UNIVERSITY OF KWAZULU-NATAL

Hybrid Development in Bottle Gourd [*Lagenaria siceraria* (Mol.) Standl.] for Drought Tolerance and Economic Traits

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Hybrid Development in Bottle Gourd [*Lagenaria siceraria* (Mol.) Standl.] for Drought Tolerance and Economic Traits

By

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Thesis abstract

Bottle gourd (*Lagenaria siceraria*) is a drought-resilient cucurbit widely cultivated in sub-Saharan Africa and Asia for diverse food, nutrition, and industry uses. Its fresh leaves and fruits are consumed as vegetables, providing essential nutrients such as vitamins B, C, and E, minerals, fiber, and amino acids. The seeds serve as a source of edible oil, dietary fiber, and phytochemicals. Bottle gourd possesses significant genetic diversity, presenting opportunities for developing high-yielding and drought-tolerant varieties. However, the crop remains under-researched, primarily cultivated by small-scale farmers using unimproved landrace varieties, resulting in low productivity. Therefore, the overall aim of this study was to develop high-yielding and drought-tolerant bottle gourd hybrids for commercialization in South Africa to enhance food and nutrition welfare and provide market opportunities to growers.

The first part of the study quantified the concentrations of cucurbitacins in diverse bottle gourd genotypes and appraised the relationship to drought tolerance. The contents of cucurbitacins B, E, and I were quantified in leaves and roots of twelve preliminarily selected bottle gourd accessions using high-performance liquid chromatography–mass spectrometry (HPLC-MS). The contents of cucurbitacins B and I were enhanced under increased drought intensity for accessions BG-48, BG-81, and GC. In all the leaf and root samples, cucurbitacin E was not detectable. This study revealed that cucurbitacins B and I are novel biochemical markers for screening drought tolerance in bottle gourd or related cucurbits.

In the second part of the study, 53 F_1 hybrids were developed and field evaluated with 12 parental accessions under NS and DS conditions in two growing seasons using a 5 × 13 α-lattice design with three replicates. Data were collected on fruit yield per plant (FYPP), seed yield per plant (SYPP) and drought tolerance indices computed. Significant interactions were detected among test genotypes and water regimes for FYPP and not for SYPP. Based on tolerance indices fourteen single cross hybrids were identified as drought tolerant including BG-27 × BG-31, BG-58 × BG-78, and BG-58 × BG-80. The newly-developed bottle gourd hybrids are recommended for cultivation in drought-prone agro-ecologies in South Africa and similar environments in SSA after multi-environment testing.

In the third part of the study, eight preliminarily selected and contrasting parents with drought tolerance were crossed using a half-diallel mating design. The 8 parents and 28 hybrids were evaluated under NS and DS conditions across two growing seasons. Data were collected on fruit yield and related traits and subjected to analysis of variance and combining ability. Significant ($p < 0.05$) specific combining ability (SCA) and general combining ability (GCA) effects were recorded for fruit yield per plant (FYPP). The SCA × environment and GCA × environment interaction effects were highly significant ($p < 0.001$) for FYPP and SYPP. The significant genotype × environment interactions suggest that genetic effects were affected by the test environment, necessitating multi-location testing across South Africa before making any recommendations for hybrid release. Parental genotypes BG-58 and GC recorded positive and significant GCA effects for FYPP under the DS condition, whereas GC recorded positive and significant GCA effects for FYPP under the NS condition. The two genotypes are ideal breeding parents for population development to select genotypes with high fruit and seed yields. Also, GCA was preponderant for FYPP, implying that selection-based breeding strategies can be effectively employed to improve fruit yields. Crosses BG-52 × BG-79, BG-80 × GC, and BG-70 × GC recorded high and positive SCA effects for FYPP and SYPP under DS condition. Crosses BG-27 × GC, BG-52 × BG-79, BG-52 × BG-58 and BG-58 × BG-80 recorded high and positive SCA effects for FYPP and SYPP under NS condition. The F_1 hybrids outperformed their parent in fruit yields, confirming the effectiveness of hybridisation for improving bottle gourd performance. The newly selected families and superior ones must be subjected to multi-environment evaluation for release and commercialization in South Africa or similar agroecologies.

The fourth part of the study determined the genotype-by-environment interactions (GEI) for fruit yield and related traits among eight selected F_1 hybrids and four checks in five contrasting environments of varying moisture conditions using a randomized complete block design with three replications. Data were collected on days to 50% male flowering (DTMF) and female flowering (DTFF), total number of fruits per hectare (TNFH) and fruit yield per hectare (FYPH) and subjected to analysis of variance, additive main effects and multiplicative interaction (AMMI) and genotype plus genotype-by-environment (GGE) biplot models. The AMMI model revealed significant ($p \leq 0.001$) effects of genotype (G), environment (E) and GEI for the studied traits. The AMMI model explained a higher (96.30%) variation for TNFH, of which G, E and GEI effects explained 49.88, 24.21 and 22.21% of the total variation, respectively. The model ascribed variations of 12.36, 73.16 and 11.41% for FYPH attributable to the G, E and GEI effects, in that order. The GGE biplot model explained 94.53 and 96.56% variations for TFYPH and FYPH, respectively. The hybrids BG-58 × BG-80, and BG-52 × BG-58 attained high and stable FYPH under both water-limited and irrigated conditions. The identified hybrids are recommended for cultivation under rainfed and irrigated conditions in

South Africa. This study offered initial insights into GEI, but broader testing across diverse locations in South Africa is recommended for more reliable conclusions.

Overall, the present study revealed that cucurbitacins B and I are the novel biochemical markers for screening drought tolerance in bottle gourd. The study developed and selected promising hybrids recommended for further testing in other provinces outside Limpopo .

Declaration

I, Phumzile Mkhize, declare the following:

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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Phumzile Mkhize

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

[redacted]

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Prof. Hussien Shimelis (Supervisor)

[redacted]

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Dr. Jacob Mashilo (Co-Supervisor)

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Dedication

This thesis is dedicated to my late mother (Nombifikile Maud Mkhize), my father (Dumezweni Mkhize), my son (Avumile Mahlatse Khanyelwa Mkhize) and my daughter (Kanalo Abusisile Zanokuhle Mkhize).

Abbreviations

AMMI	Additive main effects and multiplicative interaction
ANOVA	Analysis of variance
BG	Bottle gourd
BLUPs	Best linear unbiased predictors
BPH	Better-parent heterosis
df	Degrees of freedom
DPPH	2,2-diphenyl-1-picrylhydrazyl
DS	Drought-stress
DTFF	Days to 50% female flowering
DTMF	Days to 50% male flowering
FW	Fruit weight
FYPH	Fruit yield per hectare
FYPP	Fruit yield per plant
GCA	General combining ability
GEI	Genotype-by-environment interactions
GGE	Genotype plus genotype-by-environment
GMP	Geometric mean productivity
HM	Harmonic mean
HSW	Hundred seed weight
K1STI	Modified stress tolerance index I
K2STI	Modified stress tolerance index II
LSD	Least significant difference
MP	Mean productivity
MPH	Mid-parent heterosis

NFF	Number of female flowers
NFPP	Number of fruits per plant
NL	Number of leaves
NMF	Number of male flowers
NS	Non-stress
NSPF	Number of seeds per fruit
PC1	First principal component
PC2	Second principal component
PH	Plant height
SCA	Specific combining ability
SR	Sex ratio
SSA	sub-Saharan Africa
SSI	Stress susceptibility index
STI	Stress tolerance index
SYPP	Seed yield per plant
TNFH	Total number of fruits per hectare
TOL	Tolerance index
YI	Yield index
YSI	Yield stability index

Publications pertaining to this thesis

Chapter 1

Mkhize P., Mashilo J., Shimelis H. (2021) Progress on genetic improvement and analysis of bottle gourd [*Lagenaria siceraria* (Molina) Standl.] for agronomic traits, nutrient compositions, and stress tolerance: a review. Frontiers in Sustainable Food Systems 5: 683635: DOI: https://doi.org/10.3389/fsufs.2021.683635

Chapter 2

Mkhize P., Mashilo J., Shimelis H. (2023) Cucurbitacins B, E and I concentrations and relationship with drought tolerance in bottle gourd [*Lagenaria siceraria* (Molina) Standl.]. Plants 12:3492: DOI: https://doi.org/10.3390/plants12193492

Chapter 3

Mkhize P., Shimelis H., Mashilo J. (2025) Hybrid performance of bottle gourd [*Lagenaria siceraria*] under drought stress and non-stress conditions. Ecological Genetics and Genomics 34:100316: DOI: https://doi.org/10.1016/j.egg.2024.100316

Chapter 4

Mkhize P., Mashilo J., Shimelis H. (2023) Combining ability and heterosis among bottle gourd [*Lagenaria siceraria* (Molina) Standl.] selections for yield and related traits under drought-stressed and non-stressed conditions. Diversity 15:925: DOI: https://doi.org/10.3390/d15030371

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Introduction to thesis

Background

Bottle gourd (*Lagenaria siceraria*) is cultivated for its leaves, fruits, and seeds. In South Africa, its tender leaves are commonly consumed as a nutritious vegetable, rich in essential minerals (Modgil et al., 2004; Sithole et al., 2015; Mokganya and Tshisikhawe, 2019). The fruits are a source of vital macro- and micronutrients, amino acids, and phytochemicals, contributing both nutritional and medicinal value (Hassan et al., 2008; Sithole et al., 2015; Mahapatra et al., 2023). Some genotypes of the crop have bitter-tasting fruits due to cucurbitacins and these fruit have many medicinal properties (Attar and Ghane, 2018; Saurabh et al., 2023). The mature fruits also serve practical purposes, such as crafting household containers. The seeds are consumed as snacks, used in agro-processing for edible oil, and provide feed by-products (Hassan et al., 2008; Ogunbusola et al., 2010; Devi et al., 2023). The seeds are rich in fiber, carotene, tocopherol, sterols, and amino acids (Ogunbusola et al., 2010; Abdel-Razek et al., 2021; Devi et al., 2023). Therefore, bottle gourd holds significant potential for promoting nutrition and health. Also, the crop has niche market opportunities in sub-Saharan African (SSA), requiring the breeding of new, well-adapted, and high-yielding varieties that possess good agronomic and horticultural traits and acceptable market standards. The present study determined the combining ability and heterosis of fruit yield and related traits among South African bottle gourd accessions under drought-stressed and non-stressed conditions for breeding and variety release.

Bottle gourd is an underutilised and under-valued crop with limited research support in Africa. South Africa is one of the centres of genetic diversity of the crop. In the region, smallholder farmers mainly cultivate bottle gourd, often in homesteads with limited yield outputs. In South Africa, smallholder farmers have grown bottle gourd predominantly using low-yielding landrace varieties due to the lack of commercial cultivars released by dedicated genetic improvement programs. Production of bottle gourd in the country is constrained by a lack of improved varieties with attributes preferred by farmers and consumers. Therefore, a dedicated bottle gourd genetic improvement program is required to develop superior and high-yielding genotypes with tolerance to biotic and abiotic stresses to enhance productivity and food security. Bottle gourd is a niche opportunity crop for economic gains through product development and value additions. In South Africa, preliminary bottle gourd pre-breeding was initiated by Mashilo et al. (2016). The authors identified genetically distant and promising genotypes for breeding in South Africa or related agroecologies. The identified bottle gourd genetic resources could further be exploited to develop superior hybrids with high yields and drought tolerance to improve crop productivity under smallholder and commercial farming systems.

To improve bottle gourd productivity in South Africa, hybridisation offers a promising approach by leveraging hybrid vigour to enhance yield, stress tolerance, and adaptability. However, the success of hybrid breeding relies heavily on selecting the right and compatible parental combinations (Fasoula and Fasoula, 2002; Singh, 2005; Griffing, 1956). This is where understanding combining ability becomes crucial (Acquaah, 2007). Through combining ability analysis, breeders can identify parents with consistent, additive genetic effects and specific hybrid combinations with exceptional performance due to additive and non-additive effects (Jinks and Hayman, 1953; Allard, 1961; Acquaah, 2007). Evaluating these effects across diverse environments helps ensure the development of stable, high-yielding hybrid varieties suited for commercial cultivation. Previous bottle gourd breeding programs using this approach have already resulted in improved varieties with enhanced nutritional quality, disease resistance, and drought tolerance (Kumar et al., 2014; Janaranjani et al., 2016; Mishra et al., 2019; Quamruzzaman et al., 2020). Applying similar strategies in South Africa could significantly accelerate the development of productive, climate-resilient hybrids tailored to local agricultural needs.

Bottle gourd has unique genetic potential and adapts to grow in harsh environments characterised by poor soils, limited water, and high-temperature conditions (Mashilo et al. 2017; 2018). In arid and semi-arid production environments, the crop is grown under rainfed conditions and often exposed to severe drought and heat stress. Several studies reported high levels of drought-tolerance of the crop (Sithole and Modi, 2016; Mashilo et al., 2016, 2017; Contreras-Soto et al., 2022). These suggested valuable genes are present in *L. siceraria* for enhancing abiotic stress tolerance (Wang et al. 2024). In southern Africa, the intensity and frequency of drought have increased, impacting major crop production (Fauchereau et al. 2003). Hence, there is a need to develop drought-tolerant crop varieties such as bottle gourd for successful cultivation in water-limited environments. There are no reports on drought-tolerant bottle gourd hybrids developed for South African conditions.

Bottle gourd leaves accumulate a bitter-tasting secondary metabolite referred to as cucurbitacins in response to water stress (Mashilo et al., 2018). Cucurbitacins could serve as proxy trait in the selection of drought-tolerant bottle gourd. No studies reported if cucurbitacins accumulation may be associated with response and adaptation to drought stress. Understanding the role of cucurbitacins in drought adaptation may serve as a novel selection criterion for drought tolerance breeding in bottle gourd or related cucurbit crops. The economic opportunity of bottle gourd can be realized in South Africa through breeding high-performing and locally adapted bottle gourd varieties, targeting the needs of consumers and the marketplace.

Rationale for the study

Bottle gourd exhibits considerable genetic diversity and notable drought tolerance compared to other cucurbits, such as pumpkin (*Cucurbita maxima*) and cucumber (*Cucurbita pepo*). In sub-Saharan Africa, including South Africa, bottle gourd is cultivated using unimproved landraces that are genetically diverse and widely adapted to grow under extreme climatic conditions characterized by drought and heat stress. Bottle gourd thrived and evolved in harsh environments, making it an ideal and drought-tolerant crop for water-limited agro-ecologies for food security and livelihoods. Despite its economic potential, there are no dedicated research and development support and breeding on bottle gourd and related cucurbits. Hence, no commercial hybrid cultivars of bottle gourd have been released in South Africa.

Research and breeding efforts are essential in South Africa to develop drought-resilient cucurbit cultivars suited for dry environments. The genetic diversity, its unique biochemical composition, such as cucurbitacins, and physiological and genetic plasticity present in bottle gourd can be explored in hybrid breeding programs. Candidate parents and derived hybrids must undergo rigorous evaluation using multiple statistical analyses, such as drought tolerance index and mean productivity, to assess yield and yield-related traits for identifying drought-tolerant genetic sources.

The South African bottle gourd genetic resources were not evaluated in hybrid combinations, and no studies of combining ability analyses were conducted, which could enable the identification of superior parental lines and hybrids with desirable traits. Elite genetic resources are assessed across diverse environments to identify and recommend broadly adapted cultivars based on genotype-by-environment interaction (GEI) analysis to ascertain their suitability for release and commercialization. The present study was based on combining ability analysis of bottle gourd parental lines selected from genetically contrasting collections to develop and deploy high-yielding hybrids with drought tolerance and local adaptation.

Overall aim and objectives of the study

The aim of this study was to develop high-yielding and drought-tolerant bottle gourd hybrids for commercialization in South Africa to enhance food and nutrition welfare and provide market opportunities to growers.

Specific objectives

The specific objectives of the study were:

i) To determine the concentrations and effect of cucurbitacins in bottle gourd and their relationship with drought tolerance. to guide the selection of drought tolerant bottle gourd genotypes or related cucurbits.

ii) To determine drought tolerance among newly-developed F_1 bottle gourd hybrids. to recommend best-performers for production and commercialization in South Africa.

iii) To determine the combining ability and heterosis among selected genotypes of bottle gourd for fruit yield and related traits under drought-stressed and non-stressed conditions. to select the best parents and hybrids for breeding.

iv) To determine the genotype-by-environment interactions (GEI) for fruit yield and related traits among newly developed bottle gourd hybrids. to guide variety recommendation and registration.

Research hypotheses

I. Cucurbitacins have antioxidant potential and are associated with drought tolerance in bottle gourd.

II. There is a considerable genotypic difference among bottle gourd parental accessions and F_1 hybrids for drought tolerance.
III. Bottle gourd parental genotypes exhibit good combining ability for agronomic traits to develop better-performing F_1 hybrids exhibiting significant heterosis over their parents.
IV. Bottle gourd F_1 hybrids display varied performance for yield and related traits when evaluated under multiple environments.

Thesis outline

This thesis is composed of five chapters, each aligned with the research objectives outlined above. The referencing system used in this thesis is based on the referencing style of the journals where the papers were published. The chapters are structured as standalone research papers, either published or prepared for publication and include all essential details. While efforts have been made to minimize redundancy, the interrelated nature of the chapters has resulted in some overlap and unavoidable repetition of references and introductory material. This format reflects the standard thesis structure adopted by the University of KwaZulu-Natal.

Chapter one presents the review of the literature on the progress in genetic improvement and analysis of bottle gourd for agronomic traits, nutrient compositions, and stress tolerance, and it is published in Frontiers in Sustainable Food Systems 5: 683635: DOI: https://doi.org/10.3389/fsufs.2021.683635. The second chapter presents the relationship between cucurbitacins B, E, and I concentrations and drought tolerance. Chapter 2 is published in Plants 12:3492: DOI: https://doi.org/10.3390/plants12193492. The third chapter focuses on the hybrid performance of bottle gourd under drought stress and non-stress conditions, and it is published in Ecological Genetics and Genomics 34:100316: DOI: https://doi.org/10.1016/j.egg.2024.100316. The fourth chapter presents the combining ability and heterosis among bottle gourd selections for yield and related traits under drought-stressed and non-stressed conditions, and the chapter is published in Diversity 15:371: DOI: https://doi.org/10.3390/d15030371. The fifth chapter determined genotype-by-environment interactions for fruit yield and related traits in selected hybrids of bottle gourd (*Lagenaria siceraria*), and it is being prepared for publication.

The outline of the thesis is, therefore, as follows:

-Thesis introduction

Chapter 1: Review of literature

Chapter 2: Cucurbitacins B, E and I concentrations and relationship with drought tolerance in bottle gourd [*Lagenaria siceraria* (Molina) Standl.].

Chapter 3: Hybrid performance of bottle gourd [*Lagenaria siceraria*] under drought stress and non-stress conditions.

Chapter 4: Combining ability and heterosis among bottle gourd [*Lagenaria siceraria* (Molina) Standl.] selections for yield and related traits under drought-stressed and non-stressed conditions.

Chapter 5: Genotype-by-environment interactions for fruit yield and related traits in selected hybrids of bottle gourd (*Lagenaria siceraria*).

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Chapter 1. Review of the literature

Abstract

Bottle gourd [*Lagenaria siceraria* (Molina) Standl.] is an important multi-purpose cucurbit crop grown for its leaf, fruit, and seed. It is widely cultivated and used for human consumption in sub-Saharan Africa (SSA) providing vital human nutrition and serving a food security crop. There is wide genetic variation among bottle gourd genetic resources in Africa for diverse qualitative and quantitative attributes for effective variety design, product development, and marketing. However, the crop is under- researched and -utilized, and improved varieties are yet to be developed and commercialized in the region. Therefore, the objective of this review is to provide the progress on bottle gourd genetic improvement and genetic analysis targeting agronomic and horticultural attributes, nutritional composition, biotic, and abiotic stress tolerance to guide current and future cultivar development, germplasm access, and conservation in SSA. The first section of the chapter presents progress on breeding of bottle gourd for horticultural traits, agronomic performance, nutritional and anti-nutritional composition, and biotic and abiotic stress tolerance. This is followed by important highlights on key genetic resources of cultivated and wild bottle gourd for demand driven breeding. Lastly, the review summaries advances in bottle gourd genomics, genetic engineering and genome editing. Information presented in this chapter should aid bottle gourd breeders and agronomists to develop and deploy new generation and promising varieties with farmer- and market -preferred attributes.

Keywords: Abiotic stress, agronomic traits, biotic stress, bottle gourd, horticultural traits, watermelon

1.1. Introduction

Bottle gourd [*Lagenaria siceraria* (Molina) Standl.] is an important cucurbit crop in Sub-Saharan Africa (SSA) and is widely cultivated by smallholder growers for its nutritious fruit. Young and succulent leaves are consumed as leafy vegetables after cooking and are often used for medicinal purposes to treat headaches (Morimoto et al., 2005; Abdin et al., 2014; Gürcan et al., 2015; Mashilo et al., 2015; Behera et al., 2021; Moustafa et al., 2021). Bottle gourd fruits are harvested while tender and boiled until soft for consumption. They are a source of essential nutrients, including iron, zinc, nitrogen, manganese, copper, phosphorus, potassium, calcium, and magnesium (Attar and Ghane, 2018; Attar and Ghane, 2019; Mahapatra et al., 2023; Saurabh et al., 2023). Sugar and milk are often added to the boiled fruit to enhance taste (Barot et al., 2015). The fruit are a good source of vitamins, including B, C, and E, carbohydrates, crude proteins, crude lipids, phenols and dietary fiber (Ojiako et al., 2007; Upaganlawar and Ramchandran, 2009; Sithole and Modi, 2015; Mahapatra et al., 2023). The fruits are also an excellent source of essential amino acids, including glutamic acid, leucine acid, arginine, lysine and aspartic acid, threonine, serine, alanine, valine, phenylalanine and arginine (Chung et al., 2000; Attar and Ghane, 2018; Ogunbusola et al., 2010; Ogunbusola, 2018). Ripening seeds are often consumed alongside the tender fruits. The seeds are dried and roasted to be eaten as snacks or make flour (Barot et al., 2015). The seeds contain essential amino acids, minerals, including copper, phosphorus, zinc, iron, and magnesium and proteins (Ogunbusola et al., 2010; Ogunbusola, 2018). The seed oil contains sterols, including β-sitosterols (Abdel-Razek et al., 2021). The mature fruits are often dried and used to make traditional containers called "Kgapa" or "Sego" in the indigenous and local Sepedi language of South Africa. The containers are used to store food, grains, and water or for decorative purposes (Mashilo et al., 2015). Bottle gourd serves as a rootstock for grafted watermelon and improves fruit yield, quality, and resistance to Fusarium and Verticilium wilts (Yetişir and Sari, 2003; Çandir et al., 2013; Keinath and Hassell, 2014; Morales et al., 2023).

Young and immature fruits of bottle gourd are highly preferred in semi-urban and urban areas as fruit vegetable. In many parts of South Africa including in KwaZulu-Natal, Gauteng and Limpopo provinces, young bottle gourd fruits with varied fruit shapes are sold at retail stores with prices ranging from 1 to 3 USD per kilogram. The dried fruit of bottle gourd has a hard shell which is ideal for making custom-made containers for decoration and other household uses. For instance, a dried fruit with neck length varying between 8 and 15 cm is valued for making containers to serve traditional beer or water. Also, designer decorative materials are prepared from dried fruits which are widely used in celebration of traditional ceremonies. Small to large oval fruits with thick rind (i.e., 2 cm) and fruit neck length of ~3 cm are used to prepare decorations and containers. Immature and ripening seeds are consumed with young fruit. The seeds are rich sources of protein, amino acids, and essential micro-and-macro elements with health-promoting benefits (Ojiako and Igwe, 2007; Said et al., 2014; Sithole et al., 2015). The number of seeds per fruit is highly variable amongst bottle gourd genotypes (Morimoto et al., 2005) providing opportunities for genotype selection with high seed yield potential. Dried seeds of bottle gourd are mainly used as roasted snack.

In SSA, bottle gourd is grown predominantly by smallholder farmers using genetically diverse landrace varieties (Mashilo et al., 2016c). There is wide genetic variation among bottle gourd genetic resources in Africa for selection and ideotype breeding. Commercial varieties are yet to be developed and deployed in the region due to a lack of dedicated genetic improvement programs of the crop. As such bottle gourd is categorized as an under-utilized crop in SSA where its production is mostly practiced by small-scale farmers under low input farming systems. Also, commercial uses of the crop (e.g., as a rootstock for watermelon production) is not known in the region limiting the development of bottle gourd as functional food and commercial crop. There are no improved cultivars released in the region for food, feed, value-adding, and for rootstock in watermelon production.

There is need for concerted and collaborative research efforts on bottle gourd among plant breeders, agronomists, geneticists in the region and internationally. This will enable knowledge and germplasm sharing and innovative research to design, develop, and release promising and well-adapted bottle gourd cultivars. The next generation of bottle gourd cultivars should encompass product profiles including quality and leaf quantity, fruit, fodder, seed, nutritional compositions to serve varied value chains, and the food and feed industry. Therefore, the aim of this review is to provide progress on bottle gourd genetic improvement and genetic analysis targeting agronomic and horticultural attributes, nutritional composition, biotic and abiotic stress tolerance to guide current and future cultivar development, germplasm access, and conservation in SSA and globally.

1.2. Progress in breeding of bottle gourd

1.2.1. Fruit qualitative traits

Considerable genetic variability exists in bottle gourd genetic resources for fruit horticultural traits (Decker-Walters et al., 2001; Morimoto et al., 2005, 2006; Sivaraj and Pandravada, 2005;

Mashilo et al., 2015, 2016b, 2017b) useful for strategic breeding and cultivar development. The crop show variation for fruit shape, size, length, colour, and texture (Figure 1.1) (Morimoto et al., 2005; Sivaraj and Pandravada, 2005; Yetişir et al., 2008; Xu et al., 2014; Mashilo et al., 2015). Fruit shape is an important trait that determines usability of the crop either for food or decorative purposes. Bottle gourd fruits vary in shape such as club-shaped, globular, bottle shaped, flate, pear shaped cylindrical, elongated straight, pyriform, round (oblate), elongated curved, and oval (Sivaraj and Pandravada, 2005; Achigan-Dako et al., 2008; Yetişir et al., 2008; Mashilo et al., 2015; Kaylan et al., 2016). Fruit shape can therefore serve as a selection marker in improvement programs. There is high genetic variability for fruit shape which may provide opportunities for improving this character. Further, fruit shape is a key market-preferred trait. Therefore, knowledge on the underlying gene action conditioning fruit shape is important for developing unique and attractive fruits to increase market opportunities. Oval and pear fruit shapes in bottle gourd are controlled by a combination of one dominant and one recessive genes. Round fruit shape is controlled by two recessive genes, whereas dominant genes control the expression of long fruit shape (Kushwaha and Ram, 1996).

The fruit neck varies considerably in shape between different genotypes (Morimoto et al., 2005; Yetişir et al., 2008; Gürcan et al., 2015; Mashilo et al., 2015). In addition, fruit neck length is highly variable in bottle gourd serving as useful trait for developing bottle gourd genotypes for ornamental purposes. Fruit neck is controlled by two genes with complementary gene action in bottle gourd (Amangoua et al., 2019). Fruit colour is an important trait for developing visually attractive fruits to increase the market value. Bottle gourd fruit are characterized by smooth or "corrugated" texture or a combination of these traits (Montes-Hernandez and Eguiarte, 2002; Mladenovic et al., 2012). The immense variation in fruit horticultural traits offers breeding and market opportunities. To develop "new" genotypes of bottle gourd, crosses can be made between cultivars expressing the traits of interest (e.g., neck shape, neck length, fruit shape, and colour). This could be followed by selection of progenies showing the trait of interest in the F_2 generation to develop desirable individuals.

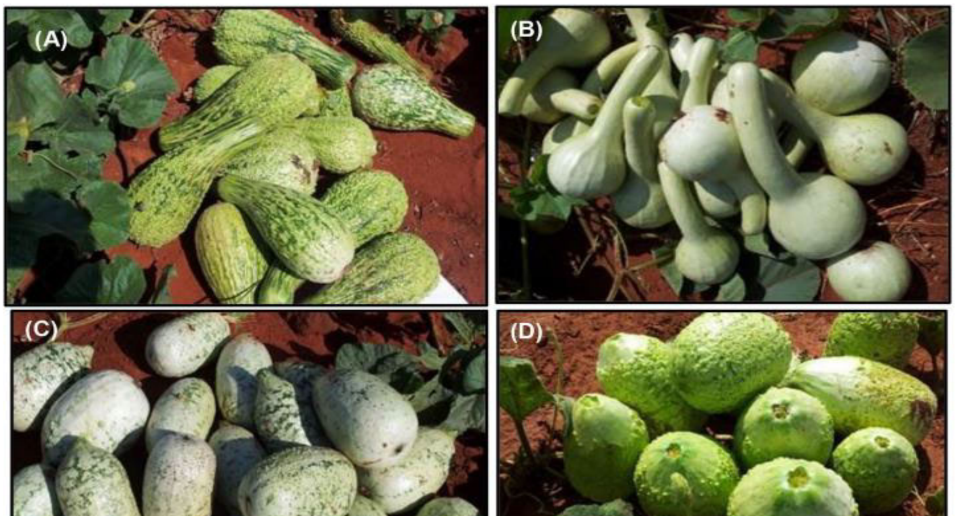

Figure 1.1. Fruit morphotypes of bottle gourd accessions cultivated in the Limpopo Province of South Africa. The bottle gourd accessions are denoted as BG series. (A) BG-67, verrucose fruit texture and club-shaped fruit used for food, (B) BG-78, light-green fruit colour and long neck used for making containers and to serving traditional sorghum-brewed beer, (C) BG-70, white-green fruit colour grown for food, (D) BG-79, cultivated for its edible fruit, (E) BG-100, commercially grown and sold in various retail outlets in KwaZulu-Natal and Gauteng provinces of South Africa, (F) BG-80, light-green fruit colour, small-neck, and corrugated skin texture, (G) BG-27, dark and light green fruit colour and slightly curved fruit neck, and (H) BG-31, dark green fruit colour and curved fruit neck (Photos provided by J. Mashilo).

The accessions were originally collected from various farmers' fields in the Limpopo Province of South Africa where cultivated forms of bottle gourd are prevalent. The collections are maintained by the Limpopo Department of Agriculture and Rural Development (LDARD). The codes are allocated by LDARD. The collections were cross-checked and compared with *Lagenaria* specimens maintained by the Agricultural Research Council-Herbarium Unit (South Africa). All collections were confirmed to be bottle gourd.

1.2.2. Agronomic performance

Extensive phenotypic variation exists for agronomic traits in bottle gourd (Decker-Walters et al., 2001; Morimoto and Mvere, 2004; Morimoto et al., 2005; Sivaraj and Pandravada, 2005; Mladenovic et al., 2012; Mashilo et al., 2015, 2017c). Trait variation may be attributed to long agricultural history, adaptation to diverse agro-ecological conditions, and selection by famers for desirable agronomic attributes and ethnobotanical utilization (Montes-Hernandez and Eguiarte, 2002). Traits including flowering time, plant height, number of main and lateral branches per plant, fruit weight, and number of fruits per plant show high levels of variation in bottle gourd (Mashilo et al., 2015, 2017c). These traits can be exploited in pre-breeding and breeding programs for cultivar development and deployment.

Variation in fruit shape and size in bottle gourd has a direct relationship with fruit yield. Several studies reported that direct selection for agronomic traits including number of female flowers per plant, number of branches per plant, number of fruits per plant, fruit width and plant height can increase fruit yield potential in bottle gourd (Narayan et al., 1996; Behera et al., 2015; Yao et al., 2015). For direct improvement of fruit yield, selection for high number of female flowers is desirable due to high correlations between these two traits (Dey et al., 2006; Mashilo et al., 2016b). To achieve this, bottle gourd with high female flowering capacity can be crossed followed by selections and advancement of progenies expressing high number of females flowers and fruit yield (Arora et al., 1982; Cramer and Wehner, 2000). To improve seed yield, fruit weight, number of seeds per fruit and hundred seed weight may serve as important attributes for hybrid development (Yao et al., 2015). Further, characters such as number of branches, plant length, number of fruits per plant, fruit weight, number of seed per fruit, hundred seed weight, sex ratio, number of male and female flowers and seed yield are highly variable and heritable (Koffi et al., 2009; Yao et al., 2015; Janaranjani et al., 2016). Hence selection for these traits enhances genetic advancement through hybrid breeding.

1.2.3. Hybrid breeding

Identification of parental genotypes with suitable agronomic and horticultural attributes is useful to develop high-performing hybrids. Various studies have identified genetically unrelated and promising genotypes possessing useful traits for hybrid breeding (Xu et al., 2011; Mashilo et al., 2015, 2017b, 2018; Yildiz et al., 2015). Hybrid vigor has been reported for important characters such as flowering time of female flowers, fruit weight (Dubey and Ram, 2007), hundred seed weight (Dubey and Ram, 2007; Janaranjani et al., 2016), days to 1st male flowering (Dubey and Ram, 2007; Janaranjani et al., 2016), sex ratio (i.e., female: male flowers), and number seeds per fruit (Dubey and Maurya, 2007; Dubey and Ram, 2007; Janaranjani et al., 2016). These findings collectively underscore the importance of strategic parent selection and hybridisation in exploiting heterosis for key economic traits, thereby laying the foundation for systematic hybrid breeding programs aimed at improving bottle gourd productivity and adaptability under diverse environmental conditions.

1.2.4. Combining ability and heterosis

Analysis of combining ability is widely used in breeding programmes to identify superior parents for cultivar development and to identify progenies with traits of interest for genetic advancement (Acquaah, 2007). General combining ability (GCA) measures the breeding value of parental genotypes and is associated with additive genetic action. Specific combining ability (SCA) measures the performance of a progeny and is associated with non-additive gene action (i.e., dominance and epistasis) (Falconer and Mackay, 1996). Both additive and non-additive gene action are reported to control inheritance of agronomic/horticultural attributes in bottle gourd (Quamruzzaman et al., 2020). For example, analysis of gene action for agronomic traits revealed that the SCA effect was greater than the GCA effect with GCA/SCA ratio of <1. This suggested the predominance of non-additive gene action over additive gene action influencing traits such as vine length, days to first male flower anthesis, days to first female flower anthesis, sex ratio, fruit length and width, fruit flesh thickness, number of seeds per fruit, hundred seed weight, and fruit yield per plant (Janaranjani et al., 2016). Therefore, improvement of agronomic traits governed by non-additive genes may be targeted through hybridization among desirable parents, which may induce high SCA effects in the progenies. Therefore, heterosis breeding is well-suited to exploit improvement of traits expressing non-additive gene action (Singh, 2005; Janaranjani et al., 2016). Heterosis for desirable traits such as earliness, number of fruits and fruit yield have been reported in bottle gourd (Singh, 2005). Exploitation of heterosis in bottle gourd is achievable due to its high cross-pollinating nature allowing recombination of favourable genes that may contribute to yield improvement (Morimoto et al., 2004).

In India, several high-yielding varieties have been developed by exploiting heterosis followed by mass selection from segregating populations (Sarao et al., 2014; Behera et al., 2015). Similarly, hybrid breeding has been explored in various cucurbit crops including bitter gourd (*Momordica charantia* L.), cucumber (*Cucumis sativus* L.), squash (*Cucurbita maxima* L.), melon (*Cucumis melo* L.), and watermelon (*Citrullus lanatus* L.) (Rhodes and Zhang, 2000; Robinson, 2000; Kohli and Vikram, 2005; Al-Mamun et al., 2016). On the contrary, a higher magnitude of GCA effect compared to SCA effect were recorded for fruit yield indicating the predominance of additive gene action controlling this trait in bottle gourd (Quamruzzaman et al., 2019, 2020). Therefore, recurrent selection would also be effective for yield improvement after crossing of parental genotypes. In SSA where bottle gourd is widely grown as food and fodder crop, exploitation of hybrid vigor is feasible in this crop, however, this has not been explored and the crop remains underutilized and unexploited in breeding programmes. As a result, there are no commercial varieties available or released for production to serve the diverse value chains of the crop in the region. Hybrid varieties of bottle gourd may play a vital role in satisfying the interest of producers and consumers. The identification and utilization of the most heterotic crosses is important for hybrid breeding. Therefore, a well-planned and dynamic bottle gourd breeding programme is needed in SSA using the available genetic resources to meet the required demand for production, value-adding, rootstock development and commercialization.

1.2.5. Nutritional and anti-nutritional compositions

In SSA and Asia, bottle gourd leaves are used a cooked leaf vegetable. The immature fruit and seed are boiled and consumed a tender vegetable (Morimoto and Mvere, 2004). The leaves contain high levels of phosphorus (P), calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) (Modgil et al., 2004; Hassan et al., 2008; Sithole et al., 2015). Potassium and Ca are the abundant mineral elements in bottle gourd leaves (Sithole et al., 2015). The fruit is vital source of amino acids notably aspartic acid (19.84–143.59µg/g), threonine (143.72–1593.36µg/g), serine (3.67–149.62µg/g), glutamic acid (25.14–431.55µg/g), alanine (11.39–222.74µg/g), valine (7.93–126.06µg/g), leucine acid (9.00–148.30µg/g), phenylalanine (14.19–177.84µg/g), lysine (6.18– 160.97µg/g), and arginine (16.15–212.42µg/g) (Wu et al., 2017a). Bottle gourd seed contains crude protein with a value of 35% and crude lipid of 39%, essential amino acids (i.e., isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, valine, alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, proline and serine), with glutamic acid (14.3 g/100 g), leucine acid (7.66 g/100 g) and aspartic acid (9.11 g/100 g) being the predominant amino acids (Hassan et al., 2008). Ogunbusola et al. (2010) reported glutamic acid content of 139–168 mg/g protein, aspartic acid (89.0–116 mg/g protein), leucine (65.8 mg/g protein), arginine (58.6 mg/g protein), and lysine (56.2 mg/g protein) in bottle gourd seed flour. The amino acid profile and protein content of bottle gourd seeds provide a low-cost protein source that meets the recommended daily allowance for both children and adults and can be effectively used to supplement cereal-based diets (Ogunbusola et al., 2010). The seeds also contain crude fat content of 47.8 g/100 g, crude protein (35.0 g/100 g), and carbohydrates (7.3 g/100 g), potassium 204.97 mg/100 g), and magnesium (33.16 mg/100 g) (Ogunbusola, 2018). Macro- and micro-nutrients and amino acid profiles present in the edible plant parts can be targeted in genetic improvement programmes for developing bio-fortified bottle gourd genotypes. Therefore, it is important to understand relationships between macro- and micro-nutrients and amino acid compositions in bottle gourd to develop breeding strategies aimed to improve nutritional profiles of the crop.

The seed also has significant amount of oil reaching up to 35%. The oil is used in the food and pharmaceutical industries. Bottle gourd oil has medicinal property for treating skin infections (Said et al., 2014). The total fatty acid content of bottle gourd seed is about 39.22% with linoleic acid (63.32%) and palmatic acid (21.36%) being the most abundant (El-dengawy et al., 2001). The nutritional value of bottle gourd may be affected by the presence of unfavourable anti-nutrients. Bottle gourd seeds contain trace amounts of tannic acid content of 1.70 mg/100 g, phytin phosphorus (0.82 mg/100 g), phytic acid (2.88 mg/100 g), and oxalate (1.58 mg/g) (Ogunbusola, 2018). Linkages of essential nutrients with anti-nutrients can reduce bioavailability and digestibility of essential mineral elements. However, the amount of anti-nutrients in bottle gourd seed is relatively low and could further be reduced through cross breeding and selection.

The leaves and fruits are rich sources of bio-active compounds such as cucurbitacins (Attar and Ghane, 2018, 2019; Chanda et al., 2019) which have various health benefits such as antioxidant, antidiabetic, anti-tumor, anti-proliferrative and anti-microbial properties (Marzouk et al., 2012; Ma et al., 2014; Ku et al., 2017; Attar and Ghane, 2019). The contents of cucubitacins type E and I have been quantified in leaves (Mashilo et al., 2018; Chanda et al., 2019). The concentration of cucurbitacin I was 0.04% w/w in mature fruit (Chanda et al., 2019). Cucurbitacin I contents of 1.00, 6.34, and 1.61 mg/g were reported in the fruit epicarp, fruit mesocarp, and seeds of wild bottle gourd, in that order (Attar and Ghane, 2018). In sweet domesticated bottle gourd, very low concentration of cucurbitacin E (0.05 mg/g) were reported, whereas cucurbitacin I ranging from 0.12 to 14 mg/g were reported (Mashilo et al., 2018). Very high concentration of cucurbitacins cause bitterness and poisoning (Puri et al., 2011; Ho et al., 2014). For example, cucurbitacin E content varying from 2 to 12.5 mg/kg is reportedly toxic to humans. Cucurbitacin C content of 1 and 2 mg/kg is reported to be toxic (Yuan et al., 2019). Hence there is a need for a strategic breeding approach to develop varieties with desirable cucurbitacin contents to reduce phyto-toxicity and to enhance the use of bottle gourd fruit for medicinal purposes. To develop bottle gourd genotypes with enhanced nutritional and phytochemical compositions, germplasm profiling for micro-and-macro nutrients, amino acids, oil and fatty acids contents, anti-nutrients, and phytochemical compounds is required. This will ascertain the levels of genotypic variation for nutritional and phyto-compounds. Association studies between nutritional, anti-nutrients, and phyto-compounds are required to develop an effective breeding programme to improve selection efficiency combing the desired traits. Planned crossing between desirable genotypes followed by assessment of gene action governing inheritance of nutritional and anti-nutritional traits in the parents and progenies is vital. Appropriate selection strategies will then be used for selection and genetic advancement of promising lines for further evaluation, release, and commercialization.

1.2.6. Biotic stress tolerance

Bottle gourd is moderately resistant to a number of viral and fungal caused diseases such as papaya ringspot, Fusarium wilt, powdery mildew, cercospora leaf spot and tobacco mosaic virus (Yetişir and Sari, 2003; Yetişir et al., 2007; Kousik et al., 2008), cucumber mosaic virus (CMV), papaya ringspot virus (PRSV-P), watermelon mosaic virus 2 (WMV-2), and zucchini yellow mosaic virus (ZYMV) (Provvidenti, 1981, 1995; Provvidenti and Gonsalves, 1984; Ling and Levi, 2007). Owing to its desirable biotic stress resistance, bottle gourd is one of the four commercial rootstocks widely used in watermelon production (Yetişir and Sari, 2003; Cohen et al., 2007; Kousik et al., 2008; Keinath and Hassell, 2014) to improve resistance to soil-borne pathogens, improve fruit yield and quality of grafted watermelon (Yetişir et al., 2008; Çandir et al., 2013). For example, sweet watermelon (cv Crimson Tide) grafted onto bottle gourd rootstocks namely FR Gold, Emphasis, 216 and Skopje bottle had increased fruit yield by 8.98, 9.64, 8.20, and 13.26 kg/m^2 compared to non-grafted plants (6.43 kg/m2) (Yetişir et al., 2003). Sweet watermelon (cv Suprit) grafted onto bottle gourd rootstocks namely: Arka Bahar and Pusa Samriddhi recorded high fruit yield at 4.1 and 4.2 kg, respectively compared with un-grafted watermelon plants (3.63 kg) (Pal et al., 2020). Grafted sweet watermelon (cv Crimson Tide) onto bottle gourd rootstock designated as 01-16, 07-06, 09- 01, 31-08, 31-09, 33-02, 33-45, 46-03, and Macis had increased the total sugar content being > 9% compared to non-grafted plants (~8%) (Çandir et al., 2013). Further, increased contents of total soluble solids to titratable acidity ratio, juice pH, citric acid, organic acid, lycopene, and carotenoid were reported on sweet watermelon grafted onto bottle gourd rootstock compared to non-grafted control (Çandir et al., 2013). Flesh firmness of 9.41 kg cm^{-2}

was reported for sweet watermelon (cv Zaojia 8424) grafted onto bottle gourd rootstock (cv Jingxinzhen 1) compared to non-grafted plants (6.64 kg cm^{-2}) (Huang et al., 2016). Transcriptome analysis of grafted watermelon onto bottle gourd rootstock revealed activation of genes involved in the increase of fruit size and rind toughness affirming the beneficial impact of bottle gourd as rootstock for improving fruit quality of grafted watermelon (Garcia-Lonazo et al., 2020). Rootstock: scion graft combinations and compatibility of genetically diverse bottle gourd and sweet watermelon genetic resources are yet to be determined for improving fruit yield and quality of grafted watermelon for commercial production. Some bottle gourd rootstocks currently used for grafted watermelon in various countries such as the USA and China include Coloso, Emphasis, Macis, Skopje, Fr Gold, Jingxinzhen no.1, WMXP3938 and WMXP3945 (Yetişir et al., 2003; Thies et al., 2010; Keinath and Hassell, 2014; Zhong et al., 2018).

Several bottle gourd genotypes resistant to powdery mildew race 1 (Kousik et al., 2008) such as PI271353, PI 271357, and PI 271359 possess moderate to high resistance to Zucchini yellow mosaic virus (Provvidenti and Gonsalves, 1984; Ling and Levi, 2007). PI 271353 was selected for its resistance against a number of viruses such as cucumber mosaic virus, squash mosaic virus, and watermelon mosaic virus (Rai et al., 2008). Some varieties such as Pusa Naveen, Pusa Santushti, Pusa Samridhi, and Pusa Sandesh reportedly had high level of resistance to cercospora leaf spot (Maheshwari et al., 2015). From these reports there is higher possibility of developing highly resistant gourd rootstocks/hybrids for watermelon production. Potential bottle gourd genotypes have been identified which may serve as useful for rootstocks commercial production of grated watermelon (Yetişir et al., 2007; Kousik et al., 2008; Keinath and Hassell, 2014). These include accessions 33-15, 31- 08, 31-09, 48-07, 07-04, 07-06, 07-42, 09-01, 35-01, and 07-06 identified in Turkey (Yetişir et al., 2007; Yetişir and Karaca, 2018) and plant introductions PI271353, PI271357, PI381840, PI273663, PI271353, PI271357, PI381840, PI273663, USVL1- 8, USVL5-5, USVL351-PMR, and USVL482-PMR identified in the USA (Kousik et al., 2008, 2018; Ling and Levi, 2013). Development of bottle gourd genotypes with multiple disease resistance could result in highly-adapted and highly performing bottle gourd genotypes for production or use as rootstocks. For example, bottle gourd genotype PI 280633 originally collected from South Africa show complete resistance to ZYMV (Ling and Levi, 2007). Furthermore, genotypes such as Emphasis, Macis, PI 27353, USVL1-8, and USVL5-5 with resistance to powdery mildew, watermelon mosaic virus, and zucchini yellow mosaic virus are useful germplasm for developing rootstocks with multiple disease tolerance. Bottle gourd hybrids such as FR-Ganggeon and FR-Sinsegye developed from crosses between FRD 22 × 963381 and FRD 2010 × 963385-2 show high levels of resistance to Fusarium wilt (Huh et al., 2012). Further, some hybrids developed in India display high levels of resistance against Fusarium wilt (Dhillon et al., 2016). To develop superior bottle gourd rootstock for watermelon production, the rooting characteristics of the cultivated accessions requires rigorous evaluations. Accessions with high deep rooting system and vining ability expressing high fruit potential can be selected for further evaluations. This is followed by grafting on widely grown watermelon cultivars followed by evaluations for yield potential and fruit quality assessment from rootstock: scion combinations. Potential accessions can then be recommended as rootstocks for commercialization and production of watermelon in SSA.

1.2.7. Abiotic stress tolerance

Bottle gourd exhibit some level of drought tolerance compared to other cucurbits (Samadia, 2002; Quamruzzaman et al., 2009; Mashilo et al., 2017a). Furthermore, the crop exhibits high salinity tolerance (Nisini et al., 2002; Yetişir and Sari, 2003; Colla et al., 2005; Yang et al., 2015; Yetişir et al., 2016) and hence widely used as rootstock for watermelon. Several drought tolerant landrace varieties have been identified (Mashilo et al., 2016a, 2017a, 2018). Also, genotypes including L28-16-3, LS28-17-2, Macis, Argentario, and Chaofeng Kangshengwang were reported to be salt tolerant (Yang et al., 2012, 2013, 2015; Yetişir and Karaca, 2018). Strategic crosses among drought tolerant landrace accessions of bottle gourd may enhance the level of drought tolerance and development of well-adapted hybrids.

Understanding of the physiological basis of drought tolerance in bottle gourd can aid effective screening and identification of novel genetic resources for breeding. Secondary metabolites such as cucurbitacins are produced in response to drought and heat stress and may also be important indicators of stress tolerance (Balkema-Boomstra et al., 2003; Mashilo et al., 2018). Cucurbitacins are a group of triterpenoids that occur exclusively in most cucurbit crops, including bottle gourd (Sharma et al., 2006; Ma et al., 2019; Semenya and Maroyi, 2019; Gong et al., 2021; Saurabh et al., 2023). However, information is scanty supporting their role in conferring drought tolerance in cucurbit crops. Mashilo et al. (2018) reported increased levels of cucurbitacin E and I in response to water stress. The authors suggested that the accumulation of cucurbitacins may be a potential physiological marker for identification and selection of bottle gourd genotypes for drought tolerance breeding. Therefore, understanding the role of cucurbitacins for abiotic stress tolerance may aid as a novel selection/breeding criterion for abiotic stress tolerance breeding in bottle gourd or related cucurbit crops. Profiling of diverse cucurbitacins should be carried out involving a broader bottle gourd genetic pool to identify novel genetic resources that can be used as mapping populations to unravel molecular mechanisms and identification of genes regulating cucurbitacin biosynthesis in bottle gourd.

1.3. Genetic resources of cultivated and wild bottle gourd

The genus *Lagenaria* comprises of the cultivated bottle gourd and five wild species including: 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; Jeffrey, 1976). Germplasm collections of the cultivated bottle gourd are available in several genebanks such in the USA, Africa (i.e., South Africa, Zimbabwe, and Kenya), Asia (i.e., China and Japan), and Turkey (Morimoto et al., 2005; Kousik et al., 2008; Yetişir et al., 2008; Xu et al., 2014). For wild species, a large set of collections of L. *sphaerica* and few collections of L. *abyssinica* and L. *breviflora* were reported in Kenya (Morimoto et al., 2005). There is generally limited data available regarding germplasm status of wild *Lagenaria* species in genebanks. Morphologically, the cultivated bottle gourd show extensive variation compared to its wild relatives for quantitative fruit traits (Morimoto et al., 2005). Reportedly, intra-specific variations exists between L. *siceraria* and L. *sphaerica*, L. *abyssinica* and L. *breviflora* (Decker-Walters et al., 2001; Morimoto et al., 2006). Within the genotypes of L. *sphaerica*, there also exists extensive genotypic variation (Morimoto et al., 2006). However, there is inconclusive evidence on phylogenic relationships among genotypes of L. *abyssinica* and L. *breviflora* (Morimoto et al., 2006) and this is yet to be resolved using diagnostic molecular markers. Further, there is limited data on the extent of introgression between the cultivated and wild species of bottle gourd. Wild species are a useful genetic resource for introgressing useful genes for biotic and abiotic stress tolerance. For example, hybrids derived between cowpea [*Vigna unguiculata* (L.) Walp.] and its wild relatives V. *umbellata* and V. *exilis* showed enhanced drought tolerance (Takahashi et al., 2015). Inter-specific hybrids of the cultivated eggplant (*Solanum melongena*) with wild species S. *insanum* displayed higher levels of drought tolerance (Kouassi et al., 2021). For effective use of wild *Lagenaria* species for breeding, there is need for comparison based on qualitative and quantitative traits using relatively large germplasm set to determine the levels of variation for these traits. Also, heat and drought tolerance, and diseases resistance studies on wild species of *Lagenaria* are required for genotype identification and selection. This information is critical to develop breeding strategies incorporating wild relatives in breeding programmes.

The domesticated bottle gourd genotypes are non-bitter, whereas wild forms are very bitter (Sivaraj and Pandravada, 2005; Morimoto et al., 2006). Fruit bitterness is associated with two RAPD markers suggesting that bitter genotypes of wild species are genetically distinctive from non-bitter cultivated types (Morimoto et al., 2006). Bitterness in the wild species is caused by accumulation of cucurbitacin I (Attar and Ghane, 2018). 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). As a result, wild species of bottle gourd may be useful sources of phytochemical compounds especially cucurbitacins for pharmaceutical applications. We propose comparative analysis of cucurbitacins profiling between cultivated and wild bottle gourd species to determine the worth of wild species as sources of phytochemical compounds. Also, there is scant information on nutritional compositions of wild bottle gourd species and its worthwhile for breeding for nutritionally-enhanced genotypes in the cultivated bottle gourd. Comparative analysis of macro and micro nutrients, amino acids, oil, and fatty acids contents between the cultivated and wild species is required to guide breeding approaches.

1.4. Advances in bottle gourd genomics

Marker-assisted breeding is a complementary tool to phenotyping allowing accelerated cultivar development and deployment. Molecular markers such as randomly amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), sequence-related amplified polymorphism (SRAP), inter-simple sequence repeat (ISSR), and single nucleotide polymorphism (SNP) markers have been used to assess genetic variability in bottle gourd and identified genetically distant genotypes for cultivar development (Provvidenti and Gonsalves, 1984; Singh et al., 2010; Xu et al., 2011; Abdin et al., 2014; Srivastava et al., 2014; Bhawna et al., 2015; Gürcan et al., 2015; Mashilo et al., 2016c). Among molecular markers deployed for genetic analysis of bottle gourd genetic resources, SSR markers show high degree of polymorphism and are highly discriminative of bottle gourd germplasm (Xu et al., 2011; Sarao et al., 2014; Bhawna et al., 2015; Mashilo et al., 2017c). Limited SSR markers have been developed for genetic differentiation of bottle gourd genetic resources. Development of SSR markers with more genomic coverage is useful to facilitate rapid screening of large bottle gourd populations including wild species for identification and selection of genetically unrelated and complementary genotypes for hybrid breeding. The marker data will complement genotype selection based on agronomic, horticultural and nutritional attributes, and biotic and abiotic stress tolerance for effective breeding.

Sequence databases that allow for high quality genome assemblies are available for bottle gourd (Xu et al., 2011; Wang et al., 2018). This includes the GourdBase, RNA-Seq, UniProt, SNAP, AUGUSTUS, PASA2 pipe-line, Pfam, MARCOIL, and cucurbit genomic databases (Xu et al., 2011; Wu et al., 2017b; Wang et al., 2018). GourdBase consists of more than 27,500 genes, over 6,000 molecular markers, fruit shape phenotypes for over 100 genetically diverse bottle gourd accessions and 56 quantitative trait loci (QLTs) controlling various traits such as umami taste, fruit shape and bitterness (Wang et al., 2018). Transcriptome sequencing data determined regulation of fruit size in bottle gourd revealing a total of 1,250 differentially expressed genes (DEG) of which 422 genes were upregulated and 828 genes were downregulated. The genes were associated with various physiological functions including cell wall metabolism, phytohormones, cell cycle, and cell division (Zhang et al., 2020a). These genomic resources present opportunities for bottle gourd genetic analysis and improvement to accelerate breeding of novel genotypes for diverse markets and consumers. Further, the available genomic resource will aid dissection of QTLs linked to agronomic and horticultural traits and nutritional attributes, and resistance to biotic and abiotic resistance to facilitate breeding and cultivar deployment.

To date, there is little information regarding QTLs controlling qualitative and quantitative traits, biotic and abiotic stress resistance in bottle gourd. Two QTLs namely Q*Bt.*1 and Q*Bt.*2 were reported to regulate bitter taste in bottle gourd (Wu et al., 2019). Thirty-seven genes conditioning plant growth and development have been identified in bottle gourd. The genes encode plant-specific transcription factors. Most of these genes were located in the nucleus, chloroplast, and mitochondria (Sidhu et al., 2020). In other cucurbit crops such as bitter gourd and *Cucurbita pepo,* QTLs have been mapped for different traits such as sex ratio (i.e., QTLs *qSR14a-F3*, *qSR14b-F3* and *qSR14b-F3*), sex expression (i.e., QTL gy/fffn/ffn), node at first pistillate flower appearance (i.e., QTL *qNPF9-F3* and *qNPF14-F2*), fruit epidermal structure (i.e., QTL *fwa* and *Wr*), immature fruit colour (i.e., w/L/H◦), and days to first pistillate flower appearance (i.e., QTL *qDPF14-F3*) (Montero-Pau et al., 2017; Cui et al., 2018; Rao et al., 2018). In addition, QTLs associated with fruit yield per plant (QTL *ypp5.1*), number of fruits per plant (QTLs *fpp5.1* and *ypp5.1*), fruit weight (QTLs *fw4.1*, *fw5.1*, *fw6.1*, and *fw12.1*), first female flower node (QTL *ffn9.1*), female flower ratio (QTL *ffr5.1* and *ffr4.1*) and fruit length (*fl1.1*) were mapped in bitter gourd (Wang and Xiang, 2013). QTLs with positive correlation with fruit shape (QTL *IFSh_3*, *IFSh_12*, *MFSh_4*, and *MFSh_5*), fruit length (QTLs *MFLe_3,* MFLe_12, *MFLe_6*, *MFLe_9*, *IPeLe_10*, *IPeLe_16*, and *MPeLe_14*), fruit colour (QTLs *ILRCo_4*, *ILRCo_10*, *ILRCo_1*, *ibRCo_4*, *ibRCo_3*, *ibRCo_12*, *laRCo_10*, *laRCo_3*, *MLRCo_4*, *MLRCo_1*, and *MLRCo_2*) were reported in *Cucurbita pepo* and other cucurbits (Montero-Pau et al., 2017). Two QTLs associated with flowering time and four QTLs linked to pollen fertility (*qPF1.1*, *qPF1.2*, *qPF3*, and *qPF7*) were mapped in hybrids developed between *Luffa acutangula* (L.) Roxb. and *L.cylindrica* (L.) Roem. (Wu et al., 2016). To develop QTLs in bottle gourd, genetic linkage maps using an F_2 population derived from desirable parental genotypes can be generated. This is followed by QTL mapping to dissect genomic regions associated with desired traits. This approach is widely undertaken in other cucurbit crops for QTL mapping and marker-assisted breeding (Wu et al., 2016; Cui et al., 2018) and provides opportunities for gene mapping in bottle gourd.

Heat and drought stress are major production constraints to bottle gourd production in arid and semi-arid environments. Seven heat-tolerant quantitative trait loci (*qHT1.1*, *qHT2.1*, *qHT2.2*, *qHT5.1*, *qHT6.1*, *qHT7.1*, and *qHT8.1*) were reported in bottle gourd. QTL *qHT2.1* was identified with major genetic effect linked to drought tolerance (Song et al., 2020). SNP markers namely SNP 2, SNP 16, and SNP 31 were associated with heat tolerance (Song et al., 2020) which are useful genomic resources for marker-assisted selection for heat tolerance in bottle gourd. Plant diseases are a major constraint in bottle gourd production (Mashilo et al., 2017b). High quality bottle gourd genome sequence facilitated mapping of a dominant monogenic locus (Prs) that conferred resistance to papaya-ringspot virus (Wu et al., 2017c). Several multiple genes such as *LsWD*, *LsGAPDH, LsH3*, *LsP4H*, and *LsCYP* associated with resistance to cucumber green mottle mosaic virus affecting bottle gourd were mapped (Zhang et al., 2018). Recently, a gene designated *HG_GLEAN_10011803* was identified and thought to be the major gene conferring resistance to Fusarium wilt in bottle gourd (Yanwei et al., 2021). High-density genetic maps have been developed employing various molecular markers including ISSR, SSR and SNPs (Xu et al., 2011, 2014; Bhawna et al., 2015). This allowed marker–trait associations using genome wide associations (GWAS) such as conditioning free glutamate content (Wu et al., 2017a). In melon and cucumber, GWAS was successfully employed to identify genomic regions associated with Fusarium wilt resistance (Joobeur et al., 2004; Zhang et al., 2014). Bottle gourd genotypes possessing resistance to economically important diseases such as zucchini yellow mosaic virus, watermelon mosaic virus, squash mosaic virus, tomato ringspot virus, tobacco mosaic virus, cucumber green mottle mosaic virus and powdery mildew have been identified (Provvidenti, 1981, 1995; Kousik et al., 2008; Ling and Levi, 2013). These are useful sources to identify candidate genes linked to disease resistance. However, QTL controlling disease resistance remains under-studied thus limiting marker-assisted breeding for biotic stress resistance of bottle gourd. Further, molecular markers linked to economically important diseases of bottle gourd remains limited for marker assisted breeding. We propose GWAS to identify SNPs and genomic regions associated with resistance to economically important diseases in bottle gourd. This will aid to design of closely associated molecular markers linked to disease resistance for effective germplasm characterization and breeding. For development of molecular markers, next-generation sequencing (NGS) technologies have been widely used in cucurbit crops such as watermelon, bitter gourd and bottle gourd to generate large amounts of sequence information for marker development (Ren et al., 2012; Zhu et al., 2016; Wu et al., 2019). NGS technology can also be applied in bottle gourd for marker development associated with disease resistance. Generally, NGS may assist in development of genomic tools for bottle gourd breeding to facilitative cultivar development with desirable traits for consumers and various industries. Cytogenetic analysis of under-researched crops such as bottle gourd is useful to achieve successful crossing, and for conventional and genomic selection programs to improve economic traits. Achigan-Dako et al. (2008) reported genome size differences reaching up to 9.5% within the cultivated genotypes of *L. siceraria* with diploid nuclear DNA value (2C) varying from 0.702 to 0.759 picograms. Generally, there is dearth of information on genome size and chromosomal variation in cultivated and wild species of bottle gourd.

1.5. Genetic engineering and genome editing

Genetic engineering is the technique of inserting new genetic information into existing cells in order to improve the expression of traits of interest. Agrobacterium tumefaciens-mediated transformation provided opportunities for incorporating useful traits in bottle gourd (Han et al., 2004, 2005; Cho et al., 2017). An Arabidopsis *CBF3/DREB1A* gene which confers cold acclimation was successfully transferred into bottle gourd using Agrobacterium-mediated transformation (Cho et al., 2017). This resulted in cold resistant transgenic plants compared to non-transgenic plants. The *CBF3/DREB1A* gene in useful for developing bottle gourd genotypes for rootstock purposes for grafting watermelon to enhance tolerance to cold temperatures. Bottle gourd expressing high levels of drought tolerance was developed incorporating the *Arabidopsis AVP1* gene that encodes a vacuolar H^+-pyrophosphatase. After 10 days of re-watering, wild-type plants showed minimal growth while the *AVP1*-expressing plants resumed rapid growth. Bottle gourd genotype expressing the *AVP1*-gene showed better recovery after 12 days of drought stress and rewatering displaying longer primary roots and more robust root systems compared to wild bottle gourd genotype (Park et al., 2014). Also, transgenic bottle gourd expressing the *Arabidopsis* H^+*- pyrophosphatase AVP1* gene showed improved salt tolerance and maintained higher relative water content under salt stress condition (Park et al., 2014). To improve the performance of bottle gourd as a rootstock, genetic engineering using Arabidopsis Ca^{2+}/H^+ exchanger *sCAX2B* resulted in enhanced Ca^{2+} substrate specificity and decreased Mn^{2+} transport capability. This resulted in watermelon/transgenic bottle gourd (scion/rootstock) combinations with higher osmotic pressure and more soluble solids suggesting that the *sCAX2B* gene improve watermelon fruit quality by effective translocation of nutrients (Han et al., 2009). Watermelon plants grafted onto transgenic bottle gourd rootstock expressing the Arabidopsis H^+-pyrophosphatase *AVP1* gene demonstrated high levels of salt tolerance than those grafted onto wild-type bottle gourd rootstock (Han et al., 2015). These studies demonstrated that genetic engineering by incorporating CBF3/DREB1A and AVP1 genes can result in bottle gourd genotypes with improved abiotic stress tolerance and agronomic performance. Success of genetic transformation depends on a reliable tissue culture regeneration system, gene construct(s), suitable vector(s) for transformation and efficient procedures to introduce desired genes into target plants. Bottle gourd cotyledons are the widely used plant tissue for genetic transformation (Han et al., 2004, 2005; Han et al., 2015).

For improving biotic stress tolerance, genetic engineering has potential for developing disease and inspect pest resistant genotypes. Transgenic watermelon lines expressing artificial microRNAs that target cucumber mosaic virus *2a/2b* genes developed via Agrobacterium mediated-transformation displayed resistance to CMV infection (Liu et al., 2016). A watermelon rootstock named “Gongdae” resistant to Cucumber Green Mottle Mosaic Virus (CGMMV) was developed using Agrobacterium mediated cDNA encoding the CGMMV coat protein gene (*CGMMV-CP*) (Park et al., 2005). Resistance to fungal disease (i.e., powdery mildew), virus diseases (i.e., cucumber green mottle mosaic, watermelon virus diseases) and insect pests (i.e., aphids and thrips) is yet to be achieved in bottle gourd through genetic engineering.

Genome editing offers great potential to develop agronomically and nutritionally superior genotypes with biotic and abiotic stress tolerance. CRISPR/Cas9 is the widely used genome editing platform allowing for substantial improvement of economic traits. In watermelon, CRISPR/Cas9 system was used to perform genome editing of *ClPDS*, a phytoene desaturase gene in watermelon which confers the albino phenotype. Transgenic watermelon plants harboured the *ClPDS* mutations and showed the albino phenotype, indicating that

CRISPR/Cas9 offers effective genome editing efficiency to develop transgenic watermelon lines with desired traits (Tian et al., 2017). CRISPR/Cas9 system was successfully employed to knockout the *Clpsk1* gene, encoding the PSK precursor, to confer enhanced Fusarium oxysporum f.sp. niveum (FON) resistance in watermelon (Zhang et al., 2020b). Development of Zucchini yellow mosaic virus and Papaya ring spot mosaic virus-W resistant cucumber lines was done using Cas9/subgenomic RNA (sgRNA) technology to disrupt the function of the recessive eIF4E (eukaryotic translation initiation factor 4E) gene (Chandrasekaran et al., 2016). Genetic modification is an effective approach for creating genetic variation for desired traits for bottle gourd improvement, however this approach has not been widely adopted. Lack of efficient protocols for transformation, effective gene delivery, selection of transformed cells, propagation, and regeneration methods are among the technical challenges limiting genetic modification in bottle gourd (Song et al., 2019). Also, given the regulatory concerns in South Africa, where all genome-edited crops are currently classified as transgenic regardless of whether foreign DNA is introduced, it is important to prioritise non-transgenic genome editing approaches for bottle gourd improvement. Such approaches can avoid introducing foreign DNA and may help align with biosafety regulations while maintaining public acceptance. Future research on bottle gourd should therefore consider different methods to ensure both innovation and regulatory compliance.

1.6. Conclusions

There is extensive genetic variation of bottle gourd in Africa for diverse qualitative and quantitative horticultural attributes for variety design, product development, and marketing. However, bottle gourd is under- researched and -utilized crop in sub-Saharan Africa. Improved varieties are yet to be developed and commercialized in the region to serve the diverse human needs and for the market-place. The present review summarized progress on bottle gourd breeding, genetic resources, and advances in bottle gourd genomics, genetic engineering and genome editing to guide cultivar development. There is need for collaborative research on bottle gourd involving plant breeders, agronomists, geneticists, and food scientists in the region and internationally for knowledge and germplasm sharing and innovative product development. The next generation of bottle gourd cultivars should encompass product profiles including the quality and quantity of leaves, fruit, fodder, seed, and nutritional composition to serve diverse value chains in both the food and feed industries. .

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Chapter 2. Cucurbitacins B, E and I concentrations and relationship with drought tolerance in bottle gourd [*Lagenaria siceraria* (Molina) Standl.]

Abstract

Bottle gourd [*Lagenaria siceraria* (Molina) Standl.]) is a relatively drought-tolerant cucurbit due to the high composition of unique biochemical compositions, including cucurbitacin. The objective of this study was to determine the concentrations of cucurbitacins in bottle gourd and their relationship to drought tolerance. The study assessed 12 bottle gourd accessions grown under two moisture levels i.e., non-stressed (NS) and drought-stressed (DS) and three drought stress intensities (i.e., mild, moderate, and severe) using a 12 × 2 × 3 factorial experiment designed in a randomized complete block design with three replications. Control studies were undertaken under glasshouse conditions. The content of cucurbitacins B, E, and I were quantified in leaves and roots using high performance liquid chromatography–mass spectrometry (HPLC-MS). The free radical scavenging activities of pure cucurbitacins B, E, and I were quantified using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and a ferrulic acid power assay (FRAP). Results revealed that cucurbitacins B and I were present in accessions BG-48, BG-58, BG-70, BG-78, BG-79, BG-81, BG-52, and GC in leaves and roots under DS condition. The contents of cucurbitacins B and I were enhanced under increased drought intensity for accessions BG-48, BG-81, and GC. In all the leaf and root samples, cucurbitacin E was not detectable. Based on the DPPH test, pure cucurbitacins I, B, and E reduced free radicals at maximum values of 78, 60, and 66%, respectively. Based on the FRAP assay, pure cucurbitacins I, B, and E had maximum ferric-reducing powers of 67, 62, and 48%, respectively. Additionally, cucurbitacin I recorded the highest antioxidant activity compared to cucurbitacins B and E. Increased cucurbitacin accumulation and antioxidant properties indicate their role in minimising cell damage caused by oxidative stress under drought-stressed environments. The present study revealed that cucurbitacins B and I serve as novel biochemical markers for screening drought tolerance in bottle gourd or related cucurbits.

Keywords: antioxidants; cucurbitacin; drought stress; high-performance liquid chromatography

2.1. Introduction

Bottle gourd shows considerable drought tolerance when compared to other cucurbits, including pumpkin (*Cucurbita maxima*) and cucumber (*Cucurbita pepo*) (Sithole and Modi, 2016). In SSA, bottle gourd production occurs under extreme climatic conditions characterized by drought and heat stress. The prevailing of the crop under harsh growing conditions made it a hardy and drought-tolerant crop (Sithole and Modi, 2016; Mashilo et al., 2016, 2017a). The ability of bottle gourd to tolerate drought stress is associated with the high composition of unique biochemical compositions, including cucurbitacin and physiological attributes (Mashilo et al., 2016, 2018). It shows diverse phenotypic attributes associated with its wide adaptation and cultivation in various agro-ecological zones globally, including in Africa, India, Turkey, the USA, and China (Morimoto and Mvere, 2004; Morimoto et al., 2005; Mashilo et al., 2017b; Yetişir and Karaca, 2018).

Cucurbit crops, including bottle gourd, produce secondary metabolites, including cucurbitacins (Morimoto et al., 2005; Mashilo et al., 2017c; Attar and Ghane, 2018; Yetişir, and Karaca, 2018; Patel et al., 2020). Cucurbitacins induce a bitter taste in the leaves, roots, and fruits of most cucurbit crops and are toxic when consumed in large quantities (Sharma et al., 2006; Cynthia et al., 2014; Verma and Jaiswal, 2015; Gong et al., 2021; Patel and Ghane, 2021; Attar et al., 2022). The following cucurbitacins are reportedly produced in cucurbits: A, B, C, D, E, F, H, I, L, Q, R, 23, 24-dihydrocucurbitacin F, dihydrocucurbitacin B, and hexanorcucurbitacin F (Chen et al., 2005; Haq et al., 2019; Dong et al., 2021; Zhang et al., 2022). The biosynthesis of cucurbitacin varies between the *Cucurbitaceae* crops and genotypes of the same species. For example, cucurbitacin B is found in melon (Ansari et al., 2019; Xu et al., 2022), C in cucumber (Shang et al., 2014), E and I in bottle gourd (Mashilo et al., 2018), E in watermelon (Gong et al., 2021), and IIa in *Hemsleya macrosperma* (Xu et al., 2022). Cucurbitacins possess various pharmacological and pharmaceutical values, including neuroprotective (Gitler et al., 2017), anti-inflammatory (Peters et al., 1997), anti-tumor (Ma et al., 2019), hepatoprotective (Wynn, 2008), hypoglycemic (Volpe et al., 2018), and anti-microbial functions (Ahmed et al., 2012; Dwijayanti et al., 2020), thus making bottle gourd consumption greatly beneficial for medicinal purposes. For example, an anti-cancer activity of cucurbitacins has been detected against cells associated with breast and ovarian cancer, hepatocellular carcinoma, human T cell leukaemia, pancreatic cancer, colon cancer, and liver carcinoma (Wakimoto et al., 2008). Other pharmacological values of bottle gourd associated with the presence of cucurbitacins include managing ulcers, diabetes, diarrhoea, and obesity (Balkema-Boomstra, et al., 2003).

Cucurbitacins are produced in cucurbits as a defence strategy against insect pests and diseases (Tewari et al., 2006). They are also produced in response to abiotic stress, including UV radiation, possibly as antioxidant molecules to reduce cell damage caused by reactive oxygen species (ROS) (i.e., free radicals such as O^2 , OH^{-}, H_2O_2, and $^1O_2$) (Mazid et al., 2011; Luo et al., 2020). In addition, cucurbitacin accumulation has been associated with increased drought tolerance (Haynes and Jones, 1975; Mashilo et al., 2018; Dwijayanti et al., 2020). Reportedly, cucumbers subjected to drought stress contained twice the amount of cucurbitacins compared to non-stressed plants (Davidovich-Rikanati et al., 2015). Mashilo et al. (2018) detected cucurbitacins E and I in bottle gourd in response to water stress. The authors reported that the enhanced production of cucurbitacins E and I under water-stressed conditions was linked to drought stress tolerance. However, there is limited information on cucurbitacins' magnitude and expression in regulating drought and other abiotic stress in cucurbits. Therefore, understanding the magnitude and association of cucurbitacins in drought adaptation may aid novel selection markers for drought tolerance breeding in bottle gourd or related cucurbits. The objective of this study was to determine the concentrations of cucurbitacins in bottle gourd and their relationship with drought tolerance.

2.2. Materials and methods

2.2.1. Plant materials

The study used 12 selected accessions of bottle gourd assigned the following codes by the Limpopo Department of Agriculture and Rural Development (LDARD) namely: BG-27, BG-31, BG-48, BG-52, BG-58, BG-67, BG-70, BG-78, BG-79, BG-80, BG-81, and GC. BG stands for botte (B) gourd (G), whereas the numbers denote the accession entry number based on the collection mission. These accessions possess varying drought tolerance levels as reported in our previous work (Mashilo et al., 2016; Mashilo et al., 2018). Furthermore, the accessions are commonly cultivated in the Limpopo Province of South Africa by small-holder farmers under dryland, high-drought, and heat stress conditions. Entry GC is grown in KwaZulu-Natal Province and sold in retail stores in South Africa. The landrace accessions are maintained by LDARD at Towoomba Agricultural Development Centre (TADC), Bela-Bela, South Africa.

2.2.2. Experimental design and sample collection

The experiment was conducted during the 2021 season under a growth chamber environment at the University of Limpopo (−25°3605400 S, 28°0 059.7600 E, 1312 m above sea level), South Africa. The study used the 12 bottle gourd accessions grown under two moisture levels (i.e., non-stressed (NS) and drought-stressed (DS)) and three drought stress intensities (i.e., mild, moderate, and severe) using a randomized complete block design with 3 replications. Seeds of the accessions were grown in a 2 L capacity polyethylene plastic pot containing approximately 2 kg of loamy soil collected from the University of Limpopo, Syferskuil Experimental farm (−23°5309.6000 S, 29°44016.8000 E, 1312 m above sea level). Three seeds were sown per accession and were watered daily to maintain soil moisture content at approximately 40% v/v field capacity. One plant was maintained per pot upon germination and three weeks after emergence. The plants were watered with tap water until a sixth fully expanded leaf was developed approximately 18 to 22 days after planting. Water was then withheld for DS treatment for the entire duration of the experiment, whereas the NS plants were supplied with water. The experimental units were without water on days 7, 14, and 21, corresponding to mild, moderate, and severe drought stress, in that order. Three fully expanded leaves were collected from each plant in DS and NS conditions. The same plants used for leaf sampling were carefully exhumed to expose the roots. The roots were gently washed with tap water to remove soil debris. The leaf and root samples were placed in 50 mL centrifuging tubes and kept at −80 °C in a −86 °C ultralow freezer until analysis.

2.2.3. Sample extraction and determination of cucurbitacins

Cucurbitacin extraction was carried out as previously outlined (Morimoto and Mvere, 2004; Chigayo et al., 2016) with some modifications. Extraction of cucurbitacins from the leaf and root samples was carried out by grinding 0.1 g of the samples to powder using a pestle and mortar in liquid nitrogen. The ground sample was transferred to a 2 mL Eppendorf tube. To each sample, 1 mL of ice-cold methanol (HPLC-grade, Sigma Aldrich) was added, and the mixture homogenised for 15 min using a mini-bead-beater (Biospec Bartlesville, OK, USA). After that, the mix was centrifuged at 14,000 × g, 15 min at 4 ◦C. The supernatant was removed and filtered through hydrophilic polypropylene membrane (13 mm × 0.2 µm) and the cucurbitacin-containing extract was stored at −20 ◦C in a freezer.

2.2.3. Detection of cucurbitacins

The cucurbitacin analyses were performed at the Department of Chemistry, School of Physical and Mineral Sciences, University of Limpopo, South Africa. Cucurbitacin analyses were performed using an SCIEX ExionLC-UV detector, LC, equipped with a Phenomenex Luna C18 2.5 µm, 2 × 100 mm column. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% of formic acid in methanol (B). Gradient elution was as follows: 0–0.5 min 20% B, 0.5–10 min 90% B, 10–12.50 min 90% B, 12.50–12.60 min 20%, 12.60–15 min 20% B (run time 15 min). Flow rate was maintained at 0.4 mL/min at 40 ◦C. Cucurbitacin identification was performed via the comparison of the retention times of the cucurbitacin B, E, and I standards detected at 230 nm. Pure cucurbitacin B, E, and I standards were purchased from Sigma-Aldrich (South Africa, South Modderfontein, Johannesburg). The samples were extracted from Luffa operculate (L.) Cohn, and isolation was conducted using a column chromatography. Molecular identification was performed with nuclear magnetic resonance. Cucurbitacins B, E, and I were quantified using an external calibration curve on a dry weight basis (Figure 2.1). Figure 2.1 shows the calibration curve constructed from peak areas of the three reference standards, i.e., cucurbitacins B, E, and I versus their concentrations, used to quantify the unknown concentrations of the cucurbitacins in leaves and roots of 12 bottle gourd genotypes grown under drought stress and non-stress conditions.

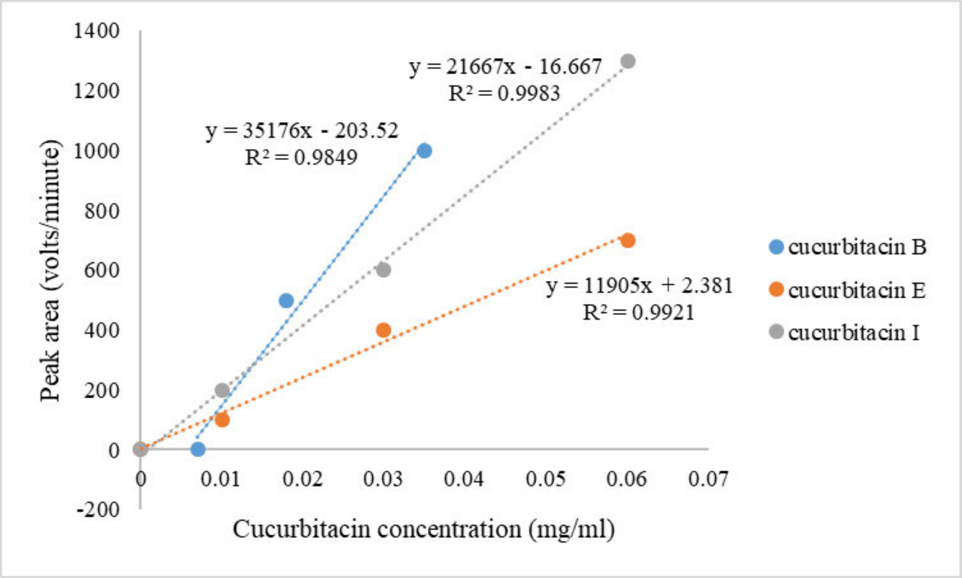

Figure 2.1. Calibration curve showing the peak areas of the standard cucurbitacins B, E, and I of a known concentration range of 0−0.1 mg/mL, used to quantify the unknown concentration of each of the cucurbitacins (B, E, and I) in the fully expanded leaf and whole root samples of 12 bottle gourds grown under drought-stressed and non-stressed conditions.

2.2.4. Quantification of antioxidant levels of pure cucurbitacins B, E, and I

2.2.4.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

Free radical scavenging activity of pure cucurbitacins B, E and I was determined using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay using the method initially reported by Chigayo et al. (2016) with some modification. Cucurbitacin standards were dissolved in methanol (HLPC grade - Sigma Aldrich) to prepare an initial 250 µg/ml solution. From the initial solution, different solutions of concentrations ranging from 250 µg/ml to 15.63 µg/ml to a final volume of 1 ml were prepared in triplicates. The concentration range consisted of the following: 250.00, 125.00, 62.50, 31.25 and 15.63 µg/ml. L-ascorbic acid was used as a standard/positive control by preparing the same concentration range as the cucurbitacin standards. To the 1 ml solutions of cucurbitacins and the positive control, 2 ml of 0.2 mmol/L DPPH solution (dissolved in methanol) was added and vortexed thoroughly. The negative control solution was prepared by adding 2 ml of 0.2 mmol/L DPPH to 1 ml of distilled water. All the mixtures were kept at room temperature, in the dark, for 30 minutes. After the elapsed time, the absorbance of each solution was measured at 517 nm using DU® 730 Life Science UV/Vis spectrophotometer (Beckman Coulter). The absorbance reading was averaged for each of the samples, and the percentage antioxidant potential (percentage inhibition) was calculated using the following formula (Bondet et al., 1997):

$$\%\, Inhibition = \left[\frac{Ac - As}{Ac}\right] \times 100$$

Where,

Ac = Absorbance of control (negative) solution

As = Absorbance of cucurbitacin solution

2.2.4.2. Ferric-reducing power assay

Evaluation of the ferric-reducing power of cucurbitacins B, E, and I was carried out as previously outlined (Benzie and Strain, 1996). with some modifications. Different concentrations of each cucurbitacin standard were prepared in triplicate to the final concentrations of 625.00, 312.50, 156.25, 78.13, and 39.06 µg/mL to a total volume of 2.5 mL. The same concentrations of L-Ascorbic acid were prepared, serving as a positive control. The different concentrations were mixed with 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (Rochelle) (1% w/v) in distilled water in test tubes. After adding each solution, the mixtures were vortexed for approximately 10 s. This was followed by incubation at 50 ◦C for 20 min. After that, 2 mL of trichloroacetic acid (Sigma) (10% w/v) in distilled water was added to each test tube. The tubes were left to stand at room temperature for approximately 20 min; during this time, the sediments settled at the bottom of the tube and the yellow top parts of the mixtures were transferred to new, clean test tubes. To the yellow solution in a new test tube, 5 mL of distilled water and 1 mL ferric chloride (0, 1% w/v) in distilled water were added, and the mixture was vortexed. The same procedure was followed for the preparation of the negative control; instead of adding cucurbitacin, distilled water was added. The colour changes for all the mixtures from yellow to green or blue was monitored and measured at a wavelength of 700 nm using DU® 730 Life Science UV/Vis spectrophotometer. The percentage increase of the potassium ferrocyanide (Fe^{2+}), which is directly proportional to the antioxidative power of the compound, was calculated using the following formula (Tannani-Spitz et al., 2007):

$$\%\ increase\ in\ potassium\ ferrocyanide\ = \left[\frac{As - Ac}{As}\right] \times 100$$

Where,
Ac = Absorbance of control (negative) solution
As = Absorbance of cucurbitacin solution

2.3. Results

2.3.1. Soil moisture content

Significant differences were observed in soil water content across the twelve accessions grown under DS conditions as presented in Figure 2.2. The NS treatment is not shown in Figure 2.2, as soil water content was maintained at field capacity (~40% v/v) throughout the experiment. Under DS conditions, a steady decrease in the soil water moisture was recorded across the different accessions. At 7 days, the soil moisture level had reached approximately 25 to 30% for most accessions. At 14 days of withholding water, the soil moisture content decreased to approximately 7 to 14% for most accessions, except for BG-81, which was at approximately

21%. At 21 days, which corresponded to the severe drought stress, the soil moisture content reached approximately 0 to 5% across the different accessions.

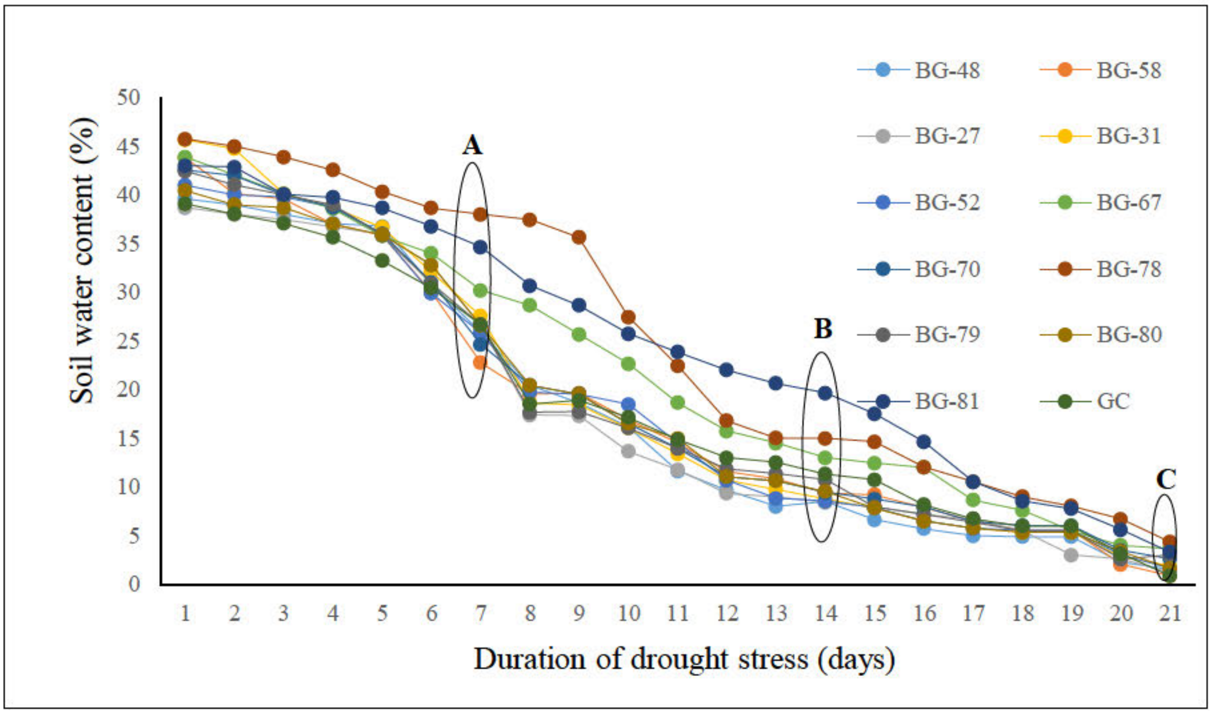

Figure 2.2. Mean soil water content (%) of the accessions grown under drought stress conditions. The areas marked A, B and C correspond with mild, moderate and severe drought stress intensity, respectively.

2.3.2. Cucurbitacins B, E, and I accumulation in bottle gourd accessions under DS and NS conditions

The contents of cucurbitacins B and I in bottle gourd leaves and roots grown under DS and NS conditions are shown in Table 2.1. Representative chromatograms for cucurbitacins B and I detected in samples are presented in Figure 2.3. Cucurbitacin E was not detected in any of the samples. Under DS condition, cucurbitacin B was detected in accessions BG-48, BG-81, BG-70, BG-78, BG-79, and GC in both root and leaf samples. Accessions BG-48, BG-81, and GC accumulated cucurbitacin B of 0.03 mg/g in the leaves under DS conditions. There were significant changes in the root cucurbitacin B concentration under increasing drought intensity for accessions BG-81, BG-48, and GC. Cucurbitacin B contents in roots for BG-81 were 0.04, 0.05 and 0.08 mg/g under mild, moderate and severe drought stress intensities, respectively. Cucurbitacin B values in the roots of BG-48 were 0.3, 0.5, and 0.6 mg/g under mild, moderate, and severe drought stress intensity, respectively. Cucurbitacin B in roots of GC were 0.03 under mild drought stress and increased to 0.04 and 0.06 mg/g under moderate and severe drought stress intensities, respectively. Under NS conditions, cucurbitacin B was not detected in the leaf samples for all accessions, whereas it was detected in the roots of accession BG-31. Cucurbitacin I was not detected in the leaves of any of the accessions under mild drought stress. However, under moderate and severe drought stress cucurbitacin I was detected for the accessions BG-48, BG-81, and GC. In the leaf samples of the accessions BG48, BG-81, and GC, drought stress did not induce significant changes in the accumulation of cucurbitacin I. Cucurbitacin I was not detected in leaf samples under NS conditions. Root cucurbitacin I content increased with increasing drought stress intensity for accessions BG-48 and GC, whereas no significant changes were observed for accessions BG-70, BG-79, and BG-81. Under the NS condition, cucurbitacin I was not detected in the root samples.

Table 2.1 Cucurbitacin B and I detections and concentrations (mg/g) in leaves and roots of bottle gourd accessions evaluated under variable drought stress intensities and non-stressed conditions.

Cucurbitacin B (mg/g)								
	Leaves			Roots			Leaves	Roots
Accession	Mild	Moderate	Severe	Mild	Moderate	Severe	NS	NS
BG-27	ND	ND	ND	ND	ND	ND	ND	ND
BG-31	ND	ND	ND	ND	ND	ND	ND	0.03
BG-48	ND	0.03	0.03	0.03	0.05	0.06	ND	ND
BG-52	ND	ND	ND	ND	ND	ND	ND	ND
BG-58	ND	ND	ND	0.03	0.03	0.03	ND	ND
BG-67	ND	ND	ND	ND	ND	ND	ND	ND
BG-70	ND	ND	ND	0.03	0.03	0.03	ND	ND
BG-78	ND	ND	ND	ND	0.03	ND	ND	ND
BG-79	ND	ND	ND	0.03	0.03	0.03	ND	ND
BG-80	ND	ND	ND	ND	ND	ND	ND	ND
BG-81	ND	0.03	0.03	0.04	0.05	0.08	ND	ND

GC	ND	0.03	0.03	0.03	0.04	0.06	ND	ND

Cucurbitacin I (mg/g)								
	Leaves			Roots			Leaves	Roots
Accession	Mild	Moderate	Severe	Mild	Moderate	Severe	NS	NS
BG-27	ND	ND	ND	ND	ND	ND	ND	ND
BG-31	ND	ND	ND	ND	ND	ND	ND	ND
BG-48	ND	0.04	0.04	0.03	0.04	0.5	ND	ND
BG-52	ND	ND	ND	0.03	0.03	0.03	ND	ND
BG-58	ND	ND	ND	0.03	ND	ND	ND	ND
BG-67	ND	ND	ND	ND	ND	ND	ND	ND
BG-70	ND	ND	ND	0.03	0.03	0.03	ND	ND
BG-78	ND	ND	ND	ND	ND	ND	ND	ND
BG-79	ND	ND	ND	0.03	0.03	0.03	ND	ND
BG-80	ND	ND	ND	ND	ND	ND	ND	ND
BG-81	ND	0.03	0.03	ND	0.04	0.04	ND	ND
GC	ND	0.03	0.04	0.04	0.05	0.07	ND	ND

ND = not detected, DS = drought stress condition, NS = non-stressed condition.

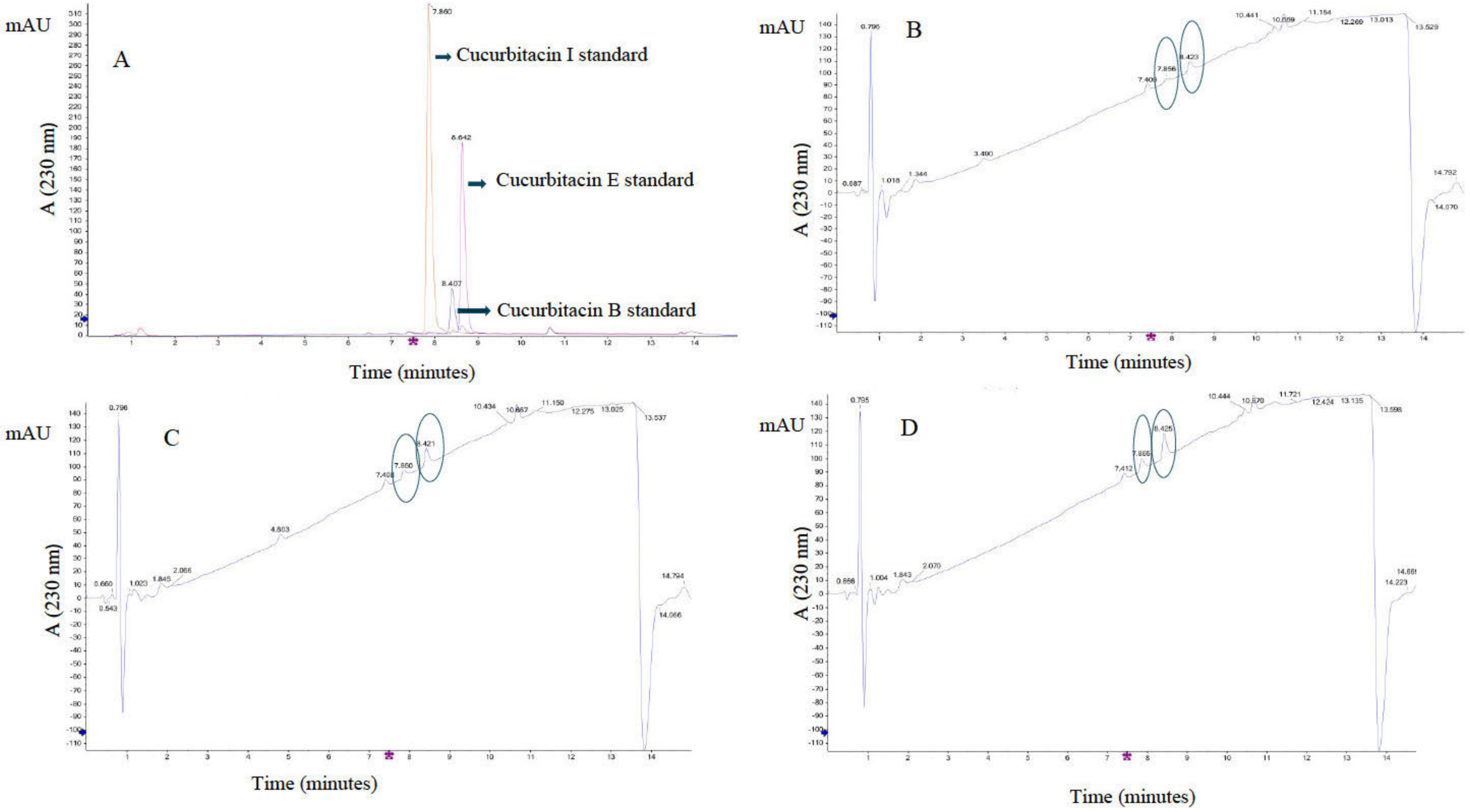

Figure 2.3. Chromatograms showing mass spectra and retention time of cucurbitacins B, E and I standards (A) and cucurbitacin B, E and I detection in leaf and root samples (B to D). mAU = milli-absorbance unit

2.3.3. Free radical scavenging activity of pure cucurbitacins B, E, and I.

Antioxidant potential of cucurbitacins B, E, and I based on the DDPH assay is presented in Figure 2.4. Cucurbitacin B recorded the lowest DPPH-reducing activities of 58 and 60% at 125 and 250 μg/mL, respectively, compared to other cucurbitacins. However, this activity was relatively higher when compared to that of the negative control, which was at a steady 12% across the different concentrations. Cucurbitacin E recorded DPPH-reducing activities of 64 and 67% at 125 and 250 μg/mL, respectively. Cucurbitacin I recorded the highest DPPH-reducing activities of 75 and 79% at 125 and 250 μg/mL, respectively, compared to the other cucurbitacins.

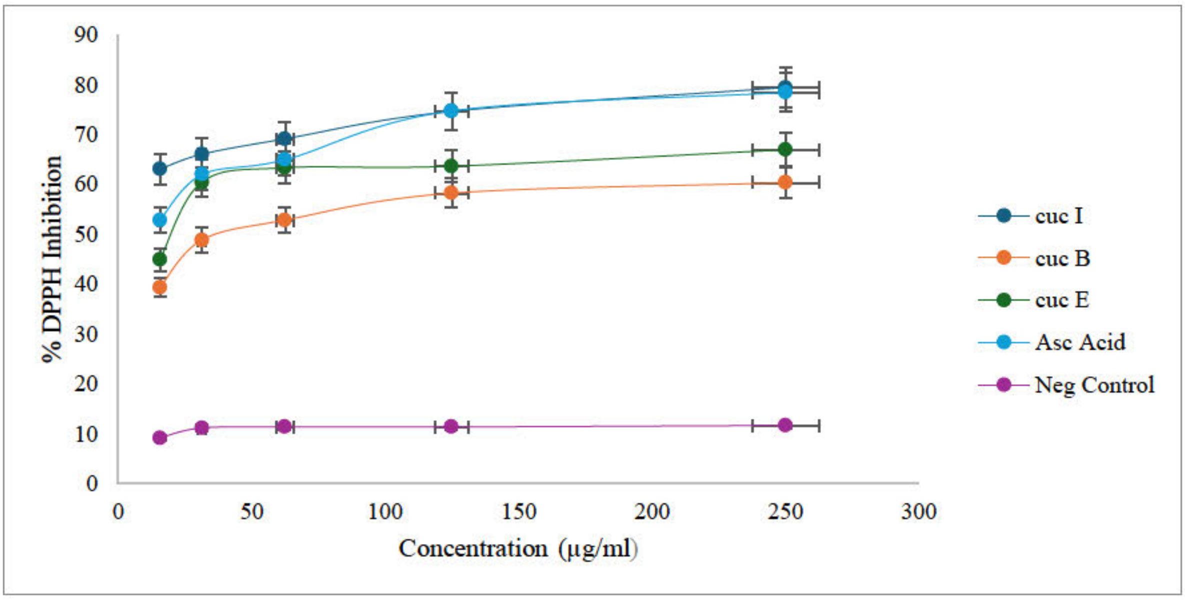

Figure 2.4. Free radical inhibiting activity of pure cucurbitacins B, E, and I based on the DPPH assay. Values are means ± standard error.

2.3.4. Ferric-reducing power of cucurbitacins B, E, and I

Antioxidative activity of cucurbitacin B, E, and I as monitored by their ability to convert potassium ferricyanide (Fe^{3+}) to Fe^{2+} is presented in Figure 2.5. A steady increase in the ferric-reducing power was recorded across the different cucurbitacins compared to the negative control. Cucurbitacin E recorded the lowest activities of 47 and 55% at 312.5 and 625 μg/mL, respectively, compared to the other cucurbitacins. However, the activity of cucurbitacin E was relatively high when compared to that of the negative control. Cucurbitacin B recorded 62% activity at 312.5 and 625 μg/mL. Cucurbitacin I recorded the highest activity of 67% at 312.5

and 625 µg/mL. The physiological model showing the synthesis and potential involvement of cucurbitacin B and I as antioxidant compounds responsible for neutralizing ROS under drought stress conditions is shown in Figure 2.6.

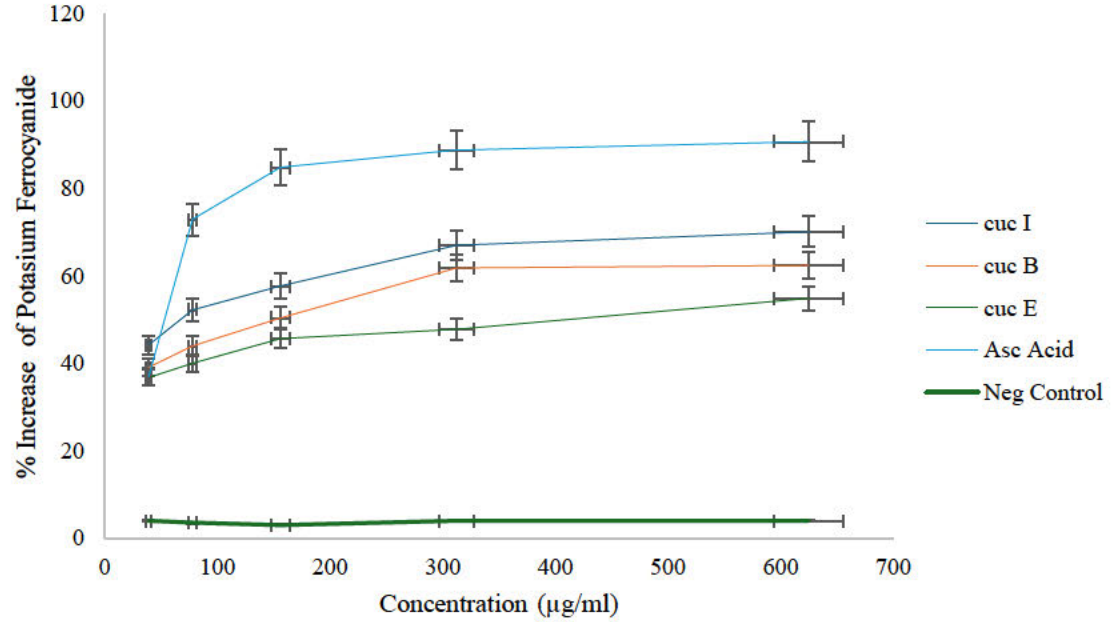

Figure 2.5. Free radical inhibiting activity of pure cucurbitacins B, E and I based on their ability to convert potassium ferricyanide $Fe^{3+}$to Fe^{2+}. Values are means ± standard error.

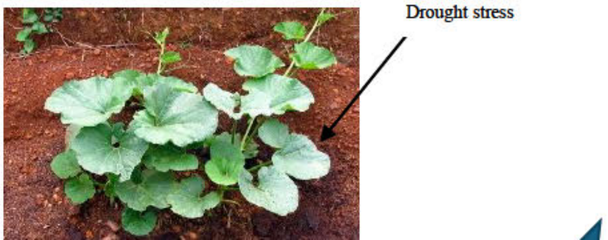

Figure 2.6. Physiological model showing critical steps involved in the synthesis of cucurbitacins B and I, chemical structures of cucurbitacins B and I, the major genes expressed, and their functions as antioxidant molecules in bottle gourd exposed to drought stress. The blue arrows represent the critical steps from drought stress exposure to protection against oxidative stress. The black arrows indicate the critical steps in cucurbitacins biosynthesis triggered by drought stress exposure, including the genes, important enzymes and molecular structures.

2.4. Discussion

In the present study, cucurbitacins B and I were detected in the leaves and roots of some accessions of bottle gourd (Table 2.1). Increased concentrations of cucurbitacins B and I were more noticeable in the roots under increased drought intensity in some accessions, including BG-81, BG-48, and GC (Table 2.1). This suggested water deficit enhances the accumulation of cucurbitacins in bottle gourd. In agreement with the previous study by Mashilo et al. (2018), the present study reported varied contents of cucurbitacin I in bottle gourd subjected to water stress. According to Davidovich-Rikanati et al. (2015) abiotic stress influences cucurbitacin biosynthesis by modulating the expression of *Bi* and/or *Bt* genes, which catalyses the breakdown of 2,3-oxidosqualene to cucurbitadienol to derive various cucurbitacins.

The biosynthesis of cucurbitacin B and I is conditioned by various genes (Figure 2.6). The exposure to drought stress probably increased cellular oxidative stress, triggering a series of biochemical reactions, including the synthesis of secondary metabolites, such as the cyclization of 2, 3-oxidosqualene by different oxidosqualene cyclases genes (Phillips et al., 2006; Tannanin-Spitz et al., 2007). This is a vital step in determining the different triterpenoid backbones (Phillips et al., 2006). There are a number of genes in the oxidosqualene cyclase family such as CmBi, which catalyse the conversion of 2,3-oxidosqualene to Cucurbitadienol (Chen et al., 2005; Thimmappa et al., 2014; Davidovich-Rikanati et al., 2015). Cucurbitadienol synthase is the key enzyme responsible for cucurbitacin biosynthesis in cucurbit crops (Shibuya et al., 2004). The activity of cucurbitadienol is controlled by the gene CYP450s (Shibuya et al., 2004). In bottle gourd, the genes Q*Bt.*1 and Q*Bt*.2 regulate cucurbitacin biosynthesis, which is dissimilar to bitterness-regulating genes in other cucurbit crops (i.e., cucumber, melon, and watermelon) (Wu et al., 2019). Therefore, cucurbitacin biosynthesis is dependent on the type of cucurbit species. The genes Q*Bt*.1 and Q*Bt*.2 could probably enhance the concentration of cucurbitacins B and I in the leaves and roots of bottle gourd (Mashilo et al., 2018) subjected to drought stress (Figure 2.6), similar to the findings of this study.

In the present study, cucurbitacin B and I did not accumulate in most of the bottle gourd accessions, suggesting accession-specific accumulation of the cucurbitacins. This is associated with a preferential selection pressure for the non-bitter types in bottle gourd for various uses, including food. For example, the tender leaves of bottle gourd are cooked as leafy vegetables by the Venda tribe of South Africa (Zhou et al., 2016). The consumption of the leaves in some parts of sub-Saharan Africa suggests possible loss of bitterness during crop domestication (Kim et al., 2018). The roots of bottle gourd are extremely bitter, an indication of high amounts of cucurbitacins. However, in the present study, cucurbitacins B and I in roots were not detected in most of the studied accessions (Table 2.1), except for BG-48, BG-53, BG-48, BG-70, and GC. This indicates that cucurbitacin accumulation is accession-specific, perhaps due to varied expression of biosynthetic genes (Davidovich-Rikanati et al., 2015). For example, the genes designated C*mBi*, C*m*710, and C*m*AC showed varied expression to regulate cucurbitacin B contents in melon (*Cucumis melo*) (Luo et al., 2020). In watermelon (*Citrullus lanantus var. lanantus*), genotypic-specific accumulation of cucurbitacins B and E has been reported (Irshad et al., 2012). Accessions BG-48, BG-81, and GC, which accumulated high amounts of cucurbitacin B and I, were previously identified as drought-tolerant by Mashilo et al. (2018), perhaps due to their cucurbitacin accumulation and other physiological mechanisms, including efficient photosynthetic capacity and photo-protection.

To determine the possible roles of cucurbitacins as antioxidant molecules, pure cucurbitacin samples were evaluated for their antioxidant activity using DPPH and FRAP assays (Figures 2.4 and 2.5). DPPH radical is a stable free radical due to its unpaired electron delocalization over the whole molecule. The donation of a proton (H^+) to this radical causes a colour change from violet to pale yellow in a solution (Ionita, 2021). The yellow colour development results from the odd electron of a nitrogen atom in DPPH being reduced by receiving a hydrogen atom from antioxidants (Gulcin and Alwasel, 2023). The Ferric-reducing ability of a compound is monitored through the reaction between the "compounds of interest"—in our case, cucurbitacin with potassium ferricyanide (Fe^{3+}) to form Fe^{2+}. The potassium ferrocyanide then reacts with ferric chloride to form a ferric-ferrous complex, a reaction monitored through the colour change from yellow to green (Oktay et al., 2003). To determine the possible roles of cucurbitacins as antioxidant molecules, pure cucurbitacin samples were evaluated for their antioxidant activity using DPPH and FRAP assays (Figures 2.4. and 2.5). Amongst the three pure cucurbitacin solutions, cucurbitacin I recovered the highest activity based on both assays, indicating potential radical-scavenging activity and conferring drought stress tolerance by reducing cell and tissue damage associated with oxidative stress under drought stress (Chanda and Dave, 2009; Elbasheir and Ndiko, 2021). Drought tolerance in maize and sorghum were associated with increased antioxidant activity which correlated with less cell damage under water-deficit conditions (Elbasheir and Ndiko, 2021). Increased antioxidant activity in wheat was also associated with drought tolerance (Wang et al., 2022). In melon (*Cucumis melo* L.), cucurbitacin B accumulation has been associated with increased antioxidative activity that enhanced tolerance to biological stress (Oktay et al., 2003). Cucurbitacin-I-, B-, and E-enriched *Cucurbitaceae* crops, such as *Blastania cerasiformis* (Stocks) A. Meeuse and B. *garcinii* (Burm. f.) Cogn., were associated with high antioxidant activity (Attar and Ghane, 2022). In citron watermelon (*Citrullus lanatus* var. *citroides*), cucurbitacin E was reported to be an inefficient scavenger of reactive oxygen species (Abdelwahab et al., 2011; Azin et al., 2021; Kang et al., 2022). In the present study, an increase in the concentrations of cucurbitacins B and I is associated with some level of drought stress tolerance in bottle gourd and serves as a potential marker for selecting drought-tolerant accessions for breeding. There is a need for genetic analysis, including the roles and expression in the biosynthesis of cucurbitacins B, E, and I under abiotic stress conditions. Additionally, the detection and possible involvement of other cucurbitacins in conferring abiotic stress tolerance in bottle gourd is paramount.

2.5. Conclusions

The present study determined the potential roles of cucurbitacins in conferring drought tolerance in bottle gourd. Accumulation of cucurbitacins B and I were identified in leaves and roots of some bottle gourd accessions subjected to drought stress. Based on the DDPH test and FRAP assays, pure cucurbitacins B, E, and I reduced free radicals, indicating their ability as antioxidant molecules in reducing cell damage caused by reactive oxygen species in drought-exposed bottle gourd crops. The present study revealed that cucurbitacins B and I are novel biochemical markers for screening drought tolerance in bottle gourd or related cucurbits.

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Chapter 3. Hybrid performance of bottle gourd [*Lagenaria siceraria*] under drought stress and non-stress conditions

Abstract

Bottle gourd (*Lagenaria siceraria* (Molina) Standl.) is one of the opportunity crops with the potential for enhancing food and income security. However, it is an undervalued crop with limited production and commercialization success attributable to a lack of dedicated research capacity and breeding efforts that develop and release varieties with valuable end-uses. Bottle gourd genetic resources are relatively tolerant to heat and drought stress, presenting opportunities for developing climate-smart and drought-resilient cultivars adapted to dry environments. Therefore, the objective of this study was to determine drought tolerance among newly-developed single cross bottle gourd hybrids to identify hybrids that outperform their parents and recommend the best performers for further testing, production and commercialization in South Africa. Fifty-three F_1 families were developed and field evaluated with 12 parental landrace accessions under non-stressed (NS) and drought-stressed (DS) conditions in two growing seasons using a 5 × 13 α-lattice design with three replicates. Data were collected on fruit yield per plant (FYPP), seed yield per plant (SYPP), and drought tolerance indices computed. Significant interactions were detected among test genotypes and water regimes for FYPP only. The mean FYPP were 0.3 and 1.0 kg, and 0.3 and 0.5 kg for SYPP under DS and NS conditions, in that order. Based on tolerance indices, the following single cross hybrids were identified as drought tolerant: BG-31 × BG-67, BG-27 × BG-31, BG-58 × BG-78, BG-31 × BG-70, BG-31 × BG-78, BG-58 × BG-67, BG-31 × BG-52, BG-52 × BG-78, BG-27 × BG-80, BG-58 × BG-81, BG-31 × BG-48, BG-67 × BG-70, BG-58 × BG-79 and BG-58 × BG-80. The newly-developed bottle gourd hybrids performed better than their parents and are recommended for cultivation in drought-prone agro-ecologies in South Africa.

Keywords: Abiotic stress, Bottle gourd, Drought tolerance indices, Yield-based selection

3.1. Introduction

Bottle gourd [*Lagenaria siceraria* (Molina) Standl., 2n = 2x = 22] is one of the opportunity cucurbit crops cultivated for its fresh and dried fruit, dry seeds and above ground biomass (Gürcan et al., 2015; Aldewy et al., 2022). The tender and freshly cooked fruit with immature seeds is used as a vegetable. The dried fruits have diverse uses, including for decoration, water bottles, grain containers, and serving beverages. Fresh and succulent leaves are cooked and consumed as leafy vegetables or used to treat various ailments (Mokganya and Tshisikhawe, 2019). Also, the seeds are dried and roasted to be consumed as snacks, or the seeds are processed into flour for animal feed (Barot et al., 2015). Fresh and dried vines are used as livestock fodder supplements.

The fresh fruit of bottle gourd is high in protein, fat, carbohydrate, fibre, ash, and energy and contains essential minerals such as iron, phosphorus, potassium, calcium and magnesium (Sithole et al. 2015; Mahapatra et al., 2022). Some bottle gourd fruits are bitter due to secondary metabolites called tetracyclic triterpenoid compounds, mainly cucurbitacins B and I (Attar and Ghane, 2018; Saurabh et al., 2023). Cucurbitacins have medicinal importance, including the treatment of cancers (Attar and Ghane, 2019; Ma et al., 2019; Moustafa et al., 2021; Patel and Ghane, 2021). The leaves are a source of essential nutrients (Sithole et al., 2015), whereas the seeds are a valuable source of protein and edible oils (Hassan 2008). The dried seeds are milled into flour and used as soup thickener in some SSA countries, including Nigeria (Maigandi and Ngang, 2002). Bottle gourd seeds are a good source of dietary fiber and phytochemicals such as carotene and tocopherol (Abdel-Razek et al., 2021; Devi et al., 2023), sterols (i.e., β-sitosterols) (Abdel-Razek et al., 2021) and amino acids (i.e., aspartic acid, arginine, leucine and lysine) (Ogunbusola et al., 2010). The health attributes of bottle gourd makes it a valuable crop for providing nutrient-dense food.

Bottle gourd is an under-utilized and under-valued cucurbit crop in its centre of origin. The crop has a wealth of genetic diversity in SSA for new variety design (Mashilo et al., 2015; Contreras-Soto et al., 2021; Mkhize et al., 2023). Elsewhere, bottle gourd hybrids with high yield potential have been developed using genetically divergent germplasm of the crop (Quamruzzaman et al., 2020; Odedara et al., 2021; Bhatt et al., 2023). Also, hybrids expressing excellent nutritional profiles, including dry matter content, ascorbic acid, reducing sugar, non-reducing sugar, total sugar and total soluble solids were derived from the varied gene pool of the crop (Bhatt et al., 2023).

Bottle gourd is a “climate-smart” crop and has the potential to grow under drought-prone agro-ecologies. It tolerates moderate to high levels of abiotic stress, including heat and drought stress (Mashilo et al., 2017; Mkhize et al. 2023). In South Africa, the crop is grown by small-holder farmers in areas with marginal rainfall and prolonged dry spells in soils with poor moisture-holding capacity (Mashilo et al., 2017, 2018; Nkosi et al., 2022). Bottle gourd is relatively drought tolerant compared to other cucurbit crops, including *Cucurbita maxima* and

C. pepo (Sithole and Modi, 2016). As a result, it has been used as a model crop to study abiotic stress tolerance (i.e., heat and drought stress) (Wang et al., 2024).

Globally water has increasingly become scarce, threatening human livelihoods and crop cultivation, especially in water-limited environments. There is a need to develop drought-adapted crop varieties with enhanced drought tolerance for sustainable food production. Bottle gourd's monoecious and cross-pollinating nature has been explored for various breeding and selection procedures, including pure line selection, pedigree selection, recurrent selection and heterosis breeding (Islam et al. 2021). Of these breeding approaches, hybrid breeding has been largely explored in improvement programs (Singh, 2004). The wide genetic variation among bottle gourd genetic resources presents opportunities for developing cultivars with high heat and drought stress tolerance. However, the crop remains under- researched and -utilized with limited production and commercialization success attributable to a lack of dedicated research capacity and investment support. Research and breeding efforts to develop drought-resilient cucurbit cultivars adapted to dry environments are gradually unfolding in South Africa. For instance, in a recent study, Mkhize et al. (2023) developed single cross hybrids of bottle gourd possessing desirable agronomic traits, including high fruit yield. The new hybrids were derived through crosses using parental landrace accessions with high fruit yield potential and drought tolerance. The developed F_1 families require rigorous testing to identify sources of drought tolerance and to assess the extent of heterosis for key agronomic traits. Therefore, this study aimed to determine drought tolerance among newly-developed single cross bottle gourd hybrids to identify hybrids that outperform their parents and recommend the best performers for production and commercialization in South Africa.

3.2. Materials and methods

3.2.1. Plant material

Fifty-three newly-developed single crosses were used in this study. The crosses were developed using 12 drought-tolerant parental accessions (Table 3.1) with diverse horticultural attributes. The parents are extensively grown by small-scale farmers in the Limpopo Province of South Africa for various purposes, including food and non-food products. In the province, the crop is grown in heat- and drought-prone environments and the selected parents have considerable drought tolerance. These accessions were sourced from the Limpopo Department of Agriculture and Rural Development (LDARD). Fifty-three F_1 hybrid families were developed at the University of Limpopo (−25˚36’54’’S, 28˚0’59.76’’ E, 1312 m above sea level), employing a half-diallel mating design (Mkhize et al. 2023). The names and attributes of the F_1 hybrids, their parental accessions, and their fruit and seed attributes are presented in Table 3.1.

Table 3.1 List and attributes of bottle gourd hybrids and accessions used in the study.

Hybrids	Code	Fruit shape	Fruit colour	Seed colour
BG-27 × BG-31	G1	Oblate	Green	Brown
BG-27 × BG-52	G2	Round	Green	Light brown
BG-27 × BG-58	G3	Pyriform	Light green	Brown

BG-27 × BG-70	G4	Elongated pyriform	Light green	Dark Brown
BG-27 × BG-79	G5	Cavate	Green	Cream
BG-27 × BG-80	G6	Cylindrical	Green	Cream
BG-27 × GC	G7	Elongated pyriform	Cream	Cream
BG-31 × BG-48	G8	Cylindrical	Green	Light brown
BG-31 × BG-70	G9	Cylindrical	Green	Cream
BG-48 × GC	G10	Elongated pyriform	Green	Brown
BG-31 × BG-52	G11	Elongated pyriform	Green	Cream
BG-48 × BG-52	G12	Elongated pyriform	Cream	Cream
BG-52 × BG-58	G13	Elongated pyriform	Cream	Cream
BG-52 × BG-70	G14	Elongated pyriform	Green	Dark Brown
BG-52 × BG-80	G15	Pyriform	Dark green	Dark Brown
BG-52 × GC	G16	Elongated pyriform	Green	Cream
BG-31 × BG-58	G17	Cylindrical	Dark green	Brown
BG-48 × BG-58	G18	Cylindrical	Dark green	Brown
BG-58 × BG-70	G19	Cylindrical	Dark green	Dark Brown
BG-31 × BG-67	G20	Oblate	Light green	Brown
BG-48 × BG-67	G21	Elongated pyriform	Green	Brown
BG-58 × BG-67	G22	Bottle shaped	Cream	Light brown
BG-67 × BG-70	G23	Cavate	Dark green	Light brown
BG-67 × BG-78	G24	Elongated pyriform	Light green	Dark Brown
BG-67 × GC	G25	Cavate	Dark green	Dark Brown
BG-70 × GC	G26	Cylindrical	Green	Dark Brown
BG-31 × BG-78	G27	Elongated	Dark green	Light brown
BG-48 × BG-78	G28	Cavate	Dark green	Brown
BG-52 × BG-78	G29	Oblate	Light green	Brown
BG-58 × BG-78	G30	Pyriform	Medium green	Brown
BG-78 × GC	G31	Cylindrical	Light green	Brown
BG-31 × BG-79	G32	Elongated pyriform	Light green	Brown
BG-52 × BG-79	G33	Elongated pyriform	Cream	Brown
BG-58 × BG-79	G34	Cavate	Dark green	Cream
BG-70 × BG-79	G35	Pyriform	Medium green	Light brown
BG-79 × BG-80	G36	Elongated pyriform	Cream	Dark Brown
BG-79 × GC	G37	Pyriform	Green	Dark Brown
BG-31 × BG-80	G38	Pyriform	Green	Brown
BG-48 × BG-80	G39	Pyriform	Green	Light brown
BG-58 × BG-80	G40	Cavate	Dark green	Dark Brown
BG-80 × GC	G41	Elongated pyriform	Cream	Brown
BG-27 × BG-81	G42	Elongated pyriform	Dark green	Cream
BG-31 × BG-81	G43	Cyndrical	Green	Light brown
BG-52 × BG-81	G44	Cyndrical	Green	Light brown
BG-58 × BG-81	G45	Cavate	Medium green	Brown
BG-67 × BG-81	G46	Pyriform	Dark green	Brown
BG-70 × BG-81	G47	Pyriform	Dark green	Brown

Table 3.1 (Continued).

Hybrids	Code	Fruit shape	Fruit colour	Seed colour
BG-78 × BG-81	G48	Elongated pyriform	Green	Cream
BG-79 × BG-81	G49	Bottle shaped	Light green	Brown
BG-80 × BG-81	G50	Cyndrical	Green	Brown
BG-81 × GC	G51	Elongated pyriform	Green	Dark Brown
BG-27 × BG-67	G52	Bottle shaped	Light green	Dark Brown

BG-27 × BG-78	G53	Elongated pyriform	Green	Cream
Parental accessions				
BG-27	G54	Cavate	Dark green	Brown
BG-31	G55	Pyriform	Light green	Brown
BG-48	G56	Cavate	Dark green	Brown
BG-52	G57	Cavate	Medium green	Brown
BG-58	G58	Elongated	Dark green	Dark Brown
BG-67	G59	Cavate	Dark green	Brown
BG-70	G60	Elongated	Dark green	Brown
BG-78	G61	Cavate	Dark green	Brown
BG-79	G62	Pyriform	Light green	Brown
BG-80	G63	Elongated	Dark green	Light brown
BG-81	G64	Pyriform	Light green	Brown
GC	G65	Pyriform	Light green	Brown

3.2.2. Experimental design and field establishment

The hybrids and parental accessions were planted during the 2020/21 and 2021/22 growing seasons under field conditions at the University of Limpopo's Syferskuil farm (−23˚53' 9.60'' S, 29˚44'16.80'' E, 1312 m above sea level) and subjected to drought-stressed (DS) and non-stressed (NS) conditions. These resulted in four testing environments, namely: E1 (DS condition during 2021 season), E2 (NS condition during 2021 season), E3 (DS condition during 2022 season and E4 (NS condition during 2022 season). During trial establishment, genotypes were established at an intra-and-inter row spacing of 3 × 2 m using a 5 × 13 α-lattice design with three replicates for each water condition. Experimental plants under DS conditions were irrigated with 27 mm of water thrice a week for two weeks to facilitate germination. When plants developed two fully expanded leaves, irrigation was withheld, and plants were grown under rainfed conditions. The plants under rainfed conditions received 243 and 198 mm of rainfall during the 2020/21 and 2021/22 growing seasons, respectively. Plants grown under the NS conditions were irrigated with 27 mm thrice a week using a sprinkler irrigation system from planting to maturity. The NS plants received a total water supply of 670 mm. Weeding was done manually as required. The maximum temperature and relative humidity at the study site ranged from 26 to 34.8 ˚C and 60 to 88%, in that order.

3.2.3. Data collection

Data were collected randomly on selected individual plants that were tagged in each block for parental genotypes and hybrids. The following data were recorded: total dried fruit weight per plant (FYPP) in kg and seed yield per plant (SYPP) in kg.

3.2.4. Data analysis

3.2.4.1. Analysis of variance

Analysis of variance was conducted to determine the effects of genotype, water conditions, and their interaction on FYPP and SYPP using GenStat version 18 (Payne et al., 2017). The Least Significant Difference (LSD) test was computed to compare treatment means at the 5% level of significance.

3.2.4.2. Drought stress intensity

Drought stress intensity was calculated based on yield performance under NS and DS conditions according to Sio-Se Mardeh et al. (2006) and Belko et al. (2014). The following formula was used:

$$\mathrm{DSI} = \frac{\mathrm{Xns} - \mathrm{Xds}}{\mathrm{Xns}}$$

Where,

DSI = drought stress intensity,

Xns = mean fruit yield averaged across all the accessions and crosses tested under NS conditions,

Xds = mean fruit yield averaged across all the accessions and crosses tested under DS conditions

3.2.5. Best linear unbiased predictors for fruit and seed yield

Best Linear Unbiased Predictors (BLUPs) for FYPP and SYPP were calculated using META-R software (Multi Environment Trail Analysis with R for Windows) Version 6.0 (Alvarado et al., 2020). The following linear model based on the lattice design was used:

$Y_{ijkl} = \mu + Gen_i + Env_j + Rep_k + Blocks_l + Block_l(Rep_k) + Gen_i \times Env_j + \varepsilon_{ijkl}$

Where,

Y_{ijkl} = response for fruit and seed yield

μ = overall mean effect,

Gen_i = genotype effect,

Env_j = environment effect,

Rep_k = replications,

$Blocks_l$ = effect of the incomplete block,

$Blocks_l$ (Rep_k) = effect of the incomplete block within replications

$Gen_i \times Env_j$ = genotype by environment interaction effect,
ε_{ijkl} = error or residual

3.2.6. Best linear unbiased predictors for drought tolerance indices

BLUPs for FYPP and SYPP across the two growing seasons were used to calculate the various drought tolerance indices. The following drought tolerance indices were computed, namely: tolerance index (TOL), mean productivity (MP), harmonic mean (HM), stress susceptibility index (SSI), geometric mean productivity (GMP), stress tolerance index (STI), yield index (YI), yield stability index (YSI), modified stress tolerance index I (K1STI), and modified stress tolerance index II (K2STI). The formulae for the different indices are presented in Table 3.2.

Table 3.2 Drought tolerance indices computed to evaluate 12 bottle gourd parental genotypes and 53 derived single cross hybrids in two growing seasons in South Africa.

Drought Tolerance Index	Abbreviation	Equation	References
Tolerance index	TOL	$Yp - Ys$	Rosielle and Hamblin, 1981.
Mean productivity	MP	$\frac{(Ys+Yp)}{2}$	Rosielle and Hamblin, 1981.
Harmonic mean	HM	$\frac{2(Yp \times Ys)}{Yp - Ys}$	Rosielle and Hamblin, 1981.
Stress susceptibility index	SSI	$\frac{1-(\frac{Ys}{Yp})}{1-(\frac{\bar{Y}s}{\bar{Y}p})}$	Fischer and Maurer, 1978.

Geometric mean productivity	GMP	$\sqrt{(Yp \times Ys)}$	Schneider et al. 1997.
Stress tolerance index	STI	$\frac{(Yp - Ys)}{(\bar{Y}p)^2}$	Schneider et al. 1997.
Yield Index	YI	$\frac{Ys}{\bar{Y}s}$	Gavuzzi et al. 1997.
Yield Stability Index	YSI	$\frac{Ys}{Yp}$	Bouslama and Schapaugh, 1984.
Modified stress tolerance index	MSTI/K$_1$STI	$\left(\frac{Yp^2}{\bar{Y}p^2}\right) \times [\frac{Yp + Ys}{\bar{Y}p^2}]$	Farshadfar and Sutka, 2003.
Modified stress tolerance index	MSTI/K$_2$STI	$\left(\frac{Ys^2}{\bar{Y}s^2}\right) \times [\frac{Yp + Ys}{\bar{Y}p^2}]$	Farshadfar and Sutka, 2003.

Ys = yield under DS condition of a genotype; Yp = yield under NS condition of a given genotype; $\bar{Y}s$ = average yield of all genotypes under DS condition; $\bar{Y}p$ = average yield of all genotypes under NS condition.

3.2.7. Genotype grouping for drought tolerance

A scatter plot was constructed using the ggplot2 package (Wickham 2016) in R version 4.1.0 (R Development Core Team, 2016) to distinguish the performance of the tested genotypes under the various test environments. The scatterplots provided information on the different levels of drought tolerance by grouping the genotypes into the four groups as follows: Group A, which comprised genotypes expressing high FYPP and SYPP under both NS and DS conditions; Group B comprise genotypes which perform good only in NS condition; Group C genotypes which are relatively higher performers under DS condition; and Group D genotypes which are low yielders under both NS and DS conditions (Fernandez, 1992).

3.3. Results

3.3.1. Analysis of variance

Analysis of variance showing the main effects of genotype, water treatment and their interaction effects for FYPP and SYPP are presented in Table 3.3. Significant ($p < 0.001$) genotypic effects were recorded for FYPP only, while significant environmental effects were recorded for both FYPP and SYPP. Genotype × environment interaction effects were significant ($p < 0.001$) for FYPP only.

Table 3.3 Mean squares and significant tests for the accessions and their 53 hybrids evaluated for fruit and seed yield per plant under non-stressed and drought-stressed conditions across two growing seasons.

Source of Variation	Traits		
	df	FYPP	SYPP
Blocks	12	0.23^{ns}	0.04^{ns}
Blocks (Reps)	24	0.13^{ns}	0.07^{ns}
Replication (Reps)	2	0.07**	0.55**
Genotype (Gen)	64	0.37**	20.06^{ns}
Environment (Env)	3	33.76**	2.93**
Gen × Env	192	0.18**	0.06^{ns}
Residual	520	0.17	0.08

Note: df = degrees of freedom; * and ** denote significant differences at 5 and 1% probability levels, respectively; ns = non-significant; FYPP = fruit yield per plant (kg); SYPP = seed yield per plant (kg).

3.3.2. Drought stress intensity

Drought stress intensity was 0.99 and 0.98 for the 2021 and 2022 growing seasons, respectively. A mean drought stress intensity of 0.97 was computed across the two seasons.

3.3.3. Fruit and seed yield performance under drought-stressed and non-stressed conditions

BLUPs estimates for FYPP and SYPP for the studied genotypes under DS and NS conditions are presented in Tables 3.4. Under DS conditions during the 2020/21 growing season, FYPP varied from 0.1 to 0.8 kg with a mean of 0.3 kg across the test genotypes. High FYPP of 0.65, 0.67, 0.83, 0.78 and 0.79 kg were recorded for crosses BG-48 × BG-58, BG-31 × BG-67, BG-58 × BG-67, BG-58 × BG-78 and BG-31 × BG-78, in that order. The parental accession BG-79 recorded FYPP of 0.7 kg. Under NS conditions during the 2020/21 growing season, FYPP ranged from 0.5 to 2.2 kg with a mean of 1.2 kg. High FYPP of 2.2, 1.5, 1.6 and 1.8 kg was recorded for the crosses BG-31 × BG-70, BG-31 × BG-52, BG-31 × BG-79 and BG-58 × BG-80, respectively. Under DS conditions during the 2020/21 growing season, SYPP ranged from 0.1 to 0.9 kg, with a mean of 0.4 kg for the tested genotypes. High SYPP of 0.9 was recorded for the crosses BG-27 × BG-79, BG-58 × BG-81 and the parental accession BG-78. Under NS conditions during the 2020/21 growing season, SYPP varied from 0.3 to 0.8 kg with a mean of 0.5 kg. Forty percent of the test genotypes recorded SYPP of > 0.6 kg.

Under DS conditions during the 2021/22 growing season, FYPP varied from 0.1 to 0.9 kg with a mean of 0.3 kg. High FYPP of 0.9 and 0.7 kg were recorded for the cross BG-27 × BG-31 and the parental accession BG-80. Low FYPP of 0.1 kg was recorded for the following crosses: BG-27 × BG-58, BG-31 × BG-48, BG-31 × BG-58, BG-48 × BG-67, BG-80 × BG-81 and BG-27 × BG-67. Under NS conditions during the 2021/22 growing season, FYPP varied from 0.3 to 2.4 kg with a mean of 1.02 kg. High FYPP were recorded for the crosses BG-27 × BG-31 (1.6 kg), BG-31 × BG-70 (1.9 kg), BG-31 × BG-52 (2.4 kg), BG-48 × BG-67 (1.7 kg) and BG-70 × BG-79 (1.8 kg). Under DS conditions during the 2021/22 growing season, SYPP varied from 0.04 to 0.5 kg with a mean of 0.2 kg. High SYPP of > 0.4 kg was recorded for 11% of the crosses. Under NS conditions during the 2021/22 growing season, SYPP varied from 0.1 to 0.9 kg with a mean of 0.4 kg. High SYPP was recorded for 43% of the crosses, of which BG-58 × BG-78 (0.6 kg) and BG-81 × GC (0.7 kg) were outstanding performers.

Across growing seasons, FYPP varied from 0.1 to 0.6 kg with a mean of 0.3 kg under DS condition. A high FYPP of 0.6 kg was recorded for the crosses BG-27 × BG-31, BG-31 × BG-70, BG-31 × BG-78 and BG-78, and the parental accession BG-80. Under NS conditions across the growing seasons, FYPP varied from 0.2 to 2.2 kg, with a mean of 0.6 kg for all test genotypes. High FYPP of 1.6, 1.7, 1.9 and 2.2 was recorded for the crosses BG-27 × BG-31, BG-31 × BG-79, BG-31 × BG-52 and BG-31 × BG-48, in that order. Under DS condition across growing seasons, SYPP varied from 0.1 to 0.7 kg with a mean of 0.3 kg. High SYPP of 0.7 kg was recorded for the crosses BG-27 × BG-80 and BG-58 × BG-81. Under NS condition across growing seasons, SYPP varied from 0.3 to 0.7 kg with a mean of 0.5 kg. High SYPP of 0.7 kg was recorded for the parental accession BG-58 (Table 3.4). The newly-developed hybrids displayed great genetic variations for fruit shape, texture and colour (Figure 3.1).

Table 3.4 BLUPs estimates of bottle gourd parental genotypes and their hybrids for fruit and seed yield per plant evaluated under drought-stressed and non-stressed conditions across two growing seasons.

	2021				2022				Mean			
	Traits											
	FYPP		SYPP		FYPP		SYPP		FYPP		SYPP	
Crosses	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS
BG-27 × BG-31	0.23	1.18	0.56	0.46	0.86	1.55	0.44	0.42	0.55	1.55	0.50	0.44
BG-27 × BG-52	0.16	0.78	0.38	0.63	0.22	0.72	0.08	0.54	0.19	0.87	0.23	0.58
BG-27 × BG-58	0.08	0.66	0.26	0.63	0.10	0.68	0.39	0.37	0.09	0.76	0.33	0.50
BG-27 × BG-70	0.27	0.79	0.22	0.58	0.47	1.23	0.29	0.18	0.37	0.98	0.25	0.38
BG-27 × BG-79	0.33	1.26	0.27	0.72	0.39	0.53	0.30	0.17	0.36	0.89	0.29	0.45
BG-27 × BG-80	0.58	1.49	0.94	0.54	0.18	0.68	0.46	0.25	0.38	1.30	0.70	0.39
BG-27 × GC	0.22	0.78	0.57	0.53	0.44	0.81	0.46	0.34	0.33	0.72	0.52	0.43
BG-31 × BG-48	0.07	1.07	0.33	0.77	0.07	1.44	0.05	0.48	0.07	1.17	0.19	0.62
BG-31 × BG-70	0.70	2.20	0.12	0.55	0.46	1.88	0.24	0.23	0.58	2.22	0.18	0.39
BG-48 × GC	0.28	1.19	0.23	0.54	0.16	1.07	0.32	0.43	0.22	1.11	0.28	0.49
BG-31 × BG-52	0.60	1.51	0.49	0.46	0.26	2.39	0.27	0.39	0.43	1.89	0.38	0.43
BG-48 × BG-52	0.12	0.72	0.42	0.61	0.35	0.72	0.40	0.40	0.24	0.62	0.41	0.50
BG-52 × BG-58	0.25	1.24	0.17	0.61	0.18	1.29	0.17	0.52	0.21	1.18	0.17	0.56

BG-52 × BG-70	0.26	0.61	0.42	0.61	0.40	0.79	0.36	0.39	0.33	0.73	0.39	0.50
BG-52 × BG-80	0.37	1.00	0.48	0.63	0.37	0.83	0.15	0.41	0.37	1.01	0.31	0.52
BG-52 × GC	0.66	1.40	0.34	0.35	0.26	0.97	0.32	0.52	0.46	0.97	0.33	0.44
BG-31 × BG-58	0.44	0.74	0.35	0.77	0.18	1.09	0.19	0.45	0.31	0.84	0.27	0.61
BG-48 × BG-58	0.65	1.33	0.26	0.51	0.09	0.82	0.27	0.37	0.37	1.47	0.26	0.44
BG-58 × BG-70	0.23	1.08	0.20	0.68	0.42	0.76	0.17	0.52	0.32	0.51	0.19	0.60
BG-31 × BG-67	0.67	0.91	0.56	0.65	0.37	0.73	0.15	0.50	0.52	0.87	0.36	0.58
BG-48 × BG-67	0.19	1.32	0.60	0.51	0.11	1.68	0.17	0.46	0.15	1.34	0.39	0.49
BG-58 × BG-67	0.83	0.99	0.27	0.65	0.20	1.03	0.12	0.37	0.52	1.02	0.20	0.51
BG-67 × BG-70	0.08	1.01	0.26	0.36	0.16	1.32	0.38	0.37	0.12	1.29	0.32	0.36
BG-67 × BG-78	0.23	0.65	0.37	0.51	0.22	1.02	0.20	0.38	0.23	0.87	0.29	0.45
BG-67 × GC	0.17	1.05	0.18	0.56	0.20	0.79	0.14	0.35	0.19	1.12	0.16	0.45
BG-70 × GC	0.12	0.96	0.25	0.67	0.53	1.31	0.21	0.42	0.33	1.16	0.23	0.55
BG-31 × BG-78	0.79	1.27	0.70	0.47	0.37	1.19	0.35	0.22	0.58	1.18	0.53	0.35
BG-48 × BG-78	0.28	1.04	0.40	0.48	0.11	0.72	0.08	0.33	0.20	0.81	0.24	0.41
BG-52 × BG-78	0.62	0.93	0.58	0.62	0.40	1.28	0.40	0.36	0.51	1.24	0.49	0.49
BG-58 × BG-78	0.78	1.04	0.62	0.39	0.35	1.29	0.42	0.62	0.57	1.04	0.52	0.50
BG-78 × GC	0.17	1.28	0.30	0.47	0.27	1.29	0.30	0.39	0.22	1.39	0.30	0.43
BG-31 × BG-79	0.24	1.59	0.32	0.61	0.33	1.21	0.43	0.48	0.28	1.70	0.38	0.55
BG-52 × BG-79	0.09	0.87	0.31	0.52	0.16	0.45	0.14	0.36	0.13	0.31	0.22	0.44
BG-79 × BG-58	0.08	1.39	0.35	0.63	0.19	0.67	0.43	0.25	0.14	0.86	0.39	0.44
BG-70 × BG-79	0.33	1.25	0.45	0.35	0.18	1.76	0.04	0.48	0.26	1.37	0.25	0.42
BG-79 × BG-80	0.21	0.52	0.23	0.62	0.66	0.34	0.21	0.14	0.44	0.45	0.22	0.38
BG-79 × GC	0.21	0.81	0.45	0.54	0.23	0.54	0.39	0.24	0.22	0.86	0.42	0.39
BG-31 × BG-80	0.37	1.12	0.07	0.42	0.21	0.65	0.24	0.24	0.29	1.02	0.15	0.33
BG-48 × BG-80	0.11	0.96	0.42	0.64	0.18	0.75	0.17	0.37	0.14	0.88	0.30	0.51

Table 3.4 (Continued).

Crosses	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS
BG-58 × BG-80	0.47	1.75	0.45	0.39	0.18	1.09	0.37	0.51	0.33	1.74	0.41	0.45
BG-80 × GC	0.12	0.72	0.21	0.55	0.18	0.25	0.16	0.40	0.15	0.22	0.18	0.48
BG-27 × BG-81	0.33	1.05	0.45	0.66	0.54	1.10	0.09	0.40	0.43	1.07	0.27	0.53
BG-31 × BG-81	0.36	0.83	0.14	0.43	0.16	0.78	0.14	0.44	0.26	0.96	0.14	0.44
BG-52 × BG-81	0.08	0.63	0.36	0.75	0.17	0.60	0.28	0.34	0.12	0.60	0.32	0.55
BG-58 × BG-81	0.38	1.07	1.00	0.38	0.22	0.96	0.37	0.39	0.30	1.26	0.68	0.38
BG-67 × BG-81	0.37	1.48	0.65	0.42	0.20	1.12	0.16	0.42	0.29	1.41	0.41	0.42
BG-70 × BG-81	0.39	1.18	0.33	0.79	0.27	1.07	0.20	0.39	0.33	1.29	0.26	0.59
BG-78 × BG-81	0.62	0.60	0.46	0.42	0.19	1.09	0.24	0.34	0.41	1.00	0.35	0.38
BG-79 × BG-81	0.45	0.76	0.42	0.41	0.33	0.79	0.16	0.37	0.39	0.72	0.29	0.39
BG-80 × BG-81	0.32	0.90	0.50	0.46	0.05	0.78	0.14	0.45	0.19	0.56	0.32	0.46
BG-81 × GC	0.54	1.16	0.65	0.37	0.21	1.04	0.07	0.66	0.38	1.26	0.36	0.52
BG-27 × BG-67	0.20	1.19	0.22	0.61	0.11	0.91	0.10	0.29	0.15	1.18	0.16	0.45
BG-27 × BG-78	0.11	0.72	0.35	0.52	0.40	0.92	0.32	0.30	0.25	0.84	0.34	0.41
Accessions												
BG-27	0.31	0.93	0.23	0.69	0.34	0.90	0.32	0.36	0.33	1.14	0.27	0.53
BG-31	0.34	0.74	0.69	0.40	0.35	0.60	0.06	0.34	0.34	0.77	0.38	0.37
BG-48	0.32	1.15	0.44	0.47	0.22	1.15	0.17	0.48	0.27	1.23	0.30	0.47
BG-52	0.19	0.95	0.25	0.61	0.26	1.91	0.28	0.45	0.22	1.57	0.27	0.53

BG-58	0.10	0.90	0.30	0.46	0.18	1.06	0.14	0.56	0.14	0.89	0.22	0.51
BG-67	0.13	1.23	0.29	0.49	0.43	1.08	0.41	0.96	0.28	1.31	0.35	0.73
BG-70	0.36	0.75	0.53	0.51	0.31	0.76	0.13	0.33	0.33	0.81	0.33	0.42
BG-78	0.58	0.73	0.90	0.62	0.07	0.97	0.13	0.50	0.33	0.82	0.52	0.56
BG-79	0.73	0.74	0.65	0.43	0.23	1.30	0.24	0.62	0.48	0.85	0.45	0.53
BG-80	0.54	0.56	0.79	0.33	0.68	1.18	0.32	0.37	0.61	0.90	0.56	0.35
BG-81	0.21	1.41	0.32	0.75	0.21	1.05	0.21	0.46	0.21	0.80	0.26	0.61
GC	0.33	0.69	0.57	0.40	0.33	0.77	0.24	0.54	0.33	0.64	0.41	0.47
Grand Mean	0.34	1.22	0.41	0.54	0.28	1.02	0.24	0.41	0.31	1.04	0.33	0.47
LSD	0.49	0.73	0.44	0.40	0.43	0.93	0.39	0.38	0.33	0.55	0.29	0.27
P-value	<.001	<.01	<.001	<.01	<0.01	<.01	<0.01	<.001	<.001	<.01	<.001	<.01

FYPP = fruit yield per plant (kg); SYPP = seed yield per plant (kg); LSD = least significant difference; *P*-value = Significant at the 5 and 1% probability level.

Figure 3.1. Morphological diversity in fruit shape, texture and colour of selected newly-developed crosses of bottle gourd hybrids. BG-31 × BG-52, green fruit colour and medium length fruit neck (A), BG-48 × BG-67, elongated pyriform and green fruit colour with dark green patches (B), BG-58 × BG-81, cavate fruit shape and medium green fruit colour with dark green patches (C), BG-78 × GC, verrucose fruit texture (D), light green fruit colour and cylindrical fruit shape, BG-58 × BG-79, dark green fruit colour and verrucose fruit texture (E), BG-52 × BG-79, cream to light green fruit colour and medium fruit neck (F), BG-31 × BG-80, pyriform fruit shape and green fruit colour (G), BG-31 × BG-81, green fruit colour with dark patches and cylindrical fruit shape (H), BG-48 × GC, elongated pyriform or club shaped fruit and verrucose fruit texture (I), BG-27 × BG-58, light green to green fruit colour (J).

3.3.4. Drought tolerance indices

Yield-based indices of drought tolerance among the tested bottle gourd genotypes are determined based on FYPP across the growing seasons are summarized in Table 3.5. Values of TOL for FYPP varied from 0.02 to 1.6, with a mean of 0.7. The cross BG-31 × BG-70 had a higher TOL value of 1.6. Eighteen percent of the studied genotypes recorded TOL values >1.0 based on FYPP. High MP values were recorded for the crosses BG-27 × BG-31 (1.05), BG-31 × BG-70 (1.40), BG-31 × BG-52 (1.16) and BG-58 × BG-80 (1.03) based on FYPP. The values of HM varied from 0.2 to 3.8, with a mean of 1.1 across the test genotypes. HM values > 2 were recorded for crosses BG-31 × BG-67, BG-58 × BG-67, BG-31 × BG-78, BG-58 × BG-78, BG-79 × BG-80, and the parental accessions BG-79 and BG-80. High SSI values were recorded for crosses BG-27 × BG-58 (1.24), BG-31 × BG-48 (1.33), BG-48 × BG-67 (1.25), BG-67 × BG-70 (1.27) and BG-27 × BG-67 (1.23). The cross BG-31 × BG-70 had a high value of 1.3 for GMP. The values of STI varied from 0.02 to 1.5, with a mean of 0.7. High STI values of 1.5, 1.4 and 1.3 were recorded for crosses BG-31 × BG-70, BG-31 × BG-52 and BG-31 × BG-79, respectively. YI varied from 0.2 to 2, with a mean of 1.0 for the assessed genotypes. Fifty one percent of the studied genotypes had the YI values > 1.0, with the highest values recorded for BG-27 × BG-31 (1.8), BG-31 × BG-70 (1.9), BG-31 × BG-67 (1.7) and BG-31 × BG-78 (1.9). YSI ranged from 0.1 to 0.9. High YSI values were recorded for crosses BG-79 × BG-81 (0.55), BG-31 × BG-67 (0.59), BG-58 × BG-70 (0.64), BG-80 × GC (0.66) and BG-79 × BG-80 (0.96). Values for MSTI/K_1STI varied from 0.02 to 11.8, with a mean of 1.7 for the studied genotypes. Crosses BG-31 × BG-70, BG-58 × BG-80 and BG-31 × BG-79 recorded the highest MSTI/K_1STI values of 11.8, 5.4 and 4.9 for MSTI/K_1STI. MSTI/K_2STI varied from 0.1 to 9.0 with a mean of 1.6. The highest values of 5.37, 5.75, 6.00 and 9.03 for MSTI/K_2STI were recorded for the genotypes BG-80, BG-31 × BG-78, BG-27 × BG-31 and BG-31 × BG-70, in that order.

Drought tolerance indices among the tested bottle gourd genotypes are determined based on SYPP across the growing seasons are summarized in Table 3.6. Cross BG-31 × BG-48 had the highest value of 0.4 of TOL. The values of MP varied from 0.2 to 0.6, with a mean of 0.4 across genotypes. A high MP value of 0.5 was recorded for 17% of the studied genotypes. Crosses BG-31 × BG-52, BG-67 × BG-70, BG-58 × BG-79, BG-58 × BG-80, BG-67 × BG-81, BG-78 × BG-81 and parental accession BG-78 had the highest values of ≥ 5.0 for HM. High SSI value of ≥ 1.0 was recorded for 73% of the studied genotypes. High values of GMP were recorded for crosses BG-58 × BG-78 (0.51) and BG-27 × BG-80 (0.52). STI varied from 1.4 to 1.9, with a mean of 0.7. Crosses BG-27 × BG-52, BG-31 × BG-48, BG-52 × BG-58, BG-31 × BG-58, BG-58 × BG-70, BG-70 × BG-81 and parental accessions BG-67 and BG-81 recorded the highest STI value exceeding 1.30. Crosses BG-27 × BG-31, BG-27 × BG-80, BG-27 × GC, BG-31 × BG-48, BG-52 × BG-78, BG-79 × GC and BG-58 × BG-81 had the highest values of YI, YSI, MSTI/K_1STI and MSTI/K_2STI.

Table 3.5 Drought tolerance indices based on fruit yield per plant for 53 crosses and 12 parental genotypes of bottle gourd assessed across two growing seasons in South Africa.

Crosses	TOL	MP	HM	SSI	GMP	STI	YI	YSI	MSTI/K1STI	MSTI/K2STI
BG-27 × BG-31	1.00	1.05	1.68	0.91	0.92	0.93	1.76	0.35	4.32	6.00
BG-27 × BG-52	0.68	0.53	0.49	1.10	0.41	0.63	0.61	0.22	0.69	0.37
BG-27 × BG-58	0.67	0.43	0.20	1.24	0.26	0.62	0.29	0.12	0.43	0.07
BG-27 × BG-70	0.61	0.68	1.19	0.88	0.61	0.57	1.20	0.38	1.13	1.81
BG-27 × BG-79	0.53	0.63	1.22	0.84	0.57	0.49	1.17	0.40	0.86	1.58
BG-27 × BG-80	0.92	0.84	1.07	1.00	0.70	0.85	1.23	0.29	2.43	2.34
BG-27 × GC	0.39	0.52	1.20	0.77	0.48	0.36	1.05	0.45	0.47	1.08
BG-31 × BG-48	1.10	0.62	0.15	1.33	0.28	1.02	0.22	0.06	1.45	0.06
BG-31 × BG-70	1.64	1.40	1.56	1.04	1.13	1.52	1.87	0.26	11.82	9.03
BG-48 × GC	0.89	0.67	0.55	1.13	0.49	0.82	0.71	0.20	1.40	0.62
BG-31 × BG-52	1.46	1.16	1.11	1.09	0.90	1.35	1.39	0.23	7.11	4.14
BG-48 × BG-52	0.39	0.43	0.75	0.88	0.38	0.36	0.76	0.38	0.29	0.46
BG-52 × BG-58	0.97	0.70	0.52	1.15	0.50	0.89	0.69	0.18	1.66	0.61
BG-52 × BG-70	0.40	0.53	1.20	0.77	0.49	0.37	1.06	0.45	0.49	1.11
BG-52 × BG-80	0.64	0.69	1.16	0.89	0.61	0.59	1.19	0.36	1.21	1.80
BG-52 × GC	0.51	0.72	1.74	0.74	0.67	0.47	1.48	0.47	1.16	2.90
BG-31 × BG-58	0.53	0.58	0.99	0.89	0.51	0.49	1.01	0.37	0.70	1.08
BG-48 × BG-58	1.10	0.92	0.99	1.05	0.74	1.02	1.20	0.25	3.41	2.45
BG-58 × BG-70	0.18	0.41	1.79	0.51	0.40	0.17	1.04	0.64	0.18	0.84
BG-31 × BG-67	0.35	0.70	2.56	0.57	0.67	0.33	1.68	0.59	0.92	3.64
BG-48 × BG-67	1.19	0.75	0.34	1.25	0.45	1.10	0.48	0.11	2.29	0.32
BG-58 × BG-67	0.51	0.77	2.08	0.70	0.72	0.47	1.66	0.50	1.37	3.93
BG-67 × BG-70	1.17	0.71	0.27	1.27	0.40	1.08	0.40	0.10	2.02	0.21
BG-67 × BG-78	0.64	0.55	0.61	1.04	0.44	0.59	0.73	0.26	0.71	0.53
BG-67 × GC	0.93	0.65	0.45	1.17	0.46	0.86	0.61	0.17	1.41	0.45

Table 3.5 (Continued).

Crosses	TOL	MP	HM	SSI	GMP	STI	YI	YSI	MSTI/K1STI	MSTI/K2STI
BG-70 × GC	0.84	0.74	0.90	1.01	0.61	0.77	1.05	0.28	1.71	1.51
BG-31 × BG-78	0.60	0.88	2.29	0.71	0.83	0.55	1.88	0.49	2.10	5.75
BG-48 × BG-78	0.62	0.50	0.51	1.07	0.40	0.57	0.63	0.24	0.57	0.37
BG-52 × BG-78	0.73	0.87	1.72	0.83	0.79	0.68	1.64	0.41	2.30	4.36
BG-58 × BG-78	0.48	0.80	2.47	0.64	0.77	0.44	1.82	0.54	1.49	4.94
BG-78 × GC	1.17	0.80	0.52	1.19	0.55	1.08	0.70	0.16	2.66	0.74
BG-31 × BG-79	1.42	0.99	0.68	1.17	0.69	1.31	0.91	0.17	4.91	1.54
BG-52 × BG-79	0.19	0.22	0.41	0.85	0.20	0.18	0.40	0.40	0.04	0.07
BG-58 × BG-79	0.73	0.50	0.32	1.19	0.34	0.67	0.44	0.16	0.64	0.18
BG-70 × BG-79	1.12	0.81	0.63	1.15	0.59	1.03	0.82	0.19	2.61	1.02
BG-79 × BG-80	0.02	0.45	3.66	0.05	0.45	0.02	1.41	0.96	0.16	1.66
BG-79 × GC	0.63	0.54	0.60	1.04	0.44	0.59	0.72	0.26	0.67	0.51
BG-31 × BG-80	0.73	0.66	0.82	1.01	0.55	0.67	0.94	0.29	1.17	1.08
BG-48 × BG-80	0.74	0.51	0.34	1.18	0.36	0.68	0.46	0.16	0.68	0.20
BG-58 × BG-80	1.41	1.03	0.80	1.14	0.75	1.31	1.05	0.19	5.36	2.13
BG-80 × GC	0.08	0.18	0.87	0.48	0.18	0.07	0.47	0.66	0.02	0.08
BG-27 × BG-81	0.64	0.75	1.46	0.84	0.68	0.59	1.40	0.40	1.48	2.72
BG-31 × BG-81	0.70	0.61	0.71	1.03	0.50	0.65	0.84	0.27	0.97	0.80
BG-52 × BG-81	0.48	0.36	0.31	1.12	0.27	0.45	0.40	0.20	0.23	0.11
BG-58 × BG-81	0.96	0.78	0.80	1.07	0.62	0.88	0.98	0.24	2.13	1.39
BG-67 × BG-81	1.13	0.85	0.71	1.12	0.63	1.04	0.92	0.20	2.89	1.33
BG-70 × BG-81	0.96	0.81	0.89	1.05	0.65	0.89	1.07	0.26	2.31	1.72
BG-78 × BG-81	0.59	0.70	1.37	0.83	0.64	0.55	1.31	0.41	1.20	2.24
BG-79 × BG-81	0.33	0.55	1.72	0.64	0.53	0.30	1.26	0.55	0.49	1.64
BG-80 × BG-81	0.37	0.37	0.55	0.94	0.32	0.34	0.60	0.33	0.20	0.24
BG-81 × GC	0.88	0.82	1.07	0.99	0.69	0.82	1.22	0.30	2.23	2.24
BG-27 × BG-67	1.03	0.67	0.35	1.23	0.42	0.95	0.49	0.13	1.59	0.30

Table 3.5 (Continued).

Crosses	TOL	MP	HM	SSI	GMP	STI	YI	YSI	MSTI/K1STI	MSTI/K2STI
BG-27 × BG-78	0.59	0.55	0.72	0.98	0.46	0.54	0.82	0.30	0.67	0.68
Accessions										
BG-27	0.82	0.73	0.91	1.01	0.61	0.75	1.05	0.29	1.63	1.49
BG-31	0.43	0.56	1.24	0.78	0.51	0.39	1.11	0.45	0.56	1.26
BG-48	0.96	0.75	0.69	1.10	0.57	0.89	0.87	0.22	1.94	1.04
BG-52	1.35	0.90	0.52	1.21	0.59	1.25	0.72	0.14	3.79	0.85
BG-58	0.75	0.52	0.33	1.19	0.35	0.69	0.45	0.16	0.70	0.20
BG-67	1.03	0.80	0.72	1.10	0.61	0.95	0.91	0.22	2.34	1.23
BG-70	0.48	0.57	1.13	0.83	0.52	0.44	1.08	0.41	0.64	1.22
BG-78	0.49	0.57	1.10	0.84	0.52	0.45	1.06	0.40	0.66	1.19
BG-79	0.37	0.67	2.21	0.61	0.64	0.34	1.55	0.56	0.83	2.99
BG-80	0.29	0.75	3.80	0.45	0.74	0.27	1.96	0.68	1.03	5.37
BG-81	0.59	0.50	0.57	1.04	0.41	0.54	0.68	0.26	0.55	0.43
GC	0.32	0.49	1.34	0.69	0.46	0.29	1.06	0.51	0.34	1.01

FYPP = fruit yield per plant; see expanded abbreviations of drought tolerant indices in Table 3.2.

Table 3.6 Drought tolerance indices based on seed yield per plant for 53 crosses and 12 parental genotypes of bottle gourd assessed across two growing in South Africa.

Crosses	TOL	MP	HM	SSI	GMP	STI	YI	YSI	MSTI/K1STI	MSTI/K2STI
BG-27 × BG-31	-0.06	0.47	-7.28	-0.46	0.47	-0.27	1.51	1.14	46.31	10.57
BG-27 × BG-52	0.35	0.41	0.76	2.02	0.37	1.60	0.70	0.39	12.67	1.96
BG-27 × BG-58	0.17	0.41	1.88	1.16	0.40	0.78	0.99	0.65	9.91	4.01
BG-27 × BG-70	0.13	0.32	1.52	1.11	0.31	0.57	0.77	0.67	12.64	1.85
BG-27 × BG-79	0.16	0.37	1.57	1.21	0.36	0.73	0.86	0.64	12.01	2.70
BG-27 × BG-80	-0.31	0.55	-1.77	-2.65	0.52	-1.41	2.13	1.80	38.08	24.62
BG-27 × GC	-0.09	0.48	-5.28	-0.65	0.47	-0.38	1.57	1.20	10.15	11.62
BG-31 × BG-48	0.43	0.41	0.55	2.32	0.34	1.96	0.58	0.30	22.91	1.33
BG-31 × BG-70	0.22	0.29	0.65	1.82	0.26	0.97	0.54	0.45	57.97	0.83
BG-48 × GC	0.21	0.38	1.27	1.44	0.37	0.95	0.83	0.57	19.27	2.61
BG-31 × BG-52	0.05	0.40	6.95	0.36	0.40	0.21	1.15	0.89	59.29	5.29
BG-48 × BG-52	0.09	0.46	4.42	0.62	0.45	0.42	1.24	0.81	7.30	6.98
BG-52 × BG-58	0.39	0.37	0.49	2.31	0.31	1.77	0.52	0.31	21.01	0.98
BG-52 × BG-70	0.11	0.44	3.59	0.72	0.44	0.49	1.18	0.78	9.77	6.14
BG-52 × BG-80	0.21	0.42	1.56	1.33	0.40	0.94	0.94	0.60	17.42	3.66
BG-52 × GC	0.10	0.38	2.79	0.79	0.38	0.47	1.01	0.76	14.89	3.83
BG-31 × BG-58	0.34	0.44	0.99	1.84	0.41	1.52	0.83	0.45	12.90	2.99
BG-48 × BG-58	0.18	0.35	1.31	1.34	0.34	0.80	0.80	0.60	31.27	2.22
BG-58 × BG-70	0.42	0.39	0.53	2.31	0.33	1.89	0.56	0.31	4.16	1.22
BG-31 × BG-67	0.22	0.47	1.90	1.26	0.45	0.98	1.09	0.62	14.70	5.45
BG-48 × BG-67	0.10	0.44	3.73	0.69	0.43	0.45	1.17	0.79	32.14	5.86
BG-58 × BG-67	0.31	0.35	0.63	2.06	0.32	1.43	0.59	0.38	15.09	1.22
BG-67 × BG-70	0.05	0.34	5.14	0.41	0.34	0.20	0.96	0.88	23.34	3.14
BG-67 × BG-78	0.16	0.37	1.59	1.20	0.36	0.73	0.87	0.64	11.40	2.75
BG-67 × GC	0.29	0.31	0.50	2.14	0.27	1.32	0.49	0.36	15.87	0.73
BG-70 × GC	0.32	0.39	0.81	1.91	0.36	1.43	0.71	0.43	21.64	1.93

Table 3.6 (Continued).

Crosses	TOL	MP	HM	SSI	GMP	STI	YI	YSI	MSTI/K1STI	MSTI/K2STI
BG-31 × BG-78	-0.18	0.44	-2.01	-1.74	0.43	-0.81	1.59	1.52	24.93	10.90
BG-48 × BG-78	0.16	0.32	1.18	1.36	0.31	0.75	0.73	0.59	8.73	1.69
BG-52 × BG-78	0.00	0.49	-27.64	-0.01	0.49	-0.01	1.48	1.00	30.95	10.68
BG-58 × BG-78	-0.02	0.51	-26.34	-0.13	0.51	-0.09	1.59	1.04	22.85	12.78
BG-78 × GC	0.13	0.37	2.02	0.99	0.36	0.58	0.91	0.70	29.09	3.03
BG-31 × BG-79	0.17	0.46	2.42	1.04	0.45	0.77	1.14	0.69	54.91	5.95
BG-52 × BG-79	0.22	0.33	0.91	1.64	0.31	0.98	0.68	0.51	1.35	1.50
BG-58 × BG-79	0.05	0.42	6.86	0.38	0.41	0.23	1.18	0.89	12.76	5.74
BG-70 × BG-79	0.17	0.33	1.18	1.38	0.32	0.78	0.74	0.59	25.62	1.81
BG-79 × BG-80	0.16	0.30	1.03	1.42	0.29	0.74	0.67	0.57	2.57	1.33
BG-79 × GC	-0.03	0.40	-10.83	-0.26	0.40	-0.14	1.27	1.08	12.13	6.42
BG-31 × BG-80	0.18	0.24	0.57	1.78	0.22	0.80	0.46	0.46	10.35	0.52
BG-48 × BG-80	0.21	0.40	1.42	1.39	0.39	0.95	0.89	0.58	12.80	3.16
BG-58 × BG-80	0.04	0.43	8.89	0.31	0.43	0.19	1.24	0.91	53.68	6.58
BG-80 × GC	0.29	0.33	0.60	2.05	0.30	1.32	0.56	0.39	0.67	1.01
BG-27 × BG-81	0.26	0.40	1.08	1.65	0.38	1.19	0.81	0.50	18.85	2.62
BG-31 × BG-81	0.30	0.29	0.43	2.24	0.25	1.34	0.43	0.33	11.11	0.54
BG-52 × BG-81	0.23	0.43	1.53	1.39	0.42	1.03	0.96	0.58	6.50	3.98
BG-58 × BG-81	-0.30	0.53	-1.75	-2.59	0.51	-1.35	2.07	1.78	34.79	22.49
BG-67 × BG-81	0.02	0.41	22.68	0.12	0.41	0.07	1.23	0.96	33.75	6.15
BG-70 × BG-81	0.33	0.43	0.95	1.85	0.40	1.49	0.80	0.44	29.33	2.70
BG-78 × BG-81	0.03	0.37	9.43	0.25	0.37	0.13	1.07	0.93	15.01	4.11
BG-79 × BG-81	0.10	0.34	2.27	0.85	0.33	0.45	0.87	0.75	7.17	2.55
BG-80 × BG-81	0.14	0.39	2.14	1.00	0.38	0.62	0.97	0.70	4.95	3.62
BG-81 × GC	0.15	0.44	2.43	0.99	0.43	0.69	1.10	0.70	28.64	5.21
BG-27 × BG-67	0.29	0.31	0.51	2.12	0.27	1.30	0.49	0.36	17.57	0.74
BG-27 × BG-78	0.07	0.37	3.91	0.57	0.37	0.32	1.02	0.83	10.84	3.83
Accessions										
BG-27	0.25	0.40	1.13	1.61	0.38	1.15	0.82	0.52	21.30	2.67

Table 3.6 (Continued).

Crosses	TOL	MP	HM	SSI	GMP	STI	YI	YSI	MSTI/K1STI	MSTI/K2STI
BG-31	-0.01	0.37	-13.90	-0.08	0.37	-0.04	1.14	1.02	9.05	4.80
BG-48	0.17	0.39	1.69	1.20	0.38	0.77	0.92	0.64	24.18	3.25
BG-52	0.27	0.40	1.05	1.68	0.38	1.21	0.80	0.50	40.49	2.55
BG-58	0.29	0.37	0.78	1.89	0.34	1.31	0.67	0.43	12.00	1.64
BG-67	0.38	0.54	1.35	1.72	0.50	1.70	1.06	0.48	37.96	5.99
BG-70	0.09	0.38	3.04	0.72	0.37	0.41	1.00	0.78	10.11	3.72
BG-78	0.04	0.54	13.76	0.25	0.54	0.19	1.56	0.93	14.77	12.92
BG-79	0.08	0.49	5.84	0.51	0.48	0.36	1.35	0.85	14.53	8.73
BG-80	-0.21	0.45	-1.86	-1.99	0.44	-0.94	1.69	1.60	14.92	12.75
BG-81	0.34	0.43	0.92	1.89	0.40	1.55	0.79	0.43	11.32	2.70
GC	0.06	0.44	5.86	0.46	0.44	0.29	1.23	0.86	7.45	6.52

SYPP = seed yield per plant; see expanded abbreviations of drought tolerant indices in Table 3.2.

3.3.5. Grouping of bottle gourd genotypes for drought tolerance

Biplots are presented in Figure 3.2 grouping the bottle gourd parental accessions and their hybrids based on FYPP and SYPP under DS and NS conditions. During the 2020/21 growing season BG-31 × BG-70 (G9) was the best performing cross for FYPP under NS and DS conditions (Group A). BG-27 × BG-67 (G52) performed best under NS condition (Group B). Crosses BG-31 × BG-67 (G20), BG-58 × BG-67 (G22), BG-58 × BG-78 (G30) and parental accession BG-79 (G62) were the best performers only under DS condition (Group C). BG-31 × BG-81 (G43) was the worst performing under DS and NS conditions (Group D) (Figure 3.2A).

During the 2021/22 growing season, cross BG-27 × BG-31 (G1) performed relatively better under DS and NS conditions (Group A) for FYPP. Crosses BG-31 × BG-52 (G11), BG-27 × GC (G7), BG-70 × BG-79 (G35), BG-48 × BG-67 (G21) performed better under NS condition (Group B), whereas BG-79 × BG-80 (G36) performed well under DS condition (Group C). Crosses BG-80 × GC (G41) and BG-67 × BG-70 (G23) produced the lowest FYPP under DS and NS conditions (Group D) (Figure 3.2B).

The two growing seasons analyses revealed that BG-27 × BG-31 (G1), BG-31 × BG-70 (G9) and BG-31 × BG-52 (11) were the best-performing cross under both DS and NS conditions for FYPP (Group A) (Figure 3.2C). Cross BG-31 × BG-79 (G32) and BG-57 performed well under NS condition (Group B). The parental accession BG-80 (G63) was the best performer under DS (Group C). Crosses BG-80 × GC (G41) and BG-52 × BG-79 (G33) showed poor performance under DS and NS conditions (Group D) (Figure 3.2C).

During the 2020/21 growing season, parental accession BG-78 (G61) performed better under DS and NS conditions for SYPP (Group A), and approximately 32% of the studied genotypes showed high performance under NS condition (Group B). Cross BG-58 × BG-81 (G45) and parental accession BG-80 (G63) performed better under DS conditions (Group C). BG-31 × BG-80 (G38), BG-31 × BG-81 (G43) and BG-67 × BG-70 (G23) had the lowest SYPP under both the DS and NS conditions (Group D) (Figure 3.2D).

During the 2021/22 growing season, cross BG-67 (G59) performed better under DS and NS for SYPP (Group A). Under NS condition, the cross BG-81 × GC (G51) showed a better performance for (Group B). Crosses BG-27 × BG-80 (G6), BG-27 × GC (G7) and BG-58 × BG-79 (G34) showed a better performance under DS condition (Group C). Poor performance under DS and NS conditions was observed from the crosses BG-27 × BG-67 (G52), BG-48 × BG-78 (G28) and parental accession BG-31 (G55) (Group D) (Figure 3.2E).

SYPP across the two growing seasons (Figure 3.2F) revealed that parental genotype BG-78 (G61) performed well under DS and NS conditions (Group A). Parental genotype BG-67 (59) was the best-performing genotype under the NS condition (Group B). Crosses BG-27 × BG-80 (G6) and BG-58 × BG-81 (G48) performed best under DS condition (Group C). The lowest value under DS and NS conditions was observed from the cross BG-31 × BG-80 (G38).

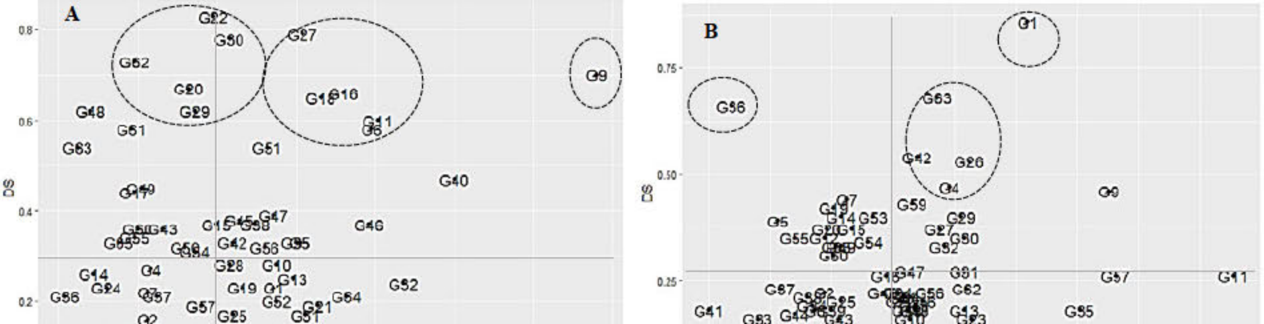

Figure 3.2. Biplots depicting grouping of bottle gourd genotypes for fruit and seed yield during different growing seasons evaluated under drought stress (DS) and non-stressed (NS) conditions. Note: Fruit yield during the 2020/21 season (A), fruit yield during 2021/2022 season (B) and fruit yield across the two growing seasons (C), seed yield during the 2020/21 season (D), seed yield during the 2021/22 season (E) and seed yield across the two growing seasons (F). Codes for the hybrids and accessions are presented in Table 3.1. The mean FYPP and SYPP values are presented by the vertical and horizontal lines on the plots. Circles in each plot show desirable selections.

3.4. Discussion

In the present study, drought tolerance was evaluated among newly developed hybrids of bottle gourd. The study revealed significant genotypic differences in the tested genotypes based on fruit yield (Table 3.3). Bottle gourd genetic resources show extensive levels of genetic diversity for fruit yield and other vital agronomic/phenotypic traits (Mashilo *et al*., 2015; Rashid et al., 2020; Mahapatra et al., 2022; Bhatt et al., 2023: Singh et al., 2023; Kumar et al., 2024). For example, the newly developed hybrids were varied for horticultural traits including fruit colour, texture and shape (Figure 3.1). Crosses BG-52 × BG-79, BG-27 × BG-70, BG-27 × GC, BG-31 × BG-52, BG-48 × BG-52, BG-52 × BG-58 and BG-52 × BG-70 had elongated pyriform shape. Round shaped fruits were observed for BG-58 × BG-81 and BG-58 × BG-79, whereas BG-31 × BG-52 had a fruit neck length. Several studies have also reported phenotypic variation in fruit characteristics in bottle gourd (Gürcan et al. 2015; Ibrahim, 2021). In addition, genetic analysis based on molecular markers revealed highly significant genetic differences in the germplasm of the crop (Contreras-Soto et al., 2021; Ibrahim, 2021; Mahapatra et al., 2022; Ibrahim et al., 2024; Kumar et al., 2024). Further, differences in fruit shape between F_1 hybrids and their parents were observed suggesting non-additive genetic effects, indicating potential heterosis. The genetic variation has allowed the selection of desirable parents, which were used in the development of superior hybrids with desirable agronomic traits, including high fruit yield, shorter days to first fruit picking, longer fruit length, maximum fruit diameter and flesh thickness, larger fruit weight, improved nodes bearing first female flower, nodes bearing first male flower, days to first male flower anthesis, and days to first female flower anthesis (Chouhan et al., 2020; Yogananda et al., 2021; Mahapatra et al., 2022; Mkhize et al., 2023; Venkatraman et al., 2024). The significant genotype × environmental interaction effects for fruit yield per plant (Table 3.3) allow the selection of suitable genotypes for either non-stressed or stressed production environments.

Genotype differentiation under drought-stressed and non-stressed conditions has been routinely conducted to identify and select drought tolerant genotypes (Clarke et al., 1992). The present study revealed crosses BG-31 × BG-70, BG-27 × BG-31, BG-58 × BG-67, BG-31 × BG-78, BG-58 × BG-78, BG-31 × BG-67, BG-58 × BG-67, BG-52 × GC, BG-58 × BG-78 and BG-52 × BG-78 as drought tolerant based on their high performance for fruit yield per plant under drought-stressed while others in both non-stressed and drought-stressed conditions (Figure 3.2). Under drought-stressed conditions, the hybrids BG-27 × BG-31, BG-31 × BG-

70, and BG-31 × BG-78 exhibited the highest fruit yield performance, with an average improvement of about 75% over all parental genotypes, except for BG-80, which exceeded the hybrids by approximately 4%. In non-stressed conditions, hybrids BG-31 × BG-79 and BG-31 × BG-52 showed a performance increase of around 15% for fruit yield compared to all parent lines. The parental genotypes BG-67 and BG-78 were highly drought tolerant based on high seed yield per plant under non-stressed and drought-stressed conditions (Figure 3.2). The accessions BG-67 and BG-78 were previously identified as drought tolerant (Mashilo et al., 2017; Mkhize et al., 2023). Also, the crosses BG-31 × BG-78, BG-27 × GC, BG-52 × BG-78, BG-58 × BG-78, BG-27 × BG-80, BG-58 × BG-81, BG-27 × BG-80 and BG-58 × BG-81 were identified as being drought tolerant based on seed yield under drought-stressed and some both non-stressed and drought-stressed conditions. Therefore, the identified newly-developed and drought tolerant crosses can be recommended for cultivation in dry production environments. The selected genotypes had the highest values for various stress tolerance indices (i.e., STI, GMP, MP and HM), indicating their high productivity (Tables 3.5 and 3.6).

Drought tolerance indices revealed extensive variation in cultivar performance (Tables 3.5 and 3.6). The drought tolerance indices identified the following drought tolerant genotypes, namely: BG-31 × BG-67, BG-27 × BG-31, BG-58 × BG-78, BG-31 × BG-70, BG-31 × BG-78 and BG-58 × BG-67 based on fruit yield per plant (Table 3.5) and BG-31 × BG-78, BG-52 × BG-78, BG-58 × BG-78, BG-27 × BG-80, BG-58 × BG-81, BG-31 × BG-48, BG-31 × BG-52, BG-67 × BG-70, BG-58 × BG-79, BG-58 × BG-80 and BG-67 × BG-81 based on seed yield per plant (Table 3.6). These agreed with the groupings of the crosses based on yield performance (Figure 3.2). All the evaluated drought tolerance indices were consistently higher in the F_1 hybrids compared to their respective parental genotypes, indicating the presence of heterotic effects and enhanced drought resilience due to hybridisation. Hence hybridisation will be recommended to improve performance of the bottle gourd varieties in South Africa. In addition, there is a need to conduct multi-environment trials to determine the genotype by environment interactions to identify stable, superior and widely adaptable genotypes with high fruit and seed yields across the test environments.

3.5. Conclusions

The current study assessed the drought tolerance of the newly-developed bottle gourd F_1 hybrids and their parental genotypes based on yield performance under non-stressed and drought-stressed conditions. Based on mean performance for fruit yield under drought-stressed conditions, the hybrids BG-27 × BG-31, BG-31 × BG-70, and BG-31 × BG-78 were the top performers, over most of the parental genotypes. Under non-stressed conditions, the hybrids BG-31 × BG-79 and BG-31 × BG-52 showed an increase in performance for fruit yield compared to all parental genotypes. Grouping biplots identified the hybrids BG-27 × BG-31, BG-31 × BG-70, and BG-31 × BG-52 as the best performers for fruit yield under both drought-stressed and non-stressed conditions. Depending on local growers' planting conditions, some of the identified hybrids can be cultivated under drought-stressed, well-watered, or both conditions. The study deployed various drought tolerant indices to identify and select the most drought tolerant genotypes which revealed crosses BG-31 × BG-67, BG-27 × BG-31, BG-58 × BG-78, BG-31 × BG-70, BG-31 × BG-78, BG-58 × BG-67, BG-31 × BG-52, BG-52 × BG-78, BG-27 × BG-80, BG-58 × BG-81, BG-31 × BG-48, BG-67 × BG-70, BG-58 × BG-79 and BG-58 × BG-80 as drought tolerant based on their high performance for fruit and seed yield. This performance was also higher than that of the parental genotypes. The identified drought-tolerant crosses are recommended for further testing and for production and commercialization in South Africa.

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Chapter 4. Combining ability and heterosis among bottle gourd [*Lagenaria siceraria* (Molina) Standl.] selections for yield and related traits under drought-stressed and non-stressed conditions

Abstract

Bottle gourd [*Lagenaria siceraria* (Molina) Standl.] is cultivated for multiple utilities, including as a leafy vegetable, for fresh and dried fruits and seeds. It is an under-researched and -utilized crop, and modern varieties are yet to be developed and deployed in sub-Saharan Africa (SSA). There is a dire need for pre-breeding and breeding of bottle gourds for commercialization in South Africa. Therefore, this study aimed to determine the combining ability and heterosis among selected genotypes of bottle gourd for fruit yield and related traits under drought-stressed and non-stressed conditions to select the best parents and hybrids. This study was conducted using parental accessions exclusively sampled and evaluated within South Africa. Eight preliminarily selected and contrasting parents with drought tolerance were crossed using a half-diallel mating design. The 8 parents and 28 crosses were evaluated under non-stressed (NS) and drought-stressed (DS) conditions across two growing seasons (2020/21 and 2021/22) using a 6 × 6 alpha lattice design with three replicates. Data were collected on fruit yield and related traits and subjected to analysis of variance, combining ability and heterosis analyses. Significant ($p < 0.05$) specific combining ability (SCA) and general combining ability (GCA) effects were computed for fruit yield per plant (FYPP). The SCA × environment and GCA × environment interaction effects were highly significant ($p < 0.001$) for FYPP and SYPP. The results suggest that genetic effects were affected by the test environment. Parental genotypes BG-58 and GC recorded positive and significant GCA effects for FYPP under the DS condition, whereas GC recorded positive and significant GCA effects for FYPP under the NS condition. The two genotypes are ideal breeding parents for population development to select genotypes with high fruit and seed yields. Crosses BG-27 × BG-79, BG-52 × BG-79, BG-70 × BG-79, BG-70 × BG-80, BG-80 × GC, and BG-70 × GC recorded high and positive SCA effects for FYPP and SYPP under DS condition. Crosses BG-52 × BG-81, BG-81 × GC, BG-27 × BG-79, BG-27 × GC, BG-79 × GC, BG-70 × BG-80, BG-58 × BG-81, BG-27 × BG-80, BG-27 × BG-58, BG-52 × BG-79, BG-52 × BG-58, BG-58 × BG-80, and BG-58 × BG-70 recorded high and positive SCA effects for FYPP and SYPP under NS condition. The patterns of hybrids with high and positive SCA arose from both tolerant and susceptible parental crosses, suggesting complex gene interactions. Even susceptible parents contributed useful alleles. Also, GCA was preponderant for FYPP, implying that selection-based breeding strategies can be effectively employed to improve fruit yields. Crosses BG-58 × BG-80, BG-27 × BG-79, BG-52 × BG-79, BG-27 × BG-52, and BG-52 × BG-80 showed high and positive mid- and better-parent heterosis under DS condition for FYPP and SYPP. Crosses BG-27 × GC, BG-79 × GC, BG-27 × BG-58, and BG-27 × BG-79 showed high and positive mid-and better parent heterosis under NS condition for FYPP and SYPP. The newly selected families are recommended for multi-environment evaluation for release and commercialization in South Africa or similar agroecologies.

Keywords: abiotic stress; bottle gourd; gene action; general combining ability; specific combining ability

4.1. Introduction

Bottle gourd [*Lagenaria siceraria* (Molina) Standl., 2n = 2x = 22] is a multi-purpose crop belonging to the *Cucurbitaceae* family. It is a highly valued food security crop in Africa and Asia (Morimoto et al., 2004, 2005; Upaganlawar and Ramchandran, 2009; Hart, 2011; Mashilo et al., 2017a). In Africa, the large and tender leaves are cooked and consumed as a leafy vegetable. The young, tender fruits are harvested and boiled until soft and consumed by adding sugar, peanuts, salt, or milk, depending on consumer preferences. The matured seeds extracted from dried fruits are processed to prepare livestock feed. The fruits of bottle gourd are an excellent source of essential macro- and micro-nutrients, including minerals (e.g., iron, phosphorus, potassium, calcium, and magnesium), vitamins B, C, and E, carbohydrates, and dietary fiber (Morimoto et al., 2004; Ojiako and Igwe, 2007; Mohale et al., 2008; Upaganlawar and Ramchandran, 2009; Hart, 2011; Sithole et al., 2015; Attar and Ghane, 2018, 2019; Mahapatra et al., 2023). The fruits are also a good source of essential amino acids, including aspartic acid, threonine, serine, glutamic acid, alanine, valine, phenylalanine, lysine and arginine, and phytochemical compounds, including phenolics, flavonoids and terpenoids (Attar and Ghane, 2018, 2019). Wild fruits of the crop are bitter due to the presence of cucurbitacins, which possess pharmaceutical values including anti-cancer and anti-diabetes (Attar and Ghane, 2018, 2019; Saurabh et al., 2023). The leaves are a good source of essential nutrients such as zinc, nitrogen, manganese, and copper (Sithole et al., 2015). The seeds are a source of crude protein, crude lipids, crude fiber, and carbohydrates (Chung et al., 2000; Deshpande et al., 2007; Ogunbusola et al., 2018). The seeds are valued for their antioxidants, sterols, and vitamins, including ascorbic acid, thiamine, riboflavin, niacin, pantothenic acid, and vitamin B-6 (Chung et al., 2000). In addition, the seeds contain essential amino acids, including glutamic acid, leucine acid, arginine, lysine, and aspartic acid (Ogunbusola et al., 2010).

Bottle gourd is one of the widely used rootstocks for grafted watermelon. It confers desirable qualities such as better fruit yield and quality and resistance to biotic (e.g., fungal and viral diseases) and abiotic (heat and drought) stress factors in grafted watermelons (Yetişir and Sari, 2003; Cohen et al., 2007; Kousik et al., 2008; Keinath and Hassell, 2014; Kousik et al., 2018; Morales et al., 2023). In Africa, bottle gourd is cultivated using genetically unimproved landrace varieties that are phenotypically and genetically diverse with low yield potential (Morimoto et al., 2005; Koffi et al., 2009; Mashilo et al., 2017a). In farmers' fields, different accessions are often planted in companion with the major crops, mainly maize, sorghum, soybeans, and rice. The landrace accessions display genetic variation and are selected by growers for their long handles/necks to make containers, and corrugated fruit with or without fruit necks are mainly for consumption. The round fruit-shaped gourds are used to make containers called "Kgapa" in the indigenous and local Sepedi language of South Africa. Planting diverse accessions in close proximity by farmers allows for cross-pollination, leading to high genetic recombination and genetic variation. The reported genetic diversity in different regions includes bottle gourd fruit having long curved-neck shapes, fruits with long straight-neck lengths, circular, oblate, pyriform, cavate, cylindrical fruit shapes, dark green fruit colour and other traits such as variation in the number of leaves and plant height (Decker-Walter et al., 2004; Morimoto et al., 2005, 2006; Ibrahim et al., 2013; Mashilo et al., 2015; 2017a, 2017b). In India, Turkey, India, China, and the USA, higher genetic variability of the crop has been reported (Decker-Walters et al., 2001; Morimoto et al., 2005, 2006; Wang et al., 2021; Bonthala et al., 2022; Mahapatra et al., 2022). The high genetic variation in bottle gourd allows for new variety designs with desirable consumer attributes, including high fruit and seed yields and other valuable traits such as fruit shape, size, colour, and neck to enhance the crop's market value in Africa.

The phenotypic variability of bottle-gourd genetic resources is documented via agronomic and horticultural traits. These include aboveground plant traits (e.g., plant height, number of primary and secondary branches), flower traits (e.g., number of male and female flowers), fruit traits (e.g., fruit shape, fruit weight, fruit yield, fruit texture, fruit colour, fruit with or without neck, fruit neck length, fruit neck bending) and seed traits (e.g., number of seeds per fruit, seed length, seed width, seed size, hundred seed weight, seed yield, seed coat colour, seed texture) (Decker-Walters et al., 2001; Morimoto et al., 2005, 2006; Mashilo et al., 2015, 2016). These traits are useful for targeted selection to develop new varieties targeting the various end-use values of the crop. For example, leaf traits such as leaf number, size, and plant height are essential to developing varieties suited for leaf vegetable and livestock fodder. Increasing the proportion of female than male flowers can aid the development of high-yielding hybrids. Increased fruit number and weight can improve fruit yield, whereas a high count of seeds per plant and increased seed weight are essential attributes to breeding high seed-yielding varieties. Some economic traits in bottle gourd are positively correlated and useful for selecting genotypes with better fruit and seed yields. For instance, fruit weight and fruit number have a direct positive effect on seed yield per fruit and fruit yield, suggesting their simultaneous selection and improvement (Pandit et al., 2009; Yao et al., 2015). These associations will allow for the breeding of bottle gourd varieties incorporating multiple traits.

Genotype selection with a desirable and complementary product profile requires progeny evaluation based on combining ability and heterosis analyses. Combining ability analysis has aided the selection of parental genotypes and progenies with high fruit yield for genetic advancement (Yadav and Kumar, 2012a; Kumar et al., 2014; Behera et al., 2016; Janaranjani et al., 2016; Mashilo et al., 2016). Fruit yield and related traits in bottle gourd were conditioned by non-additive gene action (Mahapatra et al., 2022). Complex gene action, including duplicate gene interaction, complimentary gene action, or non-allelic interaction, was reported for fruit yield in bottle gourd (Doust et al., 2014; Janaranjani et al., 2016). Yadav and Kumar, (2012b) and Mishra et al. (2019) reported high GCA compared to SCA effects for fruit yield, indicating the involvement of additive gene action conditioning their inheritance. Analysis of heterosis in bottle gourd identified the dominant form of heterosis for plant height, fruit length, and the number of branches, aiding the identification of hybrids for use in strategic breeding and variety release (Yadav and Kumar, 2012b).

Presently, in Africa, bottle gourd is an under-researched and -utilized crop, and modern varieties are yet to be developed and deployed. There is a dire need for pre-breeding and breeding bottle gourds with increased fruit and seed yield to enhance the market value of the crop. In previous studies, Mashilo et al. (2017b) identified accessions of bottle gourd with desirable agronomic attributes, including high fruit and seed yields useful for hybrid breeding. Nkosi et al. (2022) recently developed F_1 hybrids of bottle gourd derived from unimproved accessions for cultivation in the cooler environments of KwaZulu-Natal Province of South Africa. These newly developed hybrids performed better regarding fruit yield than the parental landrace accessions, indicating the possibility of developing cultivars with high yield potential and other desirable farmer-preferred traits. The next generation of improved bottle gourd varieties should comprise traits and attributes with multiple uses, including fodder, seed, and fruit, to serve varied value chains in the food, feed, and processing industries. Therefore, the objective of this study was to determine the combining ability and heterosis among selected genotypes of bottle gourd for fruit yield and related traits under drought-stressed and non-stressed conditions to select the best parents and hybrids for breeding.

4.2. Materials and methods

4.2.1. Plant material and generation of hybrids

The study used eight selected bottle gourd landrace accessions as parental genotypes for hybrid development. The selected bottle gourd accessions are widely grown in the Limpopo Province of South Africa by small-holder farmers for food (Table 4.1). The accessions are phenotypically and genetically divergent based on previous studies (Mashilo et al., 2015, 2016). Additionally, the accessions exhibit varied responses to drought stress (Mashilo et al., 2017c, 2018). The Limpopo Department of Agriculture and Rural Development maintains the landrace accessions at Toowoomba Agricultural Development Centre (TADC), Bela-Bela, South Africa.

Table 4.1 List and attributes of selected bottle gourd genotypes used in the cross.

Accession designation	Drought response	Fruit shape	Fruit neck length	Primary fruit colour
BG-27	Tolerant	Cavate	Long	Dark green
BG-52	Tolerant	Cavate	Long	Medium green
BG-58	Susceptible	Elongated	Long	Dark green
BG-70	Susceptible	Elongated	No neck	Dark green
BG-79	Tolerant	Pyriform	Short	Light green
BG-80	Susceptible	Elongated	Short	Dark green
BG-81	Susceptible	Pyriform	No neck	Light green
GC	Tolerant	Pyriform	No neck	Light green

The eight parental accessions were grown in a 5 L capacity polyethylene plastic pots under glasshouse conditions at the University of Limpopo (- 25° 36' 54" S, 28° 0' 59.76" E, 1312 m above sea level), South Africa. Five seeds per accession were sown in well-drained polyethylene plastic containing a loamy soil collected from the University of Limpopo, Syferskuil Experimental farm(-23°53'9.60" S, 29°44'16.80" E, 1312 m above sea level). Three plants were retained per accession in each pot two weeks after emergence and were watered daily to maintain soil moisture content approximately at field capacity (i.e., 40% v/v). Plants were allowed to grow until the development of male and female flowers, which occurred approximately 38 and 46 days after planting, respectively. The male flowers were brushed gently onto the female flower to ensure sufficient pollen for cross-pollination. The crosses were developed using a half-diallel mating design aiming for 28 crosses. The fully developed fruits from each of the crosses were labelled and sun-dried for up to four months. The seeds were extracted from the fruits, sun-dried, placed in labelled paper bags, and then stored in a dry, cool place for later use.

4.2.2. Study site and experimental design

Field experiments were conducted at the University of Limpopo's Syferskuil research farm, Mankweng, South Africa, during the 2020/21 and 2021/22 growing seasons. The area is characterized by sandy and loamy soils. The average rainfall received during the 2020/21 and 2021/22 growing seasons were 243 and 198 mm, respectively. The maximum temperature and relative humidity ranged from 26 to 34.8 ◦C and 60% to 88% for both growing seasons. The 8 parental genotypes and 28 successful crosses were evaluated under non-stressed (NS) and drought-stressed (DS) conditions using a 6 × 6 α-lattice design with three replications. In each block, three plants were established for parental accessions and crosses. The two water conditions and growing seasons provided four testing environments. Parents and crosses were planted at an intra-and-inter row spacing of 3 × 2 m apart. Sprinkler irrigation was used to water the plants. In the first two weeks after planting, the second true leaf stage, plants under both DS and NS conditions were watered weekly with approximately 27 mm of water. Thereafter, supplemental irrigation of approximately 27 mm of water was applied per week for plants grown under NS condition, whereas plants under DS condition were rain-fed. The total amount of water received by the plants grown under the NS condition was approximately 670 mm, whereas those under the DS condition received approximately 256 mm during the 2020/21 growing season. During the 2021/22 growing season, plants under the NS condition received approximately 680 mm of water, whereas those under the DS condition received approximately 269 mm of water. The plants under DS condition experienced a drought stress intensity of about 0.7 during the first and second seasons, which was calculated using the following formula:

$$\mathrm{DSI} = \frac{\bar{\mathrm{X}}\mathrm{ns} - \bar{\mathrm{X}}\mathrm{ds}}{\bar{\mathrm{X}}\mathrm{ns}}$$

where DSI is drought stress intensity; Xns, mean fruit yield averaged across all the genotypes tested under NS condition; Xds mean fruit yield averaged across all the genotypes tested under DS condition (Sio-Se Mardeh et al., 2006; Belko et al., 2014).

4.2.3. Data collection

Data were collected on a single randomly selected and tagged plant out of the three plants in each block for parental genotypes and crosses. The following agronomic traits were measured: total number of male and female flowers per plant, sex ratio calculated as the total number of male flowers per plant to the total number of female flowers per plant, number of leaves per plant, plant height measured from the base of the plant to the tip of the main vine in meters, number of fruits per plant, single fruit weight of dried fruit (kg), fruit circumference (cm) measured as the horizontal distance around the boundary of the fruit, fruit yield per plant (kg), number of seeds per fruit, hundred seed weight (g) and seed yield per plant (kg). The fruit-related traits were measured on a single fully developed fruit per plant.

4.2.4. Data analysis

4.2.4.1. Analysis of variance

Analysis of variance was performed using GenStat version 18 (Payne et al., 2017). The Least Significant Difference (LSD) test was computed to compare treatment means at the 5% level of significance.

4.2.4.2. Estimates of best linear unbiased predictors

Best Linear Unbiased Predictors (BLUPs) were calculated using META-R (Multi Environment Trail Analysis with R for Windows) Version 6.0 (Alvarado et al., 2020). The BLUPs estimates were computed based on the lattice design procedure using the following linear model:

$$Y_{ijkl} = \mu + Loc_i + \mathrm{Rep}_j(\mathrm{Loc}_i) + \mathrm{Block}_k(\mathrm{Loc}_i\mathrm{Rep}_j) + \mathrm{Gen}_l + \mathrm{Loc}_i \times \mathrm{Gen}_l + \varepsilon_{ijkl}$$

Where,

$Yijkl$ = the trait of interest,

μ = overall mean effect,

Loc_i = effects of the $_i$th environment,

Rep_j = effects of the $_j$th replicate,

Block_k (Rep_i) = effects of the $_k$th incomplete block within the $_j$th replicate,

$Loc_i \times Gen_l$ = environment × genotype interaction,

Gen_j = effects of the $_l$th genotype,

ε_{ijkl} = error associated with the $_i$th replication, $_j$th incomplete block and the $_k$th genotype, which is assumed to be normally and independently distributed, with mean zero and homocedastic variance $\sigma^{2}\cdot$ Genotypes, environment, and interactions were treated as random factors effects to calculate BLUPs.

4.2.5. Estimates of the GCA and SCA effects

The significant tests for GCA and SCA effects were estimated using PBTools version 1.4 (Griffing, 1956). The GCA and SCA effects and genetic variance components were estimated using AGD-R (Analysis of Genetic Designs in R) Version 5.0 (Rodríguez et al., 2018) using a half-diallel mating design, method II and model I. The analysis was performed using the following fixed-effect model:

$Y_{ijk} = \mu + g_i + g_j + s_{ij} + e_{ijk}$

Where,

Y_{ijk} = value for the *ij*th cross in the *k*th replication

μ = the population mean,

g_i and g_j = GCA effects for the *i*th and *j*th parents

sij = the SCA effect of the cross of the ith and jth parents

eijk = error term associated with the cross of the ith and jth parents in the *k*th replication

4.2.6. Gene action and heritability estimates

Broad sense heritability (h^2B), narrow sense heritability (h^2n), additive variance (σ^2A) and dominance variance (σ^2D) was calculated according to Allard (1999) using the following formula:

$$h^2B = \frac{\sigma^2 g}{\sigma^2 g + \frac{\sigma^2 ge}{nLoc} + \sigma^2 z/(nLoc\ x\ nRep)}$$

Where,

σ^2 g = genotypic variance,

$\sigma^2 z$ = error variance,

nRep = number of replicates,

$\sigma^2 ge$ = G × E interaction variance,

nLoc = number of environments in the analysis

$$\mathrm{h}^2 n = \frac{\sigma^2 A}{\sigma^2 A + \sigma^2 D + \sigma^2 E}$$

Where,

$\sigma^2 A$ = additive variance component,

$\sigma^2 D$ = dominance variance component,

$\sigma^2 E$ = environmental effect,

$$\sigma^2 A = 4\sigma^2 gca$$

where, gca = general combining ability effect

$$\sigma^2 D = 4\sigma^2 sca$$

where, sca = specific combining ability effect

4.2.7. Heterosis estimates

Mid-parent heterosis (MPH) and better-parent heterosis (BPH) were computed according to the following equations (Falconer and Mackay, 1996):

$$MPH = 100\ X\left(\frac{F1-MP}{MP}\right) \text{ and}$$

$$BPH = 100\ X\left(\frac{F1-BP}{BP}\right)$$

Where,

F1 = mean performance of F_1,

MP = mean of the two parents making the cross and

BP = mean of the better parent for that particular cross

4.2.8. Correlation analysis

The BLUPs estimates were used to compute Pearson correlation coefficients to determine the associations between assessed horticultural traits using SPSS version 25 (SPSS Inc., Chicago, IL, USA, 2018).

4.3. Results

4.3.1. Genotype, water condition, and their interaction effects

Analysis of variance showing the main effects of genotype, water conditions, and their interaction for the studied agronomic traits are shown in Table 4.2. Significant genotypic effects ($p < 0.001$) were recorded for all traits except for SR. The effects of water conditions were highly significant ($p < 0.001$) for all the traits. Genotype × environment interaction effects were significant ($p < 0.001$) for all assessed traits.

Table 4.2 Analysis of variance showing mean squares and significant tests for the parental genotypes and their crosses evaluated for agronomic/horticultural traits under non-stressed and drought-stressed conditions across two growing seasons in South Africa.

	Traits												
Source of variation	df	NMF	NFF	SR	NL	PH	NFPP	FW	FC	FYPP	NSPF	HSW	SYPP
Incomplete Block (IB)	5	8922.00**	432.2**	12.94*	11562.00^{ns}	15.68^{ns}	16.9^{ns}	0.01^{ns}	3292.20**	0.39^{ns}	157280.00**	24.83^{ns}	0.15^{ns}
IB × Replication (Reps)	10	4309.00^{ns}	129.90^{ns}	7.45^{ns}	4050.00^{ns}	68.71^{ns}	5.39^{ns}	0.00^{ns}	114.3^{ns}	0.09^{ns}	9818.00^{ns}	16.31^{ns}	0.01^{ns}
Reps	2	62.00^{ns}	55.10^{ns}	2.92^{ns}	24012.00^{ns}	57.91^{ns}	30.68^{ns}	0.00^{ns}	196.2^{ns}	0.69^{ns}	82067.00^{ns}	21.45^{ns}	0.01^{ns}
Genotype (Gen)	35	2691.80**	157.34**	7.45^{ns}	27966**	76.65**	23.68**	0.03**	1409.70**	0.69**	128815.00**	24.24**	5.24**
Environment (Env)	3	25075.00**	2958.70**	31.41**	774455**	749.79**	683.71**	0.04**	7604.5**	12.46**	1149414.00**	115.08**	9.54**
Gen × Env	105	2219.2**	104.68**	5.43^{ns}	16872**	61.67*	11.08**	0.02**	1061.6**	0.31**	75629.00**	15.22**	12.12**
Residual	287	1607	60.49	5.55	19681	45.46	4.11	0.04	674.6	0.07	62491	10.77	4.47

Note: df, degrees of freedom; * and ** denote significant differences at 5 and 1% probability levels, respectively; ns = not significant; NMF = number of male flowers per plant, NFF = the number of female flowers per plant, SR = sex ratio, NL = the number of leaves per plant, PH = plant height (m), NFPP = the number of fruits per plant, FW = fruit weight (kg/fruit), FC = fruit circumference (cm), FYPP = fruit yield per plant (kg), NSPF = the number of seeds per fruit, HSW = hundred seed weight (g/100 seed), SYPP = seed yield per plant (kg).

4.3.2. Performance of bottle gourd parents and hybrids for assessed traits.

BLUPs estimates for the assessed traits for parents and their hybrids under DS and NS conditions across the two growing seasons are presented in Tables 4.3 and 4.4, respectively. An approximately 50 % increase in the performance of the different parental genotypes and hybrids was recorded for NMF, NFF, PH, NFPP, FC, NSPF and SYPP under NS condition compared to the DS condition. Under DS condition high FYPP of > 0.4 kg was recorded for crosses BG-52 × BG-58, BG-52 × BG-79, BG-58 × BG-80 and BG-80 × GC, while a low FYPP of < 0.2 kg was recorded for approximately 18 % of the crosses. Higher FYPP of > 0.4 kg was recorded for parental genotypes GC and low FYPP of < 0.2 kg was recorded for five parental genotypes, including BG-27, BG-52, BG-79, BG-80 and BG-81. Under NS condition crosses, BG-80 × BG-81 and BG-81 × GC recorded higher FYPP of > 1.5 kg whereas BG-70 × BG-80 recorded the least FYPP of 0.4 kg. Parents BG-70 and GC recorded high FYPP of 1.6 and 2.8 kg, respectively whereas all the other parents recoded a low FYPP of < 1 kg.

Table 4.3 BLUPs estimates of bottle gourd parents and their hybrids for selected traits evaluated under drought stress conditions across two growing seasons in South Africa.

	Traits											
Genotype	NMF	NFF	SR	NL	PH	NFPP	FW	FC	FYPP	NSPF	HSW	SYPP
Crosses												
BG-27 × BG-52	47.64	8.92	6.88	88.68	4.26	2.88	0.07	59.89	0.21	144.78	15.53	0.1
BG-27 × BG-58	64.24	12.28	6.4	96.11	4.01	3.36	0.07	58.16	0.29	320.74	16.63	0.29
BG-27 × BG-70	67.73	14.78	6.14	119.84	4.54	3.52	0.08	48.12	0.35	334.75	16.38	0.24
BG-27 × BG-79	64.24	11.42	6.63	87.88	5.47	4.16	0.07	47.94	0.31	364.69	16.07	0.24
BG-27 × BG-80	88.53	11.7	7.27	59.95	2.33	2.08	0.04	41.25	0.14	359.86	16.31	0.14
BG-27 × GC	44.57	9.11	6.47	71.25	2.02	2.08	0.05	59.29	0.14	194.04	16.88	0.11
BG-52 × BG-58	72.9	10.94	7.05	144.69	7.27	5.92	0.08	58.63	0.52	459.52	16.95	0.52
BG-52 × BG-70	76.94	11.7	6.40	136.3	6.25	4.48	0.08	51.98	0.41	444.38	16.34	0.33
BG-52 × BG-80	67.17	11.8	6.51	85.94	2.46	2.72	0.07	81.99	0.20	268.1	16.49	0.16
BG-52 × GC	66.2	11.22	6.38	113.86	3.63	2.56	0.07	57.8	0.20	406.39	15.43	0.15
BG-58 × BG-70	80.99	12.86	6.54	148.57	6.41	4.96	0.07	85.92	0.39	419.27	16.95	0.4
BG-58 × GC	88.94	15.54	6.38	115.16	4.79	4.16	0.08	47.83	0.38	289.83	16.26	0.23
BG-70 × GC	59.64	9.97	6.72	106.6	2.85	2.08	0.06	35.41	0.14	227.04	16.53	0.12
BG-52 × BG-79	83.64	12.38	6.75	122.74	5.83	4.96	0.08	53.4	0.49	482.38	16.35	0.48
BG-58 × BG-79	63.54	10.65	6.59	86.59	2.99	2.4	0.06	51.67	0.18	208.05	16.86	0.12
BG-70 × BG-79	79.18	15.45	6.48	123.71	2.65	3.04	0.07	40.67	0.24	201.61	16.74	0.14
BG-79 × BG-80	46.66	9.4	6.43	118.55	4.34	3.84	0.06	53.43	0.27	144.94	16.2	0.11
BG-79 × GC	43.73	8.53	7.04	143.56	5.01	4.00	0.08	66.59	0.37	186.31	16.89	0.14
BG-58 × BG-80	61.59	11.8	6.09	143.4	7.26	5.76	0.09	71.71	0.59	224.47	17.02	0.23
BG-70 × BG-80	59.64	10.07	6.82	96.59	3.06	3.52	0.07	50.88	0.27	317.84	16.53	0.21
BG-80 × GC	81.97	14.01	6.34	124.19	6.64	5.28	0.09	65.61	0.49	273.57	16.8	0.29
BG-27 × BG-81	89.64	15.26	6.34	105.96	4.9	3.68	0.07	55.11	0.28	311.73	15.39	0.17
BG-52 × BG-81	86.71	17.47	6.21	143.89	3.24	4.16	0.07	47.71	0.29	483.18	15.35	0.30
BG-58 × BG-81	62.15	11.42	6.36	76.09	3.05	2.72	0.06	49.71	0.28	242.34	15.88	0.13
BG-70 × BG-81	41.08	8.63	6.42	124.36	4.79	3.52	0.08	51.74	0.28	150.8	16.41	0.12
BG-79 × BG-81	71.36	13.24	6.48	55.43	1.99	2.08	0.05	33.41	0.13	351.17	16.55	0.13
BG-80 × BG-81	92.43	15.93	6.72	109.35	4.62	3.36	0.07	52.13	0.25	441.81	16.68	0.25
BG-81 × GC	63.41	10.17	6.78	150.18	5.46	3.84	0.09	51.67	0.42	360.99	15.96	0.24
Parents												
BG-27	52.38	10.36	6.58	74.32	2.73	2.4	0.05	43.26	0.14	208.05	15.85	0.12
BG-52	59.36	11.9	6.19	98.21	2.77	1.92	0.04	34.57	0.13	264.23	16.59	0.11
BG-58	66.2	11.61	6.4	122.26	4.79	3.84	0.07	51.42	0.31	269.55	16.18	0.18
BG-70	50.85	9.59	6.6	98.21	3.33	4	0.06	64.24	0.21	197.42	16.04	0.14
BG-79	50.15	9.49	6.57	107.73	2.83	2.88	0.05	49.66	0.17	199.19	16.65	0.14
BG-80	73.73	12.28	6.42	132.75	4.07	3.84	0.06	73.45	0.19	269.87	16.97	0.21
BG-81	73.59	12.86	6.52	87.88	2.66	2.4	0.07	66.92	0.18	228.33	17.29	0.14
GC	94.81	16.79	6.17	187.47	6.97	5.6	0.07	77.87	0.49	432.15	16.81	0.32
Mean	67.71	11.99	6.53	111.34	4.23	3.56	0.07	55.31	0.29	296.76	16.41	0.21
LSD (%)	38	8.49	1.47	59.4	2.89	2.34	0.03	17.99	0.28	210.96	2.36	0.27
CV (%)	52.71	80.87	35.95	47.35	56.63	58.62	34.91	24.65	89.75	63.18	20.26	122.95
P-value	>0.05	<0.05	>0.05	<0.05	>0.05	<0.05	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05

NMF = number of male flowers per plant, NFF = the number of female flowers per plant, SR = sex ratio, NL = the number of leaves per plant, PH = plant height (m), NFPP = the number of fruits per plant, FW = fruit weight (kg/fruit), FC = fruit circumference (cm), FYPP = fruit yield per plant (kg), NSPF = the number of seeds per fruit, HSW = hundred seed weight (g/100 seed), SYPP = seed yield per plant (kg). CV = coefficient of variation, LSD = least significant difference.

Table 4.4 BLUPs estimates of bottle gourd parents and their hybrids for selected traits evaluated under non-stress conditions across two growing seasons in South Africa.

	Traits											
Genotype	NMF	NFF	SR	NL	PH	NFPP	FW	FC	FYPP	NSPF	HSW	SYPP
Crosses												
BG-27 × BG-52	119.07	15.60	7.84	201.55	8.70	8.96	1.08	61.54	1.75	574.16	14.38	0.64
BG-27 × BG-58	145.99	25.34	6.77	419.92	10.65	8.80	0.14	51.24	1.26	554.98	15.79	0.67
BG-27 × BG-70	144.85	18.79	7.75	209.55	8.82	7.80	0.09	50.44	0.74	773.20	13.73	0.70
BG-27 × BG-79	170.49	27.90	7.19	322.53	9.74	8.96	0.11	90.07	1.01	469.99	14.40	0.55
BG-27 × BG-80	150.41	18.79	8.19	282.54	9.59	6.97	0.09	48.55	0.77	330.24	15.09	0.30
BG-27 × GC	150.98	23.10	7.20	213.95	9.07	9.13	0.18	61.53	1.64	402.98	12.80	0.41
BG-52 × BG-58	181.88	24.70	7.60	221.55	8.98	7.97	0.13	85.21	1.06	436.98	15.98	0.45
BG-52 × BG-70	164.94	23.26	7.42	291.14	10.09	8.96	0.10	67.91	0.94	391.91	15.91	0.56
BG-52 × BG-80	133.89	17.19	7.88	328.13	8.80	7.13	0.08	53.18	0.60	546.48	16.60	0.62
BG-52 × GC	178.89	24.86	7.74	240.55	9.18	8.80	0.10	73.15	0.86	692.16	13.73	0.72
BG-58 × BG-70	145.85	19.27	7.80	201.55	9.38	8.46	0.08	55.73	0.79	354.95	15.47	0.39
BG-58 × GC	150.12	20.23	7.83	208.15	8.85	5.64	0.07	109.78	0.44	486.20	15.74	0.34
BG-70 × GC	183.17	30.93	6.98	168.16	8.67	7.97	0.14	80.08	1.09	387.56	14.07	0.42
BG-52 × BG-79	155.11	23.58	7.16	206.15	9.20	8.30	0.10	77.29	0.86	372.15	17.22	0.50
BG-58 × BG-79	156.82	21.35	7.56	301.94	18.42	9.30	0.10	78.74	0.98	574.95	13.75	0.67
BG-70 × BG-79	160.09	18.63	8.34	145.96	8.15	7.97	0.10	67.49	0.77	824.99	12.52	0.67
BG-79 × BG-80	140.72	17.35	8.80	328.93	9.49	10.79	0.11	59.93	1.26	370.96	14.19	0.51
BG-79 × GC	162.37	25.98	7.05	186.81	8.69	10.13	0.13	75.89	1.36	334.00	14.89	0.44
BG-58 × BG-80	166.79	24.54	7.28	323.53	9.71	10.96	0.11	87.89	1.22	533.24	12.13	0.61
BG-70 × BG-80	160.52	21.99	7.62	213.35	8.81	8.13	0.10	72.90	0.79	524.34	15.34	0.56
BG-80 × GC	161.94	23.58	7.33	332.53	10.02	8.46	0.12	84.15	0.99	573.96	15.06	0.67
BG-27 × BG-81	176.04	29.50	6.92	368.53	10.22	9.13	0.12	77.01	1.06	668.64	14.15	0.71
BG-52 × BG-81	165.22	25.50	7.58	387.52	11.09	7.97	0.12	81.55	0.95	668.44	12.65	0.54
BG-58 × BG-81	146.70	23.58	7.06	184.56	9.65	9.13	0.11	70.61	1.00	595.31	13.25	0.62
BG-70 × BG-81	147.84	23.26	7.12	374.33	10.40	11.29	0.13	79.49	1.53	272.13	14.09	0.41
BG-79 × BG-81	143.57	22.15	7.53	382.72	10.13	9.79	0.08	52.42	0.94	347.64	15.66	0.46
BG-80 × BG-81	162.09	26.14	7.17	302.34	10.77	10.46	0.11	72.84	1.17	598.47	13.44	0.74
BG-81 × GC	179.32	27.90	7.14	363.13	10.48	10.29	0.15	80.06	1.60	687.61	12.13	0.71
Parents												
BG-27	144.00	18.47	8.02	351.93	8.59	6.97	0.09	56.60	0.67	355.94	16.14	0.36
BG-52	133.17	17.19	7.71	290.94	8.88	7.47	0.11	68.41	0.84	400.81	15.42	0.48
BG-58	164.94	23.58	7.42	267.94	9.04	9.30	0.10	76.98	0.96	438.76	14.02	0.51
BG-70	179.89	26.14	7.38	422.92	10.26	12.29	0.12	80.78	1.59	306.13	14.76	0.52
BG-79	132.89	21.83	6.93	148.36	8.38	6.47	0.09	60.21	0.61	326.68	14.81	0.32
BG-80	157.24	20.39	7.95	176.56	8.23	7.80	0.12	85.57	0.91	407.92	15.41	0.52
BG-81	158.67	23.10	7.35	185.75	9.04	8.30	0.08	55.38	0.69	580.48	16.51	0.81
GC	184.02	26.78	7.37	441.32	11.22	13.78	0.16	80.20	2.48	364.44	14.02	0.65
Mean	157.24	22.85	7.50	277.70	9.70	8.89	0.11	71.41	1.01	486.94	14.59	0.55
LSD (%)	39.12	7.81	1.79	125.73	7.33	3.28	0.03	28.16	0.57	213.28	3.60	0.25
CV (%)	23.38	30.50	30.02	34.46	98.75	31.90	25.66	34.48	45.03	33.73	24.41	38.79
P-value	>0.05	<0.05	>0.05	<0.05	>0.05	<0.05	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05

NMF = number of male flowers per plant, NFF = the number of female flowers per plant, SR = sex ratio, NL = the number of leaves per plant, PH = plant height (m), NFPP = the number of fruits per plant, FW = fruit weight (kg/fruit), FC = fruit circumference (cm), FYPP = fruit yield per plant (kg), NSPF = the number of seeds per fruit, HSW = hundred seed weight (g/100 seed), SYPP = seed yield per plant (kg). CV = coefficient of variation, LSD = least significant difference.

4.3.3. The GCA and SCA effects

The ANOVA summary showing mean squares and significant tests for GCA and SCA effects for the assessed traits across the two growing seasons are presented in Table 4.5. The environmental effect was significant for all traits except for HSW. The genotypic effect was significant for all traits except for SR, PH, FC, and SYPP. The genotypic × environmental effect was significant for all traits except for SR, FW, FC, and NSPF. The GCA effects were significant for NFF, NFPP, FW, FC, and FYPP, whereas SCA effects were significant for NMF, NFF, NL, NFPP, FYPP, HSW, and SYPP. The GCA × environment interaction effects were significant for NMF, NFF, NL, NFPP, FYPP, HSW, and SYPP, whereas SCA × environment effects were significant for NMF, NFF, NL, PH, NFPP, FYPP, HSW, and SYPP.

Table 4.5 Analysis of variance showing mean squares and significant tests for parental genotypes and their hybrids for the assessed traits across two growing seasons in South Africa.

	Traits												
Source of variation	df	NMF	NFF	SR	NL	PH	NFPP	FW	FC	FYPP	NSPF	HSW	SYPP
Environment (Env)	3.00	25074.90**	4125.34**	31.79*	1009293.0**	785.16**	684.26**	0.051**	10863.87**	13.17**	1269586.00**	125.94^{ns}	3.23**
Env × Rep	6.00	269.09^{ns}	42.93^{ns}	2.60^{ns}	10572.48**	31.45^{ns}	12.10**	0.00^{ns}	412.96^{ns}	0.21**	25322.75^{ns}	38.04**	0.07**
Env × Rep × Block	40.00	2112.83**	145.49**	6.93^{ns}	8314.86**	48.66^{ns}	7.05**	0.00^{ns}	876.45^{ns}	0.19**	53420.55^{ns}	14.54*	0.09**
Genotype (Gen)	35.00	3966.34*	157.33*	7.45^{ns}	27965.56*	76.65^{ns}	23.68**	0.03**	1409.69^{ns}	0.69**	128815.50*	24.53*	0.15^{ns}
Gen × Env	105.00	2219.24**	104.68**	5.43^{ns}	16872.5**	61.67*	23.78**	0.00^{ns}	1061.55^{ns}	0.31**	75628.94^{ns}	15.21**	0.12**
GCA	7.00	4839.62^{ns}	229.01*	7.99^{ns}	13288.07^{ns}	52.32^{ns}	11.08**	0.03*	2902.99*	1.02*	100802.40^{ns}	11.54^{ns}	0.11^{ns}
SCA	28.00	3748.02*	139.42^{ns}	7.32^{ns}	31634.94*	82.73^{ns}	25.67*	0.02*	1036.37^{ns}	0.61**	135818.70*	27.78**	0.16^{ns}
GCA × Env	21.00	2513.755**	96.00**	4.66^{ns}	16083.49**	39.91^{ns}	23.18**	0.00^{ns}	936.45^{ns}	0.31**	53004.61^{ns}	20.88**	0.14**
SCA × Env	84.00	2145.60**	106.84**	5.62^{ns}	17081.00**	67.10*	11.65**	0.00^{ns}	1092.80^{ns}	0.31**	81285.03^{ns}	13.79*	0.11**
Residual	100.00	30125.06	26.92	5.05	464.46	43.97	2.88	0.00	-174.76	0.02	-950.83	8.75	0.01

Note: GCA = general combining ability; SCA = specific combining ability; df = degrees of freedom; * and ** denote significant difference at 5 and 1% probability levels; ns = non-significant; NMF = number of male flowers per plant; NFF = the number of female flowers per plant; SR = sex ratio; NL = the number of leaves per plant; PH = plant height (m); NFPP = the number of fruits per plant; FW = fruit weight (kg/fruit); FC = fruit circumference (cm); FYPP = fruit yield per plant (kg); NSPF = the number of seeds per fruit; HSW = hundred seed weight (g/100 seed); SYPP = seed yield per plant (kg).

4.3.4. General combining effects of parental genotypes.

General combining effects of the parental genotypes for yield and related traits under DS and NS conditions across the two growing seasons are presented in Table 4.6. The parental lines exhibited varied GCA effects for the different assessed traits. Significant positive and high positive GCA effects were considered desirable for the different traits. Under the DS condition, BG-58 and GC were the best-performing parental genotypes that recorded high positive and significant GCA effects for NL, PH, NFPP, FW, and FYPP. In addition, BG-58 recorded a high positive and significant GCA effect of 0.24 for SYPP. Under the NS condition, parental genotype GC recorded significant and high positive and GCA effects of 18.3, 3.7, 1.4, 0.02, and 0.5 for NMF, NFF, NFPP, FW, and FYPP, in that order. BG-52 and BG-79 recorded a high and negative GCA effect of 0.1 for SYPP.

Table 4.6 General combining effects for the assessed traits among the parental genotypes evaluated under drought stress and non-stress conditions across two growing seasons in South Africa.

	Traits											
Parents	NMF	NFF	SR	NL	PH	NFPP	FW	FC	FYPP	NSPF	HSW	SYPP
					Drought stress							
BG-81	15.44^{ns}	6.39**	-0.72^{ns}	-10.09ns	-1.04**	-1.01**	0.00^{ns}	-1.41^{ns}	-0.11^{ns}	35.55^{ns}	-1.80**	-0.11^{ns}
BG-27	-14.51^{ns}	-0.11^{ns}	-0.49^{ns}	-15.54**	-0.83^{ns}	-0.76^{ns}	-0.01**	-2.36^{ns}	-0.10^{ns}	-60.72^{ns}	-1.01^{ns}	-0.06^{ns}
BG-79	-13.96^{ns}	-3.16^{ns}	0.18^{ns}	-17.14**	-0.70^{ns}	-0.46^{ns}	-0.01**	-6.13**	-0.07^{ns}	-29.17^{ns}	0.67^{ns}	-0.04^{ns}
BG-52	8.14^{ns}	1.09^{ns}	0.09^{ns}	-5.49ns	-0.27^{ns}	-0.36^{ns}	0.00^{ns}	0.31^{ns}	-0.02^{ns}	71.03^{ns}	0.11^{ns}	0.11^{ns}
BG-80	-1.71^{ns}	-1.16^{ns}	0.37^{ns}	-6.39ns	-0.22^{ns}	-0.12^{ns}	0.00^{ns}	4.32^{ns}	-0.04^{ns}	-62.27^{ns}	0.97^{ns}	-0.08^{ns}
BG-58	16.39^{ns}	1.54^{ns}	-0.12^{ns}	23.76**	1.93**	1.19**	0.01**	4.16^{ns}	0.16**	17.43^{ns}	1.44^{ns}	0.24**
BG-70	-13.76^{ns}	-3.13^{ns}	0.42^{ns}	-1.69ns	-0.34^{ns}	0.44^{ns}	0.00^{ns}	-2.75^{ns}	-0.02^{ns}	-52.05^{ns}	-0.27^{ns}	-0.01^{ns}
GC	3.99^{ns}	-1.26^{ns}	0.27^{ns}	32.56**ns	1.46**	1.19**	0.02**	3.85^{ns}	0.19**	80.18^{ns}	-0.10^{ns}	0.04^{ns}
					Non-stress							
BG-81	7.24^{ns}	3.49**	-0.65**	56.38**	2.26**	1.03^{ns}	0.00^{ns}	-3.53^{ns}	0.14^{ns}	171.58**	-0.76^{ns}	0.27^{ns}
BG-27	-5.91^{ns}	-0.31^{ns}	-0.15^{ns}	5.63^{ns}	-0.07^{ns}	-0.98^{ns}	0.00^{ns}	-0.31^{ns}	-0.14^{ns}	30.73^{ns}	1.28^{ns}	0.02^{ns}
BG-79	-7.26^{ns}	-0.96^{ns}	0.20^{ns}	-13.78^{ns}	-0.84^{ns}	0.08^{ns}	-0.01**	-1.55^{ns}	-0.07^{ns}	-91.13**	0.06^{ns}	-0.11**
BG-52	-24.46**	-3.41**	-0.02^{ns}	-35.58**	-1.15**	-1.78**	-0.02**	1.20^{ns}	-0.37**	-26.23^{ns}	1.97**	-0.07**
BG-80	6.09^{ns}	-1.46^{ns}	0.62**	-26.83^{ns}	-0.94^{ns}	-0.53^{ns}	0.01**	1.43^{ns}	-0.02^{ns}	-44.48^{ns}	0.33^{ns}	-0.03^{ns}
BG-58	3.29^{ns}	0.09^{ns}	-0.04^{ns}	-14.23^{ns}	-0.22^{ns}	-0.18^{ns}	0.00^{ns}	2.07^{ns}	-0.06^{ns}	19.53^{ns}	-0.94^{ns}	-0.02^{ns}
BG-70	2.69^{ns}	-1.11^{ns}	0.44^{ns}	3.28^{ns}	0.13^{ns}	0.98^{ns}	0.00^{ns}	-0.29^{ns}	0.06^{ns}	-26.08^{ns}	-0.56^{ns}	-0.05^{ns}
GC	18.34**	3.69**	-0.39^{ns}	25.13^{ns}	0.83^{ns}	1.38**	0.02**	0.97^{ns}	0.47**	-33.93^{ns}	-1.39^{ns}	0.01^{ns}

Notes: ** denote significant at 1% probability level of t-values based on a two-tailed test, respectively; ns = non-significant; NMF = number of male flowers per plant; NFF = the number of female flowers per plant; SR = sex ratio; NL = the number of leaves per plant; PH = plant height (m); NFPP = the number of fruits per plant; FW = fruit weight (kg/fruit); FC = fruit circumference (cm); FYPP = fruit yield per plant (kg); NSPF = the number of seeds per fruit; HSW = hundred seed weight (g/100 seed); SYPP = seed yield per plant (kg).

4.3.5. Specific combining ability effects of the crosses

Specific combining ability effects of the crosses for the assessed traits under DS and NS conditions across the two growing seasons are presented in Tables 4.7 and 4.8, respectively. The SCA effect varied widely among the 28 hybrids. Under DS condition, positive and significant SCA effects of 0.4, 0.5, and 0.7 for FYPP were recorded for BG-52 × BG-79 (tolerant × tolerant parental accession) , BG-27 × BG-79 (tolerant × tolerant), and BG-70 × BG-79 (susceptible × tolerant), in that order. In addition, positive and significant SCA effects of 0.6 for SYPP were recorded for BG-70 × BG-80 (susceptible × susceptible). Under NS condition, BG-52 × BG-81 (tolerant × susceptible), BG-27 × BG-79 (tolerant × tolerant), and BG-79 × GC (tolerant × tolerant parental accession) recorded positive and significant SCA effects of 0.5, 0.6, and 1 for FYPP, in that order. Positive SCA effects of 0.2, 0.3, and 0.4 for SYPP were recorded for BG-58 × BG-81 (susceptible × susceptible), BG-52 × BG-79 (tolerant × tolerant parental accession), and BG-27 × GC (tolerant × tolerant), in that order.

Table 4.7 Specific combining effects for the studied agronomic traits among the crosses evaluated under drought-stressed conditions across two growing seasons in South Africa.

	Traits											
Cross	NMF	NFF	SR	NL	PH	NFPP	FW	FC	FYPP	NSPF	HSW	SYPP
BG-27 × BG-81	-6.50^{ns}	-6.40^{ns}	0.80^{ns}	-38.09**	-1.45^{ns}	-0.71^{ns}	-0.01^{ns}	5.44^{ns}	-0.08^{ns}	-242.12**	4.12**	0.00^{ns}
BG-79 × BG-81	49.95**	7.60^{ns}	-0.71^{ns}	35.86^{ns}	3.63**	2.54**	0.01^{ns}	4.82^{ns}	0.22^{ns}	-21.85^{ns}	-5.28**	0.02^{ns}
BG-52 × BG-81	37.90^{ns}	8.65^{ns}	-0.17^{ns}	-45.54**	-2.57^{ns}	-1.26^{ns}	-0.02**	-8.69^{ns}	-0.15^{ns}	386.60**	3.02^{ns}	0.03^{ns}
BG-80 × BG-81	21.30^{ns}	15.90**	-1.12^{ns}	-19.69^{ns}	-2.54^{ns}	-1.36^{ns}	-0.02**	-10.42^{ns}	-0.18^{ns}	77.40^{ns}	-3.33^{ns}	-0.19^{ns}
BG-58 × BG-81	38.65^{ns}	15.15**	-1.16^{ns}	21.71^{ns}	1.31^{ns}	0.49^{ns}	0.00^{ns}	2.85^{ns}	0.01^{ns}	317.20**	-0.79^{ns}	0.19^{ns}
BG-70 × BG-81	-28.45^{ns}	-6.05^{ns}	0.12^{ns}	-14.44^{ns}	-2.07^{ns}	-1.41^{ns}	-0.03**	-8.77^{ns}	-0.27^{ns}	-207.50^{ns}	-0.81^{ns}	-0.22^{ns}
BG-81 × GC	-74.30**	-15.70**	0.04^{ns}	27.01^{ns}	2.28^{ns}	0.84^{ns}	0.01^{ns}	7.57^{ns}	0.09^{ns}	-253.32**	0.26^{ns}	-0.10^{ns}
BG-27 × BG-79	-32.05^{ns}	-12.75^{ns}	1.41^{ns}	71.26**	2.87^{ns}	1.59^{ns}	0.06^{ns}	1.75^{ns}	0.45**	185.75^{ns}	-1.32^{ns}	0.27^{ns}
BG-27 × BG-52	-20.10^{ns}	-5.40^{ns}	0.74^{ns}	-22.19^{ns}	-1.72^{ns}	-1.21^{ns}	-0.01^{ns}	0.26^{ns}	-0.11^{ns}	-128.58^{ns}	0.09^{ns}	-0.12^{ns}
BG-27 × BG-80	42.35^{ns}	6.15^{ns}	-0.63^{ns}	60.91**	6.12^{ns}	3.49**	0.02**	1.68^{ns}	0.25^{ns}	185.37^{ns}	-2.23^{ns}	0.18^{ns}
BG-27 × BG-58	-66.25**	-14.10**	0.92^{ns}	-16.24^{ns}	-0.04^{ns}	-0.61^{ns}	0.00^{ns}	0.73^{ns}	-0.11^{ns}	-306.83**	1.41^{ns}	-0.29^{ns}
BG-27 × BG-70	38.60^{ns}	-1.85^{ns}	2.79**	-50.84**	-3.14^{ns}	-1.76^{ns}	-0.04^{ns}	-25.26**	-0.20^{ns}	173.47^{ns}	0.52^{ns}	-0.04^{ns}
BG-27 × GC	-12.00^{ns}	0.95^{ns}	0.02^{ns}	16.51^{ns}	-0.43^{ns}	0.34^{ns}	0.01^{ns}	9.45^{ns}	0.05^{ns}	304.77**	-0.19^{ns}	0.36^{ns}
BG-52 × BG-79	44.65^{ns}	21.80**	-3.41**	66.96**	2.41^{ns}	1.59^{ns}	0.04^{ns}	-4.85^{ns}	0.42**	194.25^{ns}	1.65^{ns}	0.24^{ns}
BG-79 × BG-80	-57.10**	-9.75^{ns}	-0.47^{ns}	-68.79**	-5.10**	-3.16**	-0.03^{ns}	12.91^{ns}	-0.40**	-271.98**	3.94**	-0.22^{ns}
BG-58 × BG-79	-25.20^{ns}	-3.30^{ns}	-0.44^{ns}	-29.99^{ns}	-2.62**	-1.81^{ns}	-0.02**	-13.40**	-0.20^{ns}	-160.18^{ns}	-1.93^{ns}	-0.16^{ns}
BG-70 × BG-79	65.70**	6.45^{ns}	1.09^{ns}	108.36^{ns}	6.57^{ns}	4.59**	0.06^{ns}	22.57**	0.69**	627.62^{ns}	0.48^{ns}	0.96^{ns}
BG-79 × GC	-60.95**	-9.30^{ns}	-0.32^{ns}	27.26^{ns}	1.84^{ns}	1.44^{ns}	0.03**	2.85^{ns}	0.15^{ns}	-191.58^{ns}	-1.59^{ns}	-0.11^{ns}
BG-52 × BG-80	-7.05^{ns}	-2.00^{ns}	0.26^{ns}	-66.39**	-4.35**	-3.46**	-0.02**	7.72^{ns}	-0.40**	-189.28^{ns}	3.97**	-0.33^{ns}
BG-52 × BG-58	38.10^{ns}	5.85^{ns}	-0.12^{ns}	-39.44^{ns}	-3.47**	-1.71^{ns}	-0.04^{ns}	-12.07^{ns}	-0.25^{ns}	-167.80^{ns}	0.76^{ns}	-0.19^{ns}
BG-52 × BG-70	-65.65**	-9.20^{ns}	0.77^{ns}	14.81^{ns}	1.11^{ns}	0.54^{ns}	0.02**	12.75^{ns}	0.11^{ns}	-330.53**	-0.55^{ns}	-0.22^{ns}
BG-52 × GC	-33.90^{ns}	-0.30^{ns}	-2.50**	-58.29**	-3.53**	-2.01^{ns}	-0.05^{ns}	-28.64^{ns}	-0.30**	-350.58**	1.32^{ns}	-0.44**
BG-58 × BG-80	-30.05ns	-7.05^{ns}	0.83^{ns}	-24.89^{ns}	-3.37**	-2.16**	0.00^{ns}	23.12**	-0.24^{ns}	-220.78**	-0.07^{ns}	-0.25^{ns}
BG-70 × BG-80	20.85ns	-1.75^{ns}	1.51^{ns}	61.96**	5.32**	3.44**	0.02**	6.00^{ns}	0.37**	272.52**	3.08^{ns}	0.63**
BG-80 × GC	62.00**	3.60^{ns}	1.40^{ns}	60.41**	5.51**	3.69**	0.04^{ns}	8.19^{ns}	0.53**	266.50**	-1.44^{ns}	0.34^{ns}
BG-58 × BG-70	-5.75ns	-2.45^{ns}	0.37^{ns}	-53.34**	-4.38**	-3.56**	-0.01^{ns}	7.09^{ns}	-0.45^{ns}	-15.23^{ns}	-2.78^{ns}	-0.33^{ns}
BG-58 × GC	10.80ns	-0.30^{ns}	0.07^{ns}	5.01^{ns}	-0.77^{ns}	-0.81^{ns}	-0.01^{ns}	1.84^{ns}	-0.13^{ns}	-50.48^{ns}	1.00^{ns}	-0.02ns
BG-70 × GC	-26.30ns	-4.50^{ns}	-2.73^{ns}	39.86^{ns}	4.29**	3.29**	0.02**	10.63^{ns}	0.44**	-89.18^{ns}	-0.05^{ns}	-0.02^{ns}

Note: ** denote significant differences at 1% probability level of t-values based on a two-tailed test, respectively; ns = non-significant; NMF = number of male flowers per plant; NFF = the number of female flowers per plant; SR = sex ratio; NL = the number of leaves per plant; PH = plant height (m); NFPP = the number of fruits per plant; FW = fruit weight (kg/fruit); FC = fruit circumference (cm); FYPP = fruit yield per plant (kg); NSPF = the number of seeds per fruit; HSW = hundred seed weight (g/100 seed); SYPP = seed yield per plant (kg).

Table 4.8 Specific combining effects for the studied agronomic traits among the crosses evaluated under non-stressed condition across two growing seasons in South Africa.

	Traits											
Cross	NMF	NFF	SR	NL	PH	NFPP	FW	FC	FYPP	NSPF	HSW	SYPP
BG-27 × BG-81	-8.32^{ns}	-3.66^{ns}	0.45^{ns}	-149.11**	-5.04**	-0.91^{ns}	-0.04**	-3.56^{ns}	-0.47**	-64.07^{ns}	5.27**	0.36^{ns}
BG-79 × BG-81	51.33**	6.14^{ns}	0.22^{ns}	30.14^{ns}	1.16^{ns}	-3.41**	0.01^{ns}	0.86^{ns}	-0.37^{ns}	201.28**	0.82^{ns}	-0.01^{ns}
BG-52 × BG-81	1.18^{ns}	5.79^{ns}	-1.47**	96.04**	2.98**	4.04**	0.01^{ns}	-0.83^{ns}	0.52**	-375.87^{ns}	1.09^{ns}	-0.38**
BG-80 × BG-81	29.38ns	10.24**	-1.21^{ns}	102.84**	3.34**	-2.11^{ns}	0.01^{ns}	-0.43^{ns}	-0.21^{ns}	332.73**	-4.86**	-0.07^{ns}
BG-58 × BG-81	-15.17^{ns}	-5.21^{ns}	1.01^{ns}	76.09^{ns}	2.31^{ns}	2.64^{ns}	0.00^{ns}	-3.02^{ns}	0.35^{ns}	10.98^{ns}	-0.58^{ns}	0.22**
BG-70 × BG-81	-43.37**	-8.26**	0.65^{ns}	-115.51**	-3.02**	-2.71^{ns}	-0.02^{ns}	1.05^{ns}	-0.42^{ns}	107.98^{ns}	-3.62^{ns}	-0.22**
BG-81 × GC	-0.27^{ns}	1.44^{ns}	-0.52^{ns}	61.49^{ns}	1.84^{ns}	1.64^{ns}	0.03**	10.48^{ns}	0.51**	-402.42^{ns}	1.32^{ns}	-0.41^{ns}
BG-27 × BG-79	-6.42^{ns}	-2.86^{ns}	0.43^{ns}	47.14^{ns}	1.46^{ns}	1.74^{ns}	0.03**	-0.98^{ns}	0.57**	253.43**	-4.72**	0.14^{ns}
BG-27 × BG-52	28.98^{ns}	-0.06^{ns}	0.96^{ns}	-49.11^{ns}	-1.87^{ns}	2.09^{ns}	-0.02^{ns}	-0.58^{ns}	0.14^{ns}	-263.37**	1.27^{ns}	-0.16**
BG-27 × BG-80	33.33^{ns}	10.09**	-1.26^{ns}	57.29^{ns}	2.31^{ns}	2.04^{ns}	0.01^{ns}	23.44**	0.27^{ns}	-116.52**	-1.69^{ns}	0.01**
BG-27 × BG-58	-92.97^{ns}	-11.46**	-0.18^{ns}	10.09^{ns}	0.90^{ns}	-0.61^{ns}	-0.03**	2.63^{ns}	-0.18^{ns}	197.08**	-0.45^{ns}	0.19**
BG-27 × BG-70	17.48^{ns}	0.09^{ns}	0.79^{ns}	4.84^{ns}	-0.82^{ns}	0.64^{ns}	0.04^{ns}	-8.60^{ns}	0.42^{ns}	-300.17**	-0.06^{ns}	-0.33**
BG-27 × GC	-24.72^{ns}	-0.96^{ns}	-0.98^{ns}	116.24**	4.13**	0.29^{ns}	0.06^{ns}	-4.52^{ns}	0.55**	254.83**	2.99^{ns}	0.38**
BG-52 × BG-79	-31.62^{ns}	-5.76^{ns}	0.27^{ns}	-96.26**	-3.29**	-2.36^{ns}	-0.03**	-11.59^{ns}	-0.45^{ns}	442.93^{ns}	-1.87^{ns}	0.33**
BG-79 × BG-80	-10.77^{ns}	1.94^{ns}	-0.77^{ns}	-24.11^{ns}	-0.65^{ns}	-0.76^{ns}	-0.03**	-1.07^{ns}	-0.51**	-152.72^{ns}	-2.28^{ns}	-0.25^{ns}
BG-58 × BG-79	-29.32^{ns}	-4.76^{ns}	-0.12^{ns}	-96.81**	-4.23**	-5.01**	-0.04^{ns}	-13.04**	-0.75**	-0.67^{ns}	0.57^{ns}	-0.09^{ns}
BG-70 × BG-79	33.88^{ns}	5.19^{ns}	-0.56^{ns}	-54.01^{ns}	1.04^{ns}	1.84^{ns}	0.01^{ns}	8.58^{ns}	0.24^{ns}	-148.07^{ns}	3.47^{ns}	-0.03^{ns}
BG-79 × GC	23.33^{ns}	0.24^{ns}	0.57^{ns}	138.74**	4.10**	4.09**	0.05^{ns}	4.43^{ns}	1.00**	-90.82^{ns}	-0.87^{ns}	0.00^{ns}
BG-52 × BG-80	13.63^{ns}	-0.31^{ns}	0.50^{ns}	53.14^{ns}	2.64^{ns}	2.74^{ns}	0.01^{ns}	-9.57^{ns}	0.31^{ns}	272.18**	-3.74^{ns}	0.43^{ns}
BG-52 × BG-58	-54.77**	-11.11**	2.14**	-87.36^{ns}	-3.63**	-2.91^{ns}	-0.01^{ns}	-4.07^{ns}	-0.45^{ns}	595.78^{ns}	-4.64**	0.33**
BG-52 × BG-70	8.08^{ns}	-0.41^{ns}	0.31^{ns}	-10.21^{ns}	-0.98^{ns}	-1.81^{ns}	0.01^{ns}	4.10^{ns}	-0.39^{ns}	-135.37^{ns}	5.24**	-0.17^{ns}
BG-52 × GC	-38.92**	-7.36^{ns}	0.57^{ns}	-47.21^{ns}	-3.52**	-0.81^{ns}	0.01^{ns}	6.62^{ns}	0.04^{ns}	-218.97**	-1.60^{ns}	-0.22**
BG-58 × BG-80	5.53^{ns}	-0.31^{ns}	0.29^{ns}	-33.46^{ns}	-1.50^{ns}	-0.56^{ns}	-0.03**	-4.70^{ns}	-0.22^{ns}	218.78**	3.00^{ns}	0.32**
BG-70 × BG-80	58.83**	5.64^{ns}	0.65^{ns}	67.44^{ns}	1.37^{ns}	1.59^{ns}	0.05^{ns}	10.47^{ns}	0.61**	-239.72**	5.93**	-0.10^{ns}
BG-80 × GC	20.43^{ns}	2.34^{ns}	-0.08^{ns}	15.94^{ns}	1.53^{ns}	1.44^{ns}	0.00^{ns}	-21.73**	0.17^{ns}	-220.62**	-0.41^{ns}	-0.14^{ns}
BG-58 × BG-70	22.78^{ns}	3.04^{ns}	-0.04^{ns}	-14.41^{ns}	0.38^{ns}	0.04^{ns}	-0.03**	-8.07^{ns}	-0.49^{ns}	297.73**	-3.49^{ns}	0.28**
BG-58 × GC	-10.52^{ns}	0.24^{ns}	-0.47^{ns}	-49.71^{ns}	-2.64**	-2.31^{ns}	0.00^{ns}	5.37^{ns}	-0.33^{ns}	-197.97**	1.15^{ns}	-0.28**
BG-70 × GC	24.28^{ns}	5.69^{ns}	-0.68^{ns}	-9.31^{ns}	2.86**	2.34^{ns}	-0.01^{ns}	10.23^{ns}	0.20^{ns}	154.03^{ns}	-6.76**	0.04^{ns}

Note: ** denote significant differences at 1% probability level of t-values based on a two-tailed test; respectively, ns = non-significant; NMF = number of male flowers per plant; NFF = the number of female flowers per plant; SR = sex ratio; NL, the number of leaves per plant; PH = plant height (m); NFPP = the number of fruits per plant; FW = fruit weight (kg/fruit); FC = fruit circumference (cm); FYPP = fruit yield per plant (kg); NSPF = the number of seeds per fruit; HSW = hundred seed weight (g/100 seed); SYPP = seed yield per plant (kg).

4.3.6. Gene action and heritability estimates

There were differences in the gene action and heritability among the assessed traits under the DS and NS conditions (Table 4.9). Under the DS condition, the broad-sense heritability (h^2B) was higher than the narrow-sense heritability (h^2n) for all traits. Under the DS condition, h^2B varied from 0.76 to 0.94 for all traits except for SR and HSW, which recorded h^2B of 0.13 and 0.23, respectively. A h^2n of zero was recorded for all traits except for NL. Similarly, under the NS condition, the h^2B was higher than the h^2n for all traits. Overall, the dominance variance (σ^2D) was higher compared to the additive variance (σ^2A) for all traits.

Table 4.9 Gene action and heritability estimates for the assessed traits under drought stress and non-stress condition across two growing seasons in South Africa.

Traits	σ^2A	σ^2D	h^2B	h^2n
		Drought stress		
NMF	0.00	1825.33	0.85	0.00
NFF	0.00	75.25	0.76	0.00
SR	0.08	0.84	0.13	0.01
NL	758.15	3548.41	0.86	0.15
PH	0.00	14.06	0.91	0.00
NFPP	0.04	7.65	0.88	0.00
FW	0.00	0.00	0.87	0.01
FC	12.35	755.56	0.94	0.02
FYPP	0.00	0.09	0.89	0.00
NSPF	0.00	69636.26	0.89	0.00
HSW	0.00	1.42	0.23	0.00
SYPP	0.00	0.09	0.85	0.00
		Non-stress		
NMF	248.28	1292.97	0.82	0.13
NFF	10.84	64.27	0.86	0.12
SR	0.50	1.53	0.23	0.06
NL	0.00	42735.77	0.95	0.00
PH	0.00	7.81	0.08	0.00
NFPP	0.35	15.35	0.89	0.02
FW	0.00	0.03	0.93	0.00
FC	83.30	988.20	0.88	0.06
FYPP	0.06	0.60	0.93	0.08
NSPF	0.00	118919.20	0.95	0.00
HSW	0.00	15.45	0.82	0.00
SYPP	0.00	0.10	0.90	0.00

Note; σ^2A = additive variance; σ^2D = dominance variance; h^2B = broad-sense heritability; h^2n = narrow-sense heritability; NMF = number of male flowers per plant; NFF = the number of female flowers per plant; SR = sex ratio; NL = the number of leaves per plant; PH = plant height (m); NFPP = the number of fruits per plant; FW = fruit weight (kg/fruit); FC = fruit circumference(cm); FYPP = fruit yield per plant (kg); NSPF = the number of seeds per fruit; HSW = hundred seed weight (g/100 seed); SYPP = seed yield per plant (kg).

4.3.7. Heterosis under drought-stressed and non-Stress conditions

Heterosis estimates for the studied traits amongst the F_1 bottle gourd evaluated under DS and NS conditions across the two growing seasons are presented in Appendix 4.1. High positive heterosis was considered desirable for the assessed traits. Under DS condition, high and positive mid-parent heterosis (MPH) of 298%, 101%, 91%, 58% and 86% for FYPP was recorded for crosses BG-58 × BG-80, BG-27 × BG-79, BG-52 × BG-79, BG-27 × BG-52 and BG-52 × BG-80, whereas better-parent heterosis (BPH) of 184%, 70%, 59%, 51% and 32% for FYPP was recorded for the same crosses, respectively. High and positive MPH of 244% and 265% and BPH of 236% and 174% for SYPP were recorded for crosses BG-70 × GC and BG-52 × BG-79, in that order. Under the NS condition, high and positive MPH of 58% and 52%, and BPH of 50% and 31% for FYPP were recorded for crosses BG-27 × GC and BG-79 × GC, in that order. At the same time, BPH of 52% and 59% for SYPP were recorded for crosses BG-27 × BG-58 and BG-27 × BG-79. In addition, MPH of 29% and 34% for SYPP were recorded for crosses BG-27 × BG-58 and BG-27 × BG-79, respectively.

4.3.8. Associations of the agronomic traits under drought and non-stressed conditions

Pearson's correlation coefficients showing the associations between the assessed traits under DS and NS conditions across the two growing seasons are presented in Table 4.10. A highly significant and moderate positive correlation was recorded between several traits. Under DS condition, significant and positive correlations were recorded between FW with FYPP ($r = 0.8$) and SYPP ($r = 0.7$). Additionally, a significant and positive correlation was recorded between FYPP and SYPP ($r = 0.8$). Whereas under NS condition, high and positive correlations were recorded between NFPP with FW ($r = 0.8$) and SYPP ($r = 0.8$). NFPP exhibited a significantly low correlation with FYPP ($r = 0.3$). FW positively correlated with FYPP ($r = 0.6$) and SYPP ($r = 0.9$). A moderate and positive correlation was recorded between FYPP and SYPP ($r = 0.5$).

Table 4.10 Pearson correlation coefficients showing associations between assessed traits among the parental genotypes and their crosses under drought-stressed (upper diagonal) and non-stressed (lower diagonal) conditions across two growing seasons in South Africa.

Traits	NMF	NFF	SR	NL	PH	NFPP	FW	FC	FYPP	NSPF	HSW	SYPP
NMF		0.866**	0.00^{ns}	0.277**	0.365**	0.378**	0.00^{ns}	0.20^{ns}	-0.06^{ns}	0.611**	-0.03^{ns}	-0.11^{ns}
NFF	0.525**		0.12^{ns}	0.333**	0.350**	0.401**	-0.06^{ns}	0.11^{ns}	-0.17^{ns}	0.577**	0.05^{ns}	-0.20^{ns}
SR	0.04^{ns}	0.21^{ns}		0.04^{ns}	0.11ns	0.06^{ns}	-0.14^{ns}	-0.03^{ns}	-0.248*	0.00ns	0.19^{ns}	-0.17^{ns}
NL	0.431**	0.08^{ns}	-0.15^{ns}		0.772**	0.712**	-0.18^{ns}	0.453**	-0.285*	0.405**	0.12^{ns}	-0.251**
PH	-0.500**	0.333**	0.286*	-0.398**		0.861**	-0.24^{ns}	0.462**	-0.339**	0.533**	0.03^{ns}	-0.22^{ns}
NFPP	-0.24^{ns}	0.511**	0.307*	-0.12^{ns}	0.866**		-0.23^{ns}	0.401**	-0.361**	0.453**	0.05^{ns}	-0.266*
FW	-0.474**	0.334**	0.382**	-0.438**	0.946**	0.783**		-0.17^{ns}	0.839**	-0.04^{ns}	-0.02^{ns}	0.683**
FC	0.690**	0.403**	-0.08^{ns}	0.598**	-0.335**	-0.02^{ns}	-0.356**		-0.282**	0.20^{ns}	-0.06^{ns}	-0.23^{ns}
FYPP	-0.13^{ns}	0.22^{ns}	0.22^{ns}	-0.22^{ns}	0.443**	0.293*	0.631**	-0.12^{ns}		-0.12^{ns}	0.08^{ns}	0.830**
NSPF	0.17^{ns}	-0.04^{ns}	0.07^{ns}	0.02^{ns}	-0.21	-0.254*	-0.17^{ns}	0.06^{ns}	-0.13^{ns}		-0.18^{ns}	-0.12^{ns}
HSW	-0.02^{ns}	0.320**	0.07^{ns}	-0.14^{ns}	0.375**	0.357**	0.327**	-0.23^{ns}	0.02^{ns}	-0.14^{ns}		0.19^{ns}
SYPP	-0.545**	0.295*	0.313**	-0.476**	0.981**	0.827**	0.969**	-0.400**	0.463**	-0.19^{ns}	0.19^{ns}	

Notes: * and ** denote significant at 5 and 1% probability level of t-values based on a two-tailed test, respectively; ns = non-significant; NMF = number of male flowers per plant; NFF = the number of female flowers per plant; SR = sex ratio; NL = the number of leaves per plant; PH = plant height (m); NFPP = the number of fruits per plant; FW = fruit weight (kg/fruit); FC = fruit circumference(cm); FYPP = fruit yield per plant (kg); NSPF = the number of seeds per fruit; HSW = hundred seed weight (g/100 seed); SYPP = seed yield per plant (kg).

4.4. Discussion

Analysis of variance revealed significant genotypic effects (Table 4.2), suggesting substantial differences among the parental genotypes and their progenies for economic traits, including fruit and seed yields. Bottle gourd is a morphologically diverse crop with variations reported for agronomic traits, including male and female flowering capacity, fruit yield and related traits, and seed yield and related traits (Falconer and Mackay, 1996; Decker-Walters et al., 2001; Morimoto et al., 2005, 2006; Mashilo et al., 2015, 2016, 2017b). Drought stress reduced flowering and fruit capacity (Tables 4.3 and 4.4). Fruit and seed yield were reduced by 71 and 62% across the tested genotypes, respectively. These indicated that bottle gourd can grow under drought-stressed environments and produce reasonable yields. The increasing drought episodes in South Africa require concerted efforts to develop drought-resilient bottle gourd varieties. The variation observed in the present study will allow for selecting of desirable genotypes for new variety design and commercialization. For example, crosses BG-52 × BG-58, BG-52 ×

BG-79, BG-58 × BG-80, BG-80 × GC, BG-80 × BG-81, and BG-81 × GC were high fruit yielders and are recommendable for further selection. Additionally, crosses BG-52 × BG-58, BG-52 × BG-79, BG-27 × BG-52, BG-27 × BG-58, BG-27 × BG-70 and BG-52 × BG-80 were the best performers for seed yield. These are ideal families for gene introgression, genetic advancement, and variety release. The significant genotype-by-environment interaction effects for the studied traits suggested environmental influence on the performance of genotypes requiring multi-environment testing to identify and recommend genotypes with specific and wide adaptation in targeted production environments.

The significant SCA effect indicated non-additive gene action for fruit and seed yield and some related agronomic traits (Tables 4.7 and 4.8). The non-additive gene action is non-fixable and challenging to transform, suggesting that such crosses should be used in direct production to increase the fruit and seed yield of bottle gourd in South Africa. There were significant GCA × environment effects for fruit and seed yields (Table 4.5). This indicated that the effects are dependent on the environment for their expression. A significant SCA × environment interaction effect existed for fruit and seed yields, suggesting that the environment played a significant role in expressing the effects. Therefore, multi-environment testing of the parents and hybrids is crucial to categorize each genotype's performance and identify genotypes with adaptation to certain environments to optimize the fruit and seed yield.

In the present study crosses with high and positive SCA effects were developed from diverse parental combinations including tolerant × tolerant, tolerant × susceptible, and even susceptible × susceptible, indicating that drought tolerance is governed by complex genetic effects such as additive, dominant, and epistatic gene actions. Notably, most of the top-performing hybrids resulted from tolerant × tolerant (dominant × dominant) crosses, suggesting a strong contribution of dominant alleles to drought tolerance. Interestingly, some hybrids from susceptible parents also showed improved performance, highlighting the presence of complementary alleles even in less tolerant genotypes (Ojas and Sprague, 1952). These findings emphasise the importance of maintaining broad genetic diversity and conducting extensive hybrid testing in drought tolerance breeding programs. Further, crosses such as BG-27 × BG-79 and BG-70 × BG-79 with high and significant SCA for fruit yield per plant were derivatives of parental genotypes BG-27, BG-70, and BG-79. Interestingly, these parents had low and non-significant GCA effects on fruit yield per plant. Previous studies in bottle gourd revealed crosses with significant SCA effects for fruit yield and yield-related traits that are derived from both or at least a parent that is a good combiner for the trait (Doust et al., 2014;

Janaranjani et al., 2016; Mishra et al., 2019; Kumar et al., 2018). The recorded high SCA effects in the current study may be due to dominant × dominant non-allelic gene interaction producing over-dominance, thus challenging to modify using breeding programs (Comstock and Robinson, 1952; Ojas and Sprague, 1952). In addition to dominance and epistasis, the SCA variation includes aberrations due to genotype × environment interactions (Begna, 2021). In the current study, crosses such as BG-81 × BG80 had a high positive and significant SCA effect for the number of female flowers. This cross manifested from good × poor general combiner parents for the trait may be attributed to favourable additive gene effects of the good general combiner parent (BG-81) and nonadditive effects of the poor general combiner (BG-80) (Ojas and Sprague, 1952). Parental genotypes such as BG-58 and GC (Table 4.6) with high GCA effects for fruit and seed yield serve as valuable germplasm for future breeding. Crosses such as BG-52 × BG-81, BG-81 × GC, BG-27 × BG-79, BG-27 × GC, BG-79 × GC, BG-70 × BG-80, BG-58 × BG-81, BG-27 × BG-80, BG-27 × BG-58, BG-52 × BG-79, BG-52 × BG-58, BG-58 × BG-80, and BG-58 × BG-70 (Tables 4.7 and 4.8) with the high positive SCA effect for fruit and seed yield are potential genetic resources for multi-environment testing for release and commercialization in South Africa.

The relative importance of general combining ability versus specific combining ability was quantified to infer the contributions of additive and dominance gene actions in controlling economic and secondary traits. Results revealed high GCA effect of approximately 56.36%, 62.17%, 52.19%, 60.00%, 73.70%, and 62.60% of the genetic variance for number of male flowers per plant, number of female flowers per plant, sex ratio, fruit weight, fruit circumference, and fruit yield per plant, respectively, is due to additive gene effects. This indicates that additive inheritance is relatively stronger than non-additive effects for these traits, making selection an effective breeding strategy. High GCA effects relative to SCA effects for fruit yield were also reported by Yadav and Kumar (2012b) and Mishra et al. (2019). Conversely, traits such as number of leaves per plant, plant height, number of fruits per plant, number of seeds per fruit, hundred seed weight, and seed yield per plant showed additive contributions of 29.57%, 38.74%, 30.15%, 42.60%, 29.40%, and 40.70%, respectively, suggesting that dominance or epistatic interactions play a major role in their inheritance. For these traits, hybrid development would likely be more beneficial than selection-based approaches. Overall, these patterns indicated that an integrated breeding approach, combining selection for additive traits and hybridisation for traits governed by dominance, would be most effective for genetic improvement in bottle gourd.

Heritability analysis is useful to provide information about the potential transmissibility of traits from parents to offspring (Abdin et al., 2014; Gouda et al., 2021). The larger broad-sense heritability compared to narrow-sense heritability for all traits (Table 4.9) indicates dominance effects. This implies that hybrid combinations may show improved performance due to heterosis, but such traits may not be reliably passed on through simple selection in breeding.

The exploitation of heterosis via intensive evaluation of hybrids identifies diverse genetic donors and allows for the identification of heterotic crosses (Behera et al., 2016; Janaranjani et al., 2016). High and positive mid- and better-parent heterosis observed in the cross BG-58 × BG-80, BG-27 × BG-79, BG-52 × BG-79, BG-27 × BG-52, and BG-52 × BG-80 for fruit yield per plant and cross, BG-70 × GC and BG-52 × BG-79 for seed yield per fruit (Appendix 4.1). Hence, these crosses are essential for strategic breeding and variety release in South Africa.

Trait correlation analyses aid in the simultaneous selection of multiple traits. The positive correlations between the number of female flowers per plant with the number of fruits per plant and the number of fruits per plant with the number of seeds per fruit, fruit yield per plant, and seed yield per fruit suggested simultaneous improvement in these traits is possible. Additionally, these suggested the linkages of desirable genes controlling the expression of the studied traits (Dey et al., 2006; Adhikari et al., 2014). Therefore, these traits are recommended for further selection in the newly developed bottle gourd hybrids to deliver varieties that meet market needs and standards and multiple crop characteristics required by South African growers.

4.5. Conclusions

The current study assessed the combining ability and heterosis for fruit yield and related traits. Crosses were made using genetically distant parents of South African bottle gourd accessions under non-stressed and drought-stressed conditions to select drought tolerant parents and new hybrids for production under water-stressed environments in South Africa or similar agroecologies globally. Drought stress reduced flowering and fruit capacity, fruit and seed yields in the presently assessed bottle gourd populations. Nevertheless, the studied genotypes produced reasonable yield levels under drought stressed conditions, indicating the possibility of breeding for enhanced drought tolerance in bottle gourds. Parental genotypes BG-58 and

GC were identified as valuable germplasm for future breeding targeting high fruit and seed yields in water-limited environments. The newly bred F_1 hybrids BG-52 × BG-81, BG-81 × GC, BG-27 × BG-79, BG-27 × GC, BG-79 × GC, BG-70 × BG-80, BG-58 × BG-81, BG-27 × BG-80, BG-27 × BG-58, BG-52 × BG-79, BG-52 × BG-58, BG-58 × BG-80, and BG-58 × BG-70 with high fruit and seed yields were drought-tolerant and are recommended for release and commercialization in South Africa following multi-environment testing. The significant G×E interaction observed suggests that hybrid performance is environment-dependent, warranting further evaluation across multiple locations in South Africa before making firm recommendations.

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Chapter 5. Genotype-by-environment interactions for fruit yield and related traits in selected hybrids of bottle gourd (*Lagenaria siceraria*)

Abstract

Bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] is a multi-purpose cucurbit crop cultivated globally. The crop in sub-Saharan Africa (SSA) shows considerable genetic diversity and resilience to drought and heat stress. However, smallholder farmers use low-yielding unimproved landrace varieties due to a lack of investment support and breeding efforts to develop and release best-performing and widely adapted hybrid varieties with desirable product profiles. Therefore, the objective of this study was to determine the genotype-by-environment interactions (GEI) for fruit yield and related traits among newly developed bottle gourd hybrids to guide variety recommendation and registration. Eight preliminarily selected F_1 hybrids and four checks were evaluated in five contrasting environments of varying moisture conditions using a randomized complete block design with three replications. Data were collected on days to 50% male flowering (DTMF) and female flowering (DTFF), total number of fruits per hectare (TNFH) and fruit yield per hectare (FYPH) and subjected to analysis of variance, additive main effects and multiplicative interaction (AMMI) and genotype plus genotype-by-environment (GGE) biplot models. The AMMI model revealed significant ($p \leq 0.001$) effects of genotype (G), environment (E) and GEI for the studied traits. The AMMI model explained a higher (96.30%) variation for TNFH, of which G, E and GEI effects explained 49.88, 24.21 and 22.21% of the total variation, respectively. The model ascribed variations of 12.36, 73.16 and 11.41% for FYPH attributable to the G, E and GEI effects, in that order. The GGE biplot model explained 94.53 and 96.56% variations for TNFH and FYPH, respectively. The hybrids BG-58 × BG-80, BG-80 × GC, BG-27 × BG-52 and BG-52 × BG-58 attained high and stable FYPH under water-limited conditions. The hybrids BG-52 × BG-58, BG-58 × BG-80 and BG-52 × BG-79 recorded high FYPH under irrigated conditions. The study was limited to one province in South Africa, and the significant G×E interactions indicate that trials must be conducted across diverse environments. Further multi-environment trials within South Africa and beyond are needed to recommend registration and commercialisation of the identified hybrids.

Keywords: Additive main effects and multiplicative interaction; bottle gourd; drought stress; GGE biplot; genotype-by-environment interaction, stability analysis

5.1. Introduction

Bottle gourd [*Lagenaria siceraria* (Mol.) Standl., 2n = 2x = 22] is a cucurbit crop with multiple utilities. It is widely cultivated for its fresh leaves, immature and matured fruit, and fresh and dry seed (Barot et al., 2015; Gürcan et al., 2015; Aldewy et al., 2022). In sub-Saharan Africa (SSA), the fruits of the non-bitter types are used for human consumption after boiling. Freshly developing leaves are cooked and consumed as leafy vegetables, whereas the seeds can be dehulled to produce flour (Ogunbusola, 2017). The fresh bottle gourd leaves are rich in potassium and calcium (Sithole et al., 2015). Appreciable amounts of phosphorus, magnesium, zinc, iron and manganese are also found in the leaves (Modgil et al., 2004; Sithole et al., 2015). The tender fruit possesses various nutrients, including carbohydrates, proteins, dietary fiber, ash, energy and vitamins (i.e., vitamin A, vitamin B-complex and vitamin C) (Sithole et al., 2015; Mahapatra et al., 2023). The young fresh fruit also contains minerals, including potassium, calcium, magnesium, phosphorus, iron and sodium, and nutrients, including antioxidants, saponins and flavonoids (Hassan et al., 2008; Sithole et al. 2015; Gajera et al., 2017; Ogunbusola, 2017; Ahmad et al., 2022; Mahapatra et al., 2023). The seeds comprise vitamins and minerals, amino acids, omega-3 fatty acids and phytochemicals such as carotene and tocopherol (Deshpande et al., 2008; Abdel-Razek et al., 2021; Devi et al., 2023). The seeds are also used in agro-processing industries as raw material for the extraction of edible seed oil and seed cake for livestock. The seed oil contains sterols (i.e., β-sitosterols) beneficial in managing cholesterol levels, prostate health, anti-inflammatory effects and immune system support (Abdel-Razek et al., 2021).

Reportedly, some genotypes have bitter fruits, which have medicinal properties due to the high contents of secondary metabolites called cucurbitacins (Attar and Ghane, 2018; Saurabh et al., 2023). For example, cucurbitacin B and I are the major compounds in bitter fruits, and the juice extracts are used to remedy various ailments such as pain, fever, pectoral cough, asthma, and other bronchial and mental health disorders (Peters et al., 1997; Wynn, 2008; Gitler et al., 2017; Volpe et al., 2018 ; Dwijayanti 2020; Moustafa et al., 2021). The juice also has anti-cancer properties and can serve as contemporary and alternative medicine (Attar and Ghane, 2019; Ma et al., 2019; Moustafa et al., 2021; Patel and Ghane, 2021). Therefore, bottle gourd can serve as a strategic and opportunity crop for overcoming malnutrition and food shortages, especially in underdeveloped countries.

Bottle gourd exhibits high tolerance to abiotic stresses, especially heat and drought stress. Hence, it can thrive when cultivated under low rainfall areas and poor soils with low moisture retention (Sithole and Modi, 2016; Mashilo et al., 2016a, 2017b). It is also tolerant to various biotic stresses such as wilting caused by *Fusarium oxysporum*, powdery mildew caused by *Erysiphe* species, cercospora leaf spot caused by *Cercospora beticola*, Zucchini yellow mosaic virus (Kousik et al., 2008; Kousik et al., 2018; Li et al., 2021), tobacco mosaic virus (Yetişir et al., 2007; Kousik et al., 2008; Wu et al., 2017), cucumber mosaic virus and watermelon mosaic virus (Ling and Levi, 2007; Ling and Levi, 2013; Zheng-gang et al., 2022). Hence, it is widely deployed as a rootstock in the production of grafted watermelon to improve tolerance to biotic and abiotic stress and fruit yield and quality (Yetişir and Sari, 2003; Yang et al., 2015; Bikdeloo et al., 2021; Morales et al., 2023).

Bottle gourd is among the important cultivated cucurbit crops in SSA. It is predominantly gown by smallholder farmers, who rely on mass selection and farm-saved seeds of unimproved and low-yielding landrace accessions. This procedure has allowed farmers to practice a long-term selection and this contributed to existence of the crop for centuries in Africa. It exhibits abundant phenotypic and genetic variation (Mashilo et al., 2015, 2017a; Nkosi et al., 2022). For example, high phenotypic variation is reported for agronomic traits including fruit shape (i.e., circular, oblate, pyriform, cavate, cylindrical fruit shapes), fruit colour (i.e., light green, no colour, dark green), fruit neck traits (i.e., long straight or curved-neck shapes), fruit circumference, plant height, fruit weight, fruit size, number of branches per plant, fruit yield and seed traits (e.g., number of seeds per fruit, seed length, seed width, seed size, hundred seed weight, seed yield, seed coat colour, seed texture) (Decker-Walters et al., 2001; Morimoto et al., 2005; Mashilo et al., 2015; Contreras-Soto et al., 2021 ; Ibrahim, 2021 ; Mahapatra et al., 2022; Kumar et al., 2024).

Despite the abundant genetic diversity of bottle gourd in SSA, the crop has not benefitted from modern breeding to develop and deploy improved varieties. The cultivated and unimproved accessions possess desirable product profiles, including biotic and abiotic stress tolerance. However, bottle gourd production and marketing are constrained by poor post-harvest shelf life of fruit, low fruit yield levels of the current varieties, fruit bitterness, poor market access and lack of value additions. Fruit post-harvest deterioration is caused by a rapid accumulation of lignin, which causes the fruit rind and fresh seeds to harden quickly (Konan et al., 2018; Ahmed et al., 2022). There is a need to improve the traditional landrace varieties through hybrid breeding to enhance economic traits through heterosis. Also, there is an increased demand for nutritionally and medicinally expectational food products derived from bottle gourd and related underutilized crops. Bottle gourd is a niche-opportunity crop with potential for high economic returns. Hence, modern variety breeding is vital to maximize its use and product development, including value-adding and as rootstock for watermelon, melons, cucumber, pumpkin and squash (Yetişir and Sari, 2003; Yang et al., 2015; Anand et al., 2021; Bikdeloo et al., 2021; Morales et al., 2023).

Candidate and elite genetic resources are evaluated across different environments to select and recommend adapted cultivars based on genotype-by-environment interaction (GEI) analysis. GEI is defined as the differential response of genotypes to varying environmental conditions and is quantified through several statistical methods, including the additive main effects and multiplicative interaction (AMMI) and genotype plus genotype-by-environment (GGE) biplot analyses (Gauch, 1992; Yan et al. 2001; Gauch et al., 2006; Yan et al., 2007; Gauch, 2013). AMMI is recommended as the most effective because of its ability to illustrate the complex interaction between genotypes and environments (Zobel et al., 1988). The AMMI model integrates the additive main effects of genotypes and environments, which are inferred using an analysis of variance (ANOVA). A principal component analysis is applied to examine the multiplicative interaction effects of genotype × environments (Zobel et al., 1988). AMMI model partitions the GEI into interpretable components while minimizing noise, making it a robust analytical method for complex GEI datasets (Zobel et al., 1988). According to Gauch (1988), AMMI is recommended for multi-environment trials as it outperforms traditional linear models by improving predictive accuracy and interpretability. Kang (1993) also emphasized its superiority in identifying genotypes with broad and specific adaptation. The graphical interpretability of genotype × environment interactions is achieved through AMMI biplots (e.g., genotype and environment scores on interaction principal component axes), which provide a robust schematic visualization of genotype-by-interaction interactions (Yan, 2001). The genotype and genotype-by-environment (GGE) biplot model is another valuable approach to visualizing and interpreting multi-environment data. GGE biplot is derived by plotting the principal components to aid visualization of genotypic performance in test environments for the selection of genotypes that are both high-yielding and stable (Yan and Tinker, 2006; Yan et al., 2007). GGE biplots are also useful for delineating mega-environments (Gauch and Zobel, 1997; Yan et al., 2006, Yan et al., 2007), genotype and environment assessment and genotype-trait relationships (Yan, 2001).

In cucurbit crops, GEI studies have been described in watermelon (Dia et al., 2016), cucumber (Barrett and Agrawal, 2004; Iwo and Odor, 2018; Ahmed et al., 2022), and ridge and spine gourds (Varalakshmi and Krishnamurthy, 2017; Tiwari and Rasogi, 2020). These studies identified genotypes with high yields and adaptation to different environments. In bottle gourd hybrid breeding, limited studies are reported on GEI to improve selection efficiency and cultivar recommendation. Pre-breeding and breeding programmes of bottle gourd have been initiated to explore its genetic diversity in South Africa, one of the centres of genetic diversity of the crop (Nkosi et al., 2022; Mkhize et al., 2023). Various genetic resources were evaluated with excellent phenotypic and agronomic traits (Decker-Walters et al., 2001; Morimoto et al., 2005; Mashilo et al., 2015; Contreras-Soto et al., 2021; Ibrahim, 2021; Mahapatra et al., 2022) and some experimental hybrids were developed (Mkhize et al., 2023). The newly bred hybrids were relatively high fruit and seed yielders and drought tolerant. The new hybrids are yet to be recommended for release and commercialization in South Africa following multi-environment trial testing. Therefore, the objective of this study was to determine the genotype-by-environment interactions (GEI) for fruit yield and related traits among newly developed bottle gourd hybrids to guide variety recommendation and registration.

5.2. Material and methods

5.2.1. Study sites

The study was conducted under field conditions across two locations of the Limpopo Province, South Africa. The first site is situated at the University of Limpopo (UL) Syferkuil research farm, Mankweng, Capricorn District Municipality, Limpopo Province (−25˚36’54’’S, 28˚0’59.76’’ E, 1312 m above sea level). The second site is at the Nkunzana village, Makhado Local Municipality, Vhembe District, Limpopo, South Africa (−23˚12’55.5’’S 30˚16’37.2’’ E). The two locations are known for their bottle gourd production and are approximately 201 Km apart. The description of the weather conditions during the experiments are presented in Table 5.1. The UL research farm is characterized by sandy and loamy soils, while the Nkunzana site has loam soils. The test genotypes were evaluated under varying watering conditions at both sites. At the UL site, the studies were conducted under three conditions: rainfed, rainfed with minimal supplemental irrigation every month at field capacity, and rainfed with optimal supplemental irrigation every week at field capacity making it three environments. Whereas two environments (rainfed and rainfed with optimal supplemental irrigation every week at field capacity were followed at the Nkunzana site). The two sites and respective moisture supply conditions resulted in five testing environments, namely: E1 (University of Limpopo research farm under rain-fed conditions), E2 (University of Limpopo research farm with rainfed and minimal supplemental irrigation conditions), E3 (University of Limpopo research farm with rainfed and optimal supplemental irrigation conditions), E4 (Nkunzana site under rain-fed conditions) and E5 (Nkunzana site under rain-fed and optimal supplemental irrigation conditions). Figure 5.1 presents the map of the study sites. Prevailing weather conditions at the test sites during the study period is presented in Table 5.1.

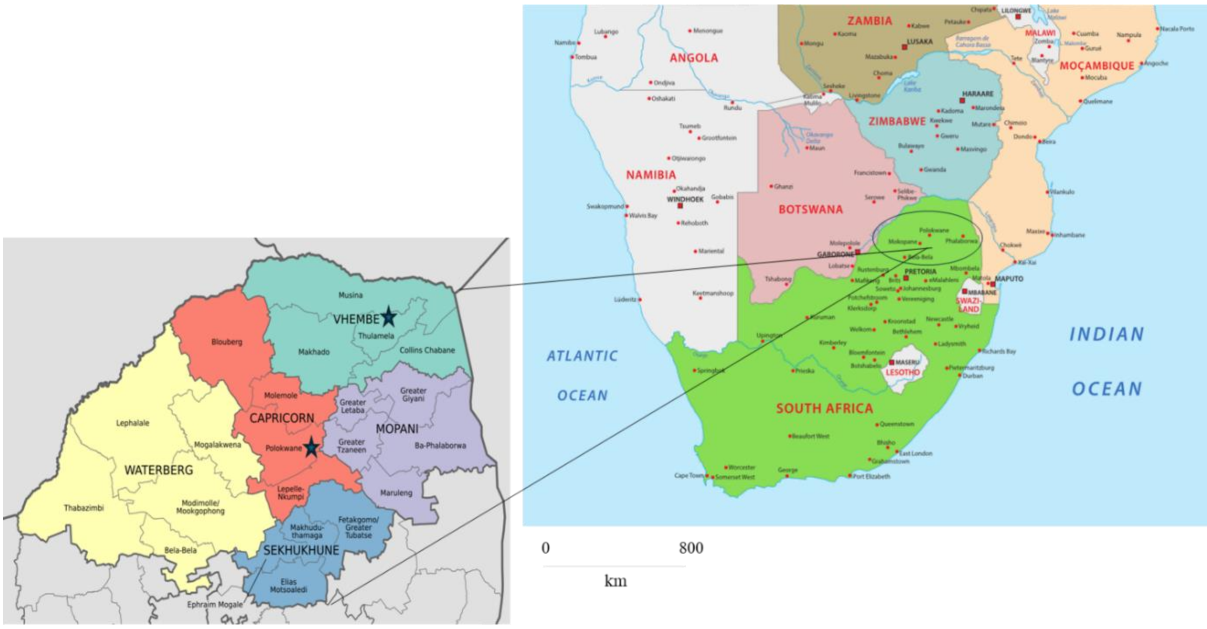

Figure 5.1. Map of the study sites in the Limpopo Province (left, marked by the solid stars) in the Republic of South Africa (right).

Table 5.1 Weather parameters in the sites during the study in 2024.

	University of Limpopo				Nkunzana			
Parameters	February	March	April	May	February	March	April	May
Tmax (˚C)	28	27	25	24	30	29	28	27
Tmin (˚C)	17	16	13	9	18	16	14	11
Precipitation (mm)	134.7	58	28	5.4	133.6	57	48	9
Humidity (%)	66	66	64	56	74	72	72	67
Wind (km/h)	18	17	16	15	8	8	7	7

Source: South African weather service (https://www.weathersa.co.za, accessed on 12/January/2025).

5.2.2. Plant material

The study used eight preliminarily bred experimental hybrids and four checks of bottle gourd. The hybrids were selected based on their high fruit-yielding capacity and drought tolerance (Mkhize et al., 2023). The test hybrids (Table 5.2) were developed from eight parental selections, namely BG-27, BG-52, BG-58, BG-70, BG-79, BG-80, BG-81 and GC. The parental genotypes used for hybrid breeding possessed varying phenotypic characteristics, including fruit shape and size, fruit colour, and high fruit and seed yields (Mashilo et al., 2015, 2016a). The parental accessions were also drought tolerant (Mashilo 2017a, 2018). The details of the test hybrids and checks with some selection criteria are presented in Table 5.2 (Mkhize 2023; 2024).

Table 5.2 Bottle gourd hybrids and parental accessions used in this study.

				FYPP		TOL
Genotypes and Pedigree	Codes	Status	Fruit shape	DS	NS	
BG-52 × BG-58	G1	Hybrid	Elongate pyriform	0.52	1.06	0.97
BG-52 × BG-79	G2	Hybrid	Elongate pyriform	0.49	0.86	0.19
BG-80 × GC	G3	Hybrid	Elongate pyriform	0.49	0.99	0.08
BG-58 × BG-80	G4	Hybrid	Cavate	0.59	1.22	0.64
BG-27 × BG-52	G5	Hybrid	Round	0.21	9.63	0.68
BG-27 × GC	G6	Hybrid	Elongate pyriform	0.14	1.64	0.39
BG-81 × GC	G7	Hybrid	Elongate pyriform	0.42	1.60	0.88
BG-80 × BG-81	G8	Hybrid	Cyndrical	0.25	1.17	0.37
BG-70	G9	Check	Pyriform	0.21	1.59	0.48
BG-81	G10	Check	Elongated	0.18	0.69	0.59
BG-80	G11	Check	Pyriform	0.19	0.91	0.29
GC	G12	Check	Elongated	0.49	2.48	0.32

FYPP, dried fruit yield per plant (kg) under drought-stressed (DS) and non-stressed (NS) conditions; TOL, drought tolerance index.

5.2.3. Experimental design and trial management

The experiments were conducted from February to May 2024. At the two locations, the F_1 hybrids and checks were established using a randomized complete block design (RCBD) with three replications. Five plants of each genotype were established in a 25 m^2 plot. Plants were established at an inter and intra spacing of approximately 5 metres. Initially two seeds were planted per hole and thinned to keep one healthy and vigorous plant two weeks after emergence. Manual weeding was done throughout the experiment at all sites. Both sites received 27 mm per week of irrigation water to promote germination and early crop establishment. At the University of Limpopo site a sprinkler irrigation system was used, whereas at the Nkunzana site a manual irrigation was done, supplying approximately 25 litres per square metre when required. Bottle gourd grows well in temperate to tropical conditions, with annual rainfall between 400-1500 mm (Haque et al., 2009). Tan et al. (2009) reported the water requirements for bottle gourd to range from 400 to 600 mm including rainfed and irrigation conditions. For experimental units marked as rainfed, plants only grew under rainfed conditions which was approximately 226 mm, which is low for bottle gourd (E1). For the experimental units under E2 and E4, the plants were provided with minimal supplemental irrigation of approximately 81 mm in both environments upon germination. The sets in E2 and E4 received a total water supply (both rainfed and supplemental irrigation) of approximately 307 and 328 mm throughout the crop cycle, respectively. The experimental units under E3 and E5 received optimal supplemental irrigation of 324 mm, and this added up with the rain water to 550 and 571 mm of the total water for E3 and E5 throughout the planting season, respectively. Applied water in E3 and E5 was within the acceptable range of the crop water requirements. Growing conditions in E1 in the University of Limpopo syferkuil research sites is regarded as drought stress, whereas E2 (University of Limpopo syferkuil research site) and E4 (Nkunzana site) are mild drought stress. Lastly, the plants in E3 and E5 at both sites were grown in non-stressed conditions.

5.2.4. Data collection

Data was collected on the following agronomic traits: days to 50% male flowering (DTMF) and female flowering (DTFF), total number of fruits per hectare (TNFH) and fruit yield per plot. 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. Number of fruits per plot was recorded when all the plants per plot had produced fruits and when 90 %

of those fruits were at the edible stage and converted to hectares. Fruit yield per plot was converted to fruit yield per hectare (FYPH, tons/ha).

5.2.5. Data analysis

5.2.5.1. Analysis of variance

Analysis of variance was performed in GenStat version 18 (Payne et al., 2017). The Least Significant Difference (LSD) test was computed to compare treatment means at the 5% level of significance. The following linear model was adopted based on the RCBD design:

$$Y_{ijkl} = \mu + G_i + E_j + R_k(E_j) + GE_{ij} + \varepsilon_{ijk}$$

Where,

$Yijkl$ = the observed value of genotype i in replication k of environment j.

μ = the grand mean,

G_i = the effect of genotype i,

E_j = the environment effect j,

$R_k(E_j)$ = the effect of the replication k in environment j,

GE_{ij} = the interaction effect of genotype i with environment j

ε_{ijkl} = the error (residual) effect of genotype i in replication k of environment j.

5.2.6. AMMI analysis

The Additive Main Effect and Multiplicative Interaction (AMMI) analysis was carried out to deduce the pattern of GEI using Genstat 23rd edition (Payne et al., 2017). The applied AMMI model, according to Zobel et al. (1988) is as follows:

$$\bar{\Upsilon}_{ijk} = \mu + G_i + E_j + \sum_{k=1}^{m} \lambda_k \sigma_{ik} \Upsilon_{jk} + \rho ij$$

Where,

$\bar{\Upsilon}_{ijk}$ = the yield of the *ith* genotype in the *jth* environment,

μ = the grand mean,

G_i = the mean of the *ith* genotype minus the grand mean,

E_j = the mean of the *jth* environment minus the grand mean,

λ_k = the square root of the eigenvalue of the *kth* IPCA axis,

σ_{ik} and γ_{jk} = the principal component scores for IPCA axis k of the *ith* genotypes and the *jth* environment,

ρ_{ij} = the deviation from the model

5.2.7. AMMI stability value analysis

AMMI stability value (ASV) was calculated to quantify and rank genotypes in terms of yield stability using the formula suggested by Purchase et al. (2000) as follows:

$$\text{AMMI Stability Value (ASV)} = \sqrt{[\frac{SSIPCA1}{SSIPCA2}]^2 + [IPCAscore]^2}$$

Where,

SS = Sum of squares,

IPCE1 = Interaction Principal Component Analysis Axis 1,

IPCE2 = Interaction Principal Component Analysis Axis 2,

The larger the IPCA score, either in a negative or positive direction, the more specifically adapted a genotype is to specific environments. Smaller ASV scores indicate a more stable genotype across environments, whereas larger ASV values indicate unstable performance (Purchase, 2000).

5.2.8. Genotype plus genotype-by-environment biplot analysis

The GGE biplot analysis based on the singular value decomposition (SVD) of the first principal component (PC1) and second principal component (PC2) was conducted in R version 4.3.2 (R Core Team, 2020) using the metan package (Yan and Tinker, 2006). The following model was adopted according to Yan et al. (2001):

$$\bar{\Upsilon}_{ij} - \mu_i - \beta_j = \sum_{k=1}^{t} \lambda_k \sigma_{ik} \Upsilon_{jk} + \varepsilon_{ij}$$

Where,

$\bar{\Upsilon}_{ij}$ = mean performance of genotype *i* in environment *j*,

μ_i = grand mean,

β_j = main effect of environment *j*,

λ_k = singular value of the k^{th} principal component (PC)

α_{ik} and γ_{jk} = scores of the i^{th} genotype and j^{th} environment, respectively, for the k^{th} PC. The term $\lambda_k\ \alpha_{ik}\ \gamma_{jk}$ is derived from the k^{th} PC of the singular value decomposition (SVD) of the matrix. The α_{ik} and γ_{jk} for k = 1, 2, 3… are referred to as "primary," "secondary," "tertiary,". effects of genotypes and environments, respectively

ε_{ij} = residual associated with genotype *i* and environment *j*.

5.3. Results

5.3.1. Effects of genotypes, environment and their interactions on fruit yield and its component traits

Analysis of variance showing effects due to genotype, environment, and their interaction effects for DTMF, DTFF, TNFH and FYPH are presented in Table 5.3. The effects of genotype, and environment were highly significant ($p < 0.001$) for all traits. Also, significant genotype × environment interaction effects were recorded for all traits.

Table 5.3 Analysis of variance showing mean squares for agronomic traits for eight hybrids and four check bottle gourd genotypes evaluated across five environments in Limpopo Province, South Africa.

		Traits			
Source of variation	Df	DTMF	DFTT	TNFH	FYPH (t/ha)
Genotype (Gen)	11	725.20***	775.90**	54160404.00**	994.00**
Environment (Env)	4	1989.60**	2467.30**	72300889.00**	16086.00**
Replications (Rep)	8	3.20^{ns}	6.70^{ns}	5041556.00^{**}	135.00^{ns}
Gen × Env	44	36.20**	40.20**	6028889.00**	2287.00**
Gen × Rep	22	4.50*	5.70^{ns}	1956525.00^{ns}	1653.00^{ns}
Residual	96	2.70	3.90	1832222.00	108.00

df, degrees of freedom; * and ** denote significant differences at 5 and 1% probability levels, respectively; ns, non-significant; DTMF, days to 50% male flowering; DTFF, days to 50% female flowering; TNFH, total number of fruits per hectare; FYPH = fruit yield per hectare (tons/ha).

5.3.2. Mean response for fruit yield and yield components

The mean values for the studied agronomic traits among bottle gourd genotypes tested in five environments is presented in Table 5.4. DTMF varied from 36 to 52, 40 to 57, 49 to 74, 37 to

66 and 47 to 71 days in E1, E2, E3, E4 and E5, respectively. Hybrid BG-27 × BG-52 recorded DTFM of 52, 56, 74, 66 and 71 in E1, E2, E3, E4 and E5, in that order. Hybrid BG-27 × GC recorded DTFM of 52, 57, 64, 63 and 63 in E1, E2, E3, E4 and E5. Relatively low DTMF of 40, 49, 37 and 47 days were recorded for check variety GC in E2, E3, E4 and E5, respectively. The mean DTFF was 44, 50, 63, 49 and 62 days in environments E1, E2, E3, E4 and E5, in that order. For DTFF, GC recorded low values of 40, 45, 54, 42 and 52 days in E1, E2, E3, E4 and E5. The hybrid BG-58 × BG-80 recorded DTFF of 41, 46, 56, 44 and 55 days in E1, E2, E3, E4 and E5. Under E1, the TNFH for BG-52 × BG-79 was 6266, but 6268 for BG-27 × BG-52 and 5466 for BG-27 × GC. In E2, the TNFH for GC was 9866, but 9200 for BG-52 × BG-58 and 9200 for BG-52 × BG-79. The checks BG-70, GC and hybrid BG-52 × BG-58 recorded high TNFH at 11866, 11896 and 11200 in E3, whereas values of 10266, 10000 and 9866 fruits were recorded for GC, BG-52 × BG-58 and BG-52 × BG-79 in E4, respectively. Hybrid BG-52 × BG-58 and BG-52 × BG-79 and GC had a high TNFH of 12266, 11733 and 12276 in E5. In E1, FYPH varied from 8 to 11, with a mean of 9 tons/ha, whereas in E2 it varied from 9 to 21 tons/ha with a mean of 14 tons/ha. In E3, FYPH varied from 14 to 52 tons/ha with a mean of 28 tons/ha, 6 to 24 with a mean of 13 tons/ha in E4. In E5, FYPH varied from 22 to 57, with a mean of 37 tons/ha. In E1, high FYPH was recorded for hybrid BG-80 × GC (12 tons/ha), BG-27 × BG-52 (11 tons/ha) and BG-80 (10 tons/ha). Under E2, check variety GC recorded FYPH of 21 tons/ha, followed by BG-58 × BG-80 (20 tons/ha). In E3, hybrids GC BG-52 × BG-58 and BG-58 × BG-80, and check variety BG-80 recorded FYPH of 52, 39, 37 and 33 tons/ha, in that order. Hybrid BG-58 × BG-80 was the best performer (24 tons/ha), followed by BG-52 × BG-58 (20 tons/ha) and check variety GC (22 tons/ha) for FYPH in E4. In E5, the high-yielding genotypes were check variety GC (57 tons/ha), and hybrids BG-52 × BG-58 (49 tons/ha), BG-52 × BG-79 (41 tons/ha) and BG-58 × BG-80 (50 tons/ha). The hybrids BG-27 × BG-52 and BG-27 × GC recorded longer DTMF of 64 and 60 days across all environments. A lower DTMF of 42 and 43 days were recorded for the check variety GC and hybrid BG-58 × BG-80 across all environments. The mean DTFF across all environments was 41. The hybrids BG-27 × BG-52 and BG-27 × GC recorded longer DTFF of 70 and 64, respectively. A relatively lower DTFF of 49 was recorded for hybrid BG-80 × BG-81. The mean TNFH across all environments was 8055. The hybrids BG-52 × BG-58 and BG-52 × BG-79 were the best performers for TNFH, whereas BG-80 × BG-81 was the worst performer. The high-yielding hybrids across all environments were BG-52 × BG-58 (27 tons/ha) and BG-58 × BG-80 (28 tons/ha), whereas BG-27 × BG-52 (14 tons/ha) was the lowest-performing hybrid.

Table 5.4 Mean values for agronomic traits among eight F_1 hybrids and four check bottle gourd genotypes evaluated under five environments in the Limpopo Province, South Africa.

Genotypes	Environments											
	E1				E2				E3			
	Traits											
	DTMF	DTFF	TNFH	FYPH	DTMF	DTFF	TNFH	FYPH	DTMF	DTFF	TNFH	FYPH
BG-52 × BG-58	38.76	42.92	4800.67	8.61	42.61	46.48	9200.48	15.51	51.62	57.65	11200.20	39.00
BG-52 × BG-79	42.39	44.02	6266.74	9.41	46.42	52.64	9200.74	11.84	62.72	68.00	10400.90	24.28
BG-80 × GC	40.08	43.88	4933.06	11.64	45.84	51.06	8133.34	10.20	53.31	57.09	8933.31	24.31
BG-58 × BG-80	36.27	41.79	4933.46	8.25	41.11	46.84	9066.28	20.97	50.00	56.04	10000.70	37.09
BG-27 × BG-52	52.28	57.67	6266.48	11.49	56.34	64.87	6800.4	11.72	74.03	79.05	5466.08	16.68
BG-27 × GC	52.07	57.08	5466.06	8.79	57.33	59.36	6400.74	9.25	64.01	70.13	6133.21	24.18
BG-81 × GC	38.73	41.00	5600.20	9.59	40.84	45.17	5600.91	12.02	64.00	73.27	5200.47	14.18
BG-80 × BG-81	37.41	41.38	4400.29	9.40	41.08	47.34	6933.35	13.63	51.17	57.23	3733.34	19.86
BG-70	38.87	40.89	4800.03	9.22	41.47	47.08	8933.05	18.62	49.16	54.41	11866.30	30.39
BG-81	38.94	42.23	4266.01	8.80	40.04	46.45	7466.00	12.68	59.46	65.26	5200.00	25.00
BG-80	44.21	47.81	4933.48	10.50	50.00	55.09	5733.34	10.97	64.37	70.21	6666.08	33.89
GC	36.85	40.94	4000.25	8.21	40.39	45.74	9866.49	21.47	49.12	54.08	11866.30	52.28
Mean	41.33	44.81	5055.56	9.49	45.28	50.53	7777.78	14.07	57.86	63.61	8055.06	28.43
LSD (5%)	31.88	33.62	230.00	35.10	23.67	47.79	211.79	58.70	32.04	25.00	170.00	12.41
CV (%)	46.28	45.04	307.02	34.45	27.03	54.02	204.33	30.76	32.91	22.92	103.10	22.41
P-value	**	**	**	**	**	**	**	**	**	**	**	**

	E4				E5				Overall			
	Traits											
Genotypes	DTMF	DTFF	TNFH	FYPH	DTMF	DTFF	TNFH	FYPH	DTMF	DTFF	TNFH	FYPH
BG-52 × BG-58	40.67	45.34	10000.00	20.40	50.00	55.62	12266.40	49.26	44.73	49.60	9493.55	26.56
BG-52 × BG-79	45.84	49.07	9866.48	14.46	62.21	67.00	11733.10	49.76	51.92	56.15	9493.59	21.95
BG-80 × GC	43.06	47.02	7333.32	7.49	51.24	57.31	8133.00	31.94	46.71	51.27	7493.21	17.12
BG-58 × BG-80	39.84	44.11	9600.29	24.37	49.19	55.03	10933.50	50.99	43.28	48.76	8906.84	28.34
BG-27 × BG-52	66.03	72.18	4266.00	6.25	71.14	76.00	5866.41	23.77	63.96	69.95	5733.07	13.98
BG-27 × GC	63.26	66.09	5600.46	10.50	63.09	69.15	7600.00	28.99	59.95	64.36	6240.09	16.34
BG-81 × GC	39.74	43.03	4800.08	8.07	65.07	71.68	6533.39	34.04	49.68	54.83	5547.01	15.58
BG-80 × BG-81	40.49	43.00	3200.34	10.36	49.05	54.46	5600.05	22.38	43.84	48.68	4773.47	15.13
BG-70	41.18	44.46	8533.39	18.15	48.00	54.10	11066.70	40.35	43.74	48.19	9039.88	23.35
BG-81	39.96	44.43	3600.05	7.57	57.10	62.19	5200.55	26.93	47.10	52.11	5146.52	16.19
BG-80	49.00	54.09	6000.16	11.31	63.64	69.00	7600.04	37.55	54.24	59.24	6186.62	20.84
GC	37.07	42.04	10266.40	22.76	47.00	46.00	12266.20	57.72	42.09	45.76	9653.12	32.49
Mean	45.42	49.89	6922.22	13.47	56.03	62.28	8733.33	37.81	49.27	41.83	7308.92	20.66
LSD (5%)	33.20	33.42	151.36	65.24	23.85	27.26	166.83	171.51	43.56	49.44	180.67	73.90
CV (CV%)	43.36	39.73	114.98	30.74	24.94	23.87	101.37	352.21	69.36	71.73	193.93	287.08
P-value	**	**	**	**	**	**	**	**	**	**	**	**

DTMF, days to 50% male flowering; DTFF, days to 50% female flowering; TNFH, total number of fruits per hectare; FYPH, fruit yield per hector; CV = coefficient of variation; LSD = least significant difference; * and ** denotes significant at the 5 and 1% probability level. E1 (University of Limpopo research farm under rain-fed conditions), E2 (University of Limpopo research farm with rainfed and minimal supplemental irrigation conditions), E3 (University of Limpopo research farm with rainfed and optimal supplemental irrigation conditions), E4 (Nkunzana village under rain-fed conditions) and E5 (Nkunzana village under rain-fed and optimal supplemental irrigation conditions).

5.3.3. The additive main effects and multiplicative interaction (AMMI) analysis

The AMMI analysis of variance revealed the assessed traits were significantly ($p \leq 0.001$) influenced by genotype, environment, and GEI effects (Table 5.5). For DTMF, the genotypes, environments, and genotype-by-environment interactions effects explained 46.01, 44.24 and 8.86% of the total explained variation, in that order. For DTFF, the total explained variation of 42.08, 48.58 and 8.71% was accounted for by genotypic, environmental and genotype-by-environment interaction effects. A high contribution to the total variation explained for TNFH was observed for genotype effects (49.98%), followed by environmental effects (24.21%) and genotype-by-environment interaction effects (22.21%). Genotype, environment, and genotype-by-environment interaction effects of 12.36, 73.16 and 11.41% were recorded for FYPH. Two significantly interactive principal component axes were inferred for DTMF and DTFF by the AMMI model. For DTMF, IPCA1 and IPCA2 accounted for 78.36 and 20.08% of the GEI variation, whereas for DTFF, IPCA1 and IPCA2 accounted for 77.42 and 18.47% of total variation. Cumulatively, a total explained variation of 98.44 and 95.89% was recorded for DTMF and DTFF, respectively. Only IPCA1 was significant for TNFH and TFYP, which accounted for 84.97 and 77.87% of the variation, respectively. AMMI analysis suggested AMMI-2 were the best-suited model for DTMF and DTFF, whereas AMMI-1 was the best-suited model for TNFH and TFYP.

Table 5.5 Additive main effects and multiplicative interaction analysis of variance for agronomic traits in bottle gourd genotypes evaluated across five test environments in the Limpopo Province, South Africa.

				Traits					
			DTMF				DTFF		
Source of variation	Df	s.s.	m.s.	Total variation explained (%)	Explained G x E (%)	s.s.	m.s.	Total variation explained (%)	Explained G x E (%)
Total	179	18317.00	102.30			20757.00	116.00		
Treatments	59	17826.00	302.10**			20173.00	341.90**		
Genotypes (G)	11	8274.00	752.20**	46.01		8535.00	775.90**	42.02	
Environments (Env)	4	7958.00	1989.60**	44.24		9869.00	2467.30**	48.58	
Reps (Env)	10	160.00	16.00**	0.89		140.00	14.00**	0.69	
G x E	44	1593.00	36.20**	8.86		1769.00	40.20**	8.71	
IPCA 1	14	1249.00	89.20**		78.36	1370.00	97.80**		77.42
IPCA 2	12	320.00	26.70**		20.08	327.00	27.20**		18.47
Residuals	18	25.00	1.40^{ns}		1.57	73.00	4.00^{ns}		4.12
Error	110	331.00	3.00			444.00	4.00		

				Traits					
			TNFH				FYPH (t/ha)		
Source of variation	Df	s.s.	m.s.	Total variation explained (%)	Explained G x E (%)	s.s.	m.s.	Total variation explained (%)	Explained G x E (%)
Total	179	1373065778.00	7670759.00			100809.00	563.00		
Treatments	59	1150239111.00	19495578.00**			85069.00	1442.00**		
G	11	595764444.00	54160404.00**	49.88		10845.00	986.00**	12.36	
Env	4	289203556.00	72300889.00**	24.21		64211.00	16053.00**	73.36	
Reps (Env)	10	44222222.00	4422222.00**	3.70		2697.00	270.22**	3.07	
G x E	44	265271111.00	6028889.00**	22.21		10013.00	228.00**	11.41	
IPCA 1	14	225413783.00	16100985.00**		84.97	7798.00	557.00**		77.87
IPCA 2	12	25349323.00	2112444.00^{ns}		9.56	1871.00	156.00^{ns}		18.75
Residuals	18	14508005.00	806000.00^{ns}		5.47	344.00	19.00^{ns}		3.38
Error	110	178604444.00	1623677.00			13043.00	119.00		

* and ** denotes significant at the 5 and 1% probability levels; df, degrees of freedom; s.s., sum of squares; m.s., mean squares; GEI, genotype-by-environment interaction; DTMF, days to 50% male flowering; DTFF, days to 50% female flowering; TNFH, total number of fruits per hectare; FYPH = Fruit yield per hectare.

5.3.4. AMMI biplots

AMMI biplots showing the relationship between genotype and test environments for the studied agronomic traits is presented in Figure 5.2. Genotypes that cluster together behave similarly, whereas environments that cluster together influence the genotypes similarly. The acute angle between the environment vectors indicates a positive correlation, and the right angle and the obtuse angle between the vectors indicate no correlation. Genotypes that are close to the origin are insensitive to environmental interactions and are broadly adapted. In contrast genotypes situated far from the origin, are sensitive to environmental effects. AMMI-2 biplots (PC1 vs PC2) for DTMF and DTFF are presented in Figures 5.2 A & B. For DTMF, genotypes BG-52 × BG-79 (G2), BG-81 (G10) and BG-80 (G11) were close to the origin indicating that they were stable. Conversely, hybrids BG-27 × BG-52 (G5) and BG-27 × GC (G6) were unstable because they were far from the biplot origin.. For DTFF, BG-80 (G11) was close to the origin, indicating that it was stable, whereas the genotypes (BG-27 × BG-52 (G5), BG-27 × GC (G6) and BG-81 × GC (G7) were located furthest from the biplot origin, indicating that they were highly sensitive to environmental effects. AMMI 1 biplot, which plots PC1 against the mean performance of genotypes and environments for TNFH and FYPH are shown in Figures 5.2 C & D. Genotypes or environments with high PC1 scores in any direction indicate high interaction and instability, whereas the opposite (i.e., low IPCA1 scores near the biplot origin) suggest low interaction and high stability. For TNFH, BG-80 × GC (G3) was closest to the biplot origin, indicating that it was stable . Hybrids BG-52 × BG-58 (G1), BG-58 × BG-80 (G4) were unstable because they were far from the biplot origin and showed specific adaption to E5 (Figure 5.2 C). For FYPH, checks BG-70 (G9), BG-80 (G11) and GC (G12) were close to the origin of the AMMI biplot, indicating that they were stable. Further, genotypes BG-81 × GC (G7), BG-80 × GC (G3), BG-27 × GC (G6) and BG-81 (G10) with moderate stability were specifically adapted to E4. Hybrids BG-27 × BG-52 (G5) and BG-80 × BG-81 (G8) were specifically adapted to E2, whereas BG-52 × BG-58 (G1) and BG-58 × BG-80 (G4) to E3. Check GC (G12) located furthest from the AMMI biplot origin indicating that it was highly unstable (Figure 5.2 D).

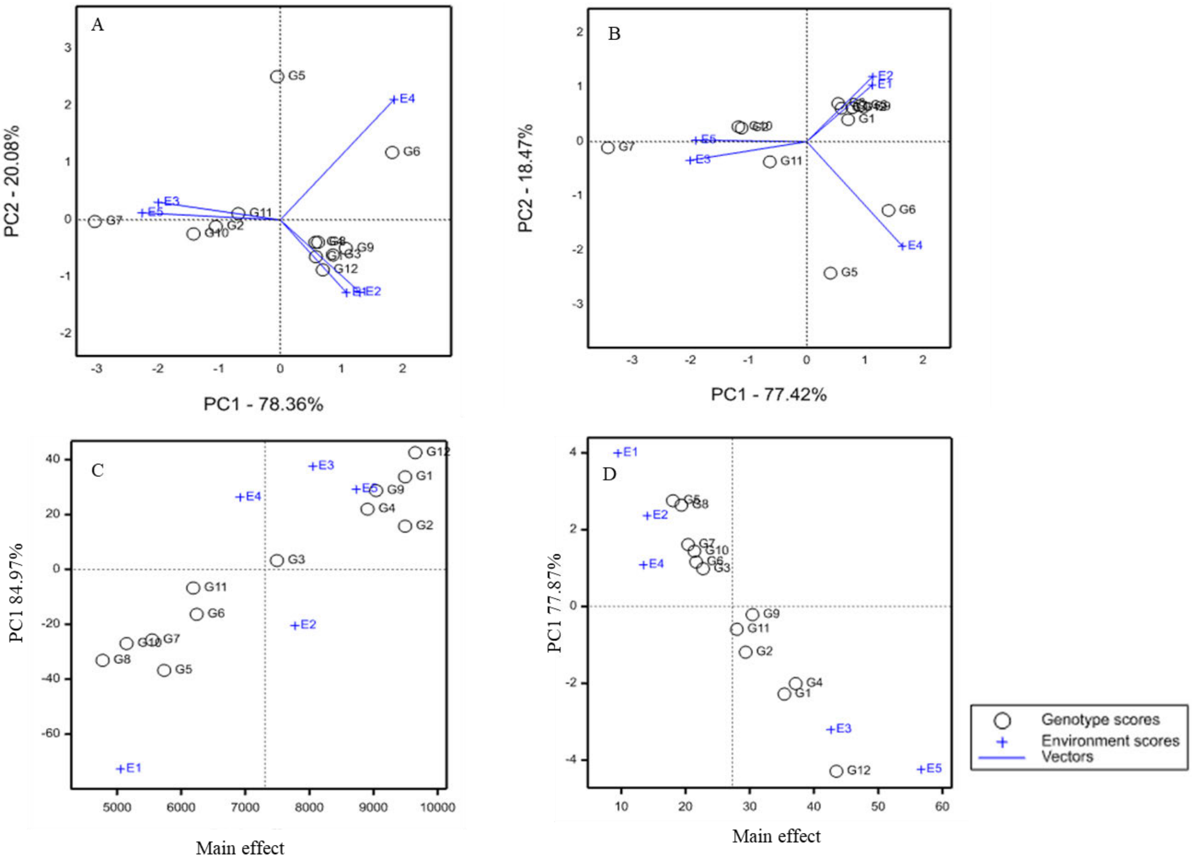

Figure 5.2. AMMI biplot showing genotypes and test environments for twelve bottle gourd genotypes assessed across five testing environments for days to 50% male flowering (A); days to 50% female flowering (B); total number of fruits per plot (C); fruit yield per hectare (ton/ha) (D). E1 (University of Limpopo site under rain-fed conditions), E2 (University of Limpopo site with rainfed and minimal supplemental irrigation), E3 (University of Limpopo site with rainfed and optimal supplemental irrigation), E4 (Nkunzana site under rain-fed conditions) and E5 (Nkunzana site under rain-fed and optimal supplemental irrigation conditions). The codes of genotypes are presented in Table 5.2.

5.3.5. Genotype ranking and AMMI stability value

The ASV for test genotypes evaluated for agronomic traits across the five environments is presented in Table 5.6. A higher mean yield and lower ASV demonstrate stable genotypic performance across the testing environments. Conversely, higher ASV indicates specific adaptation but high yield (Purchase et al., 2000). For DTMF, low ASV of < 3 was recorded for hybrids BG-58 × BG-80 (G4), BG-52 × BG-58 (G1), BG-27 × BG-52 (G5), BG-80 × BG-81 (G8), BG-80 (G11) and check variety GC (G12), indicating stable genotypic performance across the testing environments, whereas, high ASV of > 7 was recorded for hybrids BG-27 × GC (G6) and BG-81 × GC (G7) indicating specific adaptation and were high yielding. For

DTFF, low ASVs of ≤ 3 were recorded for BG-58 × BG-80 (G4), BG-80 × BG-81 (G8), BG-27 × BG-52 (G5) and check variety BG-80 indicating stable genotypic performance across the five testing environments, whereas high ASVs of > 6 were recorded for hybrids BG-27 × GC (G6) and BG-81 × GC (G7) indicating that these were high yielders. For TNFH, high ASV of > 200 was recorded for most genotypes including checks BG-81and GC, and hybrids BG-70, BG-52 × BG-58, BG-27 × BG-52, BG-81 × GC, BG-80 × BG-81, indicating specific adaptation and were high yielding. For FYPH, BG-70 (G9), BG-80 (G11) and BG-27 × GC (G6) recorded a low ASV of 0.6, 1.9 and 3.0, respectively, indicating stable genotypic performance across the testing environments. Low ASV values of 3.28 and 3.49 were recorded for BG-27 × GC and BG-80 × GC (G3) for FYPH, indicating stable genotypic performance across the testing environments. High ASV values of > 6 were recorded for BG-52 × BG-58, BG-58 × BG-80, BG-27 × BG-52, BG-80 × BG-81 and GC for FYPH, indicating specific adaptation and were high yielding.

Table 5.6 Additive main effects and multiplicative interaction rank and stability values for 12 bottle gourd genotypes assessed across five environments for agronomic traits.

Genotype	Rank	ASV	DTMF	PC1	PC2	Genotype	Rank	ASV	DTFF	PC1	PC2
G4	1.00	2.33	43.27	0.59	0.40	G8	1	2.43	48.67	0.55	0.72
G1	2.00	2.42	44.53	0.60	0.66	G4	2	2.63	48.60	0.61	0.63
G8	3.00	2.49	44.20	0.63	0.40	G11	3.00	2.75	59.40	-0.65	-0.38
G5	4.00	2.57	64.13	-0.05	-2.55	G5	4.00	3.03	70.00	0.42	-2.47
G11	5.00	2.73	54.47	-0.70	-0.11	G1	5.00	3.08	49.53	0.73	0.41
G12	6.00	2.91	42.20	0.71	0.89	G12	6.00	3.42	46.93	0.80	0.64
G3	7.00	3.49	46.53	0.88	0.63	G3	7.00	4.00	51.27	0.94	0.68
G2	8.00	4.20	51.80	-1.07	0.12	G9	8.00	4.23	48.40	1.00	0.65
G9	9.00	4.31	43.73	1.09	0.51	G2	9.00	4.80	56.27	-1.14	0.26
G10	10.00	5.66	46.93	-1.44	0.25	G10	10.00	5.04	52.33	-1.20	0.28
G6	11.00	7.40	60.20	1.87	-1.20	G6	11.00	6.17	64.47	1.43	-1.29
G7	12.00	12.08	49.53	-3.09	0.03	G7	12.00	14.65	54.80	-3.49	-0.11

Genotype	Rank	ASV	TNFH	PC1	PC2	Genotype	Rank	ASV	FYPH (ton/ha)	PC1	PC2
G3	1.00	30.30	7493.00	3.21	-9.94	G9	1.00	0.67	23.34	-0.17	-0.09
G11	2.00	64.50	6187.00	-6.79	22.52	G11	2.00	1.93	20.84	-0.41	1.07
G2	3.00	140.40	9493.00	15.71	13.17	G6	3.00	3.28	16.34	0.83	0.61
G6	4.00	146.40	6240.00	-16.37	15.67	G3	4.00	3.49	17.11	0.89	0.19
G4	5.00	195.40	8907.00	21.97	1.47	G10	5.00	4.61	16.20	1.14	1.16
G7	6.00	229.30	5547.00	-25.68	21.00	G2	6.00	4.73	21.95	-1.00	-2.65
G10	7.00	242.40	5147.00	-27.04	-30.26	G7	7.00	5.33	15.58	1.26	-1.99
G9	8.00	255.50	9040.00	28.71	-9.41	G4	8.00	6.50	28.33	-1.66	-0.56
G8	9.00	295.10	4773.00	-33.13	-16.63	G1	9.00	7.32	26.56	-1.87	0.10
G1	10.00	299.70	9493.00	33.69	4.22	G8	10.00	8.15	15.13	2.07	0.84
G5	11.00	327.40	5733.00	-36.81	-0.04	G5	11.00	8.95	13.98	2.29	0.06
G12	12.00	378.40	9653.00	42.53	-11.78	G12	12.00	13.23	32.49	-3.37	1.28

ASV, additive main effects and multiplicative interaction stability value; DTMF, days to 50% male flowering; DTFF, days to 50% female flowering; TNFH, total number of fruits per hectare; FYPH = Fruit yield per hectare; PC1, the first principal component axis; PC2, the second principal component axis.

5.3.6. Winning genotypes for assessed traits

AMMI selection of the best-performing genotypes in different environments for the evaluated traits is presented in Table 5.7. The AMMI model 2 was identified as the most predictive and accurate for DFMF and DTFF. Hence, the AMMI 2 selection was employed where BG-27 × BG-52 (G5) was a winner hybrid for DFMF in E1 and E2. Whereas BG-81 × GC (G7) won in E3 and E5, and BG-27 × GC (G6) won in E4. For DTFF, BG-27 × GC (G6) was winner in E1, E2 and E4, whereas BG-81 × GC (G7) won in E3 and E5. AMMI model 1 was identified as the most predictive for TNFPH and FYPH and hence used for selecting winner genotypes. BG-52 × BG-79 (G2) was a winner genotype in E1, whereas GC (G12) won in E2, E3, E4 and E5 for TNFPH. For FYPH, BG-70 (G9) won in E1, whereas GC (G12) won in E2, E3, E4 and E5.

Table 5.7 Additive main effects and multiplicative interaction selections showing the wining genotypes per environment among 12 bottle gourd genotypes evaluated across five test environments in the Limpopo Province, South Africa.

		DTMF		Genotypes					DTFF		Genotypes		
Environment	Mean	Score	1	2	3	4	Environment	Mean	Score	1	2	3	4
E1	41.33	1.25	G6	G5	G11	G2	E1	44.81	1.450	G5	G6	G11	G2
E2	45.28	1.50	G6	G5	G11	G2	E2	50.53	1.461	G5	G6	G11	G2
E3	57.86	-2.29	G5	G7	G6	G11	E3	63.61	-2.576	G5	G7	G6	G11
E4	45.42	2.14	G5	G6	G11	G2	E4	49.89	2.117	G5	G6	G11	G2
E5	56.58	-2.59	G5	G7	G11	G6	E5	62.28	-2.452	G5	G7	G11	G6
	TNFPH			Genotypes				FYPH			Genotypes		
Environment	Mean	Score	1	2	3	4	Environment	Mean	Score	1	2	3	4
E1	5056.00	-72.71	G2	G5	G7	G6	E1	9.49	3.99	G9	G8	G4	G5
E2	7778.00	-20.47	G12	G9	G1	G2	E2	14.07	2.37	G12	G4	G1	G9
E3	8056.00	37.60	G12	G1	G9	G2	E3	42.64	-3.203	G12	G1	G4	G11
E4	6922.00	26.31	G12	G1	G2	G9	E4	13.47	1.08	G12	G4	G1	G9
E5	8733.00	29.26	G12	G1	G2	G9	E5	56.71	-4.24	G12	G4	G2	G1

DTMF, days to 50% male flowering; DTFF, days to 50% female flowering; TNFH, total number of fruits per hectare; FYPH, fruit yield per hector; E1 (University of Limpopo site under rain-fed conditions), E2 (University of Limpopo site with rainfed and minimal supplemental irrigation), E3 (University of Limpopo site with rainfed and optimal supplemental irrigation), E4 (Nkunzana site under rain-fed conditions) and E5 (Nkunzana site under rain-fed and optimal supplemental irrigation conditions). The codes of genotypes are presented in Table 5.2.

5.3.7. Basic, mean vs. stability, ranking genotypes and “which-won-where” genotype plus genotype-by-environment (GGE) biplots

The GGE biplots for the evaluated traits among the eight hybrids and four check varieties in five environments are shown in Figures 5.3, 5.4, 5.5 and 5.6. Figure 5.3 presented the basic GGE biplots for assessed traits. The GGE model explained 99.37% of the total variation (PC1 = 86.77%; PC2 =12.60%) for DTMF (Figure 5.3 A) and 99.09% (PC1 = 85.87%; PC = 13.22%) for DTFF (Figure 5.3 B), 97.00% (93.91 and 3.09%) for TNFPH (Figure 5.3 C) and 97.56% (88.52 and 9.04%) for FYPH (Figure 5.3D), respectively. Overall, the variation explained by the GGE model exceeded 80% for all traits, indicating an excellent data fit.

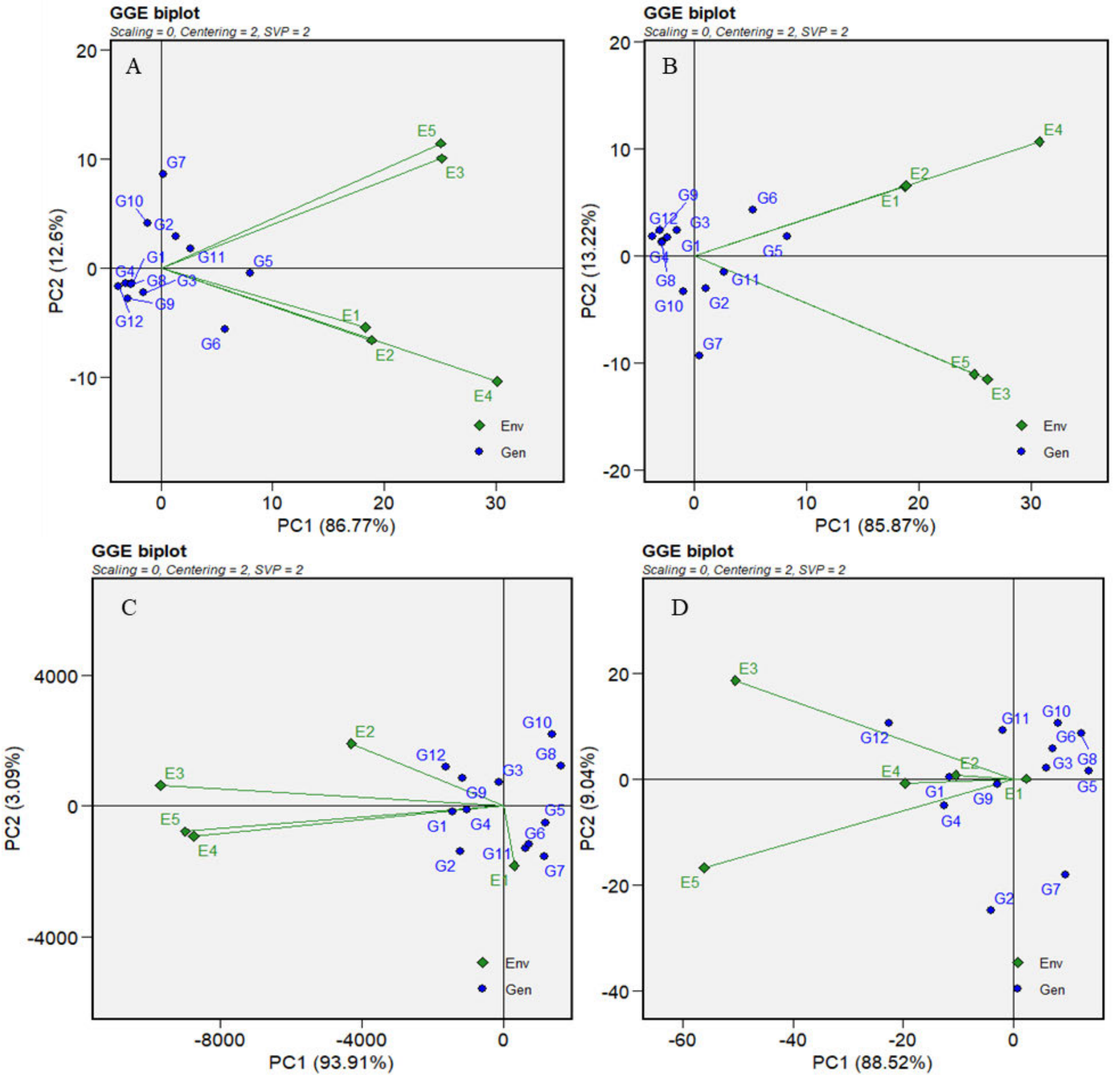

Figure 5.3. Genotype plus genotype-by-environment biplots showing genotypic performance of eight hybrids and four check bottle gourd genotypes for days to 50% male flowering (DTMF, A); days to 50% female flowering (DTFF, B); total number of fruits per hectare (TNFH, C); fruit yield (tons/ha) (FYPH, D) across five testing environments. E1 (University of Limpopo site under rain-fed conditions), E2 (University of Limpopo site with rainfed and minimal supplemental irrigation), E3 (University of Limpopo site with rainfed and optimal supplemental irrigation), E4 (Nkunzana site under rain-fed conditions) and E5 (Nkunzana site under rain-fed and optimal supplemental irrigation conditions). The codes of genotypes are presented in Table 5.2.

Figure 5.4 presented the trait mean and genotype stability based on GGE biplot for the assessed traits. The continuous green line with the arrowhead is called "average-environment axis" (AEA) and is used to classify the genotypes based on mean performance. The line perpendicular to the AEA is referred to as the line of stability. Genotypes that are closer to the

AEA (with smaller projections on the stability axis) are more stable, showing with variable performance across environments. In addition, genotypes that have larger projections along this axis exhibit greater variability in their performance, indicating lower stability (Yan and Tinker 2006). For DTMF and DTFF (Figures 5.4 A and B), BG-27 × BG-52 (G5) had a high mean performance and stability as it presented a line of stability closer to the AEA axis. Hybrid BG-27 × GC (G6) was high-yielding but unstable whereas BG-81 × GC (G7) recorded low yields and presented larger projections along the AEA axis, exhibiting greater variability in its performance. BG-80 × BG-81 (G8) and BG-81 (10) were stable performers, while BG-27 × BG-52 (G5), BG-81 × GC (G7) and BG-27 × GC (G6), and checks BG-80 (G11) and GC (G12) were unstable for TNFH. Genotypes BG-27 × BG-52 (G5), BG-27 × GC (G6) and BG-80 × GC (G3) were stable for FYPH, with shorter projections from the AEA axis. Contrastingly, BG-52 × BG-79 (G2), BG-81 × GC (G7) and BG-80 × BG-81 (G8) were unstable and best performers for FYPH with longer extensions from the AEA axis.

The genotype ranking GGE biplot is presented in Figure 5.5. In this biplot, the genotypes closer to the centre of the concentric circles are the most desirable and resemble close to that of the ideal genotype (Yan and Tinker, 2006; Yang et al., 2015). For DTMF and DTFF, hybrids BG-27 × BG-52 (G5) and BG-27 × GC (G6) were desirable genotypes. For TNFH, BG-52 × BG-58 (G1), BG-52 × BG-79 (G2) and GC (G12) were desirable. For FYPH, the genotypes closest at the centre of the concentric circles were GC (G12), followed by BG-52 × BG-58 (G1) and BG-58 × BG-80 (G4).

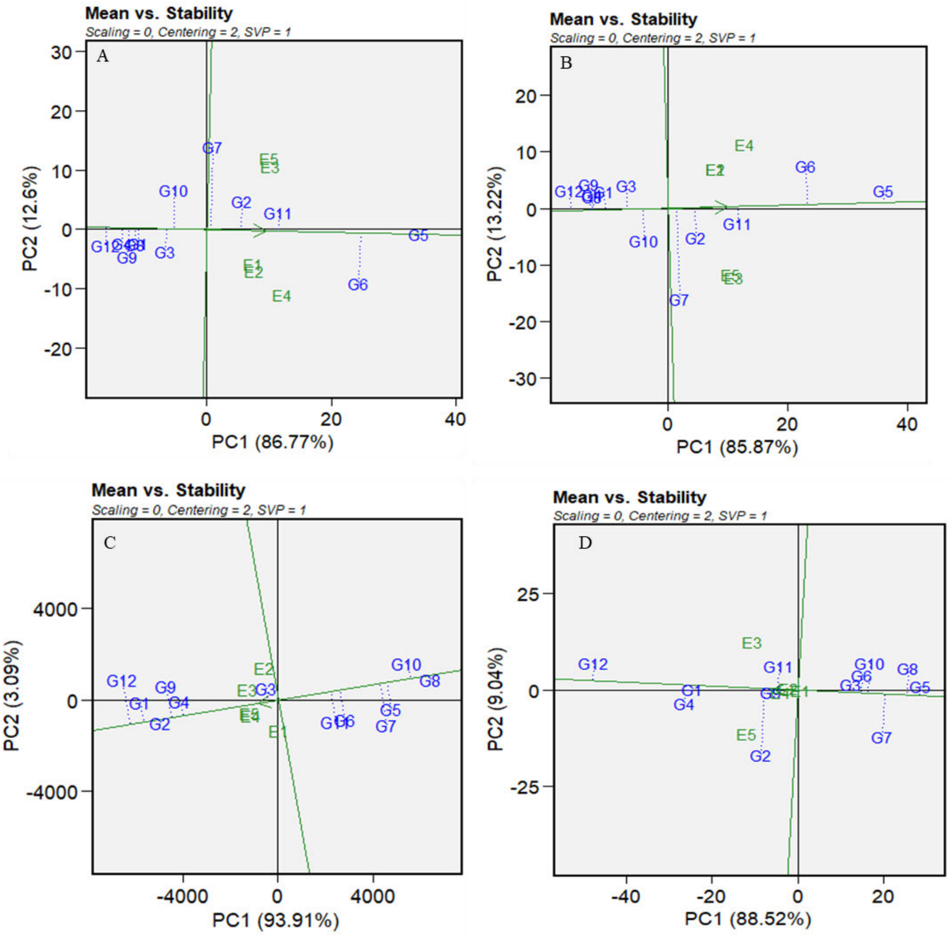

Figure 5.4. Genotype plus genotype-by-environment biplots for mean vs stability of eight hybrids and four check bottle gourd genotypes evaluated across five test environments in South Africa. Note: DTFM, Days to 50% male flowering (A); DFFF, days to 50% female flowering (B); TNFPH, total number of fruits per hectare (C); FYPH, fruit yield in tons/ha (D); PC, principal component. Environments (E1 to E5) are presented by the green rhombus: E1 (University of Limpopo site under rain-fed conditions), E2 (University of Limpopo site with rainfed and minimal supplemental irrigation), E3 (University of Limpopo site with rainfed and optimal supplemental irrigation), E4 (Nkunzana site under rain-fed conditions) and E5 (Nkunzana site under rain-fed and optimal supplemental irrigation conditions). The codes of genotypes are presented in Table 5.2.

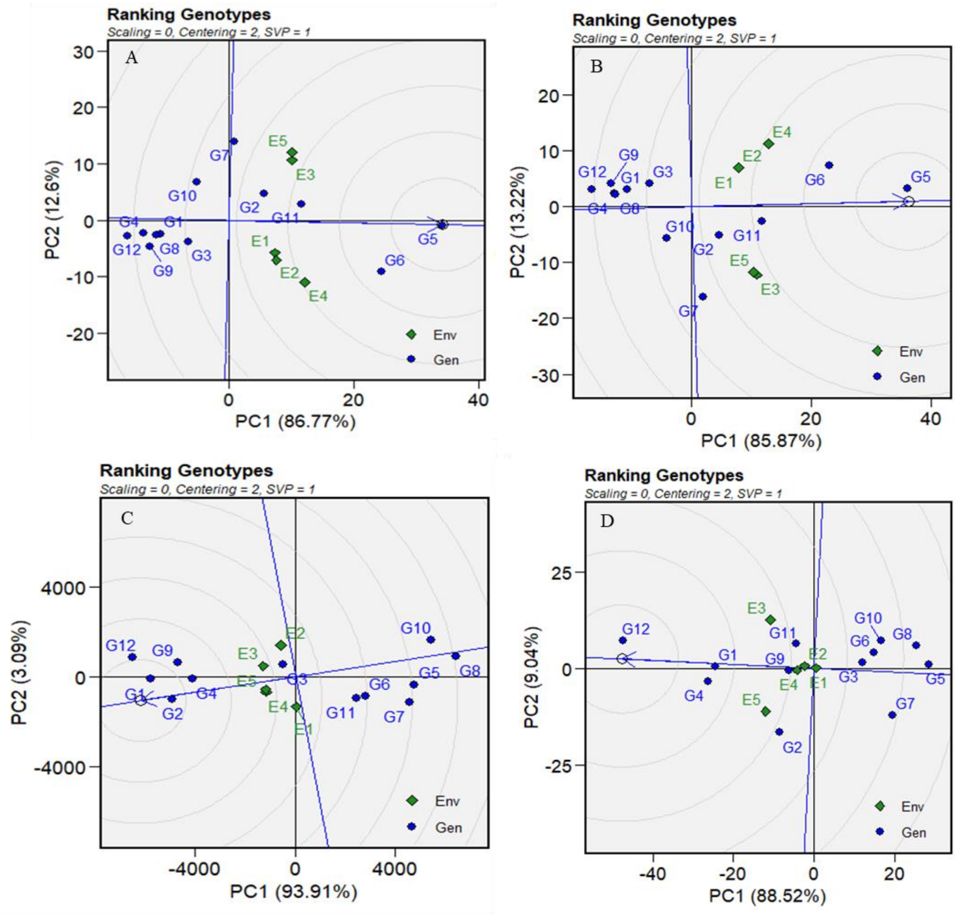

Figure 5.5. Genotype plus genotype-by-environment biplots for ranking genotypes of eight hybrids and four check bottle gourd genotypes evaluated across five test environments in South Africa. Note: DTFM, Days to 50% male flowering (A); DFFF, days to 50% female flowering (B); TNFPH, total number of fruits per hectare (C); FYPH, fruit yield in tons/ha (D); PC, principal component. E1 (University of Limpopo site under rain-fed conditions), E2 (University of Limpopo site with rainfed and minimal supplemental irrigation), E3 (University of Limpopo site with rainfed and optimal supplemental irrigation), E4 (Nkunzana site under rain-fed conditions) and E5 (Nkunzana site under rain-fed and optimal supplemental irrigation conditions). The codes of genotypes are presented in Table 5.2.

The best performing genotypes in each environment could be discerned using the “which-won-where” GGE biplot, as indicated in Figure 5.6. In the biplot, a polygon is constructed by drawing lines around the outermost genotypes located in the biplot. The points that form the corners of the polygon and the genotypes closest to an environment are the genotypes that perform the best in those environments. Each vertex of the polygon corresponds to a "winning" genotype in a specific environment (Yan and Tinker 2006). Therefore, for DTMF cross BG-27 × GC (G6) won in E1 and E4. For DTFF, G6 won in E1 and E2. For

TNFPH G12 won in E2. For FYPH, G3 and G9 won in E1, G12 in E3, G1 and G4 in E2 and E4.

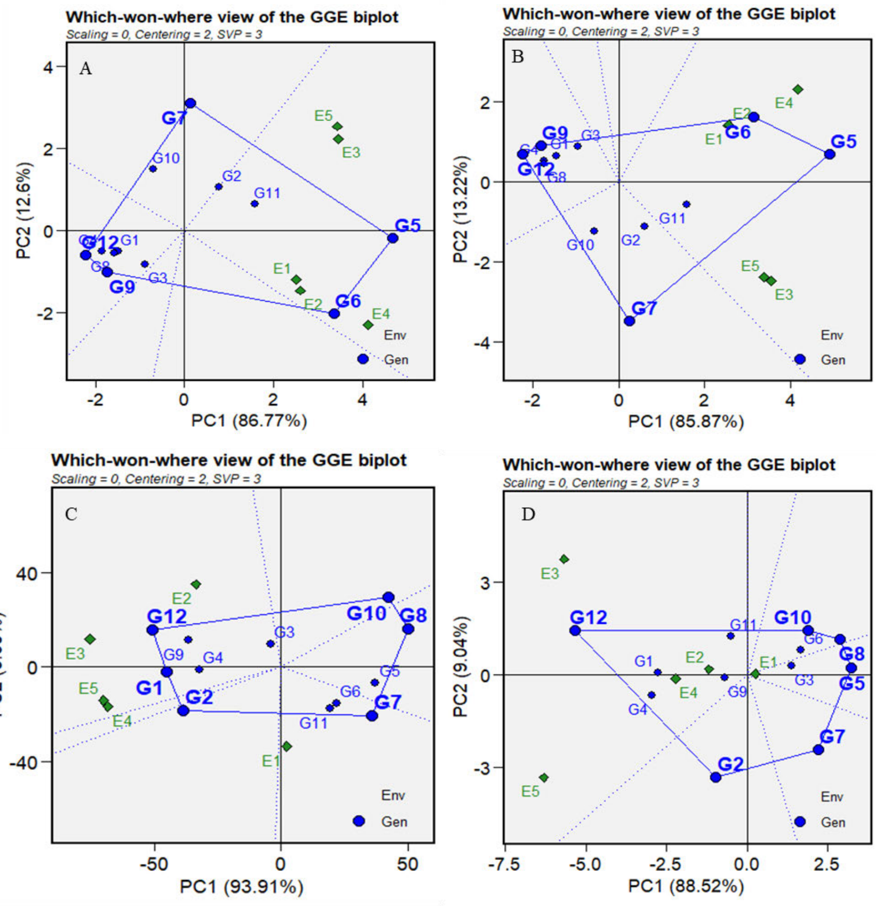

Figure 5.6. “Which-won-where” genotype plus genotype-by-environment biplots depicting best-performing eight hybrid and four check bottle gourd genotypes across five test environments in South Africa. Note DTFM, Days to 50% male flowering (A); DFFF, days to 50% female flowering (B); TNFPH, total number of fruits per hectare (C); FYPH, fruit yield in tons/ha (D); PC, principal component. E1 (University of Limpopo site under rain-fed conditions), E2 (University of Limpopo site with rainfed and minimal supplemental irrigation), E3 (University of Limpopo site with rainfed and optimal supplemental irrigation), E4 (Nkunzana site under rain-fed conditions) and E5 (Nkunzana site under rain-fed and optimal supplemental irrigation conditions). The codes of genotypes are presented in Table 5.2.

5.4. Discussion

Bottle gourd is a multi-use crop cultivated for its edible leaves, fresh and dried fruits, and seeds. It has significant genetic diversity and is tolerant to biotic and abiotic stress. It is an opportunity crop ideal for low-input agriculture and food systems to enhance food and nutrition security. In Africa, smallholder farmers primarily cultivate the crop using low yielding landrace varieties. Developing and deploying genetically improved varieties with desirable product profiles are essential for value-adding, commercialization and enhance market opportunities. The present study determined genotype-by-environment interaction for agronomic traits among newly developed F_1 hybrids of bottle gourd to identify best-performing hybrids for recommendation, registration and cultivation in South Africa. The accessions used in the present study were pre-selected and showed high fruit yield capacity, earliness, and drought tolerance.

The performance of the eight experimental hybrids and four check genotypes that were assessed varied for days to 50% male and female flowering, total number of fruits per hectare, and fruit yield per hectare (Table 5.3). These indicated phenotypic variability for assessed agronomic traits in the test germplasm. Bottle gourd is highly variable for agronomic traits, including flower and fruit traits (Decker-Walters et al., 2001; Morimoto et al., 2005; Mashilo et al., 2015; Contreras-Soto et al., 2021; Ibrahim, 2021; Mahapatra et al., 2022; Kumar et al., 2024). The variation in economic traits in the crop allowed exploitation in crop improvement programmes to develop hybrids with excellent product profiles and end-use quality traits (Janaranjani et al., 2016; Quamruzzaman et al., 2020). Further, the environment influenced the genotypic performance for the investigated traits (Table 5.3), suggesting that the best-performing genotypes could be selected by targeting specific production environments.

Earliness is an important trait that allows for early harvesting and marketing. It is a drought-avoidance strategy that enables completion of the crop lifecycle before adverse weather conditions such as drought and heat stress (Araus et al., 2002). Variable early flowering attributes (e.g. days to 50% male and female flowering) were observed among test hybrids under water-limited environments compared to optimal watering conditions (Table 5.4), suggesting that bottle gourd hybrids have buffering capacity against drought. The hybrids BG-58 × BG-80, BG-80 × BG-81 and BG-52 × BG-58 were early flowering compared to check varieties BG-81 and BG-80. Therefore, the identified short-cycle hybrids possess a drought avoidance mechanism and may be important for cultivation in drought-prone environments. Desirable and complementary alleles governing early flowering were combined in the test hybrids. The findings agreed with previous reports from combining ability studies that indicated the significance of additive and non-additive genetic effects influencing agronomic traits in bottle gourd (Janaranjani et al., 2016; Quamruzzaman et al., 2020).

Fruit yield is the key agronomic and horticultural trait in crop breeding programs. The hybrids BG-58 × BG-80, BG-80 × GC, BG-27 × BG-52, BG-80, BG-52 × BG-58 and BG-52 × BG-79 were identified as the high yielders in drought-stressed conditions and recorded fruit yield ≥ 10 tons/ha (Tables 5.4 and 5.7). Further, BG-52 × BG-58 was identified as the high yielder in drought-non-stressed conditions and recorded fruit yield ≥ 39 tons/ha. Hybrids BG-52 × BG-58 and BG-58 × BG-80 were high yielding across non-stressed and drought-stressed test environments and recorded fruit yield ≥ 27 tons/ha compared to the check varieties BG-81 (16 tons/ha), BG-80 (21 tons/ha) and BG-70 (23 tons/ha). It is worth noting that the hybrids BG-52 × BG-58 and BG-58 × BG-80 consistently performed well under both drought-stressed and non-stressed conditions. These hybrids are particularly suitable for smallholder farmers who often face unpredictable weather patterns, experiencing both drought and high rainfall within the same season or who cannot anticipate drought conditions at the time of planting bottle gourd. The high fruit yield in the new bottle gourd hybrids is attributable to desirable allelic combinations. Several studies reported that bottle gourd hybrids have better performance compared to their parental genotypes and express high heterotic effects (Quamruzzaman et al., 2009; Amangoua et al., 2018; Quamruzzaman et al., 2020). Fruit yield and related traits are controlled by complex genetic architecture and further subjected to genetic × environmental interactions.

Multi-environment trials (MET's) are essential to evaluate the performance, adaptability, and stability of genotypes for targeted production environments. In the present study, the additive main effects and multiplicative interaction (AMMI) and genotype plus genotype-by-environment (GGE) biplot analyses were deployed for MET's (Table 5.5; Figure 5.2). The AMMI analysis revealed a higher genotypic contribution to the total variation observed compared to environments and GEI effects for days to 50% male female flowering (Table 5.5). The higher genotype effects can be attributed to desirable genes in the cultivated bottle gourd genetic resources (Mashilo et al., 2015, 2016b; 2017a; Nkosi et al., 2022). For days to 50% male and female flowering and fruit yield, there was a higher environmental contribution to the total variation observed compared to genotype and GEI, indicating the importance of the environment in selection and discriminating genotypes for yield performance in bottle gourd (Table 5.5). The higher environmental contributions are expected as fruit yield is a complex trait controlled by multiple genes and interconnected component traits (i.e., number of fruits per plant, fruit length, width), which are highly affected by environmental conditions (Yogesh et al., 2009; Dias et al., 2016). Higher environmental contributions to the total variation observed in the present study are comparable to studies on related cucurbit crops, including sponge gourd (Singh et al., 2024), watermelon (Dia et al., 2016) and cucumber (Ahmed et al., 2022).

The AMMI biplots (AMMI-1 and 2) revealed the stability of the tested hybrids. Further, the AMMI stability values proposed by Purchase et al. (2000) revealed variable stability for assessed agronomic traits. A low value of ASV indicates a high relative stability and vice versa. Accordingly, the hybrids BG-58 × BG-80, BG-52 × BG-58, BG-27 × BG-52, BG-80 × BG-81, BG-80 and check variety GC were identified as stable for days to 50% male flowering, BG-58 × BG-80, BG-80 × BG-81, BG-27 × BG-52 and BG-80 for days to 50% female flowering, BG-80 × GC and BG-80 for total number of fruits per hectare, and BG-70, BG-80, BG-27 × GC and BG-80 × GC for fruit yield per hectare (Table 5.6). Stable genotypes are recommended for breeding and cultivation in multiple environments. On the contrary, hybrids with high instability for fruit yield were BG-52 × BG-58, BG-58 × BG-80, BG-27 × BG-52, BG-80 × BG-81 and GC. Theoretically, unstable genotypes have a higher-yield potential and specific adaptation to certain environments (Purchase et al., 2000). Thus, they are recommended for cultivation to improve yields in specific environments. Therefore, BG-81 × GC, BG-80 × GC, BG-27 × GC, BG-27 × BG-52 and BG-80 × BG-81 are recommended for cultivation under rainfed conditions, whereas BG-52 × BG-58 and BG-58 × BG-80 are recommended for cultivation under irrigated conditions. The results of GGE biplot analysis corroborated those of AMMI analysis. For instance, the mean vs stability GGE biplot revealed BG-27 × BG-52, BG-27 × GC and BG-80 × GC as stable for fruit yield per hectare. Further, ranking genotypes GGE biplot revealed BG-52 × BG-58 and BG-58 × BG-80 as the ideal genotypes for fruit yield. Lastly the “which-won-where” GGE biplot revealed BG-27 × GC for days to 50% male flowering, BG-27 × GC for days to 50% female flowering, GC for the total number of fruits per hectare, and BG-80 × GC and BG-70 for fruit yield per hectare. In addition, the “which-won-where” GGE biplot identified BG-80 × GC, BG-27 × GC and GC for cultivation under rainfed conditions, whereas BG-27 × GC, BG-52 × BG-58 and BG-58 × BG-80 for cultivation under both rainfed and irrigated conditions.

5.5. Conclusions

The present study evaluated the genotype-by-environment interactions among newly developed F_1 bottle gourd hybrids to guide variety recommendation and registration. The F_1 hybrids BG-58 × BG-80, BG-80 × GC, BG-27 × BG-52 and BG-52 × BG-58 attained high fruit yield per hectare under drought conditions. The hybrids BG-52 × BG-58, BG-58 × BG-80 and BG-52 × BG-79 recorded high fruit yield per hectare under irrigated conditions. The hybrids BG-52 × BG-58 and BG-58 × BG-80 performed consistently well under both drought-stressed and non-stressed conditions, making them suitable for smallholder farmers facing unpredictable rainfall. The study was limited to a single province within South Africa. Additionally, the presence of significant genotype × environment interaction effects highlights that for accurate identification of stable and high-performing genotypes, trials must be conducted across diverse environments. Therefore, it would be premature to recommend any hybrid for release or commercialisation based on the current data. The study can only support recommendations for further multi-environment trials across different provinces in South Africa, and subsequently in other sub-Saharan African countries, before considering release and commercialisation.

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Overview and implications of the study

Introduction and objectives of the study

Bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] belongs to the *Cucurbitaceae* family and provides multiple values, including food, feed, and other household and industrial products. It is widely cultivated in sub-Saharan Africa (SSA) and Asia primarily for its succulent and tender fresh leaves and fruit which are cooked and consumed as vegetables. The dried seeds are used for oil extraction or consumed as a roasted snack. Various custom-made water and food containers, and crafts and artisan home décor are prepared from dried fruits for local and regional markets. South Africa is one of the centres for genetic diversity of bottle gourd which could serve as a source of unique genetic variation for breeding, developing and deploying demand-led cultivars with enhanced productivity, product development, and abiotic and biotic stress tolerance. However, bottle gourd remains an underutilized and undervalued crop in the region and is cultivated primarily by smallholder farmers using genetically unimproved low-yielding landraces. There is a need for dedicated research and development support and genetic improvement programs of the crop for food security and enhanced livelihoods. Mashilo et al. (2015; 2016; 2017) investigated agro-morphological variability, genetic diversity, and trait association in bottle gourd landraces of South Africa. Their studies selected and delivered unique and complementary genotypes that can be explored in hybrid breeding to exploit heterosis for horticultural and agronomic traits and drought tolerance. Therefore, this study was undertaken to develop high-yielding and drought-tolerant bottle gourd hybrids for commercialization in South Africa to enhance food and nutrition welfare and provide market opportunities to growers.

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 production and research of the crop.

Research findings in brief:

Cucurbitacins B, E and I concentrations and relationship with drought tolerance in bottle gourd [*Lagenaria siceraria* (Molina) Standl.]

The concentrations of cucurbitacins were quantified in 12 preliminarily selected bottle gourd accessions using High Performance Liquid Chromatography- Mass Spectroscopy (HPLC-MS) and their antioxidant potential were determined using 2,2-diphenyl-1-picrylhydrazyl and ferrulic acid power assays. Further, the study established the relationship between cucurbitacin accumulation and drought tolerance. The main outcomes were:

- Cucurbitacins B and I were detected in accessions BG-48, BG-58, BG-70, BG-78, BG-79, BG-81, BG-52, and GC in leaves and roots under drought-stressed conditions.

- The contents of cucurbitacins B and I were enhanced by increased drought stress intensity for accessions BG-48, BG-81, and GC.
- Cucurbitacin E was not detectable in leaves and root samples of test accessions.
- Pure cucurbitacins I, B, and E reduced free radicals by 78, 60, and 66% based on the 2,2-diphenyl-1-picrylhydrazyl test.
- Pure cucurbitacins I, B, and E had maximum ferric-reducing powers of 67, 62, and 48% based on the ferrulic acid power assay.
- Cucurbitacin I recorded the highest antioxidant activity compared to cucurbitacins B and E.
- Cucurbitacins B and I serve as novel biochemical markers for screening drought tolerance in bottle gourd or related cucurbits.
- Accessions BG-48, BG-81, and GC were identified as drought-tolerant for production and breeding.

Hybrid performance of bottle gourd [*Lagenaria siceraria*] under drought stress and non-stress conditions

Fifty-three F_1 bottle gourd hybrids were developed using a half-diallel mating design. The hybrids with 12 parental accessions were field evaluated under non-stressed and drought-stressed conditions. Various drought tolerance indices were estimated based on fruit and seed yield. The main findings of this study were:

- The test accessions showed varied performances for fruit yield per plant and seed yield per plant under non-stressed and drought-stressed conditions.
- The mean fruit yield per plant were 0.3 and 1.0 kg under drought-stressed and non-stressed conditions, respectively.
- The mean seed yield per plant were 0.3 and 0.5 kg under drought-stressed and non-stressed conditions, respectively.
- Based on tolerance indices, hybrids BG-31 × BG-67, BG-27 × BG-31, BG-58 × BG-78, BG-31 × BG-70, BG-31 × BG-78, BG-58 × BG-67, BG-31 × BG-52, BG-52 × BG-78, BG-27 × BG-80, BG-58 × BG-81, BG-31 × BG-48, BG-67 × BG-70, BG-58 × BG-79 and BG-58 × BG-80 were identified as drought tolerant and best yielders.
- All the evaluated drought tolerance indices were consistently higher in the F_1 hybrids compared to their respective parental genotypes.

- Based on mean performance for fruit yield under drought-stressed conditions, the hybrids BG-27 × BG-31, BG-31 × BG-70, and BG-31 × BG-78 were the top performers.
- Under non-stressed conditions, the hybrids BG-31 × BG-79 and BG-31 × BG-52 showed an increase in performance for fruit yield compared to all parental genotypes.
- Hybrids BG-27 × BG-31, BG-31 × BG-70, and BG-31 × BG-52 were the best performers for fruit yield under both drought-stressed and non-stressed conditions.
- Parental accession BG-80 featured in the top performers under drought-stressed conditions.

Combining ability and heterosis among bottle gourd [*Lagenaria siceraria* (Molina) Standl.] selections for yield and related traits under drought-stressed and non-stressed conditions

Combining ability and heterosis analyses were carried out for 28 F_1 hybrids of bottle gourd derived using eight parental accessions using a half-diallel mating design. The hybrids and parental checks were evaluated under drought-stressed and non-stressed conditions. Data was collected for fruit yield and related traits. The primary outcomes were:

- The test genotypes showed high variability for fruit yield and related traits.
- Parental genotypes BG-58 and GC presented high general combining ability effects for fruit and seed yield, making them ideal genotypes for breeding. Crosses BG-81 × BG-52, BG-81 × GC, BG-27 × BG-79, BG-27 × GC, BG-79 × GC, BG-80 × BG-70, BG-81 × BG-58, BG-27 × BG-80, BG-27 × BG-58, BG-79 × BG-52, BG-52 × BG-58, BG-80 × BG-58, and BG-58 × BG-70 were selected displaying high and positive specific combining ability effects for fruit and seed yield. Overall, the patterns of GCA vs SCA suggested that a combined breeding strategy utilising selection for traits controlled by additive gene action and hybridisation for those influenced by dominance would be the most effective approach for genetic improvement in bottle gourd.
- High and positive mid- and better-parent heterosis were recorded in the crosses BG-80 × BG-58, BG-27 × BG-79, BG-79 × BG-52, BG-27 × BG-52, and BG-52 × BG-80 for fruit yield per plant and crosses, BG-70 × GC and BG-79 × BG-52 for seed yield per fruit, making them ideal hybrids for further multi-environment testing and production.
- Under drought-stressed conditions, there were positive correlations between fruit weight with fruit yield per plant ($r = 0.8$; $P \leq 0.001$), and fruit weight with seed yield

per plant ($r = 0.7$; $P \leq 0.001$). Additionally, a significant and positive correlation ($r = 0.8$; $P \leq 0.00$) was recorded between fruit yield per plant and seed yield per plant.

- Under non-stressed conditions, there were positive correlations between the number of fruits per plant with fruit weight ($r = 0.8$; $p \leq 0.001$) and the number of fruits per plant with seed yield per plant ($r = 0.8$; $P \leq 0.001$).

Genotype-by-environment interactions for fruit yield and related traits in selected hybrids of bottle gourd (*Lagenaria siceraria*)

Eight preliminarily selected F_1 hybrids and four check varieties were evaluated under selected environments with varied moisture regimes. Genotype-by-environment interactions were assessed for fruit yield and related traits. Data was subjected to the additive main effects and multiplicative interaction (AMMI) and genotype plus genotype-by-environment (GGE) biplot models. The main outcomes were:

- The AMMI model revealed significant ($P \leq 0.001$) effects of genotype, environment and genotype-by-environment interactions for assessed traits.
- The AMMI model explained a higher 96.30% variation for the total number of fruits per hectare, of which genotype, environment and genotype-by-environment interactions effects explained 49.88, 24.21 and 22.21% of the total variation, respectively.
- The AMMI model ascribed variations of 12.36, 73.16 and 11.41% for fruit yield per hectare attributable to the genotype, environment and genotype-by-environment interactions effects, in that order.
- The GGE model explained 94.53 and 96.56% variations for the total number of fruits per hectare and fruit yield per hectare, respectively.
- Overall, the hybrids BG-58 × BG-80, BG-80 × GC, BG-27 × BG-52 and BG-52 × BG-58 attained high and stable fruit yield per hectare under water-limited conditions and recommended for multilocation testing in different provinces in South Africa.
- Hybrids BG-52 × BG-58 and BG-58 × BG-80 performed well under both drought-stressed and non-stressed conditions.

Implications of the research findings for breeding bottle gourd, its production and drought tolerance

The following summarises the implications of the overall research findings of the study:

- Cucurbitacins B and I contents are useful biochemical markers for selecting and identifying drought-tolerant bottle gourd and related cucurbits.
- Drought tolerant hybrids such as BG-31 × BG-67, BG-27 × BG-31, BG-58 × BG-78, BG-31 × BG-70, BG-31 × BG-78, BG-58 × BG-67, BG-31 × BG-52, BG-52 × BG-78, BG-27 × BG-80, BG-58 × BG-81, BG-31 × BG-48, BG-67 × BG-70, BG-58 × BG-79 and BG-58 × BG-80 are recommended for testing beyond the Limpopo province to narrow them down to 1 to 3 best performers for registration
- Parental accessions such as BG-58 and GC with positive general combining ability effects for economic traits are ideal for future bottle gourd breeding programs.
- The following are promising F_1 hybrids: BG-52 × BG-81, BG-81 × GC, BG-27 × BG-79, BG-27 × GC, BG-79 × GC, BG-70 × BG-80, BG-58 × BG-81, BG-27 × BG-80, BG-27 × BG-58, BG-52 × BG-79, BG-52 × BG-58, BG-58 × BG-80, and BG-58 × BG-70 with high and positive specific combining ability effects and heterosis for fruit and seed yield, and drought tolerance.
- The following F_1 hybrids such as BG-58 × BG-80, BG-80 × GC, BG-27 × BG-52, BG-52 × BG-58 and BG-52 × BG-79 have stable and better performance for fruit yield per hectare.
- Overall, the mean performance revealed that the crosses BG-27 × BG-70, BG-29 × BG-79, BG-52 × BG-70, BG-58 × BG-70, BG-58 × BG-80, and BG-81 × GC were among the best-performing hybrids for fruit yield under drought-stressed conditions in both Chapters 3 and 4. Under non-stressed conditions, the top-performing crosses were BG-52 × BG-58, BG-70 × GC, BG-79 × BG-80, BG-58 × BG-80, BG-27 × BG-81, BG-58 × BG-81, BG-70 × BG-81, and BG-81 × GC, as observed in both chapters. The parental genotypes GC and BG-80 showed strong performance for fruit yield under drought-stressed conditions, while GC and BG-70 performed well under non-stressed conditions. The results suggested that both additive and dominance effects play roles in trait inheritance, supporting a combined selection and hybridisation approach for bottle gourd improvement. The study developed and selected promising hybrids that are recommended for multilocation testing in other provinces in South Africa.

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APPENDIX

Appendix 4.1 Estimates of mid parent and better parent heterosis for the studied agronomic traits under drought stress and non-stress conditions across two growing seasons in South Africa.

												Traits												
	NMF (%)		NFF (%)		SR%		NL (%)		PH (%)		NFPP (%)		FW (%)		FC (%)		FYPP (%)		NSPF (%)		HSW (%)		SYPP (%)	
Crosses	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb
Drought-Stress																								
BG-27 × BG-52	-14.74	-19.75	-19.85	-25.03	7.76	4.53	2.81	-9.70	54.89	53.85	33.35	20.01	50.93	43.38	53.91	38.45	57.78	51.07	-38.69	-45.21	-4.25	-6.38	-12.94	-14.95
BG-27 × BG-58	8.36	-2.95	11.8	5.79	-1.45	-2.77	-2.22	-21.39	6.47	-16.38	7.69	-12.5	21.07	0	22.86	13.11	28.67	-6.31	34.32	18.99	3.81	2.78	99.08	67.73
BG-27 × BG-70	173.82	69.00	39.47	12.98	-89.57	-94.52	52.97	-19.33	-29.22	-39.41	69.21	-13.34	-99.88	-99.94	89.86	-4.64	-99.9	-99.95	219.85	72.97	103.00	2.87	16.09	16.09
BG-27 × BG-79	31.23	29.31	48.14	42.65	-6.74	-6.86	38.92	22.02	49.87	36.55	10.00	-12.00	46.31	32.46	-10.48	-25.1	100.87	69.86	65.12	60.90	2.73	2.15	84.06	71.30
BG-27 × BG-80	36.63	-14.92	7.96	-12.05	-86.30	-92.64	85.13	-4.13	-21.45	-26.26	68.46	-13.34	-99.84	-99.92	172.96	37.06	-99.88	-99.94	72.23	-6.73	111.18	6.49	-4.71	-6.90
BG-27 × GC	25.32	22.65	15.00	10.20	0.88	0.80	-3.46	-18.43	96.70	93.51	57.59	44.46	45.2	40.66	3.19	-3.46	99.05	80.87	79.1	75.29	-1.12	-3.49	82.9	71.01
BG-52 × BG-58	110.76	39.16	32.32	5.56	-92.74	-96.24	255.98	94.69	74.39	29.77	378.67	146.72	-99.79	-99.89	168.08	35.55	-99.76	-99.88	308.72	120.87	109.48	6.89	254.24	198.45
BG-52 × BG-70	56.63	0.47	10.43	-16.15	-93.06	-96.41	102.7	8.55	-31.82	-49.04	108.63	8.34	-99.84	-99.92	102.04	2.44	-99.93	-99.97	61.57	-14.07	95.41	-0.38	-20.00	-33.86
BG-52 × BG-80	22.56	16.23	-0.41	-1.61	1.74	0.01	23.65	11.49	65.38	30.52	55.57	16.67	34.27	6.53	20.91	1.10	86.30	32.04	66.50	64.86	-0.30	-1.55	130.50	90.56
BG-52 × GC	21.91	13.17	9.83	-0.81	1.91	-1.27	-12.49	-12.49	-19.33	-26.05	-8.11	-32.01	34.41	16.23	65.94	27.62	17.12	-4.41	16.15	1.46	1.05	-0.65	27.18	15.84
BG-58 × BG-70	20.90	11.52	4.94	-5.65	0.00	-2.92	10.58	5.69	29.72	28.46	6.67	-11.11	55.99	43.79	37.25	16.40	30.35	13.91	75.38	53.8	-7.21	-7.38	22.41	12.00
BG-58 × GC	146.33	49.84	68.84	30.68	-86.43	-92.74	128.33	17.26	85.44	73.01	318.33	116.72	-99.76	-99.88	175.25	38.36	-99.67	-99.83	105.9	9.69	94.43	-1.99	98.81	98.81
BG-70 × GC	126.11	36.44	40.35	8.07	-90.59	-95.08	190.52	51.28	93.87	66.88	401.58	158.4	-99.82	-99.91	394.33	148.55	-99.71	-99.85	198.19	58.67	101.7	2.15	243.58	235.68
BG-79 × BG-52	42.92	26.35	16.76	6.62	3.85	2.32	11.35	0.40	43.66	21.69	26.54	24.01	22.91	10.88	-7.67	-16.88	91.23	58.74	106.6	78.96	1.54	1.10	205.29	174.04
BG-79 × BG-58	9.24	-4.01	0.91	-8.27	1.60	0.31	-24.70	-29.18	-21.58	-37.67	-28.58	-37.51	5.13	-10.88	2.24	0.49	-25.23	-41.75	-11.23	-22.82	2.73	1.26	-24.23	-32.29
BG-79 × BG-70	5.38	-33.94	-3.99	-26.48	-92.74	-96.25	122.18	17.43	-3.55	-10.52	104.39	4.17	-99.79	-99.9	156.56	29.49	-99.83	-99.91	30.13	-30.88	104.74	4.42	-18.11	-18.11
BG-79 × BG-80	101.78	19.61	71.36	33.10	-90.69	-95.12	95.86	1.19	-38.48	-44.59	55.96	-20.84	-99.81	-99.91	57.61	-20.9	-99.82	-99.91	40.73	-25.2	104.2	3.45	-7.48	-22.05
BG-79 × GC	18.05	-29.51	3.70	-19.03	-86.36	-92.68	89.80	-3.04	20.74	-9.39	96.45	0.00	-99.81	-99.9	107.08	3.91	-99.76	-99.88	1.06	-46.23	98.63	0.15	-21.91	-35.44
BG-80 × BG-58	21.97	21.13	23.65	23.03	-7.43	-7.63	39.27	33.11	136.08	118.35	67.46	44.01	73.98	62.21	25.92	11.63	208.37	184.56	13.19	12.69	4.15	2.22	64.19	63.38
BG-80 × BG-70	88.95	17.29	25.77	5.01	-90.21	-94.86	88.89	-1.64	-14.47	-20.21	73.47	-12.00	-99.82	-99.91	57.93	-20.8	-99.8	-99.9	196.5	61	103.37	3.04	62.78	51.49
BG-80 × GC	157.35	61.21	73.92	46.06	-86.58	-92.78	146.26	26.46	132.08	99.79	159.52	32.01	-99.74	-99.87	103.68	2.13	-99.57	-99.78	154.83	38.57	107.78	4.77	127.2	106.95
BG-81 × BG-27	165.06	76.30	93.61	59.08	-93.46	-96.62	101.47	7.89	9.88	-12.44	80.65	-8.00	-99.82	-99.91	70.28	-14.22	-99.87	-99.93	191.02	57.9	88.15	-4.02	8.77	-2.36

Appendix 4.1 (Continued).

Crosses	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb
BG-81 × BG-52	177.80	72.91	119.44	83.96	-91.09	-95.33	157.4	33.56	-2.88	-15.71	183.18	44.46	-99.82	-99.91	91.43	-3.91	-99.78	-99.89	347.05	142.57	81.97	-7.84	129.97	115.02
BG-81 × BG-58	97.29	23.93	42.58	20.23	-86.53	-92.76	37.87	-29.37	16.8	7.99	84.45	-5.56	-99.82	-99.91	99.48	0.11	-99.76	-99.88	123.89	21.66	89.16	-4.65	0.55	-8
BG-81 × BG-70	22.72	-18.09	10.19	-9.10	-93.38	-96.57	116.83	15.43	13.71	-14.46	138.33	22.23	-99.81	-99.9	106.37	4.2	-99.87	-99.93	39.63	-24.3	93.25	-1.49	-25.99	-33.86
BG-81 × BG-79	467.81	455.08	104.6	103.12	-94.13	-95.12	1547.92	1262.19	-36.34	-48.28	3160.13	2900.63	-99.93	-99.93	17557.92	17144.05	-99.95	-99.95	1950.07	1931.4	9363.15	7622.97	10.59	8.05
BG-81 × BG-80	535.87	450.41	152.97	147.96	-95.80	-96.41	1880.14	1467.74	-2.17	-17.54	4978.93	4438.28	-99.91	-99.91	15238.4	10627.26	-99.93	-99.94	2515.87	2503.12	6088.61	5035.16	67.3	40.95
BG-81 × GC	327.71	277.56	60.25	55.97	-95.08	-96.39	3018.07	2053.24	36.53	-2.49	5257.88	5086.77	-99.88	-99.89	15311.25	10534.18	-99.87	-99.9	2017.62	1988.21	6834.85	4813.27	63.82	35.44
										Non-stress														
BG-27 × BG-52	-49.5	-49.50	-25.83	-38.96	20.51	2.15	-23.06	-37.3	12.86	-1.77	0.11	-8.24	39.89	14.95	-16.54	-19.9	21.60	21.35	-53.58	-54.83	4.42	-1.73	-15.57	-29.96
BG-27 × BG-58	-5.49	-11.48	20.51	7.45	-12.33	-15.62	35.49	19.32	20.84	17.88	8.18	-5.37	47.96	41.05	-23.28	-33.44	54.56	31.48	39.67	26.49	4.72	-2.14	52.75	29.91
BG-27 × BG-70	-0.14	-4.35	-3.29	-7.84	2.49	2.07	6.92	-19.72	13.94	11.54	-5.63	-10.66	-11.59	-22.02	-31.7	-43.26	-2.94	-15.55	-13.53	-19.04	-4.34	-6.49	-31.43	-41.92
BG-27 × BG-79	-10.55	-19.48	-15.76	-28.11	0.57	-3.45	-45.91	-50.45	-6.42	-14.00	-18.99	-36.53	-11.59	-22.02	-26.56	-37.56	-34.16	-53.08	133.57	117.23	-11.15	-14.94	58.61	34.16
BG-27 × BG-80	-0.24	-4.85	11.14	0.00	-6.29	-10.21	-20.42	-39.21	2.83	0.32	19.60	10.02	-5.54	-12.35	9.91	8.72	7.81	6.45	-13.93	-30.58	-21.62	-22.51	-29.64	-49.2
BG-27 × GC	23.15	18.40	38.46	27.81	-3.79	-10.33	28.94	-8.35	14.79	13.34	33.4	28.63	23.11	20.58	54.22	49.59	58.08	50.35	37.7	32.04	-6.94	-10.79	59.83	51.78
BG-52 × BG-58	10.9	-1.16	9.18	-7.76	-1.18	-5.2	-44.14	-49.8	-9.35	-19.95	-23.23	-42.21	6.01	-16.55	24.58	6.25	-32.86	-57.32	21.32	19.91	5.95	-1.00	-11.24	-31.17
BG-52 × BG-70	15.49	-0.46	40.69	15.51	-7.43	-9.49	-54.07	-61.09	-13.75	-22.74	-25.03	-42.21	2.11	-14.19	7.78	-0.14	-34.17	-55.99	1.29	-3.30	-4.39	-8.73	-26.29	-35.93
BG-52 × BG-80	10.65	0.00	14.1	-1.35	-1.88	-3.75	4.19	0.07	12.62	11.65	6.94	-3.58	-1.8	-5.21	-6.59	-11.79	4.96	-1.82	-6.64	-10.68	8.08	3.18	11.78	8.40
BG-52 × GC	-14.47	-25.57	-20.65	-34.23	4.45	2.20	-8.07	-22.41	-8.00	-14.17	-27.77	-41.94	-26.41	-29.88	-28.71	-34.17	-50.11	-61.89	54.61	36.35	10.00	7.64	22.71	18.24
BG-58 × BG-70	34.47	34.33	27.43	13.91	5.69	0.34	9.51	-17.32	6.33	3.32	26.24	17.81	-2.88	-12.16	13.74	6.93	19.59	3.24	90.29	72.69	-9.13	-10.94	79.21	49.72
BG-58 × GC	2.88	-5.39	0.40	-12.45	3.96	1.53	-12.67	-28.46	-1.21	-2.06	-28.47	-32.05	-26.22	-36.47	77.36	60.46	-42.25	-47.27	-0.91	-16.24	-1.42	-4.69	-46.72	-57.5
BG-70 × GC	0.44	-7.25	2.55	-5.49	-0.41	-1.93	-13.77	-30.72	9.56	5.56	10.89	8.53	-31.36	-34.60	-27.62	-34.88	-9.54	-13.20	-12.22	-12.99	0.35	0.32	-21.2	-23.96
BG-79 × BG-52	-10.04	-13.78	-5.14	-9.78	-3.29	-3.54	-40.32	-51.25	-4.66	-10.33	-23.11	-32.47	-6.83	-14.15	-2.02	-4.33	-32.75	-45.99	-0.08	-15.18	19.66	16.67	-3.67	-4.31
BG-79 × BG-58	5.31	-4.92	-5.98	-9.48	5.39	1.93	45.06	12.69	111.52	103.79	17.93	0.00	6.99	0.00	14.78	2.28	24.72	1.82	50.23	31.04	-4.63	-7.16	60.60	30.92
BG-79 × BG-70	-6.94	-11.76	3.17	-2.98	-4.63	-4.94	-47.32	-57.67	-14.17	-22.52	-12.24	-26.53	-2.17	-20.1	-3.43	-5.36	-21.00	-45.21	-16.83	-23.88	6.22	6.22	-24.11	-32.23
BG-79 × BG-80	-0.62	-2.94	-15.26	-21.00	8.54	4.88	-34.33	-45.53	-5.63	-9.84	-6.81	-14.31	-13.65	-20.44	-16.96	-21.13	-18.24	-20.38	94.88	88.03	-14.91	-18.75	28.92	28.28
BG-79 × GC	-13.03	-14.68	-25.66	-26.42	19.17	18.63	45.00	22.76	5.02	5.01	22.68	16.1	21.01	7.46	-9.44	-22.15	52.22	30.87	-27.21	-36.09	-7.04	-14.06	-22.42	-36.63
BG-80 × BG-58	6.65	-7.28	2.33	-6.11	1.72	-1.37	13.27	-23.50	4.22	-5.32	16.84	-10.82	2.72	-11.01	24.68	8.80	11.32	-22.99	68.53	63.23	-17.96	-18.09	44.16	16.91
BG-80 × BG-70	-4.77	-10.77	-5.49	-15.89	-0.65	-4.24	-28.82	-49.55	-4.67	-14.08	-19.03	-33.82	-18.87	-18.87	-12.36	-14.81	-36.36	-49.9	46.87	28.54	1.68	-0.48	7.47	7.30
BG-80 × GC	-4.33	-9.98	-4.22	-9.78	-0.43	-0.63	9.26	-21.37	3.88	-2.30	-17.77	-31.12	17.11	-3.15	23.60	4.16	-13.03	-37.55	29.47	-1.12	-3.67	-8.79	0.52	-17.46
BG-81 × BG-27	-3.25	-4.33	11.47	10.14	-6.13	-6.19	-14.72	-16.49	-4.86	-8.95	-29.97	-33.77	-16.87	-27.19	-4.33	-4.67	-47.99	-57.40	99.43	83.47	-1.65	-4.11	21.03	8.72

Appendix 4.1 (Continued).

Crosses	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb	Hm	Hb
BG-81 × BG-52	13.89	5.07	20.81	16.84	1.82	-4.73	138.54	119.49	33.49	32.34	11.65	2.13	13.61	-1.57	11.88	-4.70	25.54	4.68	81.99	63.86	-16.26	-17.91	28.48	4.33
BG-81 × BG-58	0.64	-7.54	4.98	2.07	-1.12	-3.94	10.47	-0.65	10.79	6.75	23.64	10.02	28.35	21.46	22.19	17.28	53.57	44.31	31.25	2.55	-15.39	-19.76	9.13	-23.64
BG-81 × BG-70	-6.70	-19.66	-4.27	-13.13	-0.45	-3.41	26.96	-15.18	6.10	-7.34	11.49	-18.09	3.05	-20.1	13.23	-0.88	-1.07	-38.42	-21.25	-25.33	-2.24	-4.84	-16.78	-37.78
BG-81 × BG-79	-9.11	-9.51	1.84	-4.15	-1.59	-5.32	111.27	106.04	17.28	12.07	21.69	18.03	-19.02	-33.02	-25.62	-38.74	17.07	2.98	-29.66	-40.11	-1.88	-5.15	-31.29	-43.66
BG-81 × BG-80	-5.01	-11.92	10.84	-2.39	-6.44	-9.88	-2.14	-31.49	10.73	-4.01	-3.08	-24.12	-23.62	-33.1	-12.12	-14.88	-30.91	-52.79	54.97	46.71	-8.69	-12.82	26.8	13.74
BG-81 × GC	4.65	-2.55	11.85	4.18	-2.93	-3.05	15.82	-17.72	3.44	-6.63	-6.78	-25.33	21.40	-9.46	18.11	-0.17	0.86	-35.53	45.54	18.46	-20.55	-26.55	-2.95	-12.35

NMF = number of male flowers per plant, NFF = the number of female flowers per plant, SR = sex ratio, NL = the number of leaves per plant, PH = plant height (m), NFPP = the number of fruits per plant, FW = fruit weight (kg/fruit), FC = fruit circumference (cm), FYPP = fruit yield per plant (kg), NSPF = the number of seeds per fruit, HSW = hundred seed weight (g/100 seed), SYPP = seed yield per plant (kg), Hb = better parent, Hm = mid parent