA uses the estimated F- statistics such as F_{ST}, F_{IS}, and F_{IT} to compare the genetic structure among and within populations. For easy management and utilization, the total molecular variance was dissected into within and among population variations. The AMOVA procedure was done using GenAlex 6.5 according to Nei (1978).

3.3.3 Cluster analysis

Genetic relationships among the landraces was determined using neighbor-joining algorithm using the unweighted pair group method using arithmetic average (UPGMA) in DARwin 6.0 (Perrier and Jacquemoud-Collet, 2006). The dendogram was generated based on Jaccard's dissimilarity matrix using binary data (0 = absent, 1 = present) to capture all the alleles amplified. Bootstrap analysis was performed for node construction using 10,000 bootstrap values to estimate the reliability of the clustering pattern.

3.4 Results

3.4.1 Polymorphism and allelic diversity of SSR markers

Estimates of genetic parameters are presented in Table 3.3. The SSR markers generated a total of 86 putative alleles (different fragment sizes) among the bottle gourd landraces. Number of alleles ranged from 4 for the markers LSR074, LSR088, LSR108 and LSR112 to 11 for LSR020, with a mean of 6.14 per locus. Number of effective alleles ranged from 1.58 to 6.14 with a mean of 3.1. The value of N_e for 50% of the loci was > 2, while for 50% of the loci N_e was > 3. Allelic richness ranged from 3 to 8.9 with a mean of 5.23. Allele size ranged from 145 to 330 base pairs (bp). The highest variation in allele size was observed in marker LSR030 (155–330 bp) and LSR077 (159-320 bp), respectively. The lowest variation in allele size was observed in marker LSR108.

Shannon's information index (*I*) ranged from 0.93 to 1.99 with a mean of 1.29. Expected heterozygosity values ranged from 0.37 to 0.84 with a mean of 0.65. Marker LSR063 had the lowest, and marker LSR015 the highest expected heterozygosity values. The PIC values ranged from 0.37 for the marker LSR063 to 0.83 for marker LSR015 with a mean of 0.57. In the present study nine SSR markers used had PIC values > 0.50. These were classified as informative markers to establish the genetic relationship of bottle gourd landrace collections. The level of gene flow (N_m) between the landraces was the highest for the markers LSR040, LSR056, LSR077 and LSR109 and the lowest for the markers LSR088, LSR112 and LSR020 with a mean of 24.46. Genetic differentiation (F_{ST}) ranged from 0 to 0.05 with a mean of 0.04.

Table 3.3. Genetic parameters generated by 14 SSR markers amongst 67 landrace collections of bottle gourd from South Africa.

	Genetic parameters								
Loci	N_a	N_e	A_r	A_{sr}	*I*	H_e	*PIC*	*Nm*	*F*ST
LSR011	7	3.91	6.43	162-190	1.62	0.75	0.71	7.50	0.03
LSR015	10	6.14	8.86	146-201	1.99	0.84	0.83	6.68	0.04
LSR020	11	3.04	5.66	161-202	1.41	0.68	0.61	4.76	0.05
LSR030	8	4.14	6.83	155-330	1.69	0.76	0.73	8.29	0.03
LSR040	7	3.06	6.87	175-214	1.46	0.68	0.60	18.42	0.01
LSR047	6	2.82	5.55	145-175	1.36	0.65	0.55	8.18	0.03
LSR056	3	2.68	3.00	155-193	1.03	0.63	0.57	81.45	0.00
LSR063	5	1.58	3.84	158-204	0.77	0.37	0.37	7.95	0.03
LSR074	4	2.21	3.50	175-203	0.97	0.55	0.41	7.46	0.03
LSR077	6	3.43	5.90	159-320	1.41	0.71	0.67	60.16	0.00
LSR088	4	2.21	3.45	155-204	0.93	0.55	0.43	1.83	0.12
LSR108	4	2.21	3.50	158-177	0.97	0.55	0.41	7.46	0.03
LSR109	7	3.68	6.18	240-273	1.48	0.73	0.70	116.99	0.00
LSR112	4	2.25	3.58	157-225	0.97	0.56	0.44	2.53	0.09
Mean	6.14	3.10	5.23	-	1.29	0.65	0.57	24.26	0.04
SE	0.64	0.31	0.45	-	0.09	0.03	0.04	9.52	0.01

N_a = number of alleles per locus; N_e = number of effective alleles per locus; A_r = allelic richness; A_{sr} = Allele size range (base pairs); *I* = Shannon's information index; H_e = heterozygosity; *PIC* = polymorphic information content; *Nm* = Gene flow; F_{ST} = *g*enetic differentiation; SE = standard error.

3.4.2 Genetic variability within and among populations based on district of collection

Marked differences were detected in the mean number of alleles and private alleles per locus between the two districts (Capricorn and Vhembe) of collection (Table 3.4). Capricorn district showed the highest number of private alleles (13) than Vhembe (4). Conversely, Vhembe district showed the highest number of effective alleles (3.58) than Capricorn district (2.81). Increased allelic richness was observed at Vhembe district, while a slightly lower allelic richness was detected at Capricorn district. Shannon's information index estimates were closely similar between the two districts. Expected heterozygosity, polymorphic information content and average genetic distance were slightly higher in Vhembe than Capricorn district. Nei's unbiased genetic distance between Capricorn and Vhembe district was 0.09.

Table 3.4. Estimation of genetic parameters amongst 67 landrace collections of bottle gourd using 14 SSR markers based on districts of collection.

	Districts of collection	
Genetic parameters	Capricorn	Vhembe
Number of private alleles	13	4
Number of alleles	5.86	5.21
Allelic richness	4.91	5.43
Number of effective alleles	2.81	3.58
Shannon's information index	1.21	1.36
Heterozygosity	0.61	0.71
Polymorphic information content	0.50	0.63
Average genetic distance	0.61	0.70

3.4.3 Cluster analysis

Jaccard's coefficient of similarity values ranged from 0.07 to 1.0, with a mean of 0.63 among the 67 landraces (Data not shown). Among the test bottle gourd landraces the following pairs were identical with the lowest dissimilarity index: BG-70 and BG-77, BG-56 and BG-76, BG-51 and BG-61, BG-60 and BG-65, BG-62 and BG-81, BG-65 and BG-70 and BG-65 and BG-77. Conversely, BG-6 was distantly related to BG-28; BG-52; BG-55; BG-57, BG-58 and BG-66. The

landraces BG-4 and BG-55, BG-8 and BG-58 and BG-9 and BG-58 were also distantly related with high dissimilarity values. The landraces which were distantly related showing the highest dissimilarity index were: BG-15 with BG-45, BG-55 and BG-28; BG-18 with BG-28, BG-29, BG-42 and BG-55; BG-19 with BG-34, BG-42, and BG-55; and BG-25 with BG-55, BG-17 and BG-58. Other landraces that were distantly related included: BG-35 with BG-52; BG-58, BG-40 with BG-52 and BG-58.

The unweighted pair group method (UPGMA)-derived dendogram based on the Jaccard's dissimilarity clustering pattern using the neighbour-joining method in DARwin 6.0 classified the bottle gourd landraces into three major clusters namely: cluster I, cluster II and cluster III (Figure 3.1). Cluster I was further subdivided into two sub-clusters consisting of 27 landraces. Cluster II had three sub-clusters consisting of 35 landraces. Cluster III had two sub-clusters consisting of 5 genotypes. Based on the highest genetic dissimilarity values of landraces in different clusters, the following unique and genetically complementary landraces were selected for breeding and systematic conservation. Landraces selected from cluster I included: BG-4, BG-6, BG-8, BG-9, BG-15, BG-17, BG-18, BG-19, BG-22, BG-24, BG-25, BG-35 and BG-40; while selections from cluster II were: BG-42, BG-52, BG-55, BG-57, BG-58 and BG-66; and from cluster III: BG-23, BG-28, BG-29 and BG-34. The selected landraces have unique agronomic attributes and fruit shape (Figure 3.2). These landraces would be genetically complementary for bottle gourd breeding.

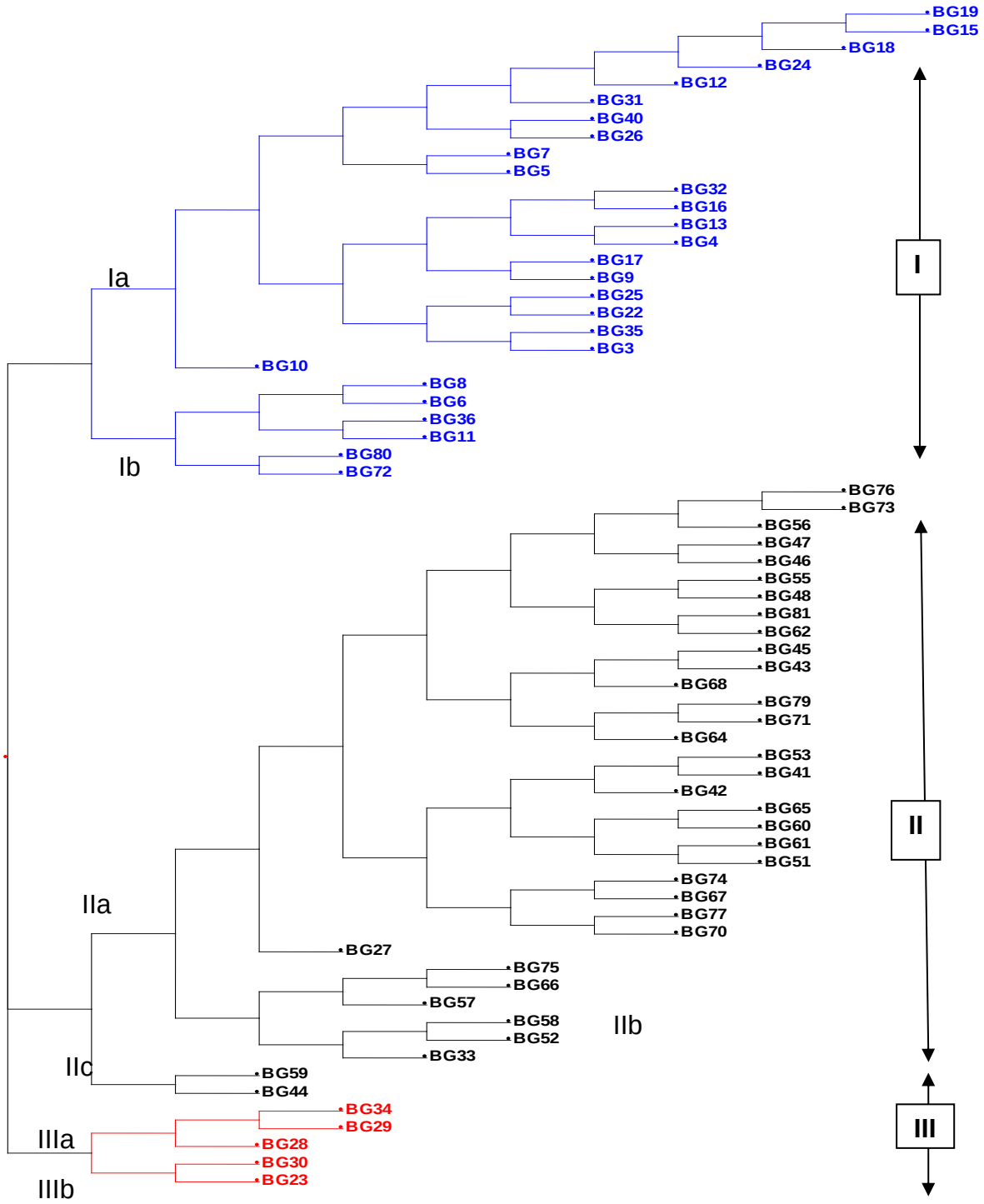

Figure 3.1. Neighbour-joining dendrogram using the unweighted pair group method (UPGMA) based on Jaccard's dissimilarity matrix revealing genetic relationships among 67 bottle gourd landraces based on SSR markers. Ia, Ib, IIa, IIb and IIc, IIIa and IIIb denote subgroups within the clusters.

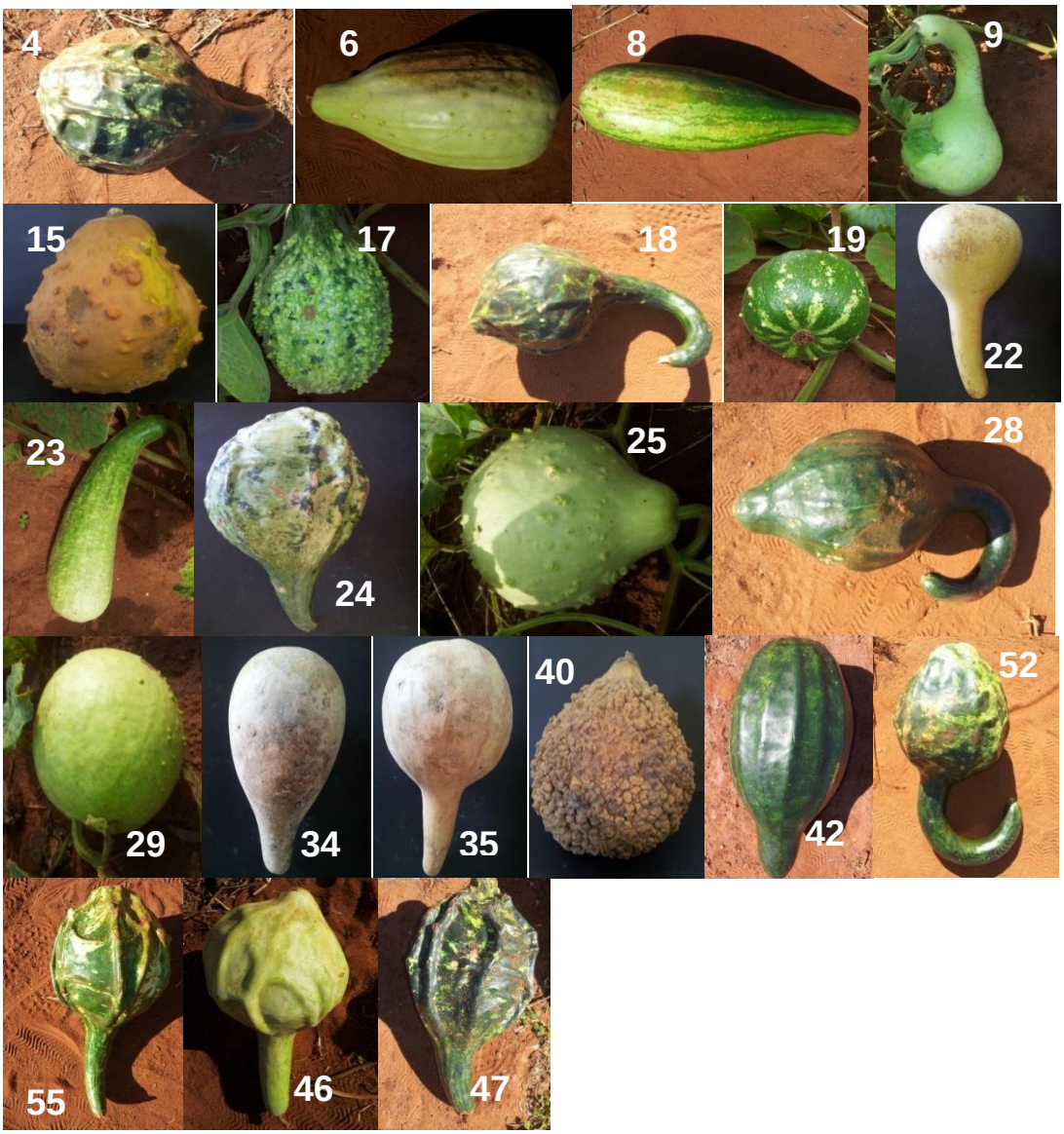

Figure 3.2. Variation in fruit architecture of bottle gourd landraces selected showing the highest dissimilarity values revealed by 14 SSR markers. Note: numbers on the photos designate entries presented in Table 3.1.

3.4.4 Analysis of molecular variance

Analysis of molecular variance revealed 79% of variation was attributable to among landraces, while 17% and 4% of the total variation was allocated to within landraces and between populations, respectively (Table 3.6).

Table 3.5. Analysis of molecular variance of 67 bottle gourd landraces studied using SSR markers.

Source of variation	df	SS	MS	Estimated variance	Percentage variance	F-Statistic
Between populations	1	12.703	12.703	0.15	4%	0.057
Among landraces	65	437.25	6.727	2.959	79%	0.001
Within landraces	67	34	0.507	0.637	17%	0.001
Total	133	483.96	3.639	3.746	100%	

df= degrees of freedom; SS= Sum of squares; MS= Mean squares

3.5 Discussion

The present study assessed genetic diversity of South African bottle gourd landraces using SSR markers. The number of alleles ranged from 4 to 11 with a mean of 6.14 per locus. This was higher than 2 to 4 alleles (average = 2.6) reported by Sarao *et al*. (2013). Using 14 polymorphic SSR loci, Xu *et al*. (2013) reported 2 to 8 alleles (mean = 3.64) per marker in 44 bottle gourd entries which is less than the current findings. Koffi *et al*. (2009) reported the number of alleles per locus varying from 1.0 to 1.4 with a mean of 1.2 suggesting a low allelic richness. Yildiz *et al*. (2015) and Bhawna *et al*. (2015a) reported the number of alleles ranging from 1 to 5 (mean= 1.64) and 2 to 4 alleles (mean = 2.84) in bottle gourd, respectively. In the current study, the number of effective alleles ranged from 1.58 to 6.14 with a mean of 3.1. These values were higher than reported by Bhawna *et al*. (2015a) with 1.46 – 3.29 (mean = 2.08). This indicates a high level of genetic diversity amongst South Africa bottle gourd landraces useful for breeding and strategic conservation.

The selected SSR markers amplified a total of 86 alleles with allele size ranges of 155 to 330 base pairs. A total of 26 alleles were reported by Sarao *et al*. (2013) and 51 alleles by Xu *et al*. (2011). The higher number of alleles generated by SSR markers in the present study suggests a wide genetic diversity that can be exploited for breeding. The high allelic number and richness in the present study implies the presence of significant genetic variation among tested landraces. This could be attributed to higher cross pollination of bottle gourd or long term selection by farmers.

Furthermore, bottle gourd is a cross-pollinated crop where recurrent exchange of gametes occurs between different genotypes (Bhawna *et al*., 2014).

Expected heterozygosity values in this study ranged from 0.37 to 0.84 with a mean of 0.657. Kofi *et al*. (2009) reported a low average heterozygosity of 0.053 with ranges of 0 to 1.4 in bottle gourd. The levels of observed heterozygosity in the present study are considerably higher than reported for wild species. Expected heterozygosity were reported to be 0.46 for annuals and 0.55 for short-lived perennials, and 0.41 for self-fertilizing, 0.60 for mixed breeding and close to 0.65 for outcrossing species of bottle gourd (Nybom, 2004). The high levels of expected heterozygosity indicate a presence of significant level of genetic diversity which will enhance selection efficiency.

In the current study the PIC values ranged from 0.37 to 0.83 (mean = 0.57). These values are comparatively higher than those reported by Sarao *et al.* (2013) with 0.23 to 0.73 and Xu *et al*. (2011) with 0.11 to 0.72 (mean = 0.4). Yildiz *et al*. (2015) reported PIC values of 0.12 to 0.52 (mean= 0.15) in bottle gourd, while Bhawna *et al*. (2015a) found PIC values of 0.05 to 0.54 (average = 0.33) using 19 SSR markers. The PIC defines a relative measure of the informativeness of a marker or discriminatory power of a polymorphic marker which depends on the number of alleles and relative frequency of an allele in the population (Gaikward *et al*., 2008; Bhawna *et al*., 2015a). In the present study, the highest PIC value was detected using marker LSR015 (0.84), closely followed by LSR020 (0.83). Also, a large proportion of the markers used exhibited a greater discriminatory power. Generally most of the markers had PIC values > 0.6 suggesting a high discriminating ability of the SSR markers for classifying the bottle gourd landraces.

A low genetic differentiation (F_{ST}) of 0.04 was found in the present study. According to Wright (1978), F_{ST} values ranging from 0–0.005 indicates low, 0.05–0.15 moderate, 0.15–0.25 high, and above 0.25 very high genetic differentiations. The low F_{ST} values in the present study could be the result of the high level of gene flow among bottle gourd landraces. The gene flow (Nm) in this study was 24.26 higher than 7.27 reported by Bhawna *et al*. (2015a). The high gene flow and low genetic differentiation between landraces in the current study may suggest a high rate of gene exchange between bottle gourd landraces due to its outcrossing. Also, high gene flow could be attributed to a high degree of movement of germplasm probably through frequent seed exchange

among farmers. Farmers recycle seed harvested from previous season, or source from neighbouring farmers or local markets for planting (Koffi *et al*., 2009). This practice results in genetic variability among individuals within populations.

The dendogram based on SSR genotyping classified the bottle gourd landraces into three main clusters revealing great genetic variation. Further, landraces were not grouped based on district of collection suggesting sharing common morphological traits. This suggests that bottle gourd use and selection must have been driven by farmers' specific socio-cultural preferences and use. For example, fruits with handles are used to make containers called "sego" for drinking water and traditional beer (Mrs Mmapitsi Kobe, personal communication) while large-oval fruits are used to make containers for storing water and food (Mrs Mahlong, personal communication). This indicates that germplasm collection programs should be based on morphological variation rather than geographical background (Yildiz *et al*., 2015). Results in this study are in agreement with the findings of Yetisir *et al*. (2008), Xu *et al*. (2011), Sarao *et al*. (2013) and Bhawna et al. (2015) who reported that clustering of bottle gourd genotypes was independent of geographical location. Decker-Walters *et al*. (2001) characterized 74 genotypes of bottle gourd from a global sample collections. The authors revealed that the lines from diverse origins (Africa, Asia and the New World) did not group based on their geographic background. Yildiz *et al*. (2015) reported that population structure analysis classified bottle gourd in two subpopulations defined by fruit shape, rather than geographical origin.

The present study estimated the Shannon's information index (*I*) with a mean value of 1.33. This was higher than 0.80 reported by Bhawna *et al*. (2015a). Genetic distance estimates ranged from 0 to 1.0, with a mean of 0.63 suggesting wide genetic diversity amongst the bottle gourd landraces. Sarao *et al*. (2014) and Xu *et al*. (2011) reported high genetic similarity coefficients of 0.96 and 0.94 in bottle gourd, in that order. Bhawna *et al*. (2015a) reported Jaccard's coefficient of similarity values ranging from 0.11 to 0.75, with a mean of 0.41 among 42 bottle gourd genotypes. These values were lower than the current study suggesting differences in bottle gourd genetic diversity.

3.6 Conclusions

The SSR analysis revealed the existence of wide genetic diversity among South African bottle gourd landraces. Unique landraces such as BG-4, BG-6, BG-8, BG-9, BG-15 from cluster I, BG-55, BG-42, BG-57 and BG-58 from cluster II, BG-28, BG-23, BG-29 and BG-34 from cluster III were selected based on their highest dissimilarity index. These could be useful for bottle gourd breeding and systematic conservation.

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Chapter 4: **Correlation and Path Coefficient Analyses of Qualitative and Quantitative Traits in Selected Bottle Gourd Landraces**

Abstract

Knowledge on associations between yield and related traits is vital to improve selection efficiency in cultivar improvement. This study determined the relationships among qualitative and quantitative traits in 36 genetically diverse bottle gourd [*Lagenaria siceraria* (Molina) Standl.] landraces using simple correlation and path analyses. Results showed significant and positive correlations between number of fruits per plant (NFPP) with number of male flowers, number of female flowers, plant height and number of branches. Number of seeds per fruit (NSPF) was significantly and positively correlated with plant height, number of male flowers, number of female flowers, number of branches and fruit weight. Qualitative traits such as fruit texture, degree of warts, fruit shape, and degree of neck bending, stem-end fruit shape and fruit neck length had significantly high and negative correlations ($P < 0.05$) with NFPP or NSPF. Path analysis revealed relatively high direct path coefficient value (0.96) between fruit weight and NSPF, while selection for increased fruit weight would bring about simultaneous and favourable selection towards increased number of female flowers and number of branches. Also, positive direct path coefficient value (0.92) was exhibited between number of female flowers and NFPP. Path analysis indicated selection for increased number of female flowers would bring about simultaneous selection of plants with higher sex ratio. Relatively high direct path coefficient value (0.47) and significant genotypic correlation were exhibited between degree of warts and NSPF. Further, positive direct path coefficient value (0.75) and non-significant negative genotypic correlation was exhibited between fruit neck length and NFPP. This study demonstrated that selection for increased fruit weight and female flowers may improve genetic gain in seed yield and fruit yield in bottle gourd breeding. Using the above analyses, the following landraces such as BG-06, BG-07, BG-09, BG-11, BG-13, BG-24 and BG-67, were selected for breeding.

Keywords: breeding, direct selection, indirect selection, *Lagenaria siceraria*

4.1 Introduction

Bottle gourd [*Lagenaria siceraria* (Molina) Standl.] is a diploid (2n = 2x = 22) vine crop mainly grown for its fruits (Beevy and Kuriachan, 1996; Achigan-Dako *et al.*, 2008) and seeds which are good source of oil and protein (Achu *et al.*, 2005; Achigan-Dako *et al.*, 2008; Loukou *et al.*, 2011). The crop is also used as decoration, household utensil and musical instruments (Jeffrey, 1976; Srivastava *et al.*, 2014). Bottle gourd has been used as a rootstock for grafting watermelon (Han *et al.*, 2009; Liu *et al.*, 2015) and in the production of seedless watermelon (Sugiyama *et al.*, 2014). However, despite its importance, the crop is considered underutilized and is under-researched.

Understanding the nature of associations among qualitative or quantitative traits is important for direct or indirect selection and consequently to improve the efficiency of selection gains in plant breeding programs (Shimelis and Hugo, 2011). Simple correlation and path coefficient analyses are useful statistical procedures to estimate the magnitude and nature of associations between selection parameters. Simple correlation analysis establishes the mutual associations of variables without regard to cause and effect. Component traits involved during selection are often inter-dependent showing direct or indirect relationships and influencing both repeatability and reliability of selection for yield and economic traits. Simple correlation coefficients can be misleading if a high correlation between two traits is a consequence of the indirect effect of other traits (Dewey and Lu, 1959).

Path coefficient analysis is a standardized partial regression which was developed by Wright (1921) and later described by several workers (Wright, 1934; Li, 1956; Bhatt, 1973; Samonte *et al.*, 1998; Mohammadi *et al.*, 2003; Toebe and Filho, 2013). Unlike simple correlation analysis, path coefficient analysis partitions the correlation coefficients into: the path coefficient that measures the direct effect of a predictor variable upon its response variable and the indirect effect(s) of a predictor variable on the response variable via other predictor variables (Dewey and Lu 1959). The proportion of variance in the response variable explained by variance in the predictor variable (partial coefficient of determination) is the square of the path coefficient. Path coefficients give the relative contribution of various yield-determining traits, enabling breeders to

decide between direct and indirect selection procedures (Ofori, 1996). Path coefficients also reduces the timeline of a selection process by guiding selections to be made on the major few traits rather than looking at several traits with little or no impact on yield or a response trait (Qaizar *et al.*, 1991).

Simple correlation and path coefficient analyses have been used in bottle gourd to determine the degree of associations amongst traits and to pinpoint their direct or indirect effect on fruit yield (Husna *et al.*, 2011; Singh *et al.*, 2012; Varpe *et al.*, 2014). Plant height, number of female flowers per plant, number of branches per plant, fruit weight and fruit length are reported to be important yield components influencing fruit yield in bottle gourd (Husna *et al.*, 2011). Typically, some bottle gourd types are grown for their seeds (Achigan-Dako *et al.*, 2008) making selection based on seed yield important. Fruit weight, number of seeds per fruit and hundred seed weight are important yield components influencing seed yield in bottle gourd (Yao *et al.*, 2015). Further, the crop exhibits great variation in fruit qualitative traits (Morimoto *et al.*, 2005; Yetişir *et al.*, 2008; Xu *et al.*, 2014) which are useful parameters for breeding and genetic analysis. However, there is no information which described selection of bottle gourd based on fruit and seed yield and qualitative traits. Whether qualitative traits relate with yield and its components response is not known. Therefore, detailed information on the nature and magnitude of association between yield and yield related traits is vital for direct or indirect selection in bottle gourd cultivar improvement. The objective of this study was to determine the relationships among qualitative and quantitative traits in 36 genetically diverse bottle gourd [*L. siceraria* (Mol.) Standl.] landraces using simple correlation and path analyses for effective selection. The information may be helpful to pinpoint the best selection criteria for strategic breeding of bottle gourd.

4.2 Materials and methods

4.2.1 Plant materials

Thirty six bottle gourd landraces collected from various geographic locations in the Limpopo Province, South Africa were used for the study (Table 4.1). Entries were collected from the following localities: Kgohloane (23°47'39.76" S; 29°22'13.45" E), Ga-Kgoroshi (23°40'57.89" S; 29°15'03.47" E), Phokwane (24°52'36.15" S; 29°44'36.15" E), Tshikonelo (22°52'49.82" S; 30°44'16.33" E), Ga-Rapitsi (23°35'48.37" S; 29°06'25.08" E) and Ga-Phasa (23°40'57.30" S; 29°15'57.30" E). Table 4.1 summarizes information related to the collection sites of landraces and fruit characters of the landraces used in this study.

Table 4.1. List of bottle gourd landraces used in the study and description of geographic location of collection sites.

Landrace	Location	District	Landrace	Location	District
BG-06	Tshikonelo	Vhembe	BG-37	Kgohloane	Capricorn
BG-07	Tshikonelo	Vhembe	BG-41	Kgohloane	Capricorn
BG-08	Tshikonelo	Vhembe	BG-42	Kgohloane	Capricorn
BG-09	Tshikonelo	Vhembe	BG-43	Kgohloane	Capricorn
BG-10	Tshikonelo	Vhembe	BG-44	Kgohloane	Capricorn
BG-11	Tshikonelo	Vhembe	BG-47	Kgohloane	Capricorn
BG-12	Tshikonelo	Vhembe	BG-52	Kgohloane	Capricorn
BG-13	Tshikonelo	Vhembe	BG-55	Kgohloane	Capricorn
BG-04	Kgohloane	Capricorn	BG-57	Kgohloane	Capricorn
BG-18	Kgohloane	Capricorn	BG-60	Kgohloane	Capricorn
BG-19	Kgohloane	Capricorn	BG-61	Kgohloane	Capricorn
BG-23	Kgohloane	Capricorn	BG-62	Kgohloane	Capricorn
BG-27	Kgohloane	Capricorn	BG-63	Kgohloane	Capricorn
BG-28	Kgohloane	Capricorn	BG-67	Ga-Rapitsi	Capricorn
BG-30	Kgohloane	Capricorn	BG-24	Ga-Phasa	Capricorn
BG-31	Kgohloane	Capricorn	BG-25	Phokwane	Sekhukhune
BG-32	Kgohloane	Capricorn	BG-16	Ga-Kgoroshi	Capricorn
BG-36	Kgohloane	Capricorn	BG-17	Ga-Kgoroshi	Capricorn

4.2.2 Study site

A field study was conducted during the 2014/2015 growing season, under dry-land conditions at Towoomba Research Station, Bela-Bela, South Africa (28º21'E, 24º25'S; 1 184 m above sea level). The area receives mean annual rainfall of 627 mm with erratic distribution. Daily average maximum and minimum temperatures range between 29.7°C and 16.5°C during the growing season and the soils are mainly Hutton and Arcadia form.

4.2.3 Experimental design and field establishment

The 36 bottle gourd landraces were evaluated using a 6 x 6 α-lattice design with two replications. Twenty seeds of each landrace were planted at an intra-and inter-row spacing of 2 m per replicate. Fertilizers were applied at the following rates: 100 kg N/ha of LAN (lime ammonium nitrate) and 165 kg P/ha SSP (single superphosphate) based on the results of a soil fertility analysis (Cedara, Pietermaritzburg) to achieve the establishment of healthy and vigorous plants. Weed control was done manually using hand-hoes and the chemical pesticide Malasol was used to control aphid infestation. A supplementary irrigation of about 27 mm was applied to the plots three times a week until maturity.

4.2.4 Data collection

Both qualitative and quantitative data were collected during the study. Descriptors of other related cucurbits were used as a reference (Maggs-Kölling *et al.*, 2000; Marr *et al.*, 2005; Morimoto *et al.*, 2005; Yetişir *et al.*, 2008; Koffi *et al.*, 2009; Aruah *et al.*, 2010). The qualitative data collected with their descriptors are summarized in Table 4.2. These traits are the most important farmers-preferred attributes of bottle gourd genotypes for various purposes. For example, fruits with straight or slightly curved necks, smooth fruit texture and cavate shaped are mostly grown and used to make containers for drinking water and traditional beer. Large-oval fruits with short and straight necks are used to make containers for storing water and food. Warted and corrugated fruits are mostly grown for food (Mapitsi Kobe and Raisibe Mahlong, personal communication). Quantitative data measured included: plant height as main-stem length (cm) measured from soil

surface to the tip of the plant; number of male flowers per plant, number of female flowers per plant, proportion of male to female flowers representing sex ratio per plant, number of branches per plant, number of fruits per plant; number of aborted fruits per plant, fruit weight (kg) of immature edible fruits, number of seeds per fruit and hundred seed weight per fruit (g). Individual measurements were collected from 10 randomly selected and tagged plants.

Table 4.2. List of qualitative traits with descriptions used in the study.

Qualitative traits	Description	Abbreviation
Primary fruit colour	1 = light green, 2 = medium green, 3 = dark green	PFC
Secondary fruit colour	1 = light green, 2 = medium green, 3 = dark green	SFC
Presence of fruit neck	0 = no, 1 = yes	PFN
Fruit texture	1 = smooth, 2 = verrucose, 3 = corrugated, 4 = verrucose + corrugated, 5 = smooth + verrucose 6 = smooth + corrugated, 7 = smooth + verrucose + corrugated	FT
Degree of warts	0 = none, 1 = few, 2 = medium, 3 = many	DW
Fruit shape	1 = oblate, 2 = circular, 3 = pyriform, 4 = elongated pyriform, 5 = cavate, 6 = cylindrical	FS
Degree of corrugation	1 = slightly, 2 = moderate, 3 = high	DW
Degree of neck bending	1 = straight, 2 = slightly curved, 3 = curved	DNB
Stem end fruit shape	1 = flat, 2 = rounded, 3 = pointed	SEFS
Fruit neck length	1 = short $\leq$ 5 cm, 2 = medium 6 -12 cm, 3 = long, 13 – 20 cm, 4 = very long > 20 cm	FNL

4.3 Data analysis

Qualitative data was analyzed using the Kruskal-Wallis non-parametric test procedure using SPSS 16.0 (SPSS 2007). Quantitative data was subjected to analysis of variance (ANOVA) using the SAS statistical program (SAS Institute 2004) and Agrobase (2005). Means were separated using the Least Significant Difference (LSD) procedure at 5% level of significance.

4.3.1 Correlation analysis

The Spearman's rank non-parametric correlation coefficients were calculated to describe the degree and pattern of associations of observed qualitative and quantitative traits of landraces. Genotypic (rg) correlations for both qualitative and quantitative data were calculated using the formula rg = $\sqrt{\delta^2_{gx} \text{ x} \delta^2_{gy}}$,respectively (Falconer and Mackey, 1996). Where δ_{gxy} is genotypic covariance and δ^2_{gx} and δ^2_{gy} are genotypic variances of trait x and y, respectively. Significance tests of the correlation coefficients were determined using the Student's t test (Snedecor and Cochran, 1989): t = r/$\sqrt{1 - r^2/n - 2}$, where r is the correlation coefficient and n is the number of observations.

4.3.2 Path coefficient analysis

Path analysis was conducted using a genotypic correlation matrix set up as A= B×C both for seed yield and fruit yield. In this matrix vector "A" represents the genotypic correlation coefficients of seed (a) or fruit yield vs. qualitative and quantitative traits. In the same vector "B" is the value of genotypic correlation for all possible combinations among the traits and vector "C", the path coefficients. The inverse of matrix B was calculated using the Matrix Inverse function (MINVERSE) of Microsoft Excel 2010. The path coefficients were calculated as the product of vector A and each row of B^{-1} using the matrix multiplication (MMULT) function of the same software. Direct and indirect path coefficients were calculated for both qualitative and quantitative traits as proposed by Wright (1921, 1934) and later described by Dewey & Lu (1959), Li (1975)

and Williams *et al.* (1990) using genotypic correlation coefficients. For path analysis of quantitative traits, number of fruits per plant (NFPP) and number of seed yield per fruit (NSPF) were considered as response variables whereas plant height (PHT), number of male flowers (NMF), number of female flowers (NFF), sex ratio (SR), number of branches (NB), number of aborted fruits (NAF), fruit weight (FW) and hundred seed weight (HSW) were considered as causal variables. For path analysis of qualitative traits, number of fruits per plant (NFPP) and number of seed per fruit (NSPF) were considered as response variables. Furthermore, primary fruit colour (PFC), secondary fruit colour (SFC), presence of fruit neck (PFN), degree of warts (DW), fruit shape (FS), degree of corrugation (DC), degree of neck bending (DNB), stem-end fruit shape (SEFS) and fruit neck length (FNL) were regarded as causal variables.

4.4 Results

4.4.1 Characterization of bottle gourd landraces using qualitative traits

Kruskal-Wallis non-parametric test revealed that the landraces had significant differences ($P < 0.05$) with respect to all of the qualitative traits assessed (Table 4.3). Mean values of landraces for common qualitative traits in bottle gourd are presented in Table 4.3. The most farmers preferred qualitative traits of bottle gourd include fruit shape, smooth, warted and corrugated skin texture. The following landraces show these traits: BG-09, BG-10 and BG-11, useful to make containers for drinking water and traditional beer (Mashilo *et al.*, 2015). The bottle gourd landraces with warted and corrugated fruit texture recommended for direct production included BG-61, BG-28, BG-18, BG-52, BG-37, BG-16, BG-62, BG-47, BG-06, BG-44, BG-55, BG-24, BG-60, BG-41, BG-57, BG-04 and BG-67 (Mashilo *et al.*, 2015).

Table 4.3. Mean response of 36 bottle gourd landraces evaluated for qualitative traits.

Landrace	PFC	SFC	PFN	FT	DW	FS	DC	NBD	SEFS	FNL
BG-06	1	0	1	6	0	4	1	1	1	2
BG-07	3	0	1	1	0	3	0	1	1	1
BG-08	1	0	0	1	0	4	0	0	1	0
BG-09	1	0	1	1	0	5	0	3	1	3
BG-10	1	0	1	1	0	5	0	2	1	3
BG-11	1	0	1	1	0	5	0	2	1	2
BG-12	2	0	1	1	0	4	0	2	1	1
BG-13	2	0	1	1	0	4	2	1	1	2
BG-04	3	1	1	3	0	5	2	2	2	2
BG-18	3	1	1	3	0	5	1	3	3	3
BG-19	3	1	0	6	1	1	0	0	1	0
BG-23	1	0	0	1	0	4	3	0	1	0
BG-27	3	0	1	4	1	5	2	2	2	3
BG-28	3	1	1	3	0	5	2	3	3	3
BG-30	3	1	1	3	0	5	1	2	1	2
BG-31	3	0	1	5	1	5	1	2	2	3
BG-32	3	1	1	3	0	5	1	2	1	2
BG-36	3	1	1	5	0	5	1	2	2	2
BG-37	3	1	1	3	0	5	1	2	3	3
BG-41	1	2	1	3	0	5	1	2	2	2
BG-42	3	1	1	3	0	5	2	2	1	2
BG-43	3	0	1	3	0	5	2	3	3	4
BG-44	3	1	1	3	0	5	1	3	3	2
BG-47	3	1	1	3	0	5	3	3	2	2
BG-52	3	1	1	3	0	5	2	3	3	3
BG-55	3	1	1	3	0	5	2	2	2	2
BG-57	2	1	1	3	0	5	2	2	2	2
BG-60	3	1	1	3	0	5	2	2	2	2
BG-61	2	1	1	3	0	5	2	2	2	2
BG-62	3	1	1	3	0	5	2	2	2	3
BG-63	3	0	1	3	0	5	2	2	3	3
BG-67	3	1	1	2	3	4	0	2	2	2
BG-24	3	1	1	3	0	5	0	1	2	2
BG-25	1	0	1	2	2	3	2	1	1	1
BG-16	3	1	1	3	0	5	0	2	3	2
BG-17	1	2	1	2	2	7	2	1	2	1
KWSL	0.002	0.001	0.001	0.001	0.005	0.001	0.005	0.001	0.007	0.002

PFC= Primary fruit colour; SFC= Secondary fruit colour; PFN = Presence of fruit neck; FT= Fruit texture; DW= Degree of warts; FS = Fruit shape; DC= Degree of corrugation; DNB = Degree of neck bending; SEFS = Stem-end fruit shape; FNL = Fruit neck length. KWSL = Kruskal-Wallis significance level.

4.4.2 Characterization of bottle gourd landraces using quantitative phenotypic traits

The analysis of variance revealed that landraces were significantly different ($P < 0.01$) with respect to quantitative phenotypic traits (data not shown). This indicates the presence of sufficient phenotypic variation among the evaluated landraces to undertake selection. Mean performance and rank amongst the bottle gourd landraces are summarized in Table 4.4. Heritability values for measured traits were relatively high (> 60%) in the landrace population. Promising landraces were identified considering three important yield determining traits in bottle gourd namely: number of fruits per plant (NFPP), fruit weight, and number of seed yield per fruit (NSPF). Top ten ranking landraces with respect to number of fruits per plant were: BG-24, BG-07, BG-13, BG-67, BG-08, BG-12, BG-37, BG-19, BG-09 and BG-06 in that order. Number of fruits per plant among this landraces ranged from 9 to 16. Landraces which produced the highest fruit weight included: BG-13, BG-67, BG-36, BG-11, BG-17, BG-09, BG-07, BG-06, BG-36 and BG-67. Fruit weight ranged from 7 to 13 kg. Top ten ranking landraces with respect to number of seeds per fruit were: BG-67, BG-13, BG-17, BG-25, BG-12, BG-19, BG-08, BG-42, BG-07 and BG-09 in that order. Number of seeds per fruit among these landraces ranged from 278 to 381.

Table 4.4. Mean response and rank (R) of 36 bottle gourd landraces evaluated for quantitative traits.

Landrace	PHT	*R*	NMF	*R*	NFF	*R*	SR	*R*	NB	*R*
BG-06	596	8	199.11	13	9.3	10	21.11	29	18.37	20
BG-07	836	1	293.31	7	14.4	2	20.25	31	30.27	3
BG-08	658	6	181.89	19	12.1	5	15.2	35	24.69	8
BG-09	554	13	315.33	5	9.5	9	33.62	17	24.91	7
BG-10	489	22	192.26	17	7.9	13	24.3	27	18.62	19
BG-11	456	25	133.96	30	4.9	20	27.39	24	11.48	32
BG-12	669	4	288.08	8	12.1	6	24.28	28	27.05	5
BG-13	660	5	380.3	1	13	3	29.07	22	26.81	6
BG-04	527	15	192.36	16	3.2	29	62.11	3	16.72	23
BG-18	418	31	160.88	23	2.1	35	82.46	2	14.65	27
BG-19	520	17	170	20	9.8	8	18.39	33	18.69	18
BG-23	325	36	105.51	35	3.1	31	35.65	16	9.42	36
BG-27	503	20	183.81	18	4.6	21	39.9	14	19.47	14
BG-28	441	29	150.66	25	3.8	23	39.77	15	15.88	24
BG-30	398	34	135.14	29	6.5	15	20.85	30	14.22	30
BG-31	488	23	152.39	24	3.3	27	46.79	10	15.8	25
BG-32	469	24	113.89	31	8.1	12	13.78	36	14.37	29
BG-36	421	30	112.95	32	2.7	34	42.16	12	11.7	31
BG-37	677	3	362.03	3	11.7	7	31.15	19	30.72	2
BG-41	495	21	150.25	26	3.5	25	43.07	11	18.16	21
BG-42	563	12	144.99	28	5.7	17	25.66	25	18.99	17
BG-43	447	27	147.51	27	5	19	30.53	21	18.02	22
BG-44	399	33	85.06	36	2.9	32	28.53	23	9.73	35
BG-47	517	18	167.23	22	3.2	28	52.07	8	20.9	12
BG-52	443	28	193.6	15	3.7	24	51.74	9	19.41	15
BG-55	452	26	206.76	12	8.2	11	25.12	26	19.02	16
BG-57	388	35	109.21	33	5.9	16	18.38	34	11.24	34
BG-60	503	19	226.5	10	4.08	22	60.93	4	21.09	11
BG-61	416	32	168.56	21	3.2	30	52.57	7	11.32	33
BG-62	581	10	222.09	11	6.7	14	33.49	18	20.12	13
BG-63	563	11	108.98	34	2	36	52.97	6	14.49	28
BG-67	711	2	374.89	2	12.2	4	30.79	20	34.1	1
BG-24	553	14	297.47	6	15.9	1	18.48	32	28.27	4
BG-25	595	9	316.16	4	2.8	33	114.88	1	23.38	9
BG-16	642	7	228.39	9	5.6	18	41.58	13	23.17	10
BG-17	525	16	197.46	14	3.4	26	58.38	5	15.44	26
Mean	525		199.14		6.56		37.98		19.19	
P-value	<.001		<.001		<.001		<.001		<.001	
R^2	0.97		0.99		0.98		0.94		0.98	
CV (%)	6.56		7.25		11.92		19.64		8.31	
$LSD_{(0.05)}$	0.61		25.48		1.32		12.6		2.87	
SED	0.36		14.91		0.78		7.46		1.68	
Heritability	0.89		0.96		0.96		0.88		0.93	

Table 4.4. (Continued)

Landrace	NFPP	*R*	NMF	*R*	NFF	*R*	SR	*R*	NB	*R*
BG-06	9.3	8	199.11	13	9.3	10	21.11	29	18.37	20
BG-07	14.4	1	293.31	7	14.4	2	20.25	31	30.27	3
BG-08	12.1	6	181.89	19	12.1	5	15.2	35	24.69	8
BG-09	9.5	13	315.33	5	9.5	9	33.62	17	24.91	7
BG-10	7.9	22	192.26	17	7.9	13	24.3	27	18.62	19
BG-11	4.9	25	133.96	30	4.9	20	27.39	24	11.48	32
BG-12	12.1	4	288.08	8	12.1	6	24.28	28	27.05	5
BG-13	13	5	380.3	1	13	3	29.07	22	26.81	6
BG-04	3.2	15	192.36	16	3.2	29	62.11	3	16.72	23
BG-18	2.1	31	160.88	23	2.1	35	82.46	2	14.65	27
BG-19	9.8	17	170	20	9.8	8	18.39	33	18.69	18
BG-23	3.1	36	105.51	35	3.1	31	35.65	16	9.42	36
BG-27	4.6	20	183.81	18	4.6	21	39.9	14	19.47	14
BG-28	3.8	29	150.66	25	3.8	23	39.77	15	15.88	24
BG-30	6.5	34	135.14	29	6.5	15	20.85	30	14.22	30
BG-31	3.3	23	152.39	24	3.3	27	46.79	10	15.8	25
BG-32	8.1	24	113.89	31	8.1	12	13.78	36	14.37	29
BG-36	2.7	30	112.95	32	2.7	34	42.16	12	11.7	31
BG-37	11.7	3	362.03	3	11.7	7	31.15	19	30.72	2
BG-41	3.5	21	150.25	26	3.5	25	43.07	11	18.16	21
BG-42	5.7	12	144.99	28	5.7	17	25.66	25	18.99	17
BG-43	5	27	147.51	27	5	19	30.53	21	18.02	22
BG-44	2.9	33	85.06	36	2.9	32	28.53	23	9.73	35
BG-47	3.2	18	167.23	22	3.2	28	52.07	8	20.9	12
BG-52	3.8	28	193.6	15	3.7	24	51.74	9	19.41	15
BG-55	8.2	26	206.76	12	8.2	11	25.12	26	19.02	16
BG-57	5.9	35	109.21	33	5.9	16	18.38	34	11.24	34
BG-60	4.08	19	226.5	10	4.08	22	60.93	4	21.09	11
BG-61	3.2	32	168.56	21	3.2	30	52.57	7	11.32	33
BG-62	6.7	10	222.09	11	6.7	14	33.49	18	20.12	13
BG-63	2	11	108.98	34	2	36	52.97	6	14.49	28
BG-67	12.2	2	374.89	2	12.2	4	30.79	20	34.1	1
BG-24	15.9	14	297.47	6	15.9	1	18.48	32	28.27	4
BG-25	2.8	9	316.16	4	2.8	33	114.88	1	23.38	9
BG-16	5.6	7	228.39	9	5.6	18	41.58	13	23.17	10
BG-17	3.4	16	197.46	14	3.4	26	58.38	5	15.44	26

Table 4.4 (Continued)

Mean	6.56	199.14	6.56	37.98	19.19
P-value	<.001	<.001	<.001	<.001	<.001
R^2	0.98	0.99	0.98	0.94	0.98
CV (%)	11.89	7.25	11.92	19.64	8.31
$LSD_{(0.05)}$	1.32	25.48	1.32	12.6	2.87
SED	0.78	14.91	0.78	7.46	1.68
Heritability	0.96	0.96	0.96	0.88	0.93

PHT = plant height in centimetre; NMF = Number of male flowers per plant; NFF = Number of female flowers per plant; SR = Sex ratio per plant; NB = Number of branches per plant; NFPP = Number of fruits per plant; NAF = Number of aborted fruits per plant; FW = Fruit weight; NSPF = Number of seeds per fruit; HSW = Hundred seed weight; R^2= Coefficient of determination; CV = Coefficient of variation; SED = Standard error of difference.

4.4.3 Correlation analysis

Spearman's rank correlation coefficients showing pair-wise associations between the assessed qualitative or quantitative phenotypic traits of the landraces are presented in Tables 4.5 and 4.6. Among qualitative traits evaluated, significant and positive correlations were observed between primary fruit colour and secondary fruit colour ($r = 0.34$, $P < 0.05$), fruit texture ($r = 0.55$, $P < 0.001$), degree of neck bending ($r = 0.39$; $P < 0.05$), stem-end fruit shape ($r = 0.53$; $P < 0.001$) and fruit neck length ($r = 0.35$, $P < 0.05$) (Table 6). Secondary fruit colour had significant and positive correlation with fruit texture ($r = 0.36$; $P < 0.05$), fruit shape ($r = 0.49$; $P < 0.01$) and stem-end fruit shape ($r = 0.43$; $P < 0.01$). Highly significant and positive correlations ($P < 0.01$) were observed between presence or absence of fruit neck with fruit shape ($r = 0.51$), degree of neck bending ($r = 0.53$), stem-end fruit shape ($r = 0.34$) and fruit neck length ($r = 0.52$). Fruit texture significantly and positively correlated ($P < 0.05$) with stem-end fruit shape ($r = 0.41$). Highly significant and positive correlations ($P < 0.01$) were observed between fruit shape with degree of neck bending ($r = 0.56$), stem-end fruit shape ($r = 0.54$) and fruit neck length ($r = 0.54$). Further, degree of neck bending significantly and positively correlated ($P < 0.001$) with stem-end fruit shape ($r = 0.58$) and fruit neck length ($r = 0.7$). There were non-significant correlations between degree of warts and corrugation with other qualitative traits. Non-significant correlations were observed between primary fruit colour with presence or absence of fruit neck, degree of warts, fruit shape and degree of corrugation. Also, correlations were not detected between presence or absence of fruit neck with fruit texture, degree of warts and degree of corrugation. Non-significant,

negative correlations were observed between fruit texture with degree of warts, fruit shape, degree of corrugation, degree of neck bending and fruit neck length.

Among quantitative traits evaluated, highly significant ($P < 0.001$) and positive correlations were observed between number of male flowers with number of female flowers ($r = 0.57$), plant height ($r = 0.74$), number of branches ($r = 0.87$), number of aborted fruits ($r = 0.42$) and number of fruits per plant ($r = 0.55$) (Table 4.6). Significantly negative correlation was observed between sex ratio (proportion of male to female flowers) with number of female flowers ($r = – 0.76$). Significant and positive correlations were observed between number of female flowers with number of branches ($P < 0.001$; $r = 0.65$), number of fruits per plant ($r = 0.94$) and number of aborted fruits ($r = 0.82$). Number of fruits per plant significantly and positively correlated with plant height ($r = 0.53$), number of male flowers ($r = 0.55$), number of female flowers ($r = 0.94$) and number of branches ($r = 0.61$). Also, sex ratio was significantly and negatively correlated with number of fruits per plant ($r = - 0.70$) and number of aborted fruits ($r = – 0.69$). Number of seeds per fruit and hundred seed weight significantly and positively correlated with plant height with $r = 0.64$ and $r = 0.45$, number of male flowers with $r = 0.62$ and $r = 0.51$, number of female flowers with $r = 0.47$ and $r = 0.36$, number of branches with $r = 0.59$ and $r = 0.42$ and fruit weight with $r = 0.79$ and $r = 0.76$, in that order.

Table 4.5. Spearman's rank correlation coefficients showing pair-wise association of qualitative traits assessed among bottle gourd landraces.

Traits	PFC	SFC	PFN	FT	DW	FS	DC	DNB	SEFS	FNL
PFC	1.00	0.34*	0.23ns	0.55**	-0.03ns	0.23ns	0.11ns	0.39*	0.53**	0.35*
SFC		1.00	0.18ns	0.36*	-0.01ns	0.49**	0.15ns	0.22ns	0.43**	-0.05ns
PFN			1.00	0.12ns	-0.11ns	0.51**	0.09ns	0.53**	0.34*	0.52**
FT				1.00	0.11ns	0.26ns	0.19ns	0.18ns	0.41*	0.30ns
DW					1.00	-0.19ns	-0.03ns	-0.30ns	-0.07ns	-0.18ns
FS						1.00	0.26ns	0.56**	0.54**	0.54**
DC							1.00	0.16ns	0.28ns	0.14ns
DNB								1.00	0.58**	0.70**
SEFS									1.00	0.56**
FNL										1.00

PFC= Primary fruit colour; SFC= Secondary fruit colour; PFN = Presence of fruit neck; FT= Fruit texture; DW= Degree of warts; FS = Fruit shape; DC= Degree of corrugation; DNB = degree of neck bending; SEFS = Stem-end fruit shape; FNL = Fruit neck length. ns= non-significant, *****significant difference at the 0.05 probability level, ******significant difference at the 0.001 probability level.

Table 4.6. Spearman's rank correlation coefficients showing pair-wise association of quantitative traits measured among bottle gourd landraces.

Traits	PHT	NMF	NFF	SR	NB	NFPP	NAF	FW	NSPF	HSW
PHT	1.00	0.74**	0.55**	-0.10ns	0.83**	0.53**	0.49**	0.58**	0.64**	0.45**
NMF		1.00	0.57**	0.05ns	0.87**	0.55**	0.42*	0.68**	0.62**	0.51**
NFF			1.00	-0.76**	0.65**	0.94**	0.82**	0.49**	0.47**	0.36*
SR				1.00	-0.11	-0.70**	-0.69**	-0.14ns	-0.10ns	-0.16ns
NB					1.00	0.61**	0.52**	0.52**	0.59**	0.42*
NFP						1.00	0.62**	0.48**	0.39*	0.41*
NAF							1.00	0.39*	0.40*	0.31
FW								1.00	0.79**	0.76**
NSPF									1.00	0.45**
HSW										1.00

PHT = plant height in centimetre; NMF = Number of male flowers per plant; NFF = Number of female flowers per plant; SR = Sex ratio per plant; NB = Number of branches per plant; NFPP = Number of fruits per plant; NAF = Number of aborted fruits per plant; FW = Fruit weight; NSPF = Number of seeds per fruit; HSW = Hundred seed weight; ns= non-significant. *****significant difference at the 0.05 probability level, ******significant difference at the 0.001 probability level.

The correlation coefficients between qualitative phenotypic traits with the two important quantitative traits (number of fruits per plant and number of seeds per fruit) are presented in Table 4.7. Non-significant correlations were recorded between number of fruits per plant and number of seeds per fruit with primary fruit colour, secondary fruit colour, presence or absence of fruit neck. Non-significant correlations were detected between number of fruits per plant with fruit texture, degree of warts and fruit shape. Significant ($P < 0.05$) negative correlations were observed between fruit texture ($r = – 0.31$), degree of warts ($r = – 0.53$) and fruit shape ($r = – 0.45$), degree of neck bending ($r = – 0.42$) and stem-end fruit shape ($r = – 0.4$) with number of seeds per fruit, in that order. However, degree of corrugation, degree of neck bending, stem-end fruit shape and fruit neck length were negatively and significantly correlated with number of fruits per plant with $r = – 0.42$, $– 0.56$, $– 0.34$ and $– 0.39$, respectively. Fruit neck length had non-significant correlation with number of seeds per fruit.

Table 4.7. Spearman's rank correlation coefficients showing pair-wise association between qualitative traits with number of fruits per plant and number of seeds per fruit in bottle gourd landraces.

Traits	PFC	SFC	PFN	FT	DW	FS	DC	DNB	SEFS	FNL
NFPP	-0.09^{ns}	-0.29^{ns}	-0.03^{ns}	-1.2^{ns}	-0.26^{ns}	-0.05^{ns}	-0.42*	-0.56**	-0.34*	-0.39*
NSPF	-0.16^{ns}	-0.28^{ns}	-0.06^{ns}	-0.34*	-0.53**	-0.45*	-0.26^{ns}	-0.42*	-0.40*	-0.24^{ns}

PFC= Primary fruit colour; SFC= Secondary fruit colour; PFN = Presence of fruit neck; FT= Fruit texture; DW= Degree of warts; FS = Fruit shape; DC= Degree of corrugation; DNB= Degree of neck bending; SEFS = Stem-end fruit shape; FNL = Fruit neck length; NFPP = Number of fruits per plant; NSPF = Number of seeds per fruit. ns= non-significant, *****significant difference at the 0.05 probability level, ******significant difference at the 0.001 probability level.

4.4.4 Path coefficient analysis of qualitative traits in bottle gourd

Path coefficient values of qualitative traits with number of seeds per fruit and number of fruits per plant as the response variate are presented in Table 4.8. High direct path coefficients were estimated between degree of warts (0.47) and fruit neck length (0.35) with number of seeds per fruit, respectively. Further, positive direct path coefficient values and non-significant negative genotypic correlation were exhibited between fruit neck length (0.75), followed by primary fruit colour (0.28) and secondary fruit colour (0.22) with number of fruits per plant, in that order.

Table 4.8. Estimates of direct (boldfaced main diagonals) and alternate/indirect path coefficient values of qualitative traits with number of seeds per fruit (top) and number of fruits per plant (bottom) in bottle gourd landraces.

Traits	PFC	SFC	PFN	FT	DW	FS	DC	DNB	SEFS	FNL	NSPF
PFC	**0.19**	0.03	0.02	-0.17	-0.04	-0.05	0.00	-0.10	-0.13	0.10	-0.16^{ns}
SFC	0.06	**0.09**	0.02	-0.13	0.02	-0.15	-0.01	-0.05	-0.11	-0.03	-0.28^{ns}
PFN	0.02	0.01	**0.19**	0.01	-0.10	-0.14	0.00	-0.14	-0.07	0.16	-0.06^{ns}
FT	0.10	0.04	-0.01	**-0.33**	0.00	-0.06	-0.01	-0.04	-0.10	0.08	-0.34*
DW	-0.02	0.00	-0.04	0.00	**0.47**	0.08	0.00	0.11	0.03	-0.11	0.53**
FS	0.03	0.04	0.09	-0.07	-0.12	**-0.30**	-0.01	-0.16	-0.13	0.18	-0.45**
DC	0.01	0.01	-0.01	-0.04	-0.05	-0.06	**-0.05**	-0.03	-0.06	0.02	-0.26^{ns}
DNB	0.06	0.01	0.09	-0.04	-0.17	-0.16	0.00	**-0.31**	-0.14	0.24	-0.42**
SEFS	0.09	0.04	0.05	-0.13	-0.05	-0.15	-0.01	-0.17	**-0.26**	0.19	-0.40**
FNL	0.05	-0.01	0.09	-0.07	0.01	-0.15	0.00	-0.21	-0.14	**0.35**	-0.24^{ns}

Traits	PFC	SFC	PFN	FT	DW	FS	DC	NBD	SEFS	FNL	NFPP
PFC	**0.28**	0.08	0.02	-0.16	0.01	-0.07	-0.04	-0.30	-0.10	0.26	-0.10^{ns}
SFC	0.10	**0.22**	0.02	-0.11	0.00	-0.16	-0.06	-0.17	-0.08	-0.04	-0.29^{ns}
PFN	0.07	0.04	**0.10**	-0.03	0.03	-0.16	-0.04	-0.41	-0.06	0.39	-0.12^{ns}
FT	0.15	0.08	0.01	**-0.30**	-0.03	-0.08	-0.08	-0.14	-0.08	0.22	-0.26^{ns}
DW	-0.01	0.00	-0.01	-0.03	**-0.23**	0.06	0.01	0.23	0.01	-0.13	-0.06^{ns}
FS	0.06	0.11	0.05	-0.08	0.04	**-0.32**	-0.11	-0.43	-0.10	0.41	-0.42*
DC	0.03	0.03	0.01	-0.06	0.01	-0.08	**-0.40**	-0.12	-0.05	0.11	-0.56**
NBD	0.11	0.05	0.05	-0.05	0.07	-0.18	-0.06	**-0.77**	-0.11	0.53	-0.34*
SEFS	0.15	0.10	0.03	-0.12	0.02	-0.17	-0.11	-0.45	**-0.18**	0.42	-0.39*
FNL	0.10	-0.01	0.05	-0.09	0.04	-0.17	-0.06	-0.54	-0.10	**0.75**	-0.09^{ns}

PFC= Primary fruit colour; SFC= Secondary fruit colour; PFN = Presence of fruit neck; FT= Fruit texture; DW= Degree of warts; FS = Fruit shape; DC= Degree of corrugation; NBD = Neck bending degree; SEFS = Stem-end fruit shape; FNL = Fruit neck length; NFPP = Number of fruits per plant. ns= non-significant, *****significant difference at the 0.05 probability level, ******significant difference at the 0.001 probability level.

4.4.5 Path coefficient analysis of quantitative traits in bottle gourd

Results on the path coefficients with number of fruits per plant as the response variable are summarized in Table 4.9. High direct path coefficient value (0.92) and highly significant genotypic correlation (r_g = 0.94, $P < 0.001$) were exhibited between the number of female flowers and number of fruits per plant. Selection for increased number of female flowers would also result in simultaneous increase in sex ratio in bottle gourd. Results on the path coefficients with number of seed per fruit as the response variable and plant height, number of male flowers, number of female flowers, sex ratio, number of branches, number of aborted fruits, fruit weight and hundred seed weight as independent variables are summarized in Table 4.10. Values of direct effects were < 1, indicating that inflation due to multicolinearity was low. Relatively high direct path coefficient

value (0.96) and highly significant genotypic correlation (r_g = 0.79, P < 0.001) were exhibited between fruit weight and number of seeds per fruit. Path analysis indicated that selection for increased fruit weight would bring about simultaneous increase in number of female flowers and number of branches (Table 4.10).

Table 4.9. Estimates of direct (boldfaced main diagonals) and alternate/indirect path coefficient values of number of fruits per plant with quantitative traits amongst bottle gourd landraces.

Traits	PHT	NMF	NFF	SR	NB	NAF	FW	NSPF	HSW	NFPP
PHT	**0.13**	0.16	0.51	0.04	0.00	-0.25	0.01	-0.05	0.03	0.53**
NMF	0.10	**0.22**	0.52	-0.02	0.00	-0.22	0.01	-0.05	0.04	0.55**
NFF	0.07	0.13	**0.92**	0.28	0.00	-0.43	0.01	-0.04	0.03	0.94**
SR	-0.01	0.01	-0.70	**-0.37**	0.00	0.37	0.00	0.01	-0.01	-0.70**
NB	0.11	0.19	0.59	0.04	**0.00**	-0.27	0.01	-0.05	0.03	0.61**
NAF	0.06	0.09	0.75	0.26	0.00	**-0.52**	0.00	-0.03	0.02	0.62**
FW	0.08	0.15	0.45	0.05	0.00	-0.21	**0.01**	-0.06	0.05	0.48**
NSPF	0.08	0.14	0.43	0.04	0.00	-0.21	0.01	**-0.08**	0.03	0.39*
HSW	0.06	0.11	0.33	0.06	0.00	-0.16	0.01	-0.03	**0.07**	0.41*

PHT (plant height) in centimeter; NMF (Number of male flowers per plant); NFF (Number of female flowers per plant); SR (Sex ratio per plant); NB (Number of branches per plant); NFPP (Number of fruits per plant); NAF (Number of aborted fruits per plant); FW (Fruit weight); NSPF (Number of seeds per fruit); HSW (hundred seed weight); NFPP= Number of fruits per plant. ns= non-significant, *****significant difference at the 0.05 probability level, ******significant difference at the 0.001 probability level.

Table 4.10. Estimates of direct (boldfaced main diagonals) and alternate/indirect path coefficient values of number of seed per fruit with quantitative traits amongst bottle gourd landraces.

Traits	PHT	NMF	NFF	SR	NB	NFPP	NAF	FW	HSW	NSPF
PHT	**0.18**	-0.20	0.36	0.01	0.33	-0.33	-0.13	0.56	-0.13	0.64**
NMF	0.13	**-0.28**	0.37	0.00	0.35	-0.34	-0.11	0.65	-0.15	0.62**
NFF	0.10	-0.16	**0.65**	0.04	0.26	-0.57	-0.21	0.47	-0.11	0.47*
SR	-0.02	-0.01	-0.49	**-0.06**	-0.04	0.43	0.18	-0.14	0.05	-0.10^{ns}
NB	0.15	-0.24	0.42	0.01	**0.40**	-0.38	-0.14	0.50	-0.12	0.60**
NFPP	0.09	-0.15	0.61	0.04	0.25	**-0.61**	-0.16	0.46	-0.12	0.39*
NAF	0.09	-0.12	0.53	0.04	0.21	-0.38	**-0.26**	0.38	-0.09	0.40*
FW	0.10	-0.19	0.32	0.01	0.21	-0.29	-0.10	**0.96**	-0.23	0.79**
HSW	0.08	-0.14	0.24	0.01	0.17	-0.25	-0.08	0.73	**-0.30**	0.45*

PHT = plant height in centimetre; NMF = Number of male flowers per plant; NFF = Number of female flowers per plant; SR = Sex ratio per plant; NB = Number of branches per plant; NFPP = Number of fruits per plant; NAF = Number of aborted fruits per plant; FW = Fruit weight; NSPF = Number of seeds per fruit; HSW = Hundred seed weight; ns = non-significant. *****significant difference at the 0.05 probability level, ******significant difference at the 0.001 probability level.

4.5 Discussion

Understanding the nature of associations among economically important traits is essential for direct or indirect selection and consequently to improve the efficiency of selection gains in plant breeding programs. In this study, correlation and path coefficient analyses were used to determine associations between qualitative and quantitative traits and consequently to determine the best selection criteria. Simple correlations among qualitative traits showed positive associations between primary fruit colour and secondary fruit colour, fruit texture, degree of neck bending, stem-end fruit shape and fruit neck length (Table 4.5). Secondary fruit colour positively correlated with fruit texture, fruit shape and stem-end fruit shape. Positive correlations were also noted between presence or absence of fruit neck with fruit shape, degree of neck bending and fruit neck length. Further, fruit texture, degree of warts, fruit shape, and degree of neck bending, stem-end fruit shape and fruit neck length were negatively correlated with number of fruits per plant and number of seeds per fruit (Table 4.7) signifying that these traits may negatively limit yield gains. Results of the present study are supported by Bahraminejad *et al*. (2011) who reported that different qualitative characteristics such as seeds with light colour and without trichome and leaves without trichome produced high seed yield. These authors suggested that linkages exist between genes controlling seed and leaf colour and seed yield. Correlations between qualitative traits have

not been reported in bottle gourd. Therefore, the present estimates may guide targeted breeding of the crop incorporating these valuable traits (Augustina *et al.*, 2013). Further, the lack of correlation between degree of warts and corrugation with other qualitative traits may be of interest for further investigation.

Simple correlation analysis among quantitative traits revealed highly significant ($P < 0.001$) and positive correlations between number of male flowers with number of female flowers, plant height ($r = 0.57$), number of branches ($r = 0.87$), number of aborted fruits ($r = 0.42$) and number of fruits per plant ($r = 0.55$) (Table 4.6). This suggests that targeted selection to improve these traits would increase fruit yield (Parhi *et al.*, 1995). Selection for these traits may ultimately improve fruit yield. Results in this study are in agreement with the report of Narayan et al. (1996), Achigan-Dako *et al*. (2006), Koffi *et al*. (2009) and Husna *et al*. (2011). The authors indicated that several quantitative traits including plant height, number of female flowers per plant and number of branches per plant were important yield components influencing fruit yield in bottle gourd. Other studies in related cucurbits (e.g. bitter gourd and cucumber) also revealed positive correlations between several traits like number of branches and number of female flowers on number of fruits per plant (Srivastava and Srivastava, 1976; Mangal *et al.*, 1983; Cramer and Wehner, 2000). Number of seeds per fruit significantly and positively correlated plant height ($r = 0.64$) and, number of male flowers ($r = 0.62$), number of female flowers ($r = 0.47$), number of branches ($r = 0.59$) and fruit weight ($r = 0.79$), respectively (Table 4.6). Correlation studies on seed yield with related traits in bottle gourd are scanty. The current study further showed that the assessed traits had relatively high heritability estimates of > 60% (Table 4.4) indicating the presence of considerable genetic variation for selection. Characters with high heritability are largely influenced by genetic effects suggesting that their direct selection could yield positive selection gains (Singh *et al.*, 2006; Bhargava *et al.*, 2007; Husna *et al.*, 2011).

Associations among traits as determined by simple correlation coefficient analysis may limit prediction on the success of selection. Therefore, correlation coefficients between various characters were partitioned into direct and indirect effects using path coefficient analysis. Path coefficient analysis is increasingly being used in plant breeding to improve selection efficiency through pinpointing traits with significant effects on yield or yield components (Puri *et al.*, 1982;

Kang *et al.*, 1983; Toebe and Filho, 2013). In this study, positive direct effect was exhibited between degree of warts and number of seeds per fruit. High direct effects were recorded between fruit neck length followed by primary and secondary fruit colour with number of fruits per plant. However, these traits were not well-correlated with the number of fruits per plant. Several qualitative traits including fruit shape, fruit colour, fruit texture and degree of warts are genetically controlled (Kushwaha and Ram, 1996; Paris and Nelson, 2004; Tiwari and Ram, 2009; Mladenovic *et al.*, 2013). The poor correlation of fruit neck length, secondary and primary fruit colour with fruit yield suggests that direct selection for these traits may not provide yield improvement.

A relatively high positive direct effect was exhibited between number of female flowers and number of fruits per plant (Table 4.9) suggesting simultaneous selection of the two traits may improve genetic gain of fruit yield in bottle gourd breeding. Number of female flowers was reported to have maximum positive direct effect on number of fruits/plant and fruit yield in bitter gourd (Srivastava and Srivastava, 1976) and bottle gourd (Narayan *et al.*, 1996). In bottle gourd, increased attempt is given towards breeding superior hybrids with high fruit yield, fruit number and high female: male flower ratio (Rakesh and Ram, 2007; Behera *et al.*, 2015). Therefore, to increase fruit yield, more female flowers are desired which is the determinant factor for increased number of fruit yield (Dey *et al.*, 2005). Significant positive correlations between female flowers and number of fruits per plant were also reported in cucumber suggesting that female sex expression has potential for increasing yield through direct selection (Cramer and Wehner, 2000; Fan *et al.*, 2006). The current analyses showed the presence of high and significant correlation between number of female flowers and number of fruits per plant ($r = 0.94$) (Table 4.5). Therefore, efforts required in breeding cultivars with a higher fruit set and yield can be achieved through selection of cultivars with increased proportion of female flowers (Arora *et al.*, 1982). Landraces which produced the highest number of female flowers and subsequent fruit set were BG-24, BG-07, BG-13, BG-67, BG-08, BG-12, BG-37, BG-19, BG-09 and BG-06. These are useful genetic resources for bottle gourd breeding emphasizing fruit yield.

High direct positive effect was exhibited between fruit weight and number of seeds per fruit allowing indirect selection for increased number of female flowers and number of branches (Table

4.10). A high path coefficient value indicates that the change will result in a proportional (or inversely proportional) change in another correlated trait, whereas a strong correlation coefficient indicates that the change will have high effect on the second trait (Cramer and Wehner, 2000). Results in this study are in agreement with Yao *et al*. (2015) who reported that fruit weight had positive high direct effect on seed yield in bottle gourd. Bottle gourd seeds are rich in essential amino acids, protein, minerals, lipids and fatty acids (Achu *et al.*, 2005; Fokou *et al.*, 2009; Essien *et al.*, 2013). Thus the seed are potentially useful for food or livestock feed (Achu *et al.*, 2005; Ojiako and Igwe, 2007; Ogunbusola *et al.*, 2010). Landraces which produced the highest fruit weight included: BG-13, BG-67, BG-36, BG-11, BG-17, BG-09, BG-07, BG-06, BG-36 and BG-67. These are useful genetic resources for bottle gourd breeding emphasizing seed yield. Knowledge of the associations among qualitative traits is important for simultaneous selections especially for bottle gourd which shows considerable variability in fruit qualitative parameters.

4.6 Conclusions

This study has demonstrated that selection for increased fruit weight and number of female flowers may improve genetic gain on number of seeds per fruit and number of fruits per plant in bottle gourd breeding. The present study further showed that selection for qualitative traits such as degree of warts may influence number of seeds per fruit whereas selection for fruit neck length may not influence number of fruits per plant in bottle gourd. Overall the correlation and path analyses allowed selection of the following landraces such as BG-06, BG-07, BG-09, BG-11, BG-13, BG-24 and BG-67 which are useful germplasm for breeding.

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Chapter 5: **Yield-Based Selection Indices for Drought Tolerance Evaluation in Selected Bottle Gourd [*Lagenaria siceraria* (Molina) Standl.] Landraces**

Abstract

Bottle gourd is an important crop in arid and semi-arid tropics where recurrent drought is the major constraint to crop production. Identification of drought tolerant bottle gourd genotypes is fundamental to enhance productivity and for effective breeding and conservation. The objective of this study was to determine drought tolerance of a diverse set of bottle gourd landraces and to identify promising genotypes for direct production or breeding. A field study was conducted using a 12 x 2 factorial experiment involving 12 bottle gourd landraces under drought-stressed (DS) and non-stressed (NS) conditions and laid out using a randomized complete block design with three replications. Important agronomic data and indices of drought tolerance were collected and subjected to analysis of variance, correlation and principal component analyses. Significant differences were observed among bottle gourd landraces with respect to edible fruit number and fruit yield under DS and NS conditions. The mean fruit number under DS and NS conditions were 15 457 and 31 088 ha^{-1}; respectively. The mean fruit yield under DS and NS were 8.75 t ha^{-1} and 22.4 t ha^{-1}, respectively. Drought stress reduced fruit number and yield by 49% and 62%, respectively. Correlation and principal component analyses revealed the significance of yield-based indices of drought tolerance such as tolerance index, geometric mean productivity, stress tolerance, mean productivity, yield index and harmonic mean which allowed discrimination of drought tolerant bottle gourd landraces. Landraces such as BG-79, BG-31, BG-67, BG-52, BG-78 and GC were identified useful for drought tolerance breeding or rootstock development programs.

Keywords: Bottle gourd, breeding, drought tolerance, landrace, drought stress

5.1 Introduction

Bottle gourd [*Lagenaria siceraria* (Molina) Standl.] is an important underutilized crop in the arid and semi-arid tropics where drought stress limits crop production and yield (Fischer and Turner, 1978; Boyer, 1982; Cattivelli *et al.*, 2008). The crop is adapted to dry areas with limited rainfall and has been reported to possess some degree of drought stress tolerance (Park *et al.*, 2014; Sithole and Modi, 2015). This is probably attributed to many years of directed selection by famers living in arid and semi-arid areas. Studies cited in the literature suggested the existence of genotypic variability in yield performance of bottle gourd under field conditions with tolerance to high temperatures and water stress (Samadia, 2002; Rakesh and Ram, 2007; Husna *et al.*, 2011). However, the underlying genotypic variability of bottle gourd with respect to drought tolerance characteristics is not well understood and has not been well-documented.

Yield levels are major indicators for the evaluation of drought tolerance characteristics. According to Blum (1988), screening for drought tolerance among genotypes must be conducted based on high performance in stressed and non-stressed environments. Consequently, genotypes that have high yields in both stressed and non-stressed conditions are considered drought tolerant. In crop improvement programmes, plant breeders use yield levels and stability under limited water regimes as a major indicator of drought tolerance. Therefore, the relative yield response of genotypes under drought-stressed and non-stressed conditions is a common parameter for identification and the selection of drought tolerant genotypes (Clarke *et al.*, 1992). Evaluation of drought tolerance of field crops has been facilitated using several yield-based selection indices under managed drought conditions (Fischer and Maurer, 1978; Rosielle and Hamblin, 1981; Mitra, 2001). These indices include: drought response index (Ouk *et al.*, 2006), drought tolerance index (Bidinger *et al.*, 1978; Fernandez, 1992), drought susceptibility index, drought stress intensity (Fischer and Maurer, 1978), mean productivity (Rosielle and Hamblin, 1981), geometric mean productivity and stress tolerance index (Kristin *et al.*, 1997). Other reported indices included yield index (Gavuzzi *et al*. (1997), yield stability index (Bouslama and Schapaugh(1984) and harmonic mean (Ganjeali *et al*. (2005). Though the literature indicated that bottle gourd grows under minimal water conditions, limited information is available on the genotypic variability of the crop for drought tolerance and associated drought tolerance characteristics. The objective of this study was

to test the applicability of a number of yield-based indices to determine drought tolerance characteristics of locally adapted bottle gourd landraces and to identify promising genotypes for direct production or breeding.

5.2 Materials and methods

5.2.1 Plant materials

Twelve selected bottle gourd landraces namely: BG-27, BG-31, BG-48, BG-52, BG-58, BG-67, BG-70, BG-78, BG-79, BG-80, BG-81, and a standard check landrace "GC" were used for the study (Table 5.1). The landraces used in this study are amongst the commonly grown in the Limpopo Province, South Africa for their edible fruits or ornamental purposes. The seeds of the landrace GC used as a check was kindly provided by Mr Clive Govender. This landrace is widely grown and marketed at various outlets such as Pick 'n Pay and fruit and vegetable stores in KwaZulu-Natal and Gauteng Provinces in South Africa.

Table 5.1. List of bottle gourd landraces used in the study with description of collection sites in two South African provinces.

		Collection sites	
Landrace	Fruit shape	Geographic coordinates	Province
BG-27	Cavate	Kgohloane (23°47'39.76" S; 29°22'13.45" E)	Limpopo
BG-31	Cavate	Kgohloane (23°47'39.76" S; 29°22'13.45" E)	Limpopo
BG-48	Cavate	Kgohloane (23°47'39.76" S; 29°22'13.45" E)	Limpopo
BG-52	Cavate	Kgohloane (23°47'39.76" S; 29°22'13.45" E)	Limpopo
BG-58	Cavate	Kgohloane (23°47'39.76" S; 29°22'13.45" E)	Limpopo
BG-67	Cavate Pyriform	Ga-Rapitsi (23°35'48.37" S; 29°06'25.08" E)	Limpopo
BG-70	Elongated	Ga-Phasa (23°40'57.30" S; 29°15'57.30" E)	Limpopo
BG-78	Cavate	Moletjie-Mabokelele (23°45'14.69" S; 29°17'36.6" E)	Limpopo
BG-79	Pyriform	Moletjie-Mabokelele (23°45'14.69" S; 29°17'36.6" E)	Limpopo
BG-80	Cavate	Moletjie-Mabokelele (23°45'14.69" S; 29°17'36.6" E)	Limpopo
BG-81	Pyriform	Moletjie-Mabokelele (23°45'14.69" S; 29°17'36.6" E)	Limpopo
GC	Pyriform	La Lucia (29°45'19.98" S; 31°04'33.41" E)	KwaZulu-Natal

5.2.2 Study site

A field study was conducted during December 2014 to March 2015 growing season (summer) under dry-land (drought-stressed) and irrigated (non-stressed) conditions at Towoomba Research Station, Bela-Bela, South Africa (28º21'E, 24º25'S; 1 184 m above sea level). The soils are of the Hutton and Arcadia form. The area usually receives mean annual rainfall of 627 mm that is erratic and poorly distributed. Daily average maximum and minimum temperatures range between 29.7°C and 16.5°C during the growing season. Climatic data during the study period was obtained from the South African Weather Service.

5.2.3 Weather conditions

In the current study, the total rainfall received during the growing season was 262 mm which was considerably lower than the long-term mean annual averages (~ 500 mm) in South Africa. Mean maximum and minimum air temperatures ranged from 26 to 35°C and 13 to 18 °C, respectively (Figure 5.1). Maximum and minimum relative humidity ranged from 74 to 91% and 14 to 66%, respectively. To quantify drought severity, drought stress intensity (DSI) was calculated according to Fischer and Maurer (1978). The DSI values vary between 0 and 1. The larger the DSI value the more severe is the stress (Belko *et al.*, 2014). Drought stress is considered severe at DSI values above 0.7 (Ramirez-Vallejo and Kelly, 1998). In the current study, drought stress intensity was moderate (0.61).

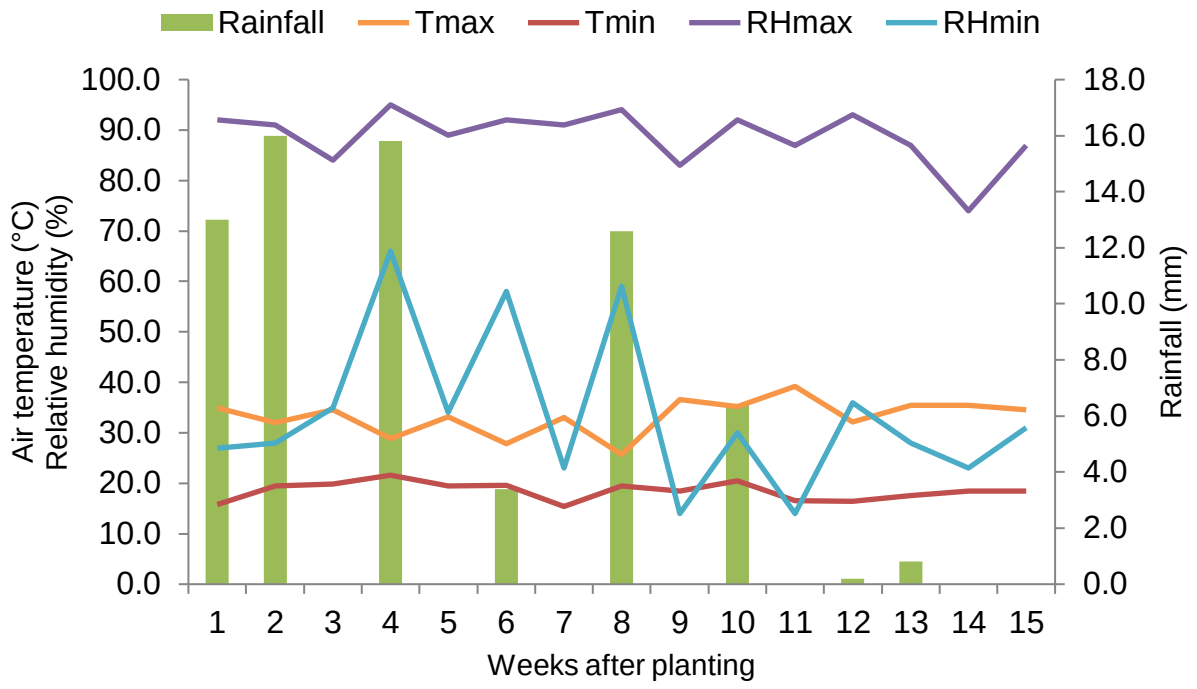

Figure 5.1. Changes in rainfall, air temperatures (Tmax=maximum temperature and Tmin=minimum temperature) and relative humidity (RHmax=maximum relative humidity and RHmin=minimum relative humidity) observed during the growing season at Towoomba Research Station.

5.2.4 Experimental design and field establishment

The study was designed using a 12 x 2 factorial experiment involving 12 bottle gourd landraces under drought-stressed (DS) and non-stressed (NS) conditions. A total of 24 treatment combinations were laid out using a randomized complete block design with three replications totalling to 72 experimental units (field plots measuring 8 m x 6 m). Twenty plants of each landrace were planted in a plot at a spacing of 2 m between rows and between plants. The experiment was established on the 11th December 2014 and terminated on the 17 March 2015. Plants subjected to drought conditions received only 262 mm of rainfall and the control (non-stressed) were provided with supplementary irrigation (27 mm three times a week using a sprinkler irrigation system) after one week of planting (vegetative stage) until maturity (reproductive stage, i.e. Irrigation was terminated when all plants have stopped producing tender edible fruits). Bottle gourd has been reported to grow well with annual rainfall ranging from 400 to 1 500 mm per annum (Haque *et al.*,

2009). The total amount of irrigation water applied to the non-stressed plants was 350 mm, providing a total volume of water of 612 mm meeting the water requirements for the crop. A soil analysis was conducted before planting for both DS and NS blocks. Results showed a pH (KCL) value of 5.94, N content of 0.05%, exchangeable K of 237 mg/kg and phosphorus of 5 mg/kg. Fertilizers were applied at the following rates: 100 kg N/ha of LAN (lime ammonium nitrate) and 165 kg P/ha SSP (single superphosphate) based on the results of a soil fertility analysis. Weed control was done manually using hand-hoes and Malasol ® (Efekto, South Africa) was used to control aphids.

5.3 Data collection

Data was collected on the following variables: Days from planting to 50% male flowering, female flowering and maturity for each landrace were determined. Days to 50% male and female flowering was recorded as number of days from planting to when about 50% of the plants showed both male and female flowers, respectively. Days to maturity was recorded when 90% of the plants produced tender edible fruits. Three successive harvests were made before plants stopped producing tender edible fruits in both stressed and non-stressed conditions. Number of fruits and fruit yield in kilograms were measured. Quantitative yield-based indices of stress tolerance such as: mean productivity (MP), tolerance index (TOL); stress susceptibility index (SSI), drought stress intensity (DSI); geometric mean productivity (GMP); stress tolerance index (STI); yield index (YI), yield stability index (YSI) and harmonic mean (HARM) were calculated using yield data under stressed and non-stressed conditions using various indices summarized in Table 5.2.

Table 5.2. Yield-based indices used for evaluating drought tolerance of bottle gourd landraces.

Indices	Formulae	Reference(s)
Mean productivity (MP)	$MP=\frac{Yns+Yds}{2}$	Rosielle and Hamblin (1981)
Drought stress intensity (DSI)	$DSI=\frac{Xns-Xds}{Xns}$	Sio-Se Mardeh *et al*. (2006)
Tolerance index (TOL)	$TOL=Yns - Yds$	Kristin *et al*. (1997) Rosielle and Hamblin (1981)
Geometric mean productivity (GMP)	$GMP=\sqrt{Yns \times Yds}$	Fernandez (1992)
Stress susceptibility index (SSI)	$SSI=\frac{Yns-Yds}{Yns \times DSI}$	Fischer and Maurer (1978)
Stress tolerance index (STI)	$STI=\frac{Yns \times Yds}{Xns^2}$	Fernandez (1992)
Yield index (YI)	$YI=\frac{Yds}{Xds}$	Gavuzzi *et al*. (1997)
Yield stability index (YSI)	$YSI=\frac{Yds}{Yns}$	Bouslama and Schapaugh (1984)
Harmonic mean (HARM)	$HARM=2\frac{Yds \times Yns}{Yds+Yns}$	Ganjeali *et al*. (2005); Ganjeali *et al*. (2011)

Y_{ds} = fruit yield of a landrace under DS conditions; Y_{ns} = fruit yield of the same landrace under NS conditions; and X_{ds} = mean fruit yield averaged across all the landraces tested under DS conditions; X_{ns} = mean fruit yield averaged across all the landraces tested under NS conditions (Belko *et al*., 2014).

5.4 Ranking of bottle gourd landraces for drought tolerance

For screening drought tolerant genotypes, a rank sum (RS) was calculated according to Farshadfar and Elyasi (2012) using the following relationship: Rank sum (RS) = Rank mean ($\bar{R}$) + Standard deviation of rank (SDR). R Standard deviation of ranks (SDR) was calculated as:

$$SDR = \sqrt{S^2 i} = \sum_{i=1}^{n} (Rij - \bar{R}i)^2 / n-1$$

Where R_{ij} is the rank of in vivo drought tolerance indicator and R_i is the mean rank across all drought tolerance indicators for the genotypes.

5.5 Data analysis

GenStat version 14th Edition (Payne *et al.*, 2011) was used to to perform analysis of variance (ANOVA). Mean comparisons among landraces were performed using the least significant difference (LSD) test procedure at 5% level of significance. The coefficients of variation (CV) were computed and expressed as a percentage (Snedecor and Cochran, 1989). Correlation analysis was performed using yield and phenological data and indices of stress tolerance to describe the pattern of associations. Significance tests of the correlation coefficients were determined using the Student's *t* test (Snedecor and Cochran 1989). Principal component analysis (PCA) was conducted using SPSS16.0. After the PCA analysis, biplots were constructed using yield in stressed and non-stressed conditions and yield–based indices of drought tolerance using appropriate factor loadings. Further, a two dimensional scatterplot was drawn using yield data under DS and NS conditions to determine genotypic variability for drought tolerance characteristics according to Fernandez *et al.* (1992).

5.6 Results

5.6.1 Effect of drought stress on genotypic performance

There was a significant ($P < 0.05$) interaction between landraces and drought with respect to fruit yield (Table 5.3). The results also showed that landraces differed significantly ($P < 0.01$) with respect to days to 50% male flowering (DMF), days to 50% female flowering (DFF) and days to maturity (DTM) ($P < 0.05$). Significant differences ($P < 0.01$) were also observed between the landraces with respect to fruit number and fruit yield. The effect of drought was significant on days to 50% flowering ($P < 0.01$), days to total maturity ($P < 0.05$), number of fruit ($P < 0.05$) and fruit yield ($P < 0.01$) (Table 5.3). A significant ($P < 0.05$) effect of the landrace × drought stress interaction on fruit yield suggested the differential response of genotypes to drought stress conditions. In turn, this suggests the presence of genetic diversity amongst the tested bottle gourd landraces for selection or direct production under dryland or irrigated environments.

Table 5.3. Mean squares for phenology, fruit number and fruit yield of bottle gourd landraces grown under non-stressed and drought-stressed conditions.

		Traits				
Source of variation	df	DMF	DFF	DTM	No. of fruits	Fruit yield
Landrace	11	24.025**	15.343**	18.809*	3.18E+08**	205.90**
Drought stresses	1	460.056**	9.389^{NS}	60.61*	4.39*E+08*	3761.59**
Landrace x drought stress	11	11.056^{NS}	3.965^{NS}	9.556^{NS}	$7.39E+08^{NS}$	102.46*
Replications	2	28.931	6.222	39.715	2.650E+07	33.49
Residual	46	6.351	4.222	8.374	5.16E+07	31.41

*Significant differences at the 0.05 probability level; **Significant differences at the 0.01 probability level; NS = Non-significant; DMF = Days to 50% male flowering; DFF = Days to 50% female flowering; DTM = Days to maturity; df = degrees of freedom.

5.6.2 Effect of drought stress on phenology of bottle gourd

Variations in days to flowering and maturity of diverse bottle gourd landraces are presented in Table 5.4. Significant differences ($P < 0.05$) were observed among the landraces for days to 50% male flowering under drought stress condition only. The landraces: BG-67, BG-78 and BG-70 had significantly lower number of days to 50% male flowering of 53, 49 and 50 days, respectively. The check landrace "GC" was late flowering (60 days of 50% male flowering) than all the landraces. On average, days to 50% male flowering were 54 and 59 days under drought stressed and non-stressed conditions, respectively. Significant differences ($P < 0.05$) were observed among the landraces with respect to days to 50% female flowering under drought stress condition only. The landraces BG-27 and BG-79 flowered in 60 days, significantly lower than days to 50% female flowering observed for BG-80, BG-81, BG-78, BG-58 and BG-67 with 67, 67, 64, 65 and 67 days, respectively. None significant differences ($P > 0.05$) were observed amongst bottle gourd landraces with respect to the number of days to maturity under both drought-stressed and non-stressed conditions. Landraces matured earlier by 9 days under DS condition. The CVs for phenological traits during this study were relatively low (1.2 to 5.3%).

Table 5.4. Time to flowering and maturity of bottle gourd landraces evaluated under drought-stressed (DS) and non-stressed (NS) conditions.

Landrace	Days to flowering (days)				Days to maturity (days)	
	Male flowers		Female flowers			
	DS	NS	DS	NS	DS	NS
BG-27	54^{cd}	59	60^{a}	64	72	82
BG-31	55^{d}	57	63^{ab}	62	68	82
BG-48	56^{d}	59	63^{ab}	66	70	75
BG-52	53^{bcd}	59	63^{ab}	64	71	78
BG-58	56^{d}	59	65^{b}	64	75	78
BG-67	53^{bcd}	61	65^{b}	65	70	82
BG-70	50^{ab}	56	63^{ab}	65	69	82
BG-78	49^{a}	59	64^{b}	64	67	78
BG-79	51^{abc}	56	60^{a}	62	72	82
BG-80	55^{d}	61	67^{b}	66	75	82
BG-81	56^{d}	60	67^{b}	66	72	82
GC	60^{e}	60	64^{ab}	66	70	75
Mean ± SE	54 ±1.9	59 ± 2.3	64 ± 1.8	65 ± 1.6	71 ± 2.6	80 ± 3.5
P – value	<.001	NS	0.02	NS	NS	NS
LSD $_{(0.05)}$	3.89	4.67	3.71	3.22	5.32	7.17
CV (%)	2.4	4.7	1.2	3.0	4.4	5.3

Means in a column followed by the same letter(s) are not significantly different at *P* = 0.05. NS= Non-significant; CV= Coefficient of variation; SE= Standard error; DS=drought stressed, NS=non-stressed.

5.6.3 Effect of drought stress on fruit number and fruit yield of bottle gourd landraces

Fruit number and fruit yield varied significantly among the bottle gourd landraces under NS and DS conditions (Table 5.5). The mean numbers of fruits under DS and NS conditions were 15 457 and 31 088 ha^{-1}; respectively. Number of fruits under DS and NS conditions ranged from 6 423 to 25 521 and 16 667 to 53 229 ha^{-1}, respectively. BG-31 and BG-78 had significantly higher number of fruits under DS conditions at 25 521 and 21058 ha^{-1}, respectively and 40 833 and 53 229 ha^{-1} under NS conditions; in that order. BG-80 had the lowest number of fruits (6 423 ha^{-1}) under DS conditions while BG-67 and BG-80 had the lowest number of fruit under NS conditions with 17 396 and 16 667 ha^{-1}, respectively. Fruit yield under both DS and NS conditions ranged from 3.48 to 12.94 and 14.2 to 44.3 t ha^{-1}, respectively. The overall mean fruit yield under NS and DS conditions were 8.75 t ha^{-1} and 22.4 t ha^{-1}, respectively. The landraces BG-78, BG-79 and GC had the highest fruit yield under DS conditions with 12.72, 12.94 and 11.94 t ha^{-1}, respectively. The

landraces BG-78 and GC had the highest fruit yield under NS conditions with 44.3 and 38.9 t ha^{-1}, respectively (Table 5.5). The landrace BG-81 had the lowest yield (3.48 t ha^{-1}) under DS condition while BG-80 had the lowest yield (14.2 t ha^{-1}) under NS condition. Drought stress reduced fruit number and fruit yield by 49 and 62%, respectively. The landraces BG-78 and BG-80 exhibited significantly reduced fruit number of 60 and 61% and fruit yield of 71 and 75%, respectively. The CVs for fruit number and yield under stressed and non-stressed conditions during this study were relatively high (29.1 to 32.6%) suggesting greater variability of the genotypes under drought conditions.

Table 5.5. Mean fruit number, fruit yield and % reduction for both fruit number and fruit yield under drought-stressed (DS) and non-stressed (NS) conditions.

	Number of fruit (ha)		Fruit yield (t ha^{-1})		Reduction (%)	
Landrace	DS	NS	DS	NS	FN	FY
BG-27	16163^{b}	34062^{bc}	7.47^{abc}	20.1^{a}	53	63
BG-31	25521^{c}	40833^{cd}	9.96^{bcd}	20.5^{a}	37	51
BG-48	13646^{ab}	28452^{abc}	7.16^{ab}	15.2^{a}	52	53
BG-52	19167^{bc}	41162^{cd}	8.88^{bcd}	32.1^{a}	53	60
BG-58	13610^{ab}	31562^{abc}	6.84^{ab}	20.8^{a}	57	67
BG-67	12985^{ab}	17396^{a}	9.39^{bcd}	19.5^{a}	25	52
BG-70	13021^{ab}	24375^{ab}	7.22^{ab}	18.1^{a}	47	60
BG-78	21058^{bc}	53229^{d}	12.72^{d}	44.3^{b}	60	71
BG-79	15173^{b}	24688^{ab}	12.94^{d}	17^{a}	39	24
BG-80	6423^{a}	16667^{a}	3.48^{a}	14.2^{a}	61	75
BG-81	14548^{b}	28750^{abc}	6.95^{ab}	17.7^{a}	49	61
GC	14167^{ab}	31875^{abc}	11.94^{cd}	38.9^{b}	56	69
Mean ± SE	15457 ± 3900	31088 ± 7380	8.75 ± 2.20	22.4 ± 6.17	49	62
P – value	0.014	0.003	0.009	<.001		
$LSD_{(0.05)}$	8088.7	15306.1	4.57	12.79		
CV (%)	30.9	29.1	30.9	32.6		

Means in a column followed by the same letter(s) are not significantly different at *P* = 0.05. NS= Non-significant; CV= Coefficient of variation, SE= Standard error; DS= drought stressed, NS= non-stressed. *Significant differences at the 0.05 probability level. **Significant differences at the 0.01 probability level

Classification of bottle gourd landraces based on fruit yield under drought stressed (DS) and non-stressed (NS) conditions are shown in Figure 5.2. BG-79, BG-31 and BG-67 produced the highest fruit yield only under DS conditions. BG-78, BG-52 and GC produced the highest fruit yield under both DS and NS conditions. BG-27, BG-48, BG-70, BG-81, BG-58 and BG-80 had the lowest fruit yields under both DS and NS conditions.

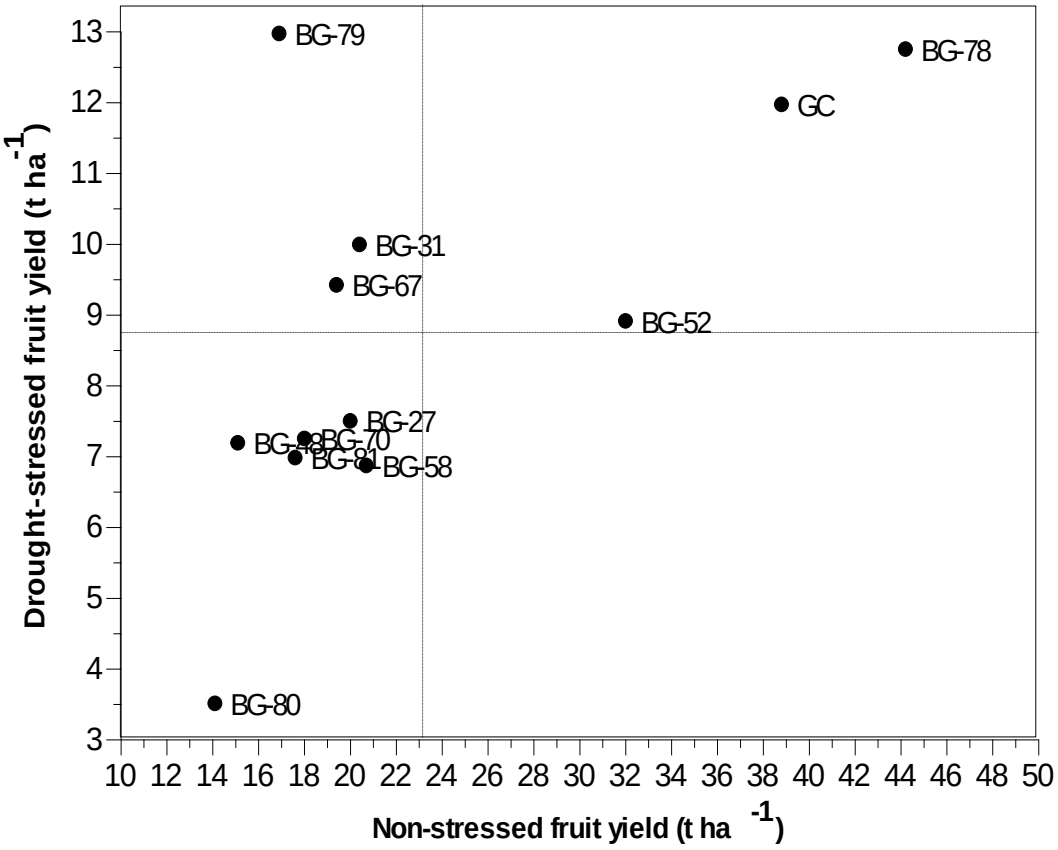

Figure 5.2. Biplot showing classification of bottle gourd landraces based on fruit yield under drought-stressed and non-stressed conditions. Dotted lines represent grand mean yields under non-stressed or drought-stressed conditions.

5.6.4 Yield-based indices of drought tolerance

Yield-based indices of drought tolerance among tested bottle gourd landraces are summarized in Table 5.6. The landraces BG-78 and GC had significantly higher values of stress tolerance index (STI), geometric mean productivity (GMP), mean productivity (MP), tolerance index (TOL), yield index (YI) and harmonic mean (HARM). The landraces BG-31, BG-48, BG-67, BG-70 and, had the highest values for yield stability index (YSI). Except BG-79, all landraces had higher values for stress susceptibility index (SSI).

Table 5.6. Yield-based indices used to evaluate drought tolerance of bottle gourd landraces.

	Indices							
Landrace	STI	GMP	MP	SSI	TOL	YI	YSI	HARM
BG-27	0.28	12.25	13.79	1.03	12.63	0.85	0.37	10.89
BG-31	0.38	14.29	15.23	0.84	10.54	1.14	0.49	13.41
BG-48	0.20	10.43	11.18	0.87	8.04	0.82	0.47	9.73
BG-52	0.53	16.88	20.49	1.19	23.22	1.01	0.28	13.91
BG-58	0.26	11.92	13.82	1.10	13.96	0.78	0.33	10.29
BG-67	0.34	13.53	14.45	0.85	10.11	1.07	0.48	12.68
BG-70	0.24	11.43	12.66	0.99	10.88	0.83	0.40	10.32
BG-78	1.05	23.74	28.51	1.17	31.58	1.45	0.29	19.76
BG-79	0.41	14.83	14.97	0.39	4.06	1.48	0.76	14.69
BG-80	0.09	7.03	8.84	1.24	10.72	0.40	0.25	5.59
BG-81	0.23	11.09	12.33	1.00	10.75	0.79	0.39	9.98
GC	0.86	21.55	25.42	1.14	26.96	1.36	0.31	18.27

STI = stress tolerance index; GMP= Geometric mean productivity; MP= Mean productivity; SSI = Stress susceptibility index; TOL = tolerance index; YI = Yield index; YSI = Yield stability index; HARM= Harmonic mean.

5.6.5 Correlation analysis

The association between drought-stressed yield (Yds) and non-stressed yield (Yns) with yield-based indices of drought tolerance is presented in Table 5.7. Stress tolerance index (STI) was strongly and positively correlated with geometric mean productivity (GMP), mean productivity (MP) tolerance index (TOL), yield index (YI), harmonic mean (HARM), drought-stressed (r = 0.79, $P < 0.001$) and non-stress (r = 0.96, $P < 0.001$) yield (Table 5.7). The correlation between stress tolerance index and geometric mean productivity was 0.99. Stress susceptibility index was negatively and significantly correlated with yield stability index (r = – 1.00; $P < 0.001$). The correlation between drought-stressed and non-stress yield was 0.63 suggesting a relatively consistent yield levels among landraces. A positive and significant correlation was observed between TOL and non-stressed yield (r = 0.96, $P < 0.001$) and a non-significant correlation between TOL and drought stress yield (r = 0.40, $P < 0.119$). A non-significant association was observed between SSI and yield under stressed and non-stressed conditions. Yield index was positively correlated with stressed (r = 1.00, $P < 0.001$) and non-stressed (r = 0.63, P = 0.024) yield while non-significant association was observed between yield stability index with stressed

and non-stressed yield. Harmonic mean was positively and significantly associated with stressed (r = 0.94, P < 0.001) and non-stressed (r = 0.86, P < 0.001) yield. Stress tolerance index was positively and significantly associated with stressed (r = 0.79, P < 0.001) and non-stressed (r = 0.96, P < 0.001) yield. Mean productivity was also positively and significantly associated with stressed (r = 0.76, P < 0.001) and non-stressed (r = 0.98, P < 0.001) yield.

Table 5.7. Correlation coefficients for pair-wise association among indices of drought tolerance with stressed and non-stressed yield 12 among bottle gourd landraces.

Indices	STI	GMP	MP	SSI	TOL	YI	YSI	HARM	Yds	Yns
STI	1.00									
GMP	0.99**	1.00								
MP	0.99**	0.99**	1.00							
SSI	0.20ns	0.11ns	0.27ns	1.00						
TOL	0.86**	0.82**	0.89**	0.66*	1.00					
YI	0.79**	0.86**	0.76**	-0.42ns	0.40ns	1.00				
YSI	-0.20ns	-0.11ns	-0.27ns	-1.00**	-0.66*	0.42ns	1.00			
HARM	0.95**	0.98**	0.94**	-0.08ns	0.69*	0.94**	0.08ns	1.00		
Yds	0.79**	0.86**	0.76**	-0.42ns	0.40NS	1.00**	0.42ns	0.94**	1.00	
Yns	0.96**	0.94**	0.98**	0.44ns	0.96**	0.63*	-0.44ns	0.86**	0.63*	1.00

STI = stress tolerance index; GMP= Geometric mean productivity; MP= Mean productivity; SSI = Stress susceptibility index; TOL = tolerance index; YI = Yield index; YSI = Yield stability index; HARM= Harmonic mean; Yds = Drought stressed yield; Yns = Non-stressed yield. *Significant differences at the 0.05 probability level. **Significant differences at the 0.01 probability level. ns = non-significant.

Correlation coefficients between phenology, yield-based indices of drought tolerance, drought stressed yield (Yds) and non-stressed yield (Yns) are presented in Table 5.8. Non-significant correlations were observed between days to 50% female flowering (DFF) with yield-based indices of drought tolerance, drought-stressed and non-stressed yields under stressed conditions and non-stressed conditions. Also, non-significant correlations were observed between days to 50% male flowering (DMF) with yield-based indices of drought tolerance, drought-stressed and non-stressed yields under stressed conditions and non-stressed conditions. Days to maturity was negatively and significantly correlated with STI (r = – 0.58, P = 0.02), YI (r = – 0.61, P = 0.02), HARM (r = – 0.65, P = 0.04) and Yds (r = – 0.61, P = 0.03) under drought stress condition while non-significant associations were observed under non-stress conditions.

Table 5.8. Correlation coefficients for pair-wise association between phenology, drought tolerance indices and fruit yield of bottle gourd landraces under stressed and non-stressed conditions.

Phenology	Stress	Indices									
		STI	GMP	MP	SSI	TOL	YI	YSI	HARM	Yds	Yns
DFF	DS	-0.13^{ns}	-0.02^{ns}	-0.13^{ns}	0.54^{ns}	0.13^{ns}	-0.47^{ns}	-0.54^{ns}	-0.32^{ns}	-0.47^{ns}	-0.02^{ns}
	NS	-0.15^{ns}	-0.03^{ns}	-0.15^{ns}	0.53^{ns}	0.14^{ns}	-0.51^{ns}	-0.53^{ns}	-0.34^{ns}	-0.51^{ns}	-0.03^{ns}
DMF	DS	-0.13^{ns}	-0.15^{ns}	-0.11^{ns}	0.24^{ns}	0.01^{ns}	-0.24^{ns}	-0.24^{ns}	-0.17^{ns}	-0.24^{ns}	-0.06^{ns}
	NS	0.01^{ns}	0.09^{ns}	0.03^{ns}	0.57^{ns}	0.27^{ns}	-0.35^{ns}	-0.57^{ns}	-0.17^{ns}	-0.35^{ns}	0.13^{ns}
DTM	DS	-0.58*	-0.55^{ns}	-0.56^{ns}	0.12^{ns}	-0.38^{ns}	-0.61*	-0.12^{ns}	-0.65*	-0.61*	-0.49^{ns}
	NS	-0.48^{ns}	-0.48^{ns}	-0.50^{ns}	-0.32^{ns}	-0.56^{ns}	-0.23^{ns}	0.32^{ns}	-0.39^{ns}	-0.23^{ns}	-0.54^{ns}

DFF = Days to 50% female flowering; DMF = Days to 50% male flowering; DTM = Days to maturity; STI = stress tolerance index; GMP = Geometric mean productivity; MP = Mean productivity; SSI = Stress susceptibility index; TOL = tolerance index; YI = Yield index; YSI = Yield stability index; HARM = Harmonic mean; Yds = drought-stressed yield; Yns = non-stressed yield. DS=drought-stressed; NS = non-stressed; NS = non-significant. *Significant differences at the 0.05 probability level.

5.6.6 Principal component analysis

To investigate the relationship among landraces and yield-based indices of stress tolerance, principal component analysis was performed. Results of the principal component analyses (PCA) revealed two PCAs which accounted for 99.72 % of total variation (Table 5.9). Stress tolerance index, geometric mean productivity, mean productivity, tolerance index, yield index, harmonic mean, drought-stressed yield (Yds) and non-stressed yield (Yns) positively correlated with PC1 which accounted for 69.68% of the total variation. Stress susceptibility index positively correlated with PC2, while yield stability index negatively correlated with PC2, which accounted for 30.04 % of the total variation. Biplot analysis was used to visualize the relationships among the yield-based indices of drought tolerance which revealed strong positive associations between yield under DS and NS conditions and TOL, HARM, GMP, MP and STI shown by the acute angles between their vectors (Figure 5.3).

Table 5.9. Principal component analysis of yield-based indices of drought tolerance of bottle gourd landraces tested under drought-stressed and non-stressed conditions.

Indices	PC1	PC2
STI	**0.97**	0.19
GMP	**0.99**	0.11
MP	**0.97**	0.26
SSI	0.01	**0.99**
TOL	**0.75**	0.65
YI	**0.91**	–0.42
YSI	-0.06	–**0.99**
HARM	**0.99**	–0.08
Yds	**0.91**	–0.42
Yns	**0.90**	0.43
Explained variance (Eigenvalue)	6.97	3.00
Proportion of total variance (%)	69.68	30.04
Cumulative variance (%)	69.68	99.72

Vector loadings > 0.7 are boldfaced. STI = stress tolerance index; GMP= Geometric mean productivity; MP = Mean productivity; SSI = Stress susceptibility index; TOL = tolerance index; YI = Yield index; YSI = Yield stability index; HARM = Harmonic mean; Yds = drought-stressed yield and Yns = non-stressed yield.

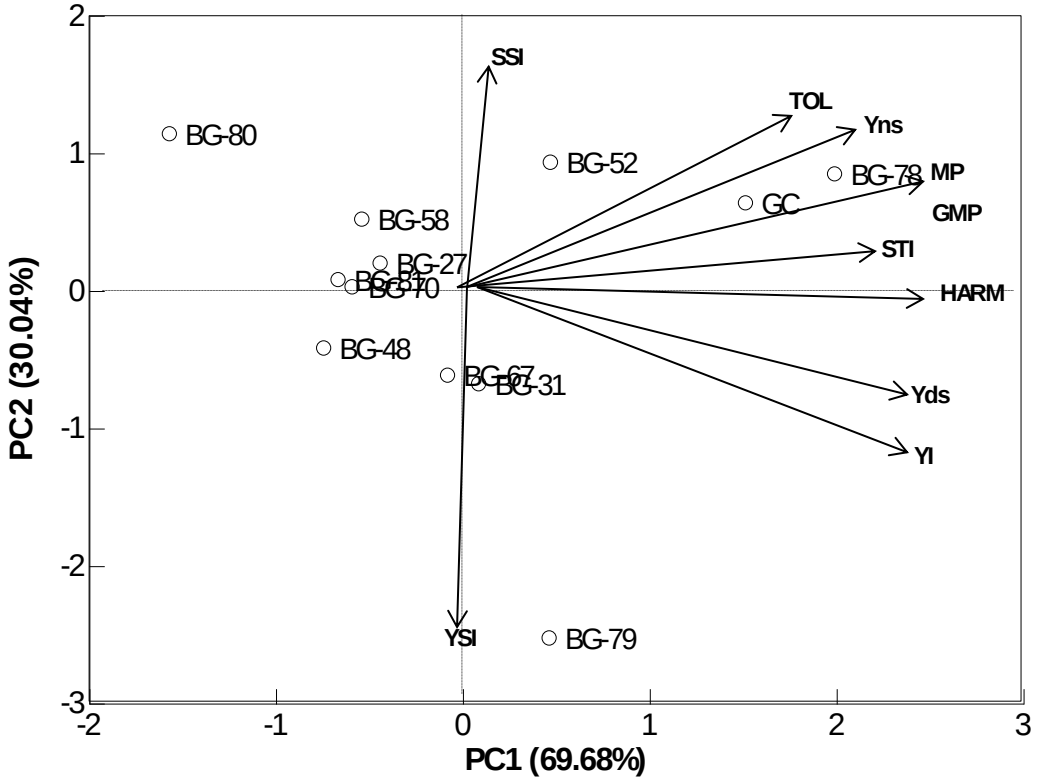

Figure 5.3. Varimax rotated principal component loadings of PC1 vs PC2 showing the relationship between yield in stress (Yds), yield in non-stress (Yns) and indices of drought tolerance of 12 bottle gourd landraces. STI = stress tolerance index; GMP= Geometric mean productivity; MP = Mean productivity; SSI = Stress susceptibility index; TOL = tolerance index; YI = Yield index; YSI = Yield stability index; HARM = Harmonic mean; Yds = drought-stressed yield and Yns = non-stressed yield.

5.6.7 Ranking of bottle gourd landraces

The estimates of yield-based indicators of drought tolerance indicated that the identification of drought-tolerant cultivars was inconsistent based on a several criterion. Different indices identified different landraces as drought tolerant. The ranking method was used to have an overall judgment on performance of landraces. To determine the most desirable drought tolerant landraces according to all indices, mean rank, standard deviation of ranks and rank sum (RS) of all drought tolerance

indices were calculated. The most drought tolerant genotypes were identified as having low RS values and these were GC, BG-78 and BG-52 (Table 5.10).

Table 5.10. Rank and rank sum ($\bar{R}$ ± SDR) of yield-based indices used to evaluate overall drought tolerance among bottle gourd landraces.

Landrace	Yns	Yds	STI	GMP	MP	SSI	TOL	YS	YSI	HARM	$\bar{R}$ ± SDR
BG-27	6	7	7	8	8	6	5	7	7	7	6.8 ± 0.92
BG-31	5	4	5	5	4	11	9	4	2	5	5.4 ± 2.63
BG-48	11	9	11	12	11	9	11	9	4	11	9.8 ± 2.3
BG-52	3	6	3	3	3	2	3	6	11	4	4.4 ± 2.67
BG-58	4	10	8	9	7	5	4	11	8	9	7.5 ± 2.46
BG-67	7	5	6	6	6	10	10	5	3	6	6.4 ± 2.17
BG-70	8	8	9	10	9	8	6	8	5	8	7.9 ± 1.45
BG-78	1	2	1	1	1	3	1	2	10	1	2.3 ± 2.79
BG-79	10	1	4	4	5	12	12	1	1	3	5.3 ± 4.42
BG-80	12	12	12	7	12	1	8	12	12	12	10 ± 3.68
BG-81	9	11	10	11	10	7	7	10	6	10	9.1 ± 1.79
GC	2	3	2	2	2	4	2	3	9	2	3.1 ± 2.18

Yns = non-stressed yield; Yds = drought-stressed yield; STI = stress tolerance index; GMP= Geometric mean productivity; MP = Mean productivity; SSI = Stress susceptibility index; TOL = tolerance index; YI = Yield index; YSI = Yield stability index; HARM = Harmonic mean; SDR = Standard deviation of ranks.

5.7 Discussion

The study revealed the presence of genetic variability for drought tolerance in bottle gourd landrace collections. Results of phenology showed that under DS conditions landraces produced male flowers 5 days earlier than under NS condition (Table 5.4). Further, early maturity was observed under DS conditions than NS conditions. This suggests that bottle gourd landraces under DS conditions accelerated flowering time and maturity to avoid terminal drought. Flowering time is an important trait related to drought adaptation, where early flowering can lead to drought escape (Araus *et al.*, 2002). Early maturity is an important drought adaptation mechanism useful in breeding for drought tolerance (Chaves *et al.*, 2003). Developing short-cycle varieties is therefore an effective drought avoidance strategy (Farooq *et al.*, 2009).

Genotypic variability for yield performance was observed under drought-stressed and non-stressed conditions, the observed variability could be useful in breeding for improved bottle gourd yield.

The landraces BG-78, BG-52 and GC had the highest fruit yield under both DS and NS conditions and can be considered drought tolerant (Table 5.5; Figure 5.1). According to Blum (1988), screening for drought tolerance among genotypes must be conducted based on high performance in stressed and non-stressed conditions. These landraces (BG-78, BG-52 and GC) would have economic yields in dry years and high performance under optimal rainfall conditions (Figure 5.5). Further, the landraces (BG-78, BG-52 and GC) had the highest values for STI, GMP, MP, TOL and HARM hence; they may be the most productive landraces under both DS and NS conditions (Table 5.6). Stress tolerance index (STI) and geometric mean productivity (GMP) have been proposed for the selection of genotypes that produce high yields under DS and NS environments (Fernandez, 1992). The higher the value of STI for a given genotype, the higher its drought tolerance and yield potential (Kumar *et al.*, 2014). Also, a relatively higher GMP value of a given genotype suggests its greater grain yield performance under both DS and NS conditions (Fernandez, 1992). Also, selection for higher MP and HARM values should increase yield in both stress and non-stress conditions (Hohls, 2001; Ganjeali *et al.*, 2005). Therefore, selection of bottle gourd landraces based on higher STI, GMP, MP, TOL and HARM will result in drought tolerance and yield improvement as they have been successfully used for selection of drought tolerance in various crop species (Ganjeali *et al.*, 2005; Ganjeali *et al.*, 2011; Belko *et al.*, 2014; Naderi and Emam, 2014).

Drought tolerance is a complex trait and its inheritance is governed by polygenes. Also it is highly influenced by genotype and environment interaction (Bahrami *et al.*, 2014). Therefore, selection under managed drought condition and use of a combination of indices may enhance breeding gains. Typically, suitable drought tolerance indices should significantly correlate with yield under stressed and non-stressed conditions (Mitra 2001). In this study, drought tolerance like STI, MP, GMP, YI and HARM correlated with yield under non-stressed and drought-stressed conditions (Table 5.7). These suggested that the drought tolerant indices used in the present study are suitable to screen drought-tolerant, high yielding bottle gourd landraces under dryland and supplemental irrigation conditions. Results in this study are in agreement with Golabadi *et al*. (2006) and Sio-Se Mardeh *et al*. (2006), Ganjeali *et al*. (2011) and Ashraf *et al*. (2015) who reported that these indices are suitable for discriminating the best genotypes under stressed and irrigated conditions. Further, a positive and significant correlation between TOL and Yns (Table 5.7) and a non-

significant correlation between TOL and Yds suggested that selection based on TOL will result in reduced yield under well-watered conditions. Similar results were reported by Clarke *et al*. (1992), Rosielle and Hamblin (1981), Sio-Se Mardeh *et al*. (2006) and Naderi and Emam (2014). A positive and significant association between Yds and Yns (r = 0.68) suggest a relatively consistent yield levels among genotypes. The current results suggested that selection of bottle gourd landraces based on yield potential would improve yield under stressed and non-stressed conditions. In contrast, Ceccarelli *et al*. (1992), Abebe *et al*. (1998), Yadav and Bhatnagar (2001) and Ganjeali *et al*. (2011) reported low to moderately association in yield performance of genotypes under stress and non-stress conditions. Rosielle and Hamblin (1981) also indicated that under most yield trials, the correlation between stressed and non-stressed yield is smaller indicating that selection for yield potential would only increase yield under non-stressed environments while the selected genotypes would perform poorly under stressed conditions.

Time to 50% male and female flowering were non-significantly associated with all yield-based indices of drought tolerance under both stressed and non-stressed conditions (Table 5.8). These suggest that the reproductive period for male and female flowering may not result in drought tolerance improvement in bottle gourd (Siddique *et al.*, 1999). Days to maturity was negatively correlated with stress tolerance index (STI), yield index (YI), harmonic mean (HARM) and drought-stressed yield (Yds) under drought stress conditions (Table 5.8). The present results suggested that breeding for bottle gourd genotypes with early maturity, increased drought-tolerance and yield potential under drought-stressed conditions is possible.

Principal component analysis indicated that PC1 explained most of the variation. Yield-based indices of stress tolerance index, GMP, MP, TOL, YS, HARM, Yds, and Yns positively correlated with PC1. Stress susceptibility index (SSI) negatively correlated with PC2 while YSI positively correlated with PC2 (Table 5.9). Thus, the first component can be considered in affecting drought tolerance and yield potential (Aliakbari *et al.*, 2014; Bahrami *et al.*, 2014; Naderi and Emam, 2014). These indices are the most important criteria to select best performing genotypes under normal and stress conditions. The second component (PC2) is regarded as drought sensitive and separates tolerant and susceptible genotypes (Aliakbari *et al.*, 2014; Bahrami *et al.*, 2014). Therefore, selection of genotypes with high PC1 and low PC2 values are suitable for both stress

and non-stress environments (Golabadi *et al.*, 2006; Aliakbari *et al.*, 2014; Naderi and Emam, 2014). In this study, landraces well-associated with PC1 values were BG-52, BG-78 and GC and those with low PC2 values were BG-31, BG-48, BG-67 and BG-79. These landraces were also the most tolerant characterized by low RS values (Table 5.10).

5.8 Conclusions

This study showed that, genetic variability for drought tolerance is present among bottle gourd landraces. Landraces such as BG-79, BG-31, BG-67, BG-52, BG-78 and GC were identified useful for drought tolerance breeding or rootstock development programs of bottle gourd. Further, yield-based indices of drought tolerant such as tolerance index, geometric mean productivity, stress tolerance, mean productivity, yield index and harmonic mean allowed discrimination of drought tolerant bottle gourd landraces.

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Chapter 6: **Bottle Gourd [*Lagenaria siceraria* (Molina) Standl.] Response to Water Stress: Relationship between Cucurbitacin Accumulation with Leaf Gas Exchange and Chlorophyll Fluorescence**

Abstract

Cucurbitacins are a group of secondary metabolites produced by cucurbits and their level of production is reported to be increased in response to water stress. The role of cucurbitacins in plant responses to water stress is not well-determined. The objective of this study was to determine the relationship between accumulation of cucurbitacins with leaf gas exchange and chlorophyll fluorescence in bottle gourd [*Lagenaria siceraria* (Molina) Standl.] under water- stress conditions. Twelve bottle-gourd landraces were investigated under water-stressed (WS) and non-stressed (NS) conditions with three replications at the Controlled Research Facility, University of KwaZulu-Natal. The results showed that stomatal conductance, transpiration rate and net CO_2 assimilation rate, ratio of CO_2 assimilation rate and intercellular CO_2, intrinsic water-use efficiency and instantaneous water-use efficiencies declined in landraces that were subjected to water stress. Intercellular CO_2 concentration, ratio of intercelluar and atmospheric CO_2 concentration increased significantly in response to water stress. The maximum PS II activity, quantum yield of PSII, photochemical quenching and non-photochemical quenching were not affected by water stress; whereas, electron transport rate, electron transport to oxygen molecules (ETR/A) and alternative electron sink (AES) declined. Cucurbitacin E and I were detected under NS and WS conditions in several bottle gourd landraces. Significant and positive correlations were observed between cucurbitacin I content with ETR/A and AES suggesting their possible role in the regulation of photorespiration and photoprotection against oxidative stress. Therefore, increased accumulation of cucurbitacin I could be considered as a tool for selection of bottle gourd genotypes for drought tolerance.

Keywords*:* breeding, drought stress, cucurbitacin, drought tolerance, physiology

6.1 Introduction

Drought is one of the leading constraints affecting global crop production and productivity (Cattivelli *et al.*, 2008). Drought tolerant crop genotypes have varied adaptation and survival mechanisms and physiological responses for under-drought stress condition. Key physiologic responses include synthesis and accumulation of compatible solutes which are referred to as osmoprotectants or osmolytes responsible in lowering of the cell water potential and in enhancing water extraction capacity in water-limited environments (Ramanjulu and Sudhakar, 2000). Among known compatible solutes, proline is the most widely distributed osmolytes, and its accumulation is involved in adaptation to water stress in plants (Ramanjulu and Sudhakar, 2000; Vendruscolo *et al.*, 2007). Transgenic soybean plants overexpressing the *Arabidopsis* Δ 1-pyrroline-5- carboxylate synthase gene, *P5CR* , showed greater tolerance to drought stress due to an increased free proline content (de Ronde *et al.*, 2004; Kocsy *et al.*, 2005). Increased proline content in wheat and mulberry plants in response to water deficit was considered an index for water stress tolerance (Ramanjulu and Sudhakar, 2000; Rampino *et al.*, 2006). Citrulline (a non-essential amino acid) accumulates exclusively in cucurbits and it has been widely reported to protect leaves from drought-induced oxidative stress by acting as a hydroxyl radical scavenger (Akashi *et al.*, 2001). Blum and Sullivan (1986) reported that plants constantly experiencing water stress may possess some unique physiological drought adaptation mechanisms.

Physiological responses of crop genotypes is an important component in crop improvement programs to improve their adaptation under water-limited environments (Subbarao *et al.*, 1995). The shikimate acid pathway is one of the important biosynthetic pathways responsible for biosynthesis of secondary metabolites in higher plants, including alkaloids (Dixon and Paiva, 1995; Scalabrin *et al.*, 2016). Plants in the *Cucurbitaceae* family produce a bitter-tasting toxic tetracyclic triterpenoid secondary metabolite commonly known as cucurbitacins (Sharma *et al.*, 2006; Sharma *et al.*, 2012; Sukhlecha, 2012). The synthesis of cucurbitacins is initiated with the cyclization of 2, 3-oxidosqualene to cucurbitadienol. Cucurbitadienol is then metabolized into different cucurbitacins by subsequent hydroxylation, acetylation and glucosylation (Chen *et al*., 2005). Cucurbitadienol, a triterpene synthesized from oxidosqualene, is the first precursor of cucurbitacins produced by a specialized oxidosqualene cyclase called cucurbitadienol synthase.

Cucurbitacins are classified into 12 types namely: A, B, C, D, E, F, I, L, 23, 24-dihydrocucurbitacin F, and hexanorcucurbitacin F. Cucurbitacins differ from each other by the presence of hydroxylation at C-2, -3,-19, -24 (cucurbitacins A, B, C and D), the presence of ketone function at C-3 (cucurbitacins A, B, C and D), double bond between C-23 (cucurbitacins B, D, E and I) and C-24 (B, D, E and I), and by the acetylation of the C-26 hydroxy group (B, D, E and I) (Greige-Gerges *et al.*, 2007).

Cucurbitacins are generally toxic to many organisms and therefore, their natural role in plants are probably related to defense against pathogens and pests (Davidovich-Rikanati *et al.*, 2015; Dube and Mashela, 2016; Shadung and Mashela, 2016). Cucurbitacins contents are reported to accumulate in response to environmental stresses including heat stress (high temperatures) and water stress (Haynes and Jones, 1975; Kano and Goto, 2003). However, the role of cucurbitacins in plant responses to water stress is not well-documented. Therefore, there is need to gain a more detailed understanding of the role of cucurbitacins in plant adaptations to water stress. Further, knowledge on their role and importance in drought adaptation may contribute to the development of reliable selection criteria for drought tolerance breeding. Therefore, the objective of this study was to determine the relationship between accumulation of cucurbitacins with leaf gas exchange and chlorophyll fluorescence in bottle gourd [*Lagenaria siceraria* (Molina) Standl.] under water-stress conditions. The underlying hypothesis was that water stress results in accumulation of cucurbitacins which may in turn regulates leaf gas exchange and chlorophyll fluorescence. Consequently, bottle gourd genotypes characterized by a higher content of cucurbitacins may be better adapted to water stress conditions.

6.2 Materials and Methods

6.2.1 Plant materials

Twelve bottle gourd landraces namely: BG-27, BG-31, BG-48, BG-52, BG-58, BG-67, BG-70, BG-78, BG-79, BG-80, BG-81 commonly grown under dry-land conditions in the Limpopo Province, South Africa, and a standard check landrace "GC" were used for this study (Table 6.1). The seeds of the landrace GC used as a check. This landrace is widely grown and marketed at

various retail outlets and fruit and vegetable stores in KwaZulu-Natal and Gauteng Provinces in South Africa. The remaining landraces were used due to their varied response to drought stress (Mashilo *et al.*, 2017) and variation in fruit characteristics such as fruit colour, shape and texture (Mashilo *et al.*, 2015; 2016).

Table 6.1. List of bottle gourd landraces used in the study and description of geographic location and coordinates of collection sites.

		Collection sites	
Landrace	Fruit shape	Geographic coordinates	Province
BG-27	Cavate	Kgohloane (23°47'39.76" S; 29°22'13.45" E)	Limpopo
BG-31	Cavate	Kgohloane (23°47'39.76" S; 29°22'13.45" E)	Limpopo
BG-48	Cavate	Kgohloane (23°47'39.76" S; 29°22'13.45" E)	Limpopo
BG-52	Cavate	Kgohloane (23°47'39.76" S; 29°22'13.45" E)	Limpopo
BG-58	Cavate	Kgohloane (23°47'39.76" S; 29°22'13.45" E)	Limpopo
BG-67	Cavate	Ga-Rapitsi (23°35'48.37" S; 29°06'25.08" E)	Limpopo
BG-70	Pyriform Elongated	Ga-Phasa (23°40'57.30" S; 29°15'57.30" E)	Limpopo
BG-78	Cavate	Moletjie-Mabokelele (23°45'14.69" S; 29°17'36.6" E)	Limpopo
BG-79	Pyriform	Moletjie-Mabokelele (23°45'14.69" S; 29°17'36.6" E)	Limpopo
BG-80	Cavate	Moletjie-Mabokelele (23°45'14.69" S; 29°17'36.6" E)	Limpopo
BG-81	Pyriform	Moletjie-Mabokelele (23°45'14.69" S; 29°17'36.6" E)	Limpopo
GC	Pyriform	La Lucia (29°45'19.98" S; 31°04'33.41" E)	KwaZulu-Natal

6.2.2 Experimental design and crop establishment

Controlled pot experiments were conducted under glasshouse conditions at the Controlled Research Facility (CEF), University of KwaZulu-Natal, Pietermaritzburg, South Africa. The study was conducted using a 12 x 2 factorial experiment laid under a completely randomized design with three replications. The 12 levels denominated bottle gourd landraces, while the 2 levels representing watering regimes (water-water-stressed and non-water-stressed conditions).

Plants were grown using a 2 L capacity drained-polyethylene plastic pots. A loamy soil of known physical properties collected from Ukulinga Research Farm, Pietermaritzburg, South Africa was used for the study. The soil was sieved through a 1 cm mesh to remove stones and clods. Each pot was filled with 2 kg of the soil. Fertilizer was applied based on soil fertility analysis using

watermelon nutrient requirements as a reference. Thus far there is no fertilizer recommendation for bottle gourd. Plants under the water-water-stressed condition were irrigated until formation of six fully expanded leaves and thereafter irrigation was withheld for 10 days before sampling. Plants in the non-water-stressed condition were watered daily to maintain soil moisture content at approximately 40% (field capacity). The mean air temperature and relative humidity inside the glasshouse were maintained at 25 ± 2 °C and 60 ± 3%, respectively.

6.3 Data collection

6.3.1 Soil moisture content monitoring

Volumetric soil water content in the upper 6-10 cm of soil on a percentage by volume were monitored using a soil moisture probe (Type ML2X attached to HH2 moisture meter, Delta devices, Cambridge, England). Ten pots for stressed and well-watered treatment were monitored for changes in soil water content.

6.3.2 Measurements of gas exchange parameters and chlorophyll fluorescence parameters

Leaf gas exchange and chlorophyll fluorescence were measured simultaneously using the LI-6400 XT Portable Photosynthesis System (Licor Bioscience, Inc. Lincoln, Nebraska, USA) fitted with an infrared gas analyzer attached to a leaf chamber fluorometer (LCF) (6400-40B, 2 cm^2 leaf area, Licor Bioscience, Inc. Lincoln, Nebraska, USA). Leaf temperature was maintained at 25°C, 400 µmol mol^{-1} of external leaf CO_2 concentration (C_a) and artificial saturating photosynthetic active radiation (PAR) was fixed at 1000 µmol m^{-2} s^{-1}, using a red-blue LED light source built into the leaf chamber fluorimeter (LCF). CO_2 was removed from external air using soda lime and mixed with pure liquid CO_2 to control leaf air CO_2 concentration in the sensor head. Flow rate was maintained at 500 µml and relative humidity maintained at 43%. The leaf-to-air vapor pressure deficit in the cuvette was maintained at 1.7 kPa to prevent stomatal closure due to the low air humidity effect. Measurements were taken between 08h00 to 11h00 h on the third half-fully expanded leaf from the tip of the plant by clamping the leaf inside the sensor head. Measurements were made from three independent plants for each genotype under non-stressed and water-stressed conditions. Gas exchange parameters such as stomatal conductance (*gs*, mmol m^{-2} s^{-1}),

photosynthetic rate/ net CO_2 assimilation rate (*A*, µmol $CO_2 m^{-2} s^{-1}$), transpiration rate (*T*, mmol H_2O m^{-2} s^{-1}), intercellular CO_2 concentration (C_i, µmol. mol m^{-1}) and ratio of intercellular and atmospheric CO_2 (C_i/C_a) concentrations. Ratio of net CO_2 assimilation rate and intercellular CO_2 concentration (A/C_i) was calculated according to Dong *et al.* (2016). Two types of water-use efficiencies were calculated: Intrinsic water use efficiency (WUE_i) calculated as the ratio of *A* and *gs* (Martin and Ruiz-Torres, 1992; Osmond *et al.*, 1999) and instantaneous water-use efficiency (WUE_{ins}), calculated as the ratio of *A* and *T* (Anyia and Herzog, 2004; de Santana *et al.*, 2015).

Chlorophyll fluorescence parameters such as minimal fluorescence (F_o') of light-adapted leaves and maximum florescence (F_m') were recorded by providing a saturating flash intensity of 1300 µmol m^{-2} s^{-1} and flash duration of 0.9 s using the LCF. The steady-state (Fs) fluorescence was also determined in light-adapted leaves under steady-state photosynthesis. The variable fluorescence was calculated in light adapted-leaves as $F_v' = F_m' - F_o'$ whereas change in fluorescence was calculated as ΔF = Fm' - Fs. Based on the measured chlorophyll fluorescence parameters some photochemical variables were calculated according to Maxwell and Johnson (2000) and Genty *et al*. (1989): The F_v'/F_m', PSII maximum efficiency which estimates the maximum quantum efficiency of PSII under light conditions (equation 1), quantum yield of PSII (Φ PSII) (equation 2), where Fs is "steady-state" fluorescence, photochemical quenching (qP) (equation 3) and non-photochemical quenching (qN) (equation 4), which measures heat dissipation of absorbed light energy. Electron transport rate (ETR) which measures the actual flux of photons (µ $molm^{-2}$ s^{-1}) driving PSII relatively to photosystem I (PS I), were calculated according to equation 5, where 0.5 is the fraction of absorbed light energy that is used by PS II, 0.84 as the fractional light absorption (PPFD) by the leaf (Baker *et al.*, 2007). The LI-6400 XT portable photosynthesis system automatically calculates these parameters. Relative measure of electron transport to oxygen molecules was calculated by the ratio of ETR/A (equation 6) (Flexas *et al.*, 2002). The alternative electron sinks (AES) was estimated by the relationship between the effective quantum efficiency of PS II and the quantum efficiency of CO_2 assimilation (A) (equation 7) (Ribeiro *et al.*, 2004).

$Fv'/Fm' = (Fm' - Fo')/Fm'$ Equation 1

Φ PSII = $(Fm' - Fs)/Fm' = \Delta F/Fm'$ Equation 2

qP = $(Fm' - Fs)/(Fm' - Fo')$ Equation 3

qN = $1 - (F_m' - F_o')/(F_m - F_o)$ Equation 4

ETR = Φ PSII x PPFD x 0.5 x 0.84 Equation 5

Electron transport to O_2 molecules = ETR/A Equation 6

AES = $\Delta F/ \Phi CO_2$; where ΦCO_2 = A/PPFD x 0.84 Equation 7

6.3.3 Determination of cucurbitacins

Cucurbitacins content was determined using a method described by Davidovich-Rikanati *et al*. (2015) with some modifications. Leaves were destructively harvested, freeze-dried and ground to powder. Leaf samples (0.2 g) were homogenized with 10 ml 80% methanol (MeOH), vortexed for 30s using a homogenizer (ULTRA-TURRAX, IKA® T25 digital, Staufen, Germany). The mixture was manually shaken using IKA® (ks 130, Staufen, Germany) for 60 min, repeating the vortex every 10 min. Debris and particles were discarded by centrifugation (5000×g) for 5 min; 1ml of each sample was then filtered through Acrodisc® syringe filters with GHP membrane, 13 mm× 0.2 μm (PALL, USA), and transferred to vials for high pressure liquid pressure liquid chromatography mass spectra (HPLC-MS) analysis. Cucurbitacin analysis was performed using a Shimadzu LCMS-2020 HPLC equipped with a Shim-pack GIST 3um C18-HP 4, 6 x 150 mm column with 0.01% aqueous formic acid B: CAN 20-40% C at 20 min, 80% C at 40 min, 90% at 50.5 min, held for 4.5 min, 20% C at 55.5 min held for 4.5 min (run time 60 min, aqc time 55 min). Flow rate was maintained at 0.5 ml/min at 40°C. Cucurbitacin identification was done by comparison of retention time and exact mass spectrum of purchased Cucurbitacins E and I standards (Sigma-Aldrich) and detected at 210 nm, with a resolution of 4nm. Quantification of

cucurbitacin E and I in samples was done using an external calibration curve on a dry weight basis, identity and purity based on retention time, peak area, UV spectra and chromatographs with authentic standards. Cucurbitacin analysis was performed at the Mass Spectroscopy laboratory, School of Chemistry, University of KwaZulu-Natal, South Africa.

6.4 Data analysis

Data on cucurbitacin content, leaf gas exchange and chlorophyll fluorescence parameters were subjected to analysis of variance using GenStat version 14th Edition (Payne *et al.*, 2011). Mean values recorded among landraces were compared using the least significant difference (LSD) test procedure at 5% level of significance. Correlation analysis was performed to describe the pattern of association between cucurbitacin content with leaf gas exchange and chlorophyll fluorescence parameters using SPSS 16.0 (SPSS, 2007). Significance tests of the correlation coefficients were determined using the Student's *t* test (Snedecor and Cochran, 1989). Principle component analysis (PCA) based on the correlation matrix was performed using SPSS 16.0. The bi-plot analysis was then used to describe and group bottle gourd genotypes for their level of drought tolerance according to Singh and Raja-Reddy (2011).

6.5 Results

6.5.1 Soil water content

Highly significant ($P < 0.001$) differences were observed between non-stressed and water-stressed treatments with respect to soil moisture content. Soil water content in the water-stressed treatments declined to approximately 10% after irrigation was withheld for 10 days. Water-stressed plants in this treatment were wilting and had drooping of leaves after 10 days of withholding water. In contrast, in the non-stressed treatments soil water content was high (above 30%) and was maintained for the entire experiment.

6.5.2 Changes in leaf gas exchange and chlorophyll fluorescence parameters among genotypes under water-stressed and non-stressed conditions

Significant differences were observed between the genotypes, water treatments and their interactions with respect to some leaf gas exchange and chlorophyll fluorescence parameters (Table 6.2). Intercellular CO_2 concentration and ratio of intercellular CO_2 and atmospheric CO_2 concentrations were not significantly affected by the interaction of the genotype by water regime suggesting that genotypes did not differ with regards to these parameters under water-stressed and non-stressed conditions (Table 6.2). Several chlorophyll fluorescence parameters were not significantly ($P < 0.05$) affected by water stress. A significant genotype x water regime effect was observed for stomatal conductance, transpiration rate, net CO_2 assimilation rate, ratio of net CO_2 assimilation rate and intercellular CO_2 concentration, intrinsic and instantaneous water use efficiencies and chlorophyll fluorescence parameters such as F_v'/F_m', ΦPS II, qP, qN, ETR, ETR/A and AES. A significant ($P < 0.05$) effect of the genotype × water stress interaction suggests differential response of genotypes under water-stressed and non-stressed conditions (Table 6.2).

Table 6.2. Analysis of variance (ANOVA) showing mean squares and significant tests of leaf gas exchange and chlorophyll fluorescence parameters of bottle gourd landraces under water-stressed and non-stressed conditions.

Leaf gas exchange parameters									
Source of variation	df	*gs*	*T*	*A*	*A/Ci*	*Ci*	*Ci/Ca*	WUE_i	WUE_{ins}
Genotype	11	0.04*	16.84*	46.8**	0.004**	5037ns	0.027ns	3057**	4.23**
Water regime	1	0.92**	535.8**	2601.8**	0.04**	78304**	0.421**	15221**	56.9**
Genotype x water regime	11	0.06**	21.12**	59.35**	0.004**	4504ns	0.026ns	2191**	3.82**
Residual	46	0.01	4.82	5.07	0.001	4405	0.025	270	0.91

Chlorophyll fluorescence parameters								
Source of variation	df	*Fv'/Fm'*	Φ PS II	qP	qN	ETR	ETR/A	AES
Genotype	11	0.02*	0.024*	0.06**	0.88*	5731*	11873**	80442**
Water regime	1	3E-05ns	0.008ns	0.05ns	0.123ns	7403*	77190**	862457**
Genotype x water regime	11	0.012*	0.047**	0.10**	0.71*	15374**	12561**	88236**
Residual	46	0.005	0.007	0.01	0.27	1628	1300	6003

gs, stomatal conductance; *T*, transpiration rate, *A*; net CO_2 assimilation rate; A/C_i, CO_2 assimilation rate/intercellular CO_2 concentration; C_i, intercellular CO_2 concentration; C_i/C_a, ratio of intercellular and atmospheric CO_2; WUE_i, intrinsic water use efficiency; WUE_{ins}, instantaneous water-use efficiency; F_v'/F_m', the efficiency of energy harvested by oxidized (open) PSII reaction centers in light-adapted leaves; Φ PSII, quantum yield of PSII; qP, photochemical quenching; qN, non-photochemical quenching; ETR, electron transport rate; ETR/A, relative measure of electron transport to oxygen molecules; AES, alternative electron sinks. * significant at the 0.05 probability level, ** significant at the 0.01 probability level, non-significant difference. df, degrees of freedom.

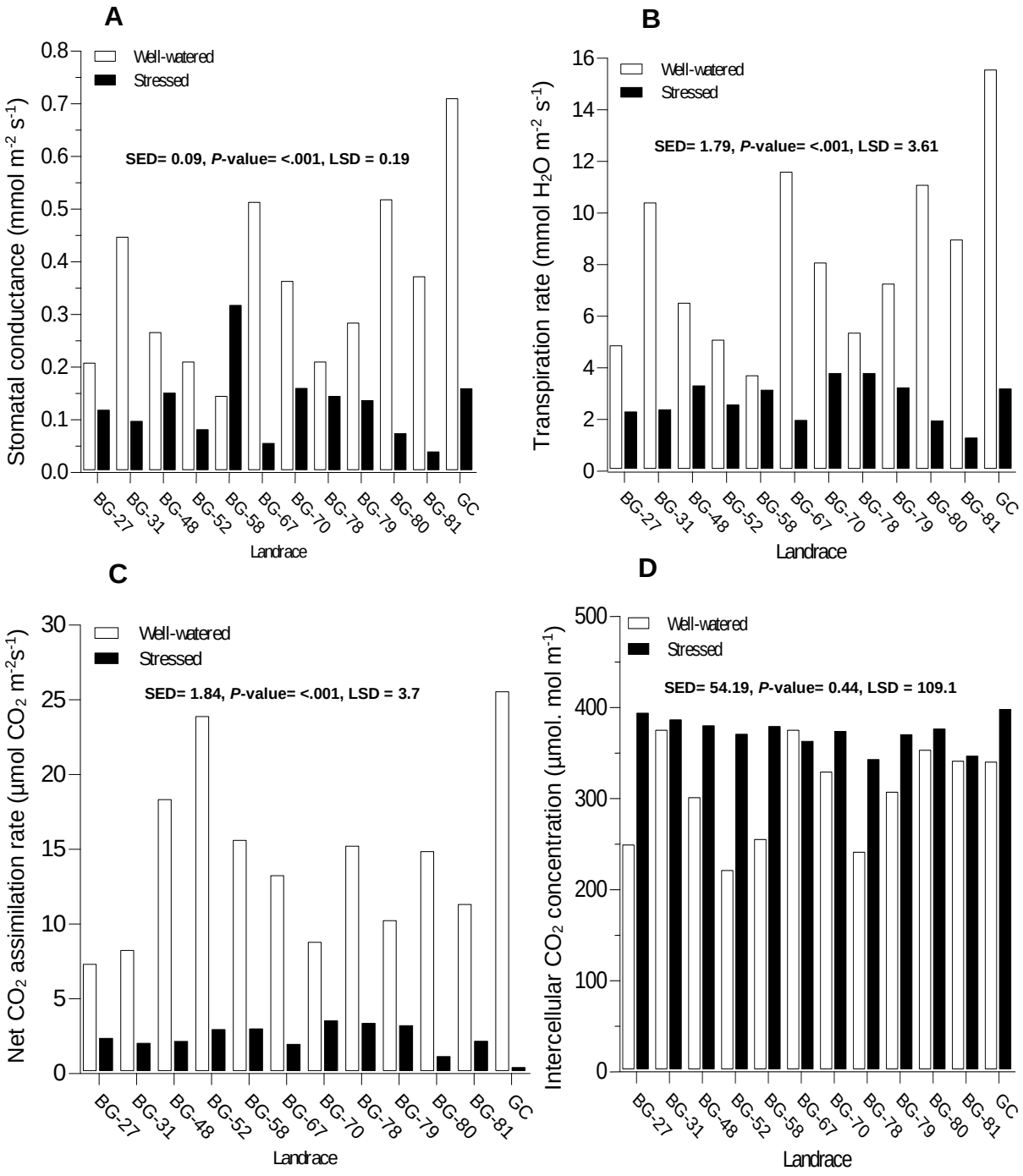
6.5.3 Changes in leaf gas exchange in response to water stress

Changes in leaf gas exchange parameters in response to water stress among the tested bottle gourd landraces are shown in Figures 6.1A-H. Under non-stressed conditions, BG-31, BG-67, BG-80 and GC maintained higher stomatal conductance (*gs*) values (> 0.4 mmol m^{-2} s^{-1}); relative to BG-27, BG-48, BG-70, BG-78, BG-79 and GC which were lower (> 0.1 mmol m^{-2} s^{-1} but < 0.4 mmol m^{-2} s^{-1}) (Figure 6.1A). Stomatal conductance values for the landraces BG-67, BG-80 and BG-81 showed a reduction of 89.5, 86.0 and 89.9% in response to water stress, which was considerably higher compared to other landraces. Clear genotypic differences were observed with respect to transpiration rates (*T*) among the landraces. Genotypes BG-27, BG-48, BG-52, BG-58, BG-70, BG-78 and BG-79 recorded *T* rate values < 10 mmol H_2O m^{-2} s^{-1}; whereas, BG-31, BG-67 and GC had values > 10 mmol H_2O m^{-2} s^{-1} under non-stressed conditions (Figure 6.1B). Water stress had a significant ($P < 0.05$) effect on lowering *T* rates and the level of reduction was higher for BG-67 (83.3 %), BG-80 (82.7 %) compared to BG-81 (85.9 %). BG-70 and BG-78 showed less reduction in *T* (53.3 and 29.4%, respectively) under water-stressed conditions. With regards to net CO_2 assimilation rate (*A*) under non-stressed conditions, BG-27, BG-31 and BG-70 recorded values <

10 µmol CO_2 m^{-2} s^{-1}; BG-67, BG-79, BG-80 and BG-81 > 10 µmol CO_2 m^{-2} s^{-1}; whereas, BG-48, BG-52, BG-58 and GC recorded values > 15 µmol CO_2 m^{-2} s^{-1} (Figure 6.1C). The reduction in *A* was significant ($P < 0.05$) and considerably severe for BG-80 and GC (92.7 and 98.7%, respectively) compared to other landraces under water stress conditions.

Generally, the mean leaf stomatal conductance and transpiration rates averaged across all genotypes followed a pattern similar to the net assimilation rate. The reduction in stomatal conductance due to water stress was probably responsible for the decline in transpiration and net assimilation rates. Intercellular CO_2 concentration increased significantly ($P < 0.05$) in response to water stress, despite the reduction in stomatal conductance and transpiration rate; however, no significant ($P > 0.05$) differences with respect to intercellular CO_2 concentration were observed among landraces under both non-stressed and water-stressed conditions (Figure 6.1D). Highly significant ($P < 0.001$) values with respect to the net assimilation rate/intercellular CO_2 ratio (A/C_i) were observed for BG-58, BG-79 and GC under non-stressed condition compared to other landraces (Figure 6.1E). Under water-stressed condition, BG-52, BG-58, BG-78 and BG-79 exhibited significantly ($P < 0.05$) higher A/C_i (> 0.007 µmol. mol m^{-1}) values. Ratio of intercellular to atmospheric CO_2 concentration (C_i/C_a) was significantly higher ($P < 0.05$) higher under water-stressed than non-stressed conditions; however, non-significant ($P > 0.05$) differences were observed among landraces under both conditions (Figure 6.1F). Water stress significantly ($P < 0.05$) reduced intrinsic water use efficiency (WUE) by 57.6% across all genotypes. BG-48, BG-52, BG-58 and BG-78 maintained significantly higher WUE_i values [> 70 (CO_2) m^{-2} (H_2O)] under non-stressed conditions which compared to other landraces. Under water-stressed condition, BG-67 and BG-81 showed a significant improvement in WUE_i by 38 and 64.5 %, respectively compared to non-stressed plants; whereas, BG-31 and BG-70 exhibited less reduction in WUE_i (14.4 and 8.8 %, respectively) (Figure 6.1G). The highest reduction in WUE_i was observed for BG-58 (89.9%) and GC (93.5 %), respectively. Further, water stress significantly reduced instantaneous water use efficiency (WUE_{ins}) by 69.3% across all genotypes (Figure 6.1H). BG-31, BG-52 and BG-58 showed significantly higher WUE_{ins} (> 3 µmol (CO_2) m^{-2} (H_2O) under non-stressed conditions. Under water stress conditions, BG-81 showed significantly higher WUE_{ins}, an improvement of 30.2%; whereas, GC showed severe reduction (92.6 %) in WUE_{ins}. Generally,

bottle gourd landraces showed variable responses with respect to leaf gas exchange parameters under both non-stressed and water-stressed conditions.



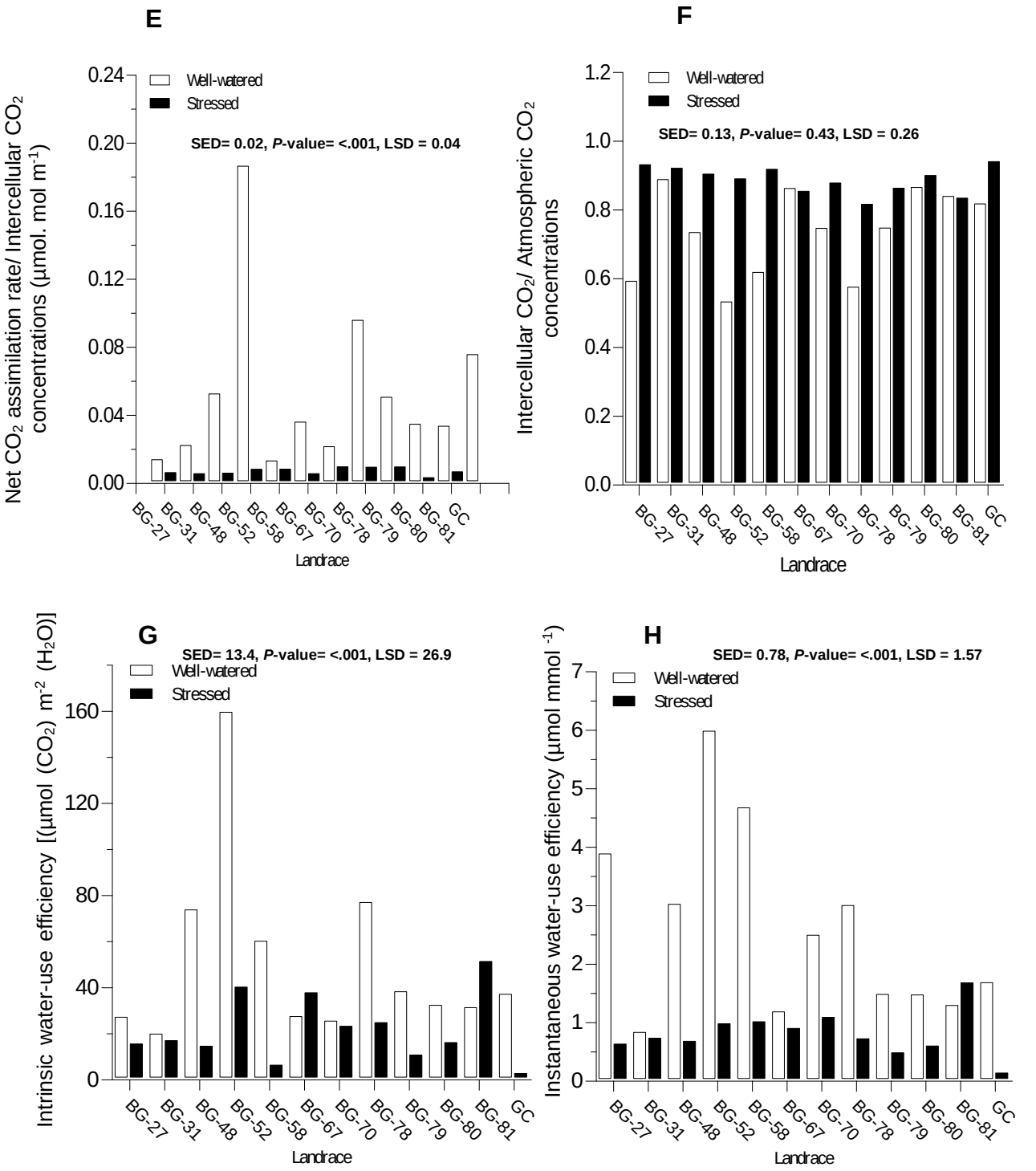
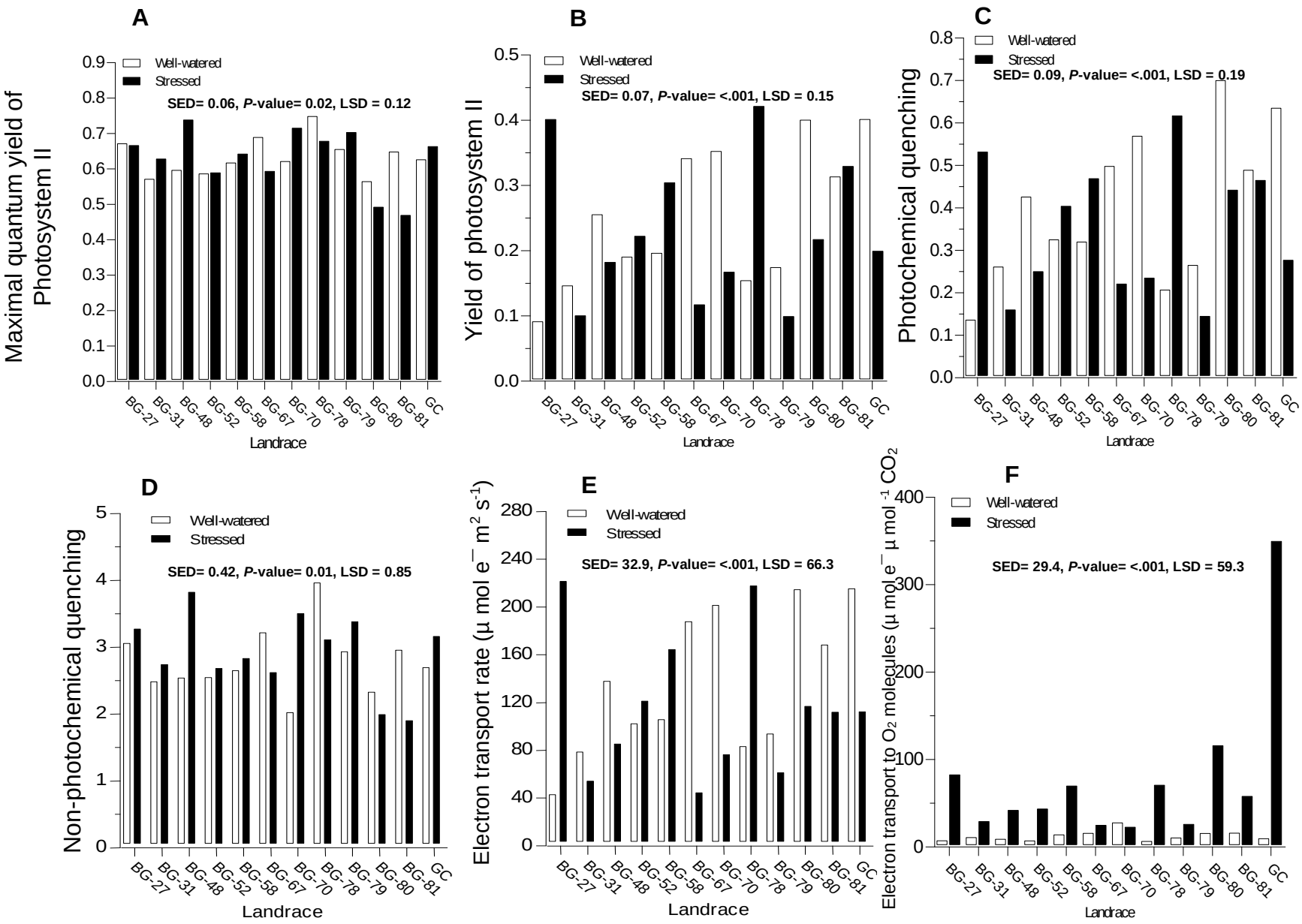


Figure 6.1. Effect of water stress on leaf gas exchange parameters among 12 bottle gourd landraces subjected to non-stressed and water-stressed conditions. Stomatal conductance (A); transpiration rate (B); net CO_2 assimilation rate (C); CO_2 concentration in the intercellular airspaces (D); ratio of CO_2 assimilation rate and intercellular CO_2 concentrations (E); ratio of intercellular and atmospheric CO_2 concentration (F); intrinsic water-use efficiency (G) and instantaneous water-use efficiency (H).

6.5.4 Changes in photosynthetic efficiency (chlorophyll fluorescence) among bottle gourd landraces in response to water stress

The effect of water stress on bottle gourd landraces in relation to photosynthetic efficiency was evaluated (Figures 6.2; A-G). Maximum quantum efficiency of PSII (F_v'/F_m') did not vary significantly among the tested landraces under non-stressed condition (Figure 6.2A). However, significant ($P < 0.05$) differences among the tested landraces were observed under water-stressed condition. BG-81 and BG-80 showed significantly reduced photosynthetic efficiency with F_v'/F_m' values of 0.47 and 0.49, respectively; whereas, BG-31, BG-48, BG-67, BG-70, BG-79 and GC showed significantly higher F_v'/F_m' values (≥ 0.6) (Figure 6.2B). Quantum yield of photosystem II (Φ PSII) varied significantly ($P < 0.05$) among the tested landraces under both non-stressed and water-stressed conditions (Figure 6.2B). BG-67, BG-70, BG-80 and GC exhibited significantly higher ΦPSII values (> 0.3) compared to other landraces under non-stressed condition. On the contrary, BG-27, BG-52, BG-58, BG-78 and BG-81 showed increased quantum yield of ΦPSII (≥ 0.3) under water-stressed condition. Photochemical quenching (qP) was significantly increased by water stress among the following landraces: BG-27, BG-52, BG-58 and BG-78 (> 0.4); whereas, it was reduced for BG-31, BG-48, BG-67, BG-70, BG-79, BG-80 and GC (< 3) (Figure 6.2C). On a percentage basis, increase in qP varied from 31.9 % (BG-58) to 66.7% (BG-78) under water-stressed condition. Non-photochemical quenching (qN) was increased by water stress for all landraces except BG-70, BG-78, BG-80 and BG-81 (Figure 6.2D). Also, varying genotypic responses with respect to qN were observed under non-stressed condition. Electron transport rate (ETR) increased significantly for BG-27 (81.1%), BG-52 (15.8%), BG-58 (35.9%) and BG-78 (62.1%) under water-stressed condition; but was reduced for the other landraces. The landraces varied under non-stressed condition with respect to ETR. The landraces BG-67, BG-70, BG-80 and GC exhibited ETR values > 150 µ mol e^- m^2 s^{-1}; whereas, BG-27, BG-31 and BG-79 showed ETR values > 90 µ mol e^- m^2 s^{-1} under non-stressed condition (Figure 6.2E). Water stress increased the relative measure of electron transport to oxygen molecules (ETR/A) ratio significantly ($P <$. 001) for BG-27, BG-80 and GC (Figure 6.2F). Water stress significantly increased alternative electron sinks (AES) in relation to non-stressed plants (Figure 6.2G). BG-27, BG-58 and GC showed significantly higher AES values compared to other landraces under water-stressed condition.


A
Maximal quantum yield of Photosystem II
Well-watered
Stressed
SED= 0.06, P-value= 0.02, LSD = 0.12
Landrace
B
Yield of photosystem II
SED= 0.07, P-value= <.001, LSD = 0.15
C
Photochemical quenching
SED= 0.09, P-value= <.001, LSD = 0.19
D
Non-photochemical quenching
SED= 0.42, P-value= 0.01, LSD = 0.85
E
Electron transport rate (µ mol e⁻ m² s⁻¹)
SED= 32.9, P-value= <.001, LSD = 66.3
F
Electron transport to O2 molecules (µ mol e⁻ µ mol⁻¹ CO2
SED= 29.4, P-value= <.001, LSD = 59.3
BG-27
BG-31
BG-48
BG-52
BG-58
BG-67
BG-70
BG-78
BG-79
BG-80
BG-81
GC

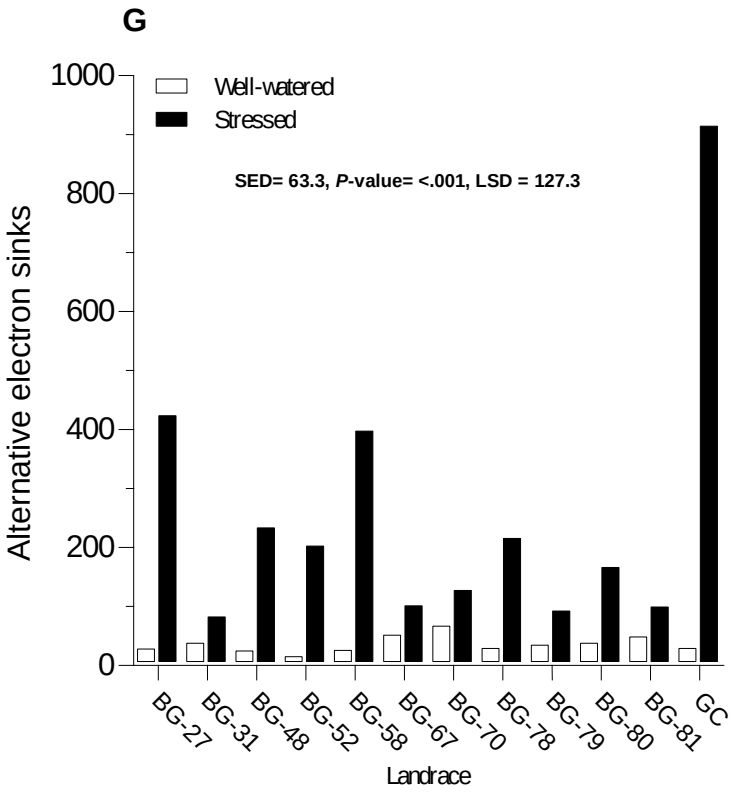


Figure 6.2. Effect of water stress on chlorophyll fluorescence parameters in light-adapted leaves of 12 bottle gourd landraces subjected to non-stressed and water-stressed conditions. Maximal quantum yield of photochemistry of photosystem II (A); quantum yield of photosystem II (B); photochemical quenching (C); non-photochemical quenching (D); electron transport rate (E); electron transport to O_2 molecules (F) and alternative electron sinks (G).

6.5.5 Cucurbitacin E and I accumulation in bottle gourd landraces under non-stressed and water-stressed conditions

Cucurbitacin E and I accumulation among the bottle under non-stressed and water-stressed conditions are shown in Table 6.3. Representative chromatograms are shown in Figure 6.3. Under non-stressed condition, cucurbitacin E was only detected in BG-81 whereas it was not detected in other landraces. Under water-stressed condition, cucurbitacin E was only detected in BG-58 and BG-81. Cucurbitacin I was only detected in BG-27 and BG-31 under non-stressed condition, whereas, it was detected in BG-27, BG-31, BG-67, BG-70, BG-78, BG-79 and GC under water-stressed condition.

Table 6.3. Summary of cucurbitacins E and I detection and concentration in 12 bottle gourd landraces subjected to non-stressed and water-stressed conditions.

	Cucurbitacin E (mg/g)		Cucurbitacin I (mg/g)	
Landrace	NS	WS	NS	WS
BG-27	ND	ND	0.28	0.17
BG-31	ND	ND	0.20	0.10
BG-48	ND	ND	ND	ND
BG-52	ND	ND	ND	ND
BG-58	ND	0.06	ND	ND
BG-67	ND	ND	ND	ND
BG-70	ND	ND	ND	14.0
BG-72	ND	ND	ND	ND
BG-78	ND	ND	ND	ND
BG-79	ND	ND	ND	0.08
BG-81	0.05	0.05	ND	0.12
GC	ND	ND	ND	0.26

ND = Not detected NS=non-stressed, WS= water-stressed

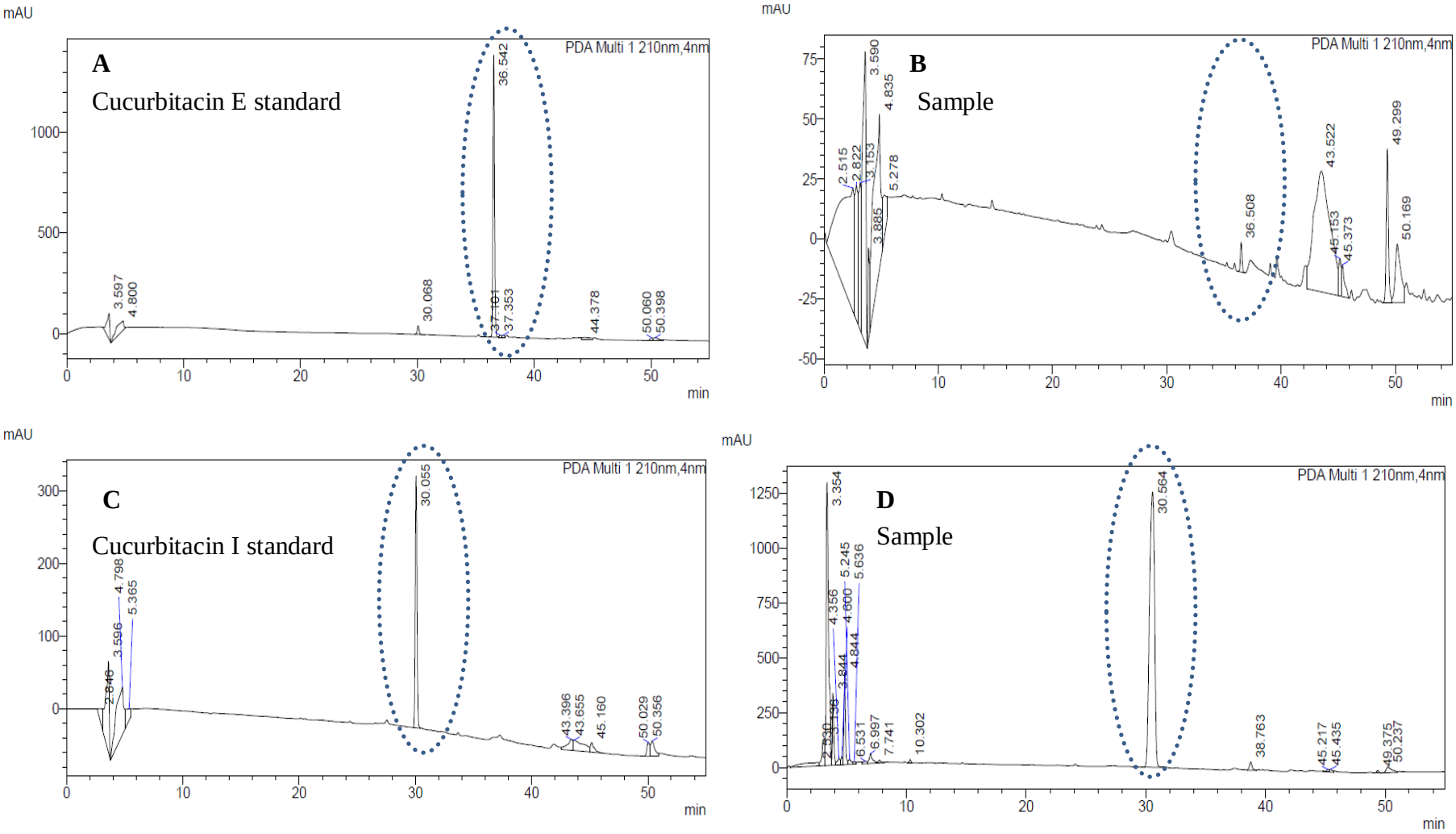


Figure 6.3. Chromatograms showing mass spectra and retention time of cucurbitacin E standard (A), detection of cucurbitacin E in sample (B), cucurbitacin I standard (C) and detection of cucurbitacin I in sample (D).

6.5.6 Correlation analysis between cucurbitacin content with leaf gas exchange and chlorophyll fluorescence parameters

Pearson correlation coefficients showing pair-wise associations between cucurbitacin I with leaf gas exchange and chlorophyll fluorescence parameters are presented in Tables 6.4 and 6.5, respectively. Non-significant correlations ($P > 0.05$) were observed between cucurbitacin I and all leaf gas exchange parameters under water-stressed condition (Table 6.4). Also, non-significant correlations ($P > 0.05$) were observed between cucurbitacin I with F_v'/F_m', ΦPS II, qP, qN and ETR under water-stressed condition. However, there were significant correlations ($P < 0.05$) between cucurbitacin I with ETR/A ($r = 0.88$; $P = 0.02$) and AES ($r = 0.86$; $P = 0.03$).

Table 6.4. Pearson coefficients showing pair-wise association between cucurbitacin I with leaf gas exchange parameters under water-stressed condition among 12 bottle gourd landraces that were detected for cucurbitacin I.

Parameter	*gs*	*T*	*A*	C_i	A/C_i	C_i/C_a	IWUE	WUE_{ins}
Cucurbitacin I	0.54^{ns}	0.73^{ns}	0.72^{ns}	-0.14^{ns}	0.71^{ns}	-0.28^{ns}	0.06^{ns}	0.25^{ns}

gs = stomatal conductance; *T* = transpiration rate; *A* = net CO_2 assimilation rate; A/C_i = CO_2 assimilation rate/intercellular CO_2 concentration; C_i = intercellular CO_2 concentration; C_i/C_a = ratio of intercellular and atmospheric CO_2 concentrations; WUE_i = intrinsic water use efficiency; WUE_{ins} = instantaneous water-use efficiency; ns = non-significant.

Table 6.5. Pearson coefficients showing pair-wise association between cucurbitacin I with chlorophyll fluorescence parameters under water-stressed condition among 12 bottle gourd landraces that were detected for cucurbitacin I.

Parameter	F_v'/F_m'	ΦPS II	qP	qN	ETR	ETR/A	AES
Cucurbitacin I	0.05^{ns}	0.25^{ns}	0.23^{ns}	-0.71^{ns}	0.32^{ns}	0.88^{*}	0.86^{*}

F_v'/F_m' = the efficiency of energy harvested by oxidized (open) PSII reaction centers in light-adapted leaves; Φ PSII = quantum yield of PSII; qP = photochemical quenching; qN = non-photochemical quenching; ETR = electron transport rate; ETR/A = relative measure of electron transport to oxygen molecules; AES = alternative electron sinks; ns= non-significant. *****significant difference at the 0.05 probability level.

6.5.7 Principal component analysis

Principal component analyses (PCA) of leaf gas exchange and chlorophyll fluorescence parameters under non-stressed water-stressed conditions are presented in Table 6.6. Under non-stressed condition, PCA revealed five PCs which accounted for 97.4 % of total variation. The

parameters *gs*, *T*, C_i, C_i/C_a, WUE_{ins}, positively correlated with PC1 which accounted for 48.2% of the total variation, while Φ PS II, qP, ETR and ETR/A positively correlated with PC2, which accounted for 21.9 % of the total variation. The parameters *A*, A/C_i and WUE_i positively correlated with PC 3; F_v'/F_m', qN positively correlated with PC 4 whereas AES positively correlated with PC 5 which accounted for 12.8, 7.5 and 7.1 of total variances, respectively. Under water-stressed condition, *A*, A/C_i, WUE_i and WUE_{ins} negatively correlated with PC 1 whereas C_i, C_iC_a, ETR/A and AES positively correlated with PC 1 which accounted for 36.3% of total variation. *gs*, *T*, F_v'/F_m' and qN positively correlated with PC 2 which accounted for 26.01% of total variation. ΦPS II, qP, and ETR positively correlated with PC 3 which accounted for 21.6% of total variation.

Table 6.6. Principal component analysis showing eigenvectors, eigenvalues, and percent variance of leaf gas exchange and chlorophyll fluorescence parameters of 12 bottle gourd landraces under non-stressed and water-stressed conditions.

	Non-stressed					Water-stressed		
Parameters	PC 1	PC 2	PC 3	PC 4	PC 5	PC 1	PC 2	PC 3
gs	**0.88**	0.37	0.02	-0.07	0.21	0.20	**0.73**	0.30
T	**0.91**	0.34	0.03	-0.05	0.18	-0.03	**0.92**	0.05
A	0.05	0.22	**0.81**	-0.07	0.51	**-0.82**	0.50	0.15
C_i	**0.84**	0.29	-0.34	-0.18	-0.22	**0.81**	0.29	-0.23
A/C_i	-0.18	-0.05	**0.96**	0.06	-0.03	**-0.85**	0.45	0.12
C_iC_a	**0.86**	0.26	-0.31	-0.20	-0.19	**0.81**	0.21	-0.11
WUE_i	-0.51	-0.10	**0.84**	-0.09	-0.01	**-0.65**	-0.64	0.03
WUE_{ins}	**-0.85**	-0.12	0.39	-0.13	0.26	**-0.69**	-0.42	0.16
F_v'/F_m'	-0.14	-0.06	-0.11	**0.97**	-0.02	0.04	**0.95**	-0.15
ΦPSII	0.47	**0.86**	0.06	-0.11	0.11	-0.10	-0.04	**0.97**
qP	0.45	**0.83**	0.07	-0.25	0.10	-0.11	-0.18	**0.96**
qN	-0.02	-0.34	0.12	**0.92**	-0.01	0.06	**0.90**	-0.16
ETR	0.45	**0.88**	0.05	-0.12	0.08	0.06	0.15	**0.95**
ETR/A	-0.02	**0.75**	-0.50	-0.22	-0.28	**0.84**	-0.05	0.22
AES	-0.07	0.03	0.10	-0.02	**0.99**	**0.76**	0.27	0.42
Explained variance (eigenvalue)	7.23	3.28	1.92	1.12	1.06	5.44	3.90	3.24
Proportion of total variance (%)	48.20	21.90	12.78	7.45	7.09	36.29	26.01	21.57
Cumulative variance (%)	48.20	70.10	82.88	90.33	97.42	36.29	62.30	83.87

Vector loadings ≥ 0.7 are boldfaced. *gs* = stomatal conductance; *T* = transpiration rate; *A* = net CO_2 assimilation rate; A/C_i = CO_2 assimilation rate/intercellular CO_2 concentration; C_i = intercellular CO_2 concentration; C_i/C_a = ratio of intercellular and atmospheric CO_2 concentrations; WUE_i = intrinsic water use efficiency; WUE_{ins} = instantaneous water-use efficiency; F_v'/F_m' = the efficiency of energy harvested by oxidized (open) PSII reaction centers in light-adapted leaves; Φ PSII = quantum yield of PSII; qP = photochemical quenching; qN = non-photochemical quenching; ETR = electron transport rate; ETR/A = relative measure of electron transport to oxygen molecules; AES = alternative electron sinks.

6.5.8 Principal component bi-plot analysis

Principal component biplots were used to visualize the relationships among bottle gourd landraces with leaf gas exchange and chlorophyll fluorescence parameters under non-stressed and water-stressed conditions (Figure 6.4 A & B). Smaller angles between dimension vectors in the same direction indicated high correlation of the variables in terms of discriminating genotypes. Genotypes excelling in a particular trait were plotted closer to the vector line and further in the direction of that particular vector. Under non-stressed condition, 4 bottle gourd landraces (i.e., BG-80, BG-81, BG-67 and GC) were differentiated by high *gs*, *T*, *A*, C_i and C_iC_a. Genotypes BG-48, BG-52, BG-58 and BG-78 exhibited high values of WUE_i and WUE_{ins} under water-stressed condition (Figure 6.4A). With regards to chlorophyll fluorescence parameters, BG-80, BG-81, BG-67 and GC showed better performance for $F_v'F_m'$, ΦPS II, qP and ETR and can be regarded as productive landraces under non-stressed condition. Under water-stressed condition, BG-48, BG-58, BG-70, BG-78 and BG-79 showed consistently higher values for *gs*, *T*, *A*, A/C_i, $F_v'F_m'$ and qN and may be regarded as drought tolerant. BG-52, BG-67 and BG-81 showed higher values for WUE_i and WUE_{ins} under water stress condition (Figure 6.4B).

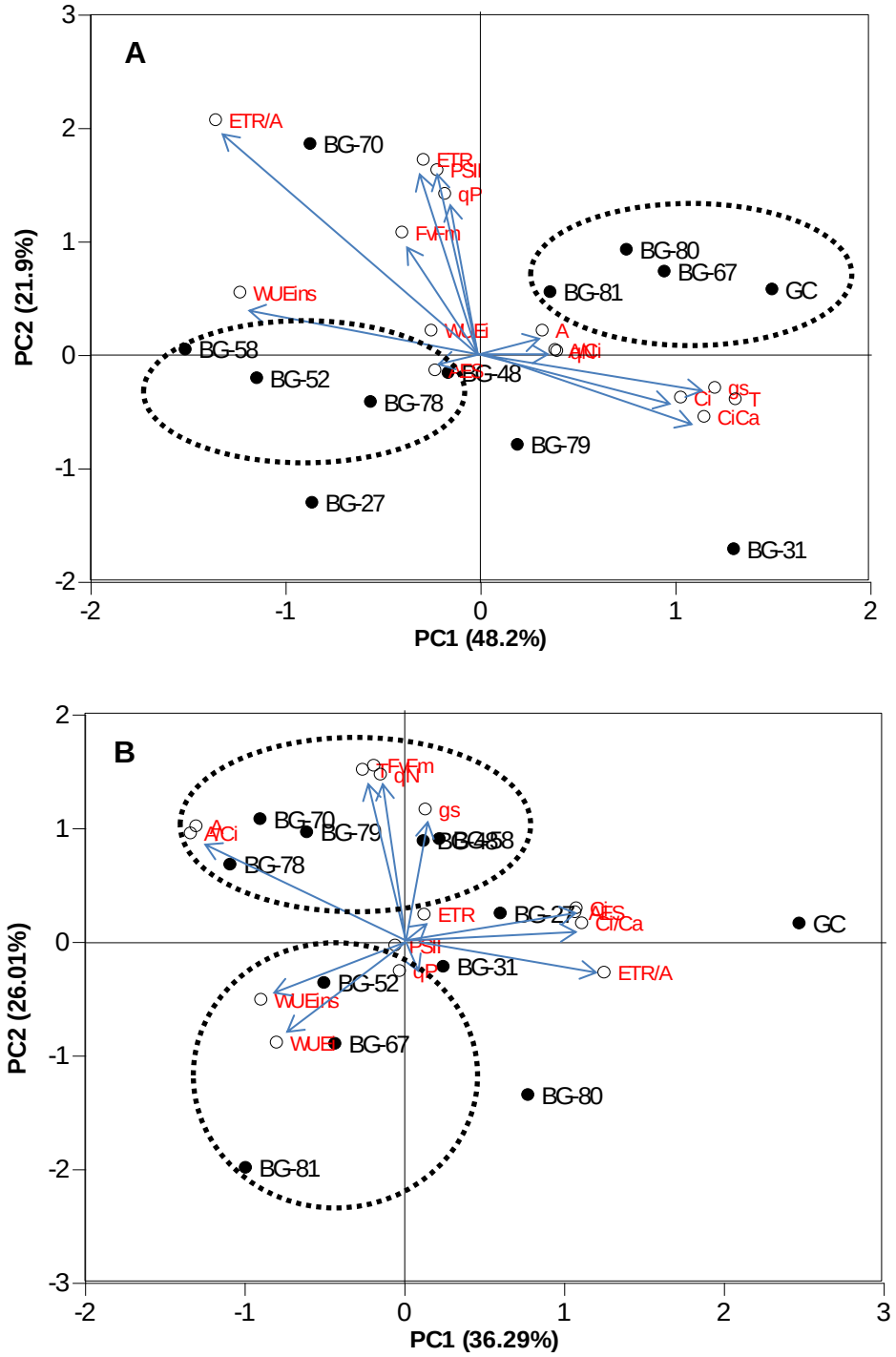


Figure 6.4. Varimax rotated principal component biplot loadings of PC1 vs. PC2 showing the grouping of bottle gourd landraces based on gas exchange and chlorophyll fluorescence parameters under non-stressed (A) and water-stressed (B) conditions. *gs* = stomatal conductance; *T* = transpiration rate; *A* = net CO_2 assimilation rate; A/C_i = CO_2 assimilation rate/intercellular CO_2 concentration; C_i = intercellular CO_2 concentration; C_i/C_a = ratio of intercellular and atmospheric CO_2 concentrations; WUE_i = intrinsic water use efficiency; WUE_{ins} = instantaneous water-use efficiency; F_v'/F_m' = the efficiency of energy harvested by oxidized (open) PSII reaction centers in light-adapted leaves; Φ PSII = quantum yield of PSII; qP = photochemical quenching; qN = non-photochemical quenching; ETR = electron transport rate; ETR/A = relative measure of electron transport to oxygen molecules; AES = alternative electron sinks.

6.6 Discussion

Knowledge of the physiological basis that may explain plant responses and adaptations to drought stress conditions could be important for drought tolerance breeding (Subbarao *et al.*, 1995). This study determined the dynamics of leaf gas exchange and chlorophyll fluorescence parameters in bottle gourd subjected to water stress. The study also related these changes to the accumulation of cucurbitacins E and I. In the present study, water stress reduced stomatal conductance (*gs*), transpiration rate (*T)* and the net CO_2 assimilation rate (*A*) (Figure 6.1A, B and C). Stomatal closure has been reported to cause a decline in intercellular CO_2 concentration (Cornic, 2000); but, results of the present study revealed that water-stressed plants had increased C_i concentration irrespectively of reduced *gs, T* and *A* (Figure 6.1D). Similar results where C_i increased were observed in water water-stressed cowpea (Singh and Raja Reddy, 2011), maize (Cruz de Carvalho *et al.*, 2011) and wheat (Guan *et al.*, 2014). Reduction in *A* was 84% which was more than the mean reduction in stomatal conductance (65%) which could be attributed to stomatal (i.e. low *g*s) and non-stomatal limitations (i.e. low A/C_i) (Dong *et al.*, 2016). Increased C_i/C_a (Figure 6.1F) in the current study further confirms the role of non-stomatal limitations of photosynthesis (Reddy *et al.*, 2004; Galle *et al.*, 2007; Singh and Raja Reddy, 2011; Flexas *et al.*, 2012).

Water use efficiency is an important physiological adaptation mechanism for improving crop productivity under water-limited conditions (Medrano *et al.*, 2015). In the current study, water stress reduced WUE_i (58%) for all genotypes; however, BG-31, BG-67, BG-70 were less affected; whereas, an increase was observed for BG-81 (Figure 6.1G). Further, water stress reduced WUE_{ins} by 74%, but, BG-81 showed higher WUE_{ins}; whereas, BG-31 and BG-67 were not affected (Figure 6.1H). Genotypic variability in WUE_i and WUE_{ins} among bottle gourd landraces could probably be due to variations in *A*, *gs* and *T* (Masle *et al.*, 2005; Erice *et al.*, 2011). Current findings further suggest that a certain threshold level is present where *gs* and *T* should decline before WUE is increased for different bottle gourd genotypes (Singh and Raja Reddy, 2011).

Chlorophyll fluorescence measurements are a direct indicator of the photosynthetic activity (Lichtenthaler and Babani, 2000) and allows estimation of the degree of injuries of photosystem II and to study the protection mechanisms involved in the removal of the excess of excitation energy from the photosynthetic apparatus (Araus *et al.*, 1998; Lu and Zhang,

1999). The present findings showed that water stress had no significant effect on F_v'/F_m' suggesting that the tested landraces are fairly tolerant to water stress (Hura *et al.*, 2009b). The quantum yield of PSII (ΦPSII) allows determination of the effectiveness of excitation energy utilized by chlorophyll a to drive of photosynthesis (Maxwell and Johnson, 2000. In the current study, water stress had no significant effect on ΦPSII. However, significant genotypic differences were recorded under water stress conditions (Figure 6.2B). Bottle gourd landraces such as BG-27, BG-52, BG-58, BG-78 and BG-81 showed increased ΦPSII yield compared to other landraces (Figure 6.2B). Two processes that quench the level of chlorophyll fluorescence in the light referred as photochemical (qP) and non-photochemical (qN) quenching were determined in the current study. qP is as an indicator of the proportion of open PSII reaction centers (Genty *et al.*, 1989; Maxwell and Johnson, 2000) and the energy used for driving photosynthesis (Hazrati *et al.*, 2016). qP values can be used to estimate the fraction of the reduction state of the primary quinone electron acceptor of photosytem II (QA), which reflects the excitation pressure on photosystem II. A decrease in qP values indicates an increase in the fraction of the reduction state of QA of PS II, which suggest increased susceptibility to photo-inhibition (Guan *et al.*, 2014). BG-27, BG-52, BG-58, BG-78 and BG-81 had higher qP values under water stress suggesting these landraces kept more PSII centers in an open state so that more excitation energy can be used for electron transport (Figure 6.2C) (Maxwell and Johnson, 2000) compared to other landraces.

Non-photochemical quenching (qN) is another important mechanism to prevent or alleviate damage caused by excessive light energy reaching the photosynthetic apparatus (Maxwell and Johnson, 2000). Non-photochemical quenching measures energy emitted as heat in the photosystem II (Baker and Rosenqvist, 2004). BG-48, BG-70, BG-79 and GC showed higher values of qN during water stress (Figure 6.2D) suggesting an efficient removal of excess excitation energy via thermal dissipation and thereby protecting leaves better from photo-damage (Souza *et al.*, 2004; Mo *et al.*, 2016). Electron transport rate declined due to water stress for BG-48, BG-70, BG-79 and GC whereas it was increased for BG-27, BG-58 and BG-78 (Figure 6.2E). Reduced stomatal conductance imposed on photosynthesis normally results in the reduction of the rate of consumption of ATP and NADPH for CO_2 assimilation, which could result in reduced rate of linear electron transport rate (Maxwell and Johnson, 2000). Increased ETR is indicative of an important mechanism for photo-inhibition protection (Singh and Raja Reddy, 2011; Yi *et al.*, 2016). An increase in ETR/A in the current study was observed

and indicates a relative increase in photorespiration (Wingler *et al.*, 1999; Singh and Raja Reddy, 2011).

The photo-respiratory pathway can function as alternative sinks for photon energy when photosynthetic CO_2 assimilation rate is restricted reducing the possible effects of oxidative stress (Lawlor and Cornic, 2002). We therefore speculate that the bottle gourd landraces evaluated in the current study possessed enhanced electron transport rate such as photorespiration during water stress (Yi *et al.*, 2016). An increase in AES was observed for all landraces under water stress conditions. Further, BG-27, BG-58 and GC showed significantly higher AES values compared to other landraces (Figure 6.2G). The ability to maintain or increase AES is indicative of drought tolerance (Rivas *et al.*, 2016) and is regarded as a protective strategy to avoid excess of energy at PSII thus reducing oxidative damage of the chloroplast (Ribeiro *et al.*, 2004; Santos *et al.*, 2009). According to Pinheiro and Chaves (2011), when CO_2 assimilation restriction is accompanied by an increase in an alternative electron sink (e.g. photorespiration or photophosphorylation), the non-cyclic electron transport reduction will be proportionally smaller than the decrease in the rate of CO_2 assimilation. Foyer and Mullineaux (1994) further reported that increased AES activity is related to the capacity of protective processes, such as the antioxidant system, photorespiration and photophosphorylation which probably alleviated the effects of excess light damage on photosynthetic apparatus among the bottle gourd landraces (Niyogi, 2000).

Biotic and abiotic stresses induce the accumulation of a wide variety of plant secondary metabolites, including alkaloids (Gregianini *et al.*, 2003; do Nascimento and Fett-Neto, 2010; de Costa *et al.*, 2013). Cucurbitacin concentrations are reported to be increased by water stress in cucurbits, suggesting their involvement of in drought tolerance. However, limited information is available concerning cucurbitacin composition and content in cucurbits and their correlation to drought tolerance. Findings of the current study showed that cucurbitacin E and I were detected in some cultivars whilst not detected in some (Table 6.3). This can be explained by the fact that farmers may have selected for non-bitterness since all the genotypes studied are used for food, either for consumption of leaves and fruits. Another possible reason could be that the level of water stress applied in the current study was not severe enough to trigger the enzyme/s related to accumulation of cucurbitacins for some genotypes. Several genes controlling accumulation of cucurbitacins have been reported namely: *bi (bi-1)* making fruit

and foliage bitter free and *Bt (Bt-1)* making the fruit highly bitter (Zhang *et al.*, 2013). The activity of these enzymes are stimulated or suppressed under environmental conditions (e.g. cool weather) (Mukherjee *et al.*, 2013).

Following our hypothesis that drought tolerance may be correlated to cucurbitacins accumulation, we performed correlation analysis between accumulated cucurbitacins I with leaf gas exchange and chlorophyll fluorescence parameters. Non-significant but strong and positive correlations were observed between cucurbitacin I with *T*, *A* and A/C_i suggesting possible involvement in the regulation of transpiration and photosynthesis possibly acting as an osmoprotectant under water stress condition. Since ETR/A and AES are indicative of photorespiration and protective process including antioxidant system; respectively, positive and strong correlations between cucurbitacin I with ETR/A and AES suggest their involvement in the regulation of these processes during water stress (Table 6.5). Potential involvement of alkaloids in plant defense against osmotic/oxidative stress damage, possibly contributing to detoxification of reactive oxygen species has been reported (do Nascimento *et al.*, 2013). Therefore, cucurbitacin I might be a considered as a useful tool for effective screening of drought tolerance. Possible elucidation of cucurbitacin E's involvement in drought adaptation could not be made because it was detected in only two landraces (n = 2) under water stress condition, therefore, failing to satisfy requirements for correlation analysis.

Principal component analysis was used to select genotypes for drought tolerance based on leaf gas exchange and chlorophyll fluorescence parameters. Landraces BG-48, BG-58, BG-70, BG-78 and BG-79 showed higher values for *gs*, *T*, *A*, F_v'/F_m' and qN and identified as the most drought tolerant (Efeoglu *et al.*, 2009; Hura *et al.*, 2009a; Cruz de Carvalho *et al.*, 2011; Pereira *et al.*, 2016). BG-70 and BG-79 also showed increased concentration of cucurbitacin I under water stress condition reiterating the fact that cucurbitacin accumulation could be a possible indicator of drought tolerance and an effective selection criterion for identifying drought tolerant genotypes.

6.7 Conclusions

The present study determined drought tolerance of selected bottle gourd landraces based on leaf gas exchange, chlorophyll fluorescence and cucurbitacin content. Positive and strong correlations between cucurbitacin I with several leaf gas exchange and chlorophyll

fluorescence parameters were detected and may serve as selection criteria for drought tolerance breeding.

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An overview of the research findings

Introduction and objectives of the study

Knowledge of the genetic diversity present in plant genetic resources is fundamental in crop improvement and conservation programs. This is especially important in 'underutilized' crop species such as in bottle gourd, which is largely domesticated using unimproved genetically diverse landraces (Aliyu *et al.*, 2016). Bottle gourd [*Lagenaria siceraria* (Molina.) Standl.] is an important underutilized and under-researched crop in Limpopo Province and other drier areas of South Africa where its cultivated using landrace varieties and wild relatives. There is no formal bottle gourd genetic improvement program in the country. This may negatively impact on breeding and strategic conservation of the crop and its subsequent market value. Bottle gourd landraces represent local varieties that have evolved largely through random natural crosses, natural selection or artificial selection by farmers for various uses including for food and to manufacture household utensils. To fully harness the agronomic or horticultural use of this crop and for targeted breeding, knowledge of its genetic diversity is important. Bottle gourd landraces can be exploited in breeding programs through systematic genetic characterization and using farmers and market preferred-attributes. Therefore, the main aim of this study was to initiate a bottle gourd pre-breeding programme in South Africa for breeding and systematic conservation of this potential crop aimed at developing unique and improved cultivars.

This overview compares the original study objectives with the research findings relative to each objective. Finally, the implications of the findings are presented in terms of their contributions to the future of bottle gourd breeding.

Objectives of the study were:

1. To determine the genetic diversity of bottle gourd landrace collections from the Limpopo Province, South Africa using qualitative and quantitative morphological traits.
2. To determine genetic diversity of bottle gourd landraces from the Limpopo Province, South Africa, using single sequence repeats (SSR) markers.

3. To use correlation and path coefficient analysis to determine level of association between qualitative and quantitative traits and subsequently to select suitable parents for breeding.
4. To determine drought tolerance of a diverse set of bottle gourd landraces and to identify promising genotypes for direct production or breeding.
5. To determine the relationship between accumulation of cucurbitacins with leaf gas exchange and chlorophyll fluorescence in bottle gourd under water stress conditions.

Research findings in brief:

Assessment of genetic diversity among bottle gourd [*Lagenaria siceraria* (Molina) Standl.] landraces using qualitative and quantitative traits

Thirty six bottle gourd landraces were phenotyped in the field using qualitative and quantitative morphological traits. The main outcomes were:

- A wide range of variation was observed for qualitative and quantitative traits among the tested bottle gourd landraces.
- Bottle gourd andraces such as BG-16, BG-25, BG-09, BG-37 and BG-10 showed suitable qualitative traits such as fruit shape, fruit colour and fruit texture.
- Landraces such as BG-07, BG-13, BG-67, BG-12, BG-09 and BG-06 showed suitable quantitative traits such as increased fruit yield and number of male and female flowers.

Genetic diversity of bottle gourd [*Lagenaria siceraria* (Molina) Standl.] landraces revealed by simple sequence repeat markers

Sixty seven genetically diverse bottle gourd landraces were genotyped using 14 selected polymorphic simple sequence repeat (SSR) markers. The main outcomes were:

- The SSR markers identified a total 86 putative alleles with an average of 6.14 alleles per locus and the PIC value range of 0.37 to 0.83 with a mean of 0.57.
- Jaccard's coefficient of similarity values ranged from 0.00 to 1.00, with a mean of 0.63 revealing great genetic diversity among the bottle gourd landrace population.
- The mean gene diversity was 0.65, which partitioned 79%, 17% and 4% of the variation to among landraces, within landraces and between populations, respectively.

- Landraces from different collection sites clustered together indicating the existence of high level of gene flow among collection sites which is attributed to seed exchange among farmers.
- Cluster analysis identified three main genetic groups revealing the presence of high genetic differentiation among the bottle gourd landraces.
- Genetically unique landraces such as BG-4, BG-6, BG-8, BG-9, BG-15, BG-55, BG-42, BG-57, BG-58, BG-28, BG-23, BG-29 and BG-34 were selected based on their high dissimilarity values and unique fruit qualitative traits.

Correlation and path coefficient analyses of qualitative and quantitative traits in selected bottle gourd landraces

Relationships among qualitative and quantitative traits in 36 genetically diverse bottle gourd landraces were studied using simple correlation and path coefficient analyses. The main outcomes were:

- Significant and positive correlations between number of fruits per plant with number of male flowers, number of female flowers, plant height and number of branches.
- Number of seeds per fruit was significantly and positively correlated with plant height, number of male flowers, number of female flowers, number of branches and fruit weight.
- Qualitative traits such as fruit texture, degree of warts, fruit shape, and degree of neck bending, stem-end fruit shape and fruit neck length had significantly high and negative correlations with number of fruits per plant or number of seeds per fruit.
- Path analysis revealed that direct selection for increased fruit weight and number of female flowers would increase in fruit and seed yield.
- Selection for warted fruit types may increase genetic gain in seed yield.
- Landraces such as BG-06, BG-07, BG-09, BG-11, BG-13, BG-24 and BG-67 were selected for breeding showing suitable qualitative (i.e. fruit texture and degree of warts) and quantitative traits (i.e. high number of female flowers and fruit yield).

Yield-based selection indices for drought tolerance evaluation in selected bottle gourd [*Lagenaria siceraria* (Molina) Standl.] landraces

Drought tolerance of 12 selected genetically diverse bottle gourd landraces was determined under non-stressed and non-stressed conditions using drought tolerance indices. The main outcomes were:

- Landraces exhibited highly significant variations for fruit number and fruit yield under non-stressed and drought-stressed conditions.
- Landraces BG-79, BG-31 and BG-67 were identified as suitable novel genotypes with high fruit yield for production only under drought-stressed condition.
- Landraces BG-78, GC and BG-52 were identified as suitable genotypes with high fruit yield for production under both non-stressed and drought-stressed conditions.
- The identified could be used as parents for drought tolerance breeding.

Bottle gourd [*Lagenaria siceraria* (Molina) Standl.] response to water stress: relationship between cucurbitacin accumulation with leaf gas exchange and chlorophyll fluorescence

Twelve genetically diverse bottle gourd landraces were grown under non-stressed and water-stressed conditions. A pot study was conducted at the Controlled Research Facility, University of KwaZulu-Natal under glasshouse conditions. Leaf gas exchange and chlorophyll fluorescence measurements were recorded and correlated with cucurbitacin content. The main outcomes were:

- Stomatal conductance, transpiration rate and net CO_2 assimilation rate, ratio of CO_2 assimilation rate and intercellular CO_2, intrinsic water-use efficiency and instantaneous water-use efficiencies declined in landraces that were subjected to water stress.
- Intercellular CO_2 concentration, ratio of intercelluar and atmospheric CO_2 concentration increased significantly in all landraces subjected to water stress.
- The maximum PS II activity, quantum yield of PSII, photochemical quenching and non-photochemical quenching were not affected by water stress; whereas, electron transport rate, electron transport to oxygen molecules (ETR/A) and alternative electron sink (AES) were reduced by water stress.
- Cucurbitacin E and I were detected under WS condition in several bottle gourd landraces. Significant and positive correlations were observed between cucurbitacin I

content with ETR/A and AES suggesting their possible role in the regulation of photorespiration and photoprotection against oxidative stress.

- Accumulation of cucurbitacin I could be considered as a tool for selection of bottle gourd genotypes for drought tolerance.
- Landraces BG-48, BG-58, BG-70, BG-78 and BG-79 were identified as drought tolerant genotypes.

Implications of the research findings for breeding bottle gourd

The following implications for breeding were noted:

- Genotypes that possessed unique qualitative and quantitative traits identified can be utilized for targeted breeding to create new varieties with improved agronomic and horticultural traits according to the needs of the farmers and market.
- The high polymorphism of SSR markers used in the current study implies their usefulness in genetic analysis of bottle gourd. Genetically unique genotypes identified through SSR analysis could be used as parents in controlled crosses aiming at developing improved bottle gourd varieties.
- Genotypes identified as drought tolerant can be recommended for direct production or as parents for drought tolerance breeding.
- Cucurbitacins accumulation could be used as a tool for selection and identification of bottle gourd genotypes for drought tolerance or susceptibility.