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Genetic Characterization of Citron Watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.) and Development of Experimental Hybrids

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Genetic Characterization of Citron Watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.) and Development of Experimental Hybrids

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Thesis abstract

Citron watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.) is indigenous to sub-Saharan Africa (SSA) with multiple uses, including human food and animal feed. Its succulent leaves are used as leafy vegetables, while the ripened yellow and orange-fleshed fruits are used to prepare various traditional dishes, and the seeds are roasted and consumed as snack. It is an emerging potential rootstock for producing grafted sweet watermelon (*Citrulus lanatus* var. *lanatus*) to improve fruit yield and biotic and abiotic stress tolerance. It is also a source of novel genes for breeding in sweet watermelon to improve fruit yield, quality and disease resistance. Citron watermelon in SSA is mainly cultivated using unimproved landrace varieties. Landraces exhibit marked phenotypic variation for fruit shape, size, skin colour patterns, and seed coat colours. Phenotypic and genetic variation among South African citron watermelon landraces is yet to be systematically assessed for diverse use and cultivar design. The overall goal of this study was to initiate a pre-breeding program for citron watermelon through identification and selection of unique and complementary genotypes for production, value-adding and breeding. The specific objectives of this study were:

i. To determine the extent of genetic diversity among South African citron watermelon landrace accessions using selected simple sequence repeat (SSR) markers to identify genetically divergent accessions for trait integration and variety development;
ii. To assess the phenotypic diversity of citron watermelon landrace accessions of South Africa and to select desirable genotypes with suitable agronomic and horticultural traits for direct production, breeding and conservation;
iii. To estimate variance components, heritability and genetic advance of phenotypic traits in citron watermelon to guide the selection of superior genotypes for direct production and breeding;
iv. To determine the combining ability and hybrid performance of citron watermelon genotypes for agronomic traits for breeding.

In the first study, 48 citron watermelon landrace collections widely grown in the Limpopo Province of South Africa were genotyped using 11 selected SSR markers.

The SSR markers amplified a total of 24 alleles, with a mean expected heterozygosity value of 0.38, indicating moderate genetic diversity among the studied accessions. Analysis of molecular variance attributed 8%, 75%, and 17% of the molecular variation between populations, among accessions and within accessions, respectively. Three distinctive genetic groups were identified based on cluster analysis. The following distantly related genotypes are recommended as breeding parents namely: WWM03, WWM04, WWM15, WWM16, WWM18, WWM22, WWM23, WWM24, WWM25, WWM26, WWM28, WWM33, WWM34, WWM35, WWM38, WWM39, WWM41, WWM66, WWM76, WWM78, WWM81, WWM84, WWM86 and WWM89 (selections from Cluster I), WWM14, WWM37, WWM42, WWM44, WWM46, WWM50, WWM65, WWM79, WWM85 and WWM87 (Cluster II), and WWM38, WWM47 and WWM48 (Cluster III). These are useful parental lines for pre-breeding to develop and release new varieties with multiple uses.

In the second study, 36 selected citron watermelon landrace accessions were evaluated under field conditions across two environments using a 6 × 6 lattice design with three replicates. Data on key qualitative and quantitative traits were collected and subjected to non-parametric and parametric statistical analyses. The accessions showed wide phenotypic variation and unique traits for genetic improvement. Positive and significant correlations *($p < 0.001$)* were recorded between total fruit yield per plant with plant height ($r = 0.64$), number of harvestable fruits ($r = 0.70$), number of marketable fruits ($r = 0.73$) and marketable fruit yield ($r = 0.96$). Seed yield per plant positively and significantly *($p < 0.001$)* correlated with number of male flowers ($r = 0.68$), plant height ($r = 0.61$) and total fruit yield ($r = 0.79$). Principal component analysis identified nine components which accounted for 86.38% of total variation amongst accessions for assessed phenotypic traits. The study recommended citron watermelon accessions such as WWM14, WWM16, WWM39, WWM41, WWM67 and WWM79 for use as leafy vegetables owing to their profuse branching ability and longer vine production. Whereas accessions including WWM03, WWM17, WWM35, WWM40, WWM50, WWM67, WWM79 and WWM85 are selected with larger fruit size. Accessions WWM05 and WWM09 are sour-flesh types which are suitable genetic stocks for breeding sweet-and-sour and sweet dessert watermelons. Orange-fleshed accessions such as WWM03, WWM04, WWM46, WWM64, WWM66 and WWM67 are recommended for fresh consumption, cooking, processing or variety design.

Accessions WWM02, WWM03, WWM08, WWM14, WWM16, WWM23, WWM38, WWM40, WWM41 and WWM67 have red and white seed coat colour which are superior selections to prepare roasted citron watermelon seed snack.

In the third study, variance components, heritability and genetic gains of phenotypic traits were estimated involving 36 accessions of citron watermelon grown under field conditions across two test environments using a 6 × 6 lattice design with three replicates. High broad-sense heritability and genetic advance as percent of the mean were recorded for fruit length at 83.86 and 4730.45%, seed length (77.73 and 1731.27%), hundred seed weight (73.73 and 4027.36%), fruit diameter (70.44 and 2949.64%) and fruit weight (70.39 and 8490.05%), respectively. Step-wise regression analysis revealed marketable fruit yield and total number of fruits per plant explaining 89% ($R^2 = 0.89$) of total variation for total fruit yield per plant, whereas number of seed per fruit and hundred seed weight explained 92% ($R^2 = 0.92$) of total variation for seed yield per fruit. Citron watermelon landrace accessions WWM03, WWM14, WWM16, WWM39, WWM65, WWM67 and WWM79 with high total fruit yield and seed yield per fruit were selected for production or breeding programme.

In the fourth study, five selected parental genotypes were crossed in a 5 × 5 half-diallel mating design to develop 10 hybrids. The 15 families (five parents and 10 F_1 hybrids) were evaluated across two environments using a randomized complete block design (RCBD) with three replications. General combining ability (GCA) and specific combining ability (SCA) effects were significant *($p < 0.001$)* for most traits. Environment × GCA was non-significant, whereas Environment × SCA effects were significant *($p < 0.001$)* for most traits. The ratios of GCA/SCA variances were less than unity for most traits, indicating non-additive gene action of the traits. Broad-sense heritability varied from low to moderate, implying variable selection response of the assessed traits among the F1 hybrids. The parental genotypes WWM16 with positive GCA effects for fruit and seed yield and WWM66, with positive GCA effects for the number of seeds per fruit and seed yield, were identified for hybrid breeding. The following F_1 hybrids, namely: WWM04 × WWM16, WWM03 × WWM66 and WWM16 × WWM50 with positive SCA effects on total fruit yield per plant and marketable fruit yield per plant, and WWM04 × WWM50, WWM03 × WWM16 and WWM03 × WWM66 with positive SCA effects for number of seeds per fruit and total seed yield were identified. The study identified novel and best-performing F1 hybrids of citron watermelon for economic traits and are recommended for multi-environmental evaluations, variety registration and commercialization.

Overall, the study revealed genetic and phenotypic variation in citron watermelon to select and recommend suitable genotypes for production and for breeding new generation varieties based on market needs and consumer preferences. The study recommends accessions such as WWM14, WWM16, WWM39, WWM64, WWM67, WWM76 and WWM79 with high fruit yield, and WWM03, WWM04, WWM14, WWM15, WWM16, WWM24, WWM28, WWM37, WWM46, WWM66 and WWM68 exhibiting high fruit and seed yield for breeding or direct production. The parents WWM04, WWM03 and WWM16 were identified as good combiners for fruit or seed yield and related-component traits for future breeding. The F_1 hybrids derived from these parents, including WWM04 × WWM16, WWM03 × WWM16, WWM03 × WWM66, WWM16 × WWM50, and WWM04 × WWM50 were best performing for economic traits and new breeding population development.

Declaration

I, Mantlo Richard Ngwepe, 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 other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
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Signed

[redacted]

Mantlo Richard Ngwepe

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

[redacted]

Prof. Hussein Shimelis (Supervisor)

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Dr. Jacob Mashilo (Co-Supervisor)

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Dedication

This thesis is dedicated to my wife (Andronica Mokgatjane Ngwepe), my son (Lefa Maphuti Ngwepe) and my daughter (Keabetswe Phaladi Ngwepe).

Abbreviations

ACCI	African Centre for Crop Improvement
AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
ARC	Agricultural Research Council
BLUPs	Best linear unbiased predictors
CAPS	Cleavage Amplified Polymorphic Sequence
CTAB	Cetyl-tetramethyl ammonium bromide
DNA	Deoxyribonucleic acid
GA	Genetic advance
GAM	Genetic advance as percent of the mean
GBS	Genotyping by Sequencing
GCA	General combining ability
GCV	Genotypic coefficient of variance
EST-SSR	Expressed Sequence Tag - Derived Simple Sequence Repeats
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
HFO-TAG	High Frequency Oligonucleotides-Targeting Active Gene
LDARD	Limpopo Department of Agriculture and Rural Development
LSD	Least significant difference
PCA	Principal component analysis
PCR	Polymerase chain reaction
PCV	Phenotypic coefficient of variance
PIC	Polymorphic information content
QTL	Quantitative trait loci
RAPD	Random Amplified Polymorphic DNA
SCA	Specific combining ability
SNP	Single Nucleotide Polymorphism
SSA	Sub-Saharan Africa
SSR	Simple sequence repeat markers
SAWS	South African Weather Services
TADC	Towoomba Agricultural Development Centre
UKZN	University of KwaZulu-Natal

Publications pertaining to this thesis

Chapter 1

Ngwepe, R.M., Mashilo, J., and Shimelis, H. 2019. Progress in genetic improvement of citron watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.): a review. *Genetic Resources and Crop Evolution*, 66, 735-758. DOI: https://link.springer.com/article/10.1007/s10722-018-0724-4

Chapter 2

Ngwepe, R.M., Shimelis, H., Mashilo, J., and Shayanowako A.I.T. 2023. Characterization of genetic diversity of citron watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.) landrace accessions of South Africa through simple sequence repeat markers*. Journal of Crop Improvement*, 37 (3), 378-394 DOI: https://doi.org/10.1080/15427528.2022.2096164

Chapter 3

Ngwepe, R.M., Shimelis, H., and Mashilo, J. 2021. Variation in South African citron watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.) landraces assessed through qualitative and quantitative phenotypic traits. *Genetic Resources and Crop Evolution*, 68, 2495–2520. DOI: https://doi.org/10.1007/s10722-021-01145-0

Chapter 4

Ngwepe, R.M., Shimelis, H., and Mashilo, J. 2021. Estimates of the variance components, heritability and genetic gains of phenotypic traits in citron watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.). *Plant Breeding*, 140 (5), 953– 967. DOI: https://doi.org/10.1111/pbr.12958

Chapter 5

Ngwepe, R.M., Shimelis, H., and Mashilo, J. 2023. Combining ability and hybrid performance in citron watermelon (*Citrullus lanatus* var. *citroides*) for agronomic traits. *Euphytica.* 219, 118.DOI: https://doi.org/10.1007/s10681-023-03246-6

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Introduction to thesis

Background

Citron watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.) is a trailing/creeping monoecious crop belonging to the Cucurbitaceae family. It is an important crop serving for human consumption, particularly in sub-Saharan Africa. For example, in South Africa, fresh succulent leaves of citron watermelon are cooked as a leafy vegetable to prepare local food referred to as "Morogo" (i.e., "Mogoro wa Talane", "Mogoro wa Motshatsha" or "Morogo wa Mokhusa) in the South African indigenous Sepedi language. Morogo is consumed with maize, sorghum or millet porridge (Franz, 1971; Fox and Norwood, 1982; Maggs-Kolling and Christiansen, 2003; Schippers, 2002). The leaves are rich in protein (23%), essential minerals (i.e., N, P, K, Ca, Mg, Na, Zn and Fe) and flavonoids (Jarret and Levy, 2012; Ogbuji et al., 2012).

The ripened fruits have yellow and orange flesh colour and are used to derive various food products such as a soup referred to as "Mokgapu or Kgodu" and porridge called "Thophi" or "Semphemphe" which are native names referred to these products by the Bapedi tribe of the Limpopo Province of South Africa (Ngwepe et al., 2019, 2021). The fruit flesh contains health-promoting carotenoids (e.g., prolycopene, phytoene, and ζ-carotene, violaxanthin, neoxanthin and β-carotene) (Tadmor et al., 2005; Bang et al., 2010, 2007; Davis et al., 2007), Vitamins A, B6, C, lycopene, and antioxidants. The seeds are rich in micro- and macro-nutrients and are mainly prepared and consumed as roasted snacks (Mashilo et al., 2016; Ngwepe et al., 2021; Mandizvo et al., 2022). Seeds are valued as a source of unsaturated fatty acids, including palmitic acid (10.6%), stearic acid (8.05%), oleic acid (16.42%) and linoleic acid (63.37%), and oil content of approximately 22.6% (Jarret and Levi, 2012). The nutritional attributes of citron watermelon's leaves, fruit and seeds make it an excellent food crop for promoting a balanced diet and combating food and nutrition crises faced by a vast population in SSA.

Citron watermelon is originated in Africa (Dane and Lang, 2004; Dane and Liu, 2007), and this assumption is supported by the extensive phenotypic diversity of the crop currently present in the region (Mujaju et al., 2010, 2013; Achigan-Dako et al., 2015; Mashilo et al., 2017). The observed phenotypic variation is useful for breeding in this crop to develop varieties with farmer and consumer-preferred traits (Ngwepe et al., 2021). The crop is mainly cultivated by smallholder farmers in sub-Saharan Africa (SSA) using unimproved landrace varieties due to a lack of improved varieties from a dedicated breeding programme. Therefore, there is a need to determine the magnitude of phenotypic and genetic variation in citron watermelon genetic resources and explore variety design and development according to the needs of clients and markets in SSA and other similar agro-ecological zones.

The crop has a niche market potential in SSA, and thus developing client-oriented varieties can accelerate efforts for increased market value and consumption. Also, citron watermelon is a valuable source of genes for introgression in sweet watermelon and has aided the breeding of high-performing and better-quality varieties in the USA and China (Gao et al., 2018; Rivera-Burgos et al., 2021; Mahapatra et al., 2023). It is also explored as an alternative rootstock for grafted watermelon delivering better quality fruit and enhancing biotic and abiotic stress tolerance (Cohen et al., 2014; Thies et al., 2015; Ngwepe et al., 2019). As a result, some accessions sourced from Africa have been released directly for commercialization as rootstocks for grafted watermelon in the USA (Cohen et al., 2014; Thies et al., 2015). Yet the crop's commercial and economic values remain relatively unexplored in SSA. There is a need for collection, strategic conservation and characterization of the genetic resources of citron watermelon, to aid in the identification and selection of better-suited varieties based on the utilities of the crop. Also, there is a need to explore the breeding value of citron germplasm resources targeting the improvement of agronomic and horticultural attributes.

Rationale for the study

Global food demand, population pressure and climate change are leading to food insecurity requiring innovative strategies to harness food production and supply, particularly in SSA (Chivenge et al., 2015). In SSA, many adapted indigenous crops such as citron watermelon, has been neglected, underutilized and under-researched (Laghetti and Hammer, 2007; Mashilo et al., 2017). Furthermore, there is a significant decline in crop diversity in agricultural production systems and reduced genetic diversity of important cucurbit crops such as citron watermelon (Chivenge et al., 2015).

Neglected and underutilized crops are adapted to local growing conditions and require low production inputs such as fertilizers, pesticides and/or irrigation. Therefore, underutilized crops such as citron watermelon should be promoted as alternative crops to ensure food security and nutritional needs of rural communities (Modi et al., 2006), especially in SSA, where the crop is largely cultivated and consumed. Also, the crop has served as a source of valuable genes for introgression and breeding in sweet watermelon (Gao et al., 2018; Rivera-Burgos et al., 2021; Mahapatra et al., 2023). It has also aided in the development of genomic resources (i.e., molecular markers and genetic linkage maps) and gene discovery in sweet watermelon for effective breeding (Gao et al., 2018; Sun et al., 2020).

Inter-specific breeding in citron watermelon has also been explored mostly for rootstock development to produce grafted sweet watermelon to enhance disease resistance and improve fruit yield and quality (Thies et al., 2015; Mahapatra et al., 2023). Despite the genetic value of citron watermelon for breeding, the crop has received limited research priority towards its genetic improvement, and as a result, a very limited number of improved varieties has been released for cultivation and commercialization in SSA. Therefore, citron watermelon consumption and production can be enhanced by developing and selecting superior and high-yielding genotypes with enhanced resistance to biotic and abiotic stresses. Such developed varieties should also possess desirable agronomic and horticultural traits that growers, consumers and markets desire.

Overall aim and objectives of the study

The overall goal of this study was to initiate a pre-breeding program for citron watermelon through identification and selection of unique and complementary genotypes for production, value-adding and breeding.

Specific objectives

The specific objectives of this study were**:**

I. To determine the extent of genetic diversity among South African citron watermelon landrace accessions using selected simple sequence repeat (SSR) markers to identify genetically divergent accessions for trait integration and variety development;

II. To assess the phenotypic diversity of citron watermelon landrace accessions of South Africa and to select desirable genotypes with suitable agronomic and horticultural traits for direct production, breeding and conservation;

III. To estimate variance components, heritability and genetic advance of phenotypic traits in citron watermelon to guide the selection of superior genotypes for direct production and breeding

IV. To determine the combining ability and hybrid performance of citron watermelon genotypes for agronomic traits for breeding.

Research hypotheses

I. Simple sequence repeat (SSR) markers will reveal genotypic variability and genetic diversity present among the South African citron watermelon landrace accessions for selection.

II. Phenotyping citron watermelon landrace accessions can assist in selecting accessions with suitable agronomic and horticultural attributes for direct production, conservation and breeding.

III. Estimating variance components, heritability and genetic advance of phenotypic traits in citron watermelon landrace accessions is effective to guiding the selection of superior accessions for hybrid breeding.

IV. Citron watermelon parental genotypes exhibit good combining ability for agronomic traits to develop better-performing F_1 hybrids.

Thesis outline

This thesis consists of five chapters according to the research objectives outlined above. The chapters are written as discrete research papers published or intended for publication containing all the necessary information. Due to their interdependence, there are some overlaps and unavoidable repetition of references and introductory information between the chapters. This is the dominant thesis format adopted by the University of KwaZulu-Natal.

Chapter 1 presents a review of the literature on progress in the genetic improvement of citron watermelon, and it was published in *Genetic Resources and Crop Evolution*, 66, 735-758. DOI: https://link.springer.com/article/10.1007/s10722-018-0724-4. Chapter 2 focuses on characterization of the genetic diversity of citron watermelon landrace accessions of South Africa through simple sequence repeat markers, and it was published in *Journal of Crop Improvement*, 37 (3), 378-394 DOI: https://doi.org/10.1080/15427528.2022.2096164. Chapter 3 highlights the variation in South African citron watermelon landraces, assessed through qualitative and quantitative phenotypic traits and published in *Genetic Resources and Crop Evolution*, 68, 2495–2520. DOI: https://doi.org/10.1007/s10722-021-01145-0. Chapter 4 estimated variance components, heritability and genetic gains of phenotypic traits in citron watermelon and was published in *Plant Breeding*, 140 (5), 953– 967. DOI: https://doi.org/10.1111/pbr.12958 . Chapter 5, which determined combining ability and hybrid performance in citron watermelon for agronomic traits, and it was published in *Euphytica*. 219, 118. DOI: https://doi.org/10.1007/s10681-023-03246-6.

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

- Thesis introduction
1. **Chapter 1**: Review of Literature
2. **Chapter 2**: Characterization of genetic diversity of citron watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.) landrace accessions of South Africa through simple sequence repeat markers.
3. **Chapter 3**: Variation in South African citron watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.) landrace accessions assessed through qualitative and quantitative phenotypic traits.
4. **Chapter 4**: Estimates of the variance components, heritability and genetic gains of phenotypic traits in citron watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.).
5. **Chapter 5**: Combining ability and hybrid performance in citron watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.) for agronomic traits.
- Overview and implications of the study

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Chapter 1. Review of the literature

Abstract

Citron watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.) is an important crop belonging to the Cucurbitaceae family. It possesses novel genes for biotic and abiotic stress tolerance and horticultural traits useful for breeding including sweet dessert watermelon. However, there is a lack of research priority limiting the genetic improvement and cultivar deployment of citron watermelon. Genetically diverse citron watermelon genetic resources are important to identify and select suitable genotypes for cultivar development incorporating horticultural attributes according to the needs and preferences of clients and marketplace. The objective of this review was to present progress on genetic improvement of citron watermelon with emphasis on cultivar development with biotic and abiotic stress tolerance and desired horticultural attributes. Further, the review highlighted genetic and molecular resources in citron watermelon and other cross-compatible cucurbits that may aid in accelerated breeding and cultivar release. Finally, the chapter summarized research focus areas with potential to enhance effective utilization of citron watermelon genetic resources for targeted breeding of this crop or related taxa such as dessert watermelon.

Keywords: *Abiotic and biotic stresses, Breeding, Citrullus lanatus, Citron Watermelon, Cucurbits, Genetic improvement*

1.1. Introduction

Citron watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.) is viny creeping monoecious crop belonging to the Cucurbitaceae family. The genus *Citrullus* include approximately seven species namely *Citrullus lanatus, Citrullus mucosospermus, Citrullus amarus, Citrullus ecirrhosus, Citrullus rehmii, Citrullus colocynthis, and Citrullus naudinianus* (Chomicki and Renner, 2015). The species *C. lanatus* comprises of two botanical varieties namely: subsp. *vulgaris* (*Schrad. ex Eckl. et Zeyh.*). var. *lanatus* (dessert watermelon) and subsp*. lanatus.* var. *citroides* (*L.H. Bailey*) *Mansf. ex Greb.*) (Citron watermelon) (Laghetti and Hammer, 2007; Chomicki and Renner, 2015). Citron watermelon is neither sweet nor bitter and is known by several names such as “cow-melon”, “tsamma melon” and “camel melon” (Robinson and Decker-Walters, 1997; Shaik et al., 2017). Citron watermelon is called “Lerotse” in the local Sepedi language of Limpopo province of South Africa.

The Southern African region is reported to be the center of diversity and probably the center of origin of most of the *Citrullus* species (Dane and Lang, 2004; Robinson and Decker-Walters, 1997). Contrastingly, Chomicki and Renner (2015) reported West Africa to be the origin of the egusi-type dessert watermelon (*C. mucosospermus*). Further, citron watermelon is thought to be the wild progenitor of the cultivated watermelon (Navot and Zamir, 1987). Citron watermelon is monoecious with male and female flowers borne on the same plant rendering high level of cross pollination (Wehner, 2008). This can promote development of new breeding populations and to select superior ideotypes with increased heterosis (hybrid vigour). Improved cultivars with high yield, early maturity and desired horticultural attributes (e.g., fruit shape, flesh colour) and resistance to biotic and abiotic stress factors can be developed using genetically divergent and complementary citron watermelon genotypes. However, hybrid citron watermelon breeding has yet to be explored in recombination of useful genes for cultivar development and deployment in sub-Saharan Africa (SSA).

In SSA, citron watermelon is mostly grown for its large yellow and orange-fleshed fruits. In the region the fruits are consumed after cooking various traditional dishes. For instance, traditional cuisines locally referred to as “Kgodu or Mokgapu”, “Semphemphe”, and “Thophi”, in the local South African Sepedi language, are prepared from citron watermelon. The fruits are also used as livestock feed (Jarret et al., 1997; Laghetti and Hammer, 2007; Nantoumé et al., 2013). The leaves are bitter-tasting and serve as a leafy vegetable after cooking and often consumed with maize-meal porridge (Fox and Norwood, 1982; Schippers, 2002; Maggs-Kolling and Christiansen, 2003). The seeds can be roasted and eaten as snack in SSA.

Sweet dessert watermelon has a narrow genetic base (Szamosi et al., 2009; Solmaz et al., 2010; Nimmakayala et al., 2014) attributed to many years of domestication and selection for its desirable horticultural traits such as red-scarlet flesh colour and sweetness (Levi et al., 2001a; Hwang et al., 2011; Lambel et al., 2014; Nimmakayala et al., 2014). For example, in the USA continued use of cultivars such ‘Charleston Gray’, ‘Jubilee’, ‘Crimson Sweet’, ‘Sugar Baby’ and ‘Sugarlee’ which are reported to have a narrow genetic base for breeding, resulted in reduced gene diversity among American watermelon accessions (Wang et al., 2015; Zhang et al., 2016). This has resulted in susceptibility of the crop to random and emerging biotic and abiotic stresses (Levi et al., 2001a; Mo et al., 2016). The low levels of biotic and abiotic stress resistance in dessert watermelon suggest that during many years of cultivation, genes conferring resistance were compromised in favour of selection for red sweet watermelon to satisfy market demands (Levi, et al., 2012).

Conversely, citron watermelon exhibits wider genetic variation (Levi et al., 2000; Dane and Lang, 2004; Levi and Thomas, 2005; Dane and Liu, 2007,) suggesting its genetic worthiness as a source of valuable genes for breeding (Levi et al., 2001b). As a result, incorporation of genes from citron watermelon is proposed as an effective approach for enhancing biotic and abiotic stress tolerance or increasing the levels of genetic diversity in dessert watermelon (Hwang et al., 2011).

Citrulus lanatus var*. lanatus* and *C. lanatus* var. *citroides* are genetically cross compatible (Levi et al., 2001a; McGregor and Waters, 2013; Rivera-Burgos et al., 2021) and the two varieties can be readily crossed (Levi et al., 2001a, 2014; Wechter et al., 2012). This makes the latter an important genetic resource for sweet dessert watermelon breeding (Norton et al., 1995). However, the genetic potential of citron watermelon as useful source of novel genes for breeding is not well explored. There is a need for a dedicated pre-breeding and breeding of citron watermelon for cultivar development. This will allow identification, selection and development of high yielding superior genotypes with enhanced resistance to biotic and abiotic stresses and improved horticultural attributes (e.g., nutritional quality). This will also aid in the development of highly adapted, high-performing dessert watermelon × citron watermelon interspecific crosses with enhanced resistance to multiple stress factors. This review summarized the progress on genetic improvement of citron watermelon to guide cultivar design and development with biotic and abiotic stress resistance and horticultural traits.

1.2. Citron watermelon genetic resources

World's citron watermelon genetic resources held by various institutions has been previously documented (McGregor, 2012). Table 1.1 provides an updated list, information on gene-banks and institutions currently maintaining citron watermelon germplasm. These germplasms provide an important raw material of useful traits such biotic and abiotic stress tolerance and horticultural traits for genetic improvement in this crop or related cucurbit crops such as sweet dessert watermelon (Zhang et al., 2016). The United States Department of Agriculture, Plant Genetic Resource Conservation Unit (USDA-PGRCU) holds the highest collection of citron watermelon germplasm (e.g., approximately 130 accessions) mostly collected from Southern African countries such as Zimbabwe, South Africa, Botswana, Zambia and Swaziland (Levi et al., 2001b, 2014; Wechter et al., 2012). In Southern Africa, citron watermelon genetic resources are preserved by the Southern African Development Community (SADC) Plant Genetic Resources Centre and the National Plant Genetic Resources Centre Regional Network (Mujaju et al., 2010, 2013). Recently, about 70 citron watermelon landrace varieties were collected by Dr. Jacob Mashilo, from diverse districts (e.g., Waterberg, Capricorn, Vhembe and Sekhukhune) of South Africa. These genetic resources are maintained at Towoomba Agricultural Development Centre (TADC), Bela-Bela, South Africa. Farmers in SSA often grow several citron watermelon and dessert watermelon landrace varieties in the same field. This could have rendered possible outcrossing resulting in tremendous genetic diversity between the two *C. lanatus* varieties (Jarret et al., 1997; Levi et al., 2012; Mashilo et al., 2017). Therefore, there is a need to continuously search, collect and strategically conserve "new" ideotypes that could have been preserved and developed through unconscious selection by farmers.

Table 1.1 Citron watermelon germplasm maintained at various institutions globally.

Institution	Country	No. of citron germplasm held	Reference
Beijing Vegetable Research Center	China	58	Zhang et al. (2016)
Towoomba Agricultural Development Centre (TADC)	South Africa	34	Mashilo et al. (2017)
United States Department of Agriculture, Plant Genetic Resource Conservation Unit (PGRCU)	USA	130	Levi et al. (2012)
Institut d'Economie Rural/CRRA-Sotuba	Mali	7	Nantoumé et al. (2013)
The National Engineering Research Center for Vegetables	China	-	Zhang et al. (2011)
National Plant Genetic Resources Center of Zimbabwe	Zimbabwe	62	Mujaju et al. (2010)
University of Cukurova	Turkey	7	Ocal et al. (2014)
Plant Germplasm Bank of the University of Pavia	Italy	5	Guzzon et al. (2017)
Beijing academy of Agriculture and Forestry Science	China	-	Zhang et al. (2011)
Genebank of the Leibniz Institute of Plant Genetics and Crop Plant Research	Germany	-	Achigan-Dako et al. (2015)
National Watermelon and Melon Germplasm Resource Library	China	-	Dou *et al*. (2018)

-, unknown number of collections held

Phenotypic and genotypic analysis of the available citron watermelon genetic resources maintained at various genebanks/institutions or those maintained in farmer's fields should aid in the identification and selection of promising citron watermelon genotypes with suitable agronomic and horticultural traits. This will facilitate effective utilization and developing breeding populations for strategic genetic improvement in this crop or related cucurbits (Nantoumé et al., 2013; Mashilo et al., 2017).

1.3. Genetic diversity in citron watermelon

1.3.1. Phenotypic diversity based on qualitative traits

Citron watermelon exhibits phenotypic diversity for important qualitative traits such as leaf shape, fruit shape and colour, rind stripe patterns, seed shape, seed coat colour and flesh colour (Figures 1.1, 1.2, 1.3, 1.4 & 1.5) (Maggs-Kölling et al., 2000; Achigan-Dako et al., 2015; Mashilo et al., 2016, 2017; Guzzon et al., 2017). Leaf shape in citron watermelon is categorized into lobed and non-lobed types. The degree of leaf lobation varies from tri-lobate to penta-lobate with wide, round and smooth lobes or narrow serrated lobes (Laghetti and Hammer, 2007) (Figure 1.1). Non-lobed leaf shape is a unique morphological trait, since most watermelon varieties (e.g., both sweet dessert and citron) exhibit lobed leaf shape (Wei et al., 2017). A single dominant gene designated as *ClLL1* has been reported to control lobed leaf shape in dessert watermelon (Wei et al., 2017). Fruit shape is elliptic, broad elliptic and elongated elliptic, whereas, fruit colour is either dark green or light green. Rind stripe patters in this crop range from none to narrow, medium or reticulated patchy stripes which vary in colour from green or dark green (Figures 1.2 and 1.3) (Szamosi et al., 2009; Mashilo et al., 2016; Wechter et al., 2016). Flesh colour vary from green-white for non-cooking types referred to as “Kiti” (Figure 1.2) in the South African local Sepedi language (Szamosi et al., 2009; Liu et al., 2013; Ren et al., 2014; Achigan-Dako et al., 2015; Guo et al., 2015; Singh et al., 2017; Zhu et al., 2017) and orange to yellow fleshed for cooking citron types (Figure 1.3) (Kousik et al., 2009; Achigan-Dako et al., 2015; Wechter et al., 2016; Mashilo et al., 2016, 2017).

Seed coat colour vary from cream, dark brown, black, grey, green, red/maroon with black spots and brown with black spots (Figure 1.5) (Achigan-Dako et al., 2015; Mashilo et al., 2016, 2017; Guzzon et al., 2017). Genetic variation in qualitative traits in citron watermelon is important for genetic analysis or breeding including sweet dessert watermelon. In sweet dessert watermelon, important breeding objectives include developing desirable cultivars with phenotypes such as ideal fruit shape, rind patterns and flesh colour based on market demands.

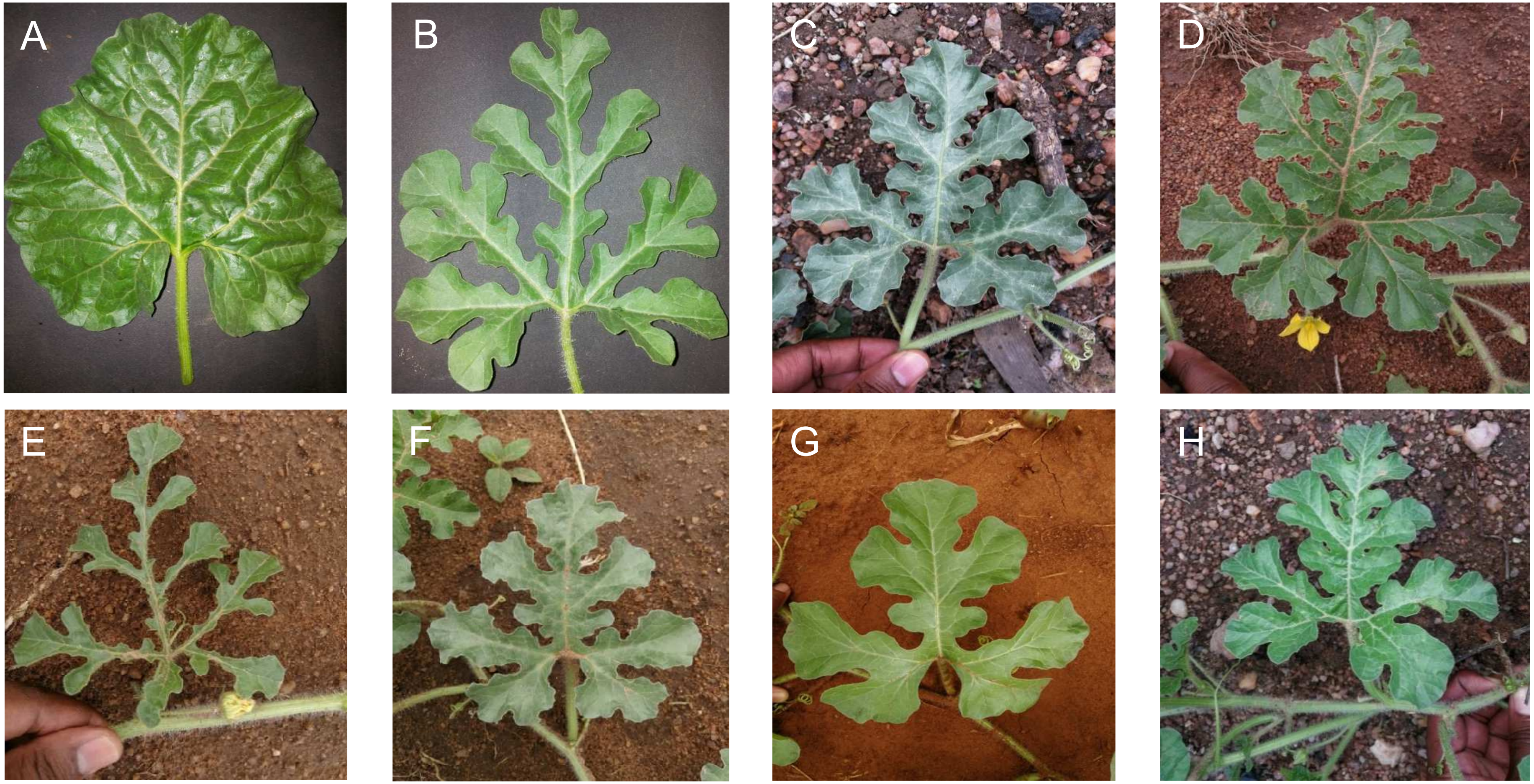

Figure 1.1: Variation in leaf morphology in citron watermelon. A = non-lobed; B, C, D, E, F, H and H = Lobed Leaves with broad and narrow serrated lobes.

Citron watermelon germplasm may provide useful phenotypic variation and horticultural traits for breeding (Mujaju et al., 2013). Genetic variation in fruit traits like fruit size and shape, fruit colour and skin colour patterns, rind thickness, leaf structure and shape, suggests genetic plasticity of the crop to incorporating useful genes (Mujaju et al., 2013).

There is generally limited information detailing phenotypic variation among citron watermelon genetic resources (Levi et al., 2001a; Mujaju et al., 2013). Further, genetic control and mapping of useful fruit traits in citron watermelon is not well-determined and understood. In dessert watermelon quantitative trait loci (QTLs) and genes controlling horticultural attributes including leaf shape (Wei et al., 2017), fruit size (Cheng et al., 2016), fruit length, fruit width, fruit shape, rind thickness (Reddy et al., 2013, 2014), flesh colour (Gusmini and Wehner, 2006; Bang et al., 2010; Cheng et al., 2016) and rind stripe patterns (Gusmini and Wehner, 2006; Cheng et al., 2016), were previously identified. Also, genes conditioning carotene content (Bang et al., 2007; Branham et al., 2017a), sugar profile (Guo et al., 2013), fatty acid profiles (Meru and McGregor, 2014), oil content of seeds (Prothro et al., 2012), flesh bitterness (Cheng et al., 2016) have also been mapped. Understanding the number of genes involved and pattern of inheritance controlling qualitative traits may enhance selection response through phenotypic or genomic tools.

Non-sweet citron and sweet dessert watermelon are two divergent *Citrullus* var. *lanatus* varieties (Jarret et al., 1997; Levi et al., 2012; Mashilo et al., 2016) and genetic mechanisms controlling qualitative traits cannot be assumed to be similar. Levi et al. (2012) observed major differences in ribosomal DNA gene configuration, suggesting substantial differences between dessert and citron watermelon genomic structure. Chloroplast and mitochondrial genomes between dessert and citron watermelon have also been reported to be different (Levi and Thomas, 2005). Further, Reddy et al. (2013) reported the presence of wide chromosomal structural differences between dessert and citron watermelon. Therefore, it can be assumed that genes controlling important horticultural traits in citron watermelon may differ from those in dessert watermelon; however, this assumption remains to be further validated by scientific studies.

Figure 1.2: White-green flesh coloured and small-sized citron watermelon types.

1.3.2. Phenotypic diversity based on quantitative trait

Morphological description of citron watermelon genetic resources were provided by several workers employing quantitative traits (Maggs-Kölling et al., 2000; Szamosi et al., 2009; Achigan-Dako et al., 2015). Important quantitative traits such days to flowering and maturity, fruit length, fruit width, fruit weight, rind thickness and thousand seed weight were reported to be highly variable in citron watermelon (Maggs-Kölling et al., 2000; Achigan-Dako et al., 2015). Hashizume et al. (2003) further reported that citron watermelons collected from South Africa showed a wide diversity in fruit shape (e.g., round, oval, conical or flat-shaped similar to pumpkin), rind colour and stripe patterns, flesh and seed coat colour. However, such studies have not identified or selected promising genotypes to develop breeding populations for cultivar development. Further, genetic variation of other important yield related traits such as number of male flowers per plant, number of female flowers per plant, plant height, number of branches per plant, number of fruits per plant need to the determined for effective selection and breeding (Mashilo et al., 2015). Such traits can be used as direct or indirect selection criteria for improving fruit and seed yield in citron watermelon. As a continuous effort to facilitative efficient utilization of citron germplasm, research on phenotypic description within this crop needs to be further explored to unravel novel genetic variation which may be applied for breeding or direct production.

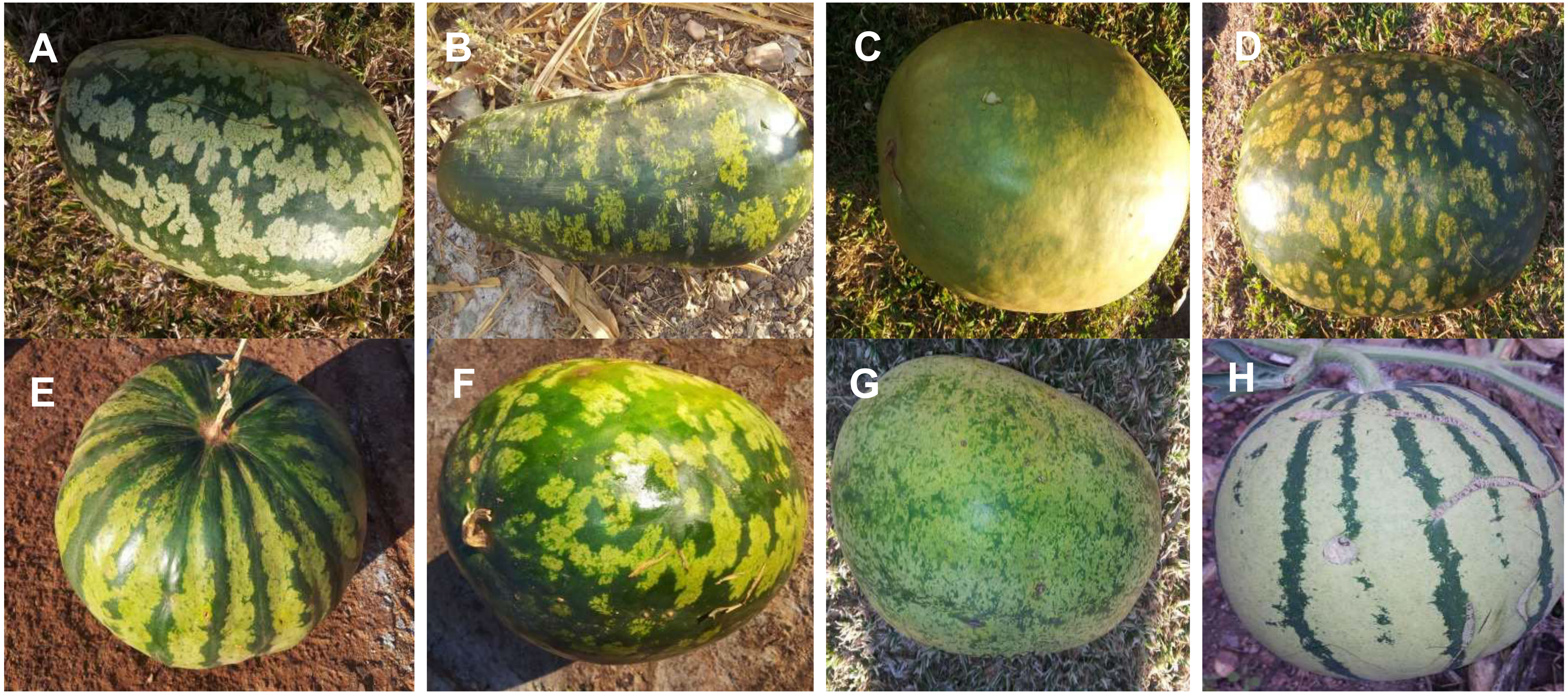

Figure 1.3: Variation for fruit shape, fruit colour and rind stripe patterns of citron watermelon. Fruit shape ranges from round (E, H, L, M), elliptic (N) and elongated (A, B & G). Fruit colour vary from light green (C, G, I & J) and dark green (L, M), Some fruits have grooves on entire fruit (I), Rind stripes range from very narrow, narrow, broad (M), medium (E & H) and reticulated (A, D, F & K), intensity of green colour of stripes vary from very light, light, medium (M), dark and very dark green (L).

Figure. 1.3: (Continued). Variation for fruit shape, fruit colour and rind stripe patterns of citron watermelon. Fruit shape ranges from round (E, H, L, M), elliptic (N) and elongated (A, B & G). Fruit colour vary from light green (C, G, I & J) and dark green (L, M), Some fruits have grooves on entire fruit (I), Rind stripes range from very narrow, narrow, broad (M), medium (E & H) and reticulated (A, D, F & K), intensity of green colour of stripes vary from very light, light, medium (M), dark and very dark green (L).

Figure 1.4: Variation in flesh colour of citron watermelon. A = yellow flesh, B = orange flesh and C = a mixture of yellow and orange flesh.

1.3.3. Genetic diversity based on molecular markers

The level of genetic diversity in citron watermelon has been previously studied using various molecular markers (Table 1.2). Commonly used molecular markers include simple sequence repeat (SSR) markers, expressed sequence tag (EST)-derived simple sequence repeats (EST-SSR) markers, random amplified polymorphic DNA (RAPD) markers, high frequency oligonucleotides (HFO) markers (Levi et al., 2001b, 2012; Mujaju et al., 2010) and peroxidase gene markers (Ocal et al., 2014). These studies revealed high genetic diversity among the studied citron watermelon genotypes useful for improved vigour, yield, biotic and abiotic stress tolerance.

Furthermore, some studies have also detailed that cooking citron watermelon (e.g., yellow- and orange-fleshed) or green-white-fleshed types (Levi et al., 2012) were investigated. The white- to green-fleshed citron watermelon types are characterized by small fruits (≤ 10 cm in length), thin rinds and limited variation in seed coat colours (i.e., mostly dark brown, green or black seeds), fruit shape and rind stripe patterns (Figure 1.2). Fruit size has recently become an important trait for breeders due to increased consumer preference for smaller sized watermelons (Gusmini and Wehner, 2007). Small-sized citron watermelons should be useful genetic resource for breeding and development of small-sized sweet dessert watermelon cultivars in future. The orange- and yellow-fleshed citron types are large fruited, with thick rinds and exhibits considerable phenotypic variation in fruit shape, colour, rind stripe patterns and seed coat colour (Figure 1.3). Whether phenotypic differences between citron types with contrasting flesh colours (white-green vs. orange or yellow) and fruit shapes (large-sized vs small-sized) are indicative of genetic differences at molecular level remains unknown.

Table1.2 Molecular marker type used, number of accessions examined with corresponding country of origin or study country in genetic diversity analysis of citron watermelon.

Marker type	No. of accessions	Country of origin of accessions	Study country	References
PCR-RFLP & SA	70	India (1), Namibia (22), Botswana (1) South Africa (18), Swaziland (3) Zambia (2), Zimbabwe (18), Zaire (2) Spain (2), Yugoslavia (2) USA (3)	USA	Dane and Liu (2007)
SSR	34	South Africa	South Africa	Mashilo et al. (2017)
RAPD	12	Zaire (1), Namibia (1), South Africa (8) Zimbabwe (1), Afghanistan (1)	USA	Levi et al. (2001a)
HFO-TAG	74	Zaire (1), South Africa (19), Zimbabwe (22) SwaZiland (4), Namibia (1), Zambia (4) Iran (1), Afghanistan (1)	USA USA	Levi et al. (2012)
SSR	58	Zaire (1), Zambia (4), Spain (1), Canada (1) South Africa (10), Serbia & Montenego (1) Zimbabwe (33), Rupland (1), Umbekant (7) Ukraine (1), Libya (1), Congo (1), USA (1)	USA	Zhang et al. (2016)
Phenotypic traits	21	Namibia (20), Zimbabwe (1)	Namibia	Maggs-Kolling et al. (2000)
Phenotypic traits	42	Congo (1), Egypt (1), India (1), Namibia (1) Russia (1), South Africa (10), Switzerland (1) Unknown country (1), Yemen (1), Zaire (1) Zimbabwe (1)	Benin	Achigan-Dako et al. (2015)

Table 1.2 (Continued).

Marker type	No. of accessions	Country of origin of accessions	Study country	References
Phenotypic traits	3	Hungary (2), Romania (1)	Hungary	Szamosi et al. (2009)
RAPD	6	South Africa (3), Zimbabwe (1), France (1) USA (1)	Turkey	Solmaz et al. (2010)
EST-SSR	11	Zimbabwe	Zimbabwe	Mujaju et al. (2013)
SSR	7	Mali	Mali	Nantoumé et al. (2013)
RAPD & SSR	50	Zimbabwe	Zimbabwe	Mujaju et al. (2010)
Peroxidase gene Markers	7	Spain (1), USA (2), Zimbabwe (2), France (1) Hungary (1)	Turkey	Ocal et al. (2014)
SSR		Botswana (2), South Africa (1), Zimbabwe (1)	USA	Jarret et al. (1997)
SSR		South Africa (10), Zimbabwe (2)	China	Zhu et al. (2016)

PCR-RFLP = Reverse transcriptase reaction, random fragment length polymorphism, SA = Sequence analysis, SSR = Simple sequence repeat markers, RAPD = Random amplified polymorphic DNA, HFO-TAG = High frequency oligonucleotides: targeting active gene markers, EST-SSR = Expressed sequence tag (EST)-derived simple sequence repeats

Figure 1.5: Variation in seed coat colours among citron watermelon genetic resources. A = black, B = dark brown with dark spots, C = green, D = maroon with dark spots, E = maroon, F = cream/light brown, G = red, H = brown, I = grey.

1.4. Breeding progress in citron watermelon

1.4.1. Biotic stress tolerance

Citron watermelon genotypes with resistance to several diseases have been identified and reported elsewhere (McGregor, 2012; Levi et al., 2017). A comprehensive list of citron watermelon genotypes is presented in Table 1.3. These genotypes are useful raw material for disease resistance breeding, gene mapping or studying the genetic control of disease resistance and resistance mechanisms in citron watermelon (Cohen et al., 2014). Majority of unique citron germplasm originated from sub-Saharan Africa, with countries such as South Africa and Zimbabwe possessing the largest number of genotypes showing durable disease resistance (Table 1.3). Various studies also reported that citron watermelon germplasm from SSA have been used in disease resistance breeding programs in the USA (Gusmini et al., 2005; Tetteh et al., 2010; Wechter et al., 2012; Ma and Wehner, 2015; Thies et al., 2015a).

Citron watermelon harbours novel genes for biotic stress tolerance breeding for sweet dessert watermelon (Edelstein et al., 2014; Fredes et al., 2017). Functional genes were reported from citron watermelon for resistance to the major economically diseases of sweet dessert watermelon. These diseases include such as root-knot nematodes [*Meloidogyne incognita*] (Thies and Levi, 2007; Thies et al., 2010, 2015b, 2016; Levi et al., 2012), fruit rot caused by *Phytophthora capsica* (Kousik et al., 2012), gummy stem blight [*Didymella bryoniae* (Auersw.)] (Gusmini et al., 2017), papaya ringspot virus type-W (Strange et al., 2002), powdery mildew [*Podosphaera xanthii* (Castagne) Braun & Shishkoff] (Thomas et al., 2005; Tetteh et al., 2010), *Fusarium* wilt (Wechter et al., 2012), bacterial fruit blotch (*Pseudomonas avenae ssp. Citrulli)* (Ma and Wehner, 2015; Choi et al., 2016*)* and *Zucchini* yellow mosaic virus (Provvidenti, 1991). It has varied fruit quality traits (e.g. thicker rind, lower sugar content and low total soluble solids) compared with other cucurbit rootstocks. Consequently, citron watermelon is used as a rootstock for dessert watermelon breeding and production (Edelstein et al., 2014; Fredes et al., 2017). Therefore, citron watermelon genetic resources from sub-Saharan Africa have proved to be a rich source of resistance/tolerance to many diseases, underlining the importance of conservation and utilization of germplasm from this region (McGregor, 2012).

Citron watermelon genotypes with improved disease resistance have been developed. For instance, genotypes USVL246-FR2 and USVL252-FR2 exhibited higher level of resistance to *Fusarium* wilt race 2. These were bred using accessions PI 482246 and PI 482252 each expressing high level of resistance to *Fusarium* wilt race 2 (Wechter et al., 2016). An autotetraploid (4N = 44) germplasm line “USVL-360” was developed from the citron watermelon accession PI 299379 which was resistant to anthracnose races 1 and 2 and *Fusarium* wilt race 2 (Wechter et al., 2012; Levi et al., 2014).

Table 1.3 Citron watermelon genotypes with resistance to economically important diseases of sweet dessert watermelon.

Genotypes	Country of origin	Disease	Reference
PI 255136, PI 270564, PI 271769, **PI 271779**	South Africa	FW Race 2	Wechter et al. (2012); Dane and Liu (2007)
PI 296335, PI 296339, **PI 299379**	South Africa		Wechter et al. (2012); Dane and Liu (2007)
PI 482246, PI 482252, PI 482273, PI 482276, **PI 482308**	Zimbabwe	FW Race 2	Wechter et al. (2012); Boyhan et al. (2003)
PI 482277, PI 482279, **PI 482282**, **PI 482331**	Zimbabwe		Wechter et al. (2012)
PI 457916	Liberia,	FW, SRKN, MJ	Cohen et al. (2014)
PI 459075	Botswana	FW, SRKN, MJ	Cohen et al. (2014)
PI 482303, **PI 482338**, PI 482326, PI 482319, PI 255137	-	MA race 2 & 3	Thies et al. (2007)
PI 482259, PI 542119, PI 244017, PI 485583, PI 542114	-		Thies et al. (2007)
PI 189225	Zimbabwe	SRKN	Thies et al. (2016)
PI 244017, PI 244018	South Africa		
PI 482259, PI 482324	Zimbabwe		Thies et al. (2007)
PI 482326, PI 482307, PI 482311, PI 482302	Zimbabwe	PM Race 2W	Tetteh et al. (2010); Thomas et al. (2005)
PI 482298, **PI 482282**, PI 482286, **PI 482338**, **PI 482322**	Zimbabwe		Ben-Naim and Cohen (2015)
PI 482246, PI 482283, **PI 189225**, PI 225557, PI 482312	Zimbabwe		
PI 500354	Zambia		Tetteh et al. (2010); Thomas et al. (2005)
PI 271711, PI 296341	South Africa		
PI 295850	-		Tetteh et al. (2010); Thomas et al. (2005)
PI 596667	-		
PI 270563	-		Tetteh et al. (2010); Thomas et al. (2005)
PI 299378	-		Tetteh et al. (2010); Thomas et al. (2005)
PI 532624	-		

Table 1.3 (Continued),

Genotypes	Country of origin	Disease	Reference
PI 482379, PI482276, PI 482315, PI 482283, PI 482284, PI 482257, PI 482267, PI 482342, PI 482294, PI 482357,	Zimbabwe	GSB	Gusmini et al. (2005); Boyhan et al. (1994)
PI 482297, PI 482374, PI 482307, PI 482343, PI 482293	Zimbabwe		Gusmini et al. (2005)
PI 482260, PI 482326	Zimbabwe		Gusmini et al. (2005)
PI 189225	Zimbabwe		Gusmini et al. (2005)
PI 271771, **PI 271778**, **PI 244019**, PI 296332, PI 296339, PI 296337	South Africa		Gusmini et al. (2005)
PI 271770, PI 296343, PI 271773, PI 296342, PI 274035	South Africa		Gusmini et al. (2005)
PI 490383, PI 490375, PI 490384, PI 490376	Mali		Gusmini et al. (2005)
PI 512398, PI 512388, PI 512361	Spain		Gusmini et al. (2005)
PI 379243, PI 357678	Yugoslavia		Gusmini et al. (2005)
PI 279461, PI 319237, PI 227203	Japan		Gusmini et al. (2005)
PI 254774	Senegal		Gusmini et al. (2005)
PI 164248	Liberia		Gusmini et al. (2005)
PI 249009	Nigeria		Gusmini et al. (2005)
PI 211915	Iran		Gusmini et al. (2005)
PI 508443	Korea		Gusmini et al. (2005)
PI 542214, PI 542123	Botswana		Gusmini et al. (2005)
PI 241689	Chile		Gusmini et al. (2005)
PI 500312, PI 500334, PI 500335	Zambia		Gusmini et al. (2005)
PI 271982	Somalia		Gusmini et al. (2005)
PI 247398	Greece		Gusmini et al. (2005)
PI 195771	Guatemala		Gusmini et al. (2005)
PI 470248	Indonesia		Gusmini et al. (2005)
PI 277979	Turkey		Gusmini et al. (2005)
PI 270546	Ghana		Gusmini et al. (2005)

Table 1.3 (Continued).

Genotypes	Country of origin	Disease	Reference
PI 482322, PI 482252, PI 482265, PI 482277, PI 482283	Zimbabwe	BFB	Ma and Wehner (2015)
PI 482309, PI 482319, PI 482324, **PI 482331**	Zimbabwe		Ma and Wehner (2015)
PI 482342,	Zimbabwe		Ma and Wehner (2015)
PI 271770, PI 596665, PI 596666, PI 596668, PI 596669	South Africa		Ma and Wehner (2015)
PI 500354	Zambia		Ma and Wehner (2015)
PI 485583	Botswana		Ma and Wehner (2015)
PI 532738	Zaire		Ma and Wehner (2015)
PI 189225, **PI 244018**, PI 482559, PI 482324, PI 244017		MI	Thies et al. (2016)
PI 500331	Zambia	PM Race 1W	Davis et al. (2007)
PI 482333, PI 482312, **PI 482308**	Zimbabwe		Davis et al. (2007)
PI 525083	Egypt		Davis et al. (2007)
PI 482308, PI 482276, PI 482299	Zimbabwe	ZYMV	Boyhan et al. (2003); Provvidenti (1991) Guner (2004); Gusmini et al. (2005)
PI 244018, PI 244017, **PI 244019**, PI 255137	South Africa	PRSV–W	Strange et al. (2002)
PI 482299, PI 482322, PI 482379	Zimbabwe		Strange et al. (2002)
PI 485583	Botswana		Strange et al. (2002)
PI 270562, PI 270563, PI 271775, **PI 271779**, PI 299379	South Africa	AR–1	Levi et al. (2001a); Sowell et al. (1980)
PI 271778, PI 271779, **PI 299379**	South Africa	AR–2	Levi et al. (2001a)
PI 482252	Zimbabwe	WMV	
PI 255137, **PI 244018**, **PI 244019**	South Africa	WMV	Gillaspie and Wright (1993)
PI 189225	Zaire	FR	Kousik et al. (2012)
PI 500354	Zambia	SqVYV	Kousik et al. (2009)

AR–1 = anthracnose races 1; AR–2 = anthracnose race 2; BFB = bacterial fruit blotch; FW = *Fusarium* wilt; GSB = gummy stem blight; PM = powdery mildew; PRSV–W = papaya ringspot virus—watermelon strain; RKN = root knot nematodes; WMV = watermelon mosaic virus; ZYMV = *Zucchini* yellow mosaic virus; FR = Fruit rot, SqVYV = squash vein yellowing virus; *MJ = Meloidogyne javanica; MI = Meloidogyne incognita; MA = Meloidogyne arenaria.* Genotypes boldfaced are resistant to several diseases, – = unknown

Further, citron watermelon has been used as rootstock for sweet dessert watermelon production, resulting in higher yields compared to other cucurbit rootstocks under root-knot nematodes infestation (Thies et al., 2010, 2015a, 2016). The United States Department of Agriculture, Agricultural Research Services (USDA-ARS) has developed several citron watermelon rootstocks recommended for sweet dessert watermelon cultivation (Table 1.4). Citron watermelon F_1 hybrid rootstocks have also been developed which had high level of resistance to root-knot nematodes equal or greater than that of their respective parents. This suggest that the genes for disease resistance could be pyramided in the progeny to produce even greater levels of disease resistance for development of highly tolerant root-knot nematodes *citroides* rootstocks (Thies et al., 2015b). However, the currently available number of citron watermelon rootstocks are limited and further studies are needed to identify additional potential germplasm for rootstock development (Thies et al., 2010).

Citron watermelon F_1 hybrid rootstocks resistant to other economically important diseases of sweet dessert watermelon have not yet been developed. Additionally, there are no F_1 hybrids developed with multiple disease resistance for use as rootstocks for sustainable dessert watermelon production. Several attempts have been made to study the genetic inheritance of major diseases of watermelon such as *Fusarium* wilt races 0, 1 and 2 (Zhang and Rhodes, 1993), *Zucchini* yellow mosaic virus (Provvidenti, 1991), watermelon mosaic virus (Xu et al., 2004), powdery mildew race 2 (Kim et al., 2013; Tetteh et al., 2010; Ben-Naim and Cohen, 2015), anthracnose race 2 (Suvanprakorn and Norton, 1980) and gummy stem blight (Gusmini et al., 2017).

Genetic control of these diseases was mostly studied using elite sweet watermelon genotypes (Tetteh et al., 2013) or citron × dessert watermelon interspecific crosses (Tetteh et al., 2013). In these crosses citron watermelon was used as the resistant parent and sweet dessert watermelon as the susceptible parent. Some studies have reported the inheritance of genes controlling disease resistance between citron × dessert watermelon crosses (Gusmini et al., 2017).

Table 1.4 Citron watermelon genotypes and F$_1$ hybrids developed for use as rootstock for sweet dessert watermelon cultivation.

Genotype	Country of origin	Resistance	References
BGV0005167	Spain	-	Fredes et al. (2017)
PI 296341-FR	South Africa	*Fusarium* wilt race 2 *Fusarium* crown rot	Cohen et al. (2014)
PI 457916	Liberia	*Fusarium* wilt race 2 *Fusarium* crown rot	Cohen et al. (2014)
PI 482318		*Meloidogyne javanica*	Cohen et al. (2014)
PI 459075	Botswana	*Fusarium* wilt race 2	Cohen et al. (2014)
RKVL-318, RKVL-301, RKVL-315, RKVL-16 RKVL-302	USA	Southern root knot nematodes	Thies et al. (2010, 2015)
F$_1$ hybrid rootstocks			
RKVL 301 × 316, RKVL 301 × 318, RKVL 318 × 301, RKVL 318 × 317 RKVL 316 × RKVL 301, RKVL 316 × 317, RKVL 316 × 318, RKVL 301 × 317 RKVL 317 × 318	USA	Southern root knot nematodes	Thies et al. (2015b)

Resistance to *Fusarium* wilt race 2 was reported to be governed by at least a pair of recessive gene, with a dominant gene from a susceptible parent being epistatic to the recessive gene. The resistance gene was detected in the citron accession PI 296341-FR. Segregation distortion have been observed in the F_2 mapping population developed from a cross between PI 296341-FR and 'New Hampshire Midget' (Hawkins et al., 2001a). Mapping studies have also shown the presence of variable segregation patterns which were non-Mendelian between dessert and citron watermelon crosses (Levi et al., 2002).

There are limited studies that investigated inheritance of major disease resistance genes between elite citron watermelon genotypes. Investigating the inheritance patterns between closely related *citroides* genotypes may provide breeding populations with Mendelian segregation pattern for most phenotypic markers (Branham et al., 2017a). For example, citron genotypes PI 482283 and PI 526233 were highly resistant to gummy stem blight (Gusmini et al., 2017) than PI 189225 and PI 271778. This may reveal a different genetic control of gummy stem blight in citron watermelon. Citron watermelon genotypes USVL246-FR2 and USVL252-FR2 which were resistant to *Fusarium* wilt race 2 (Wechter et al., 2016) and PI 542114 (USVL114) (Branham et al., 2017a) susceptible to *Fusarium* wilt race 2 can be useful genetic material in studying the inheritance of this disease in citron watermelon. Such studies may also be extended to other major diseases of watermelon (Table 1.2). Genetic populations developed in this scheme may be useful in the development of accurate genetic linkage maps and for locating most QTL associated with resistance to major diseases of watermelon (Branham et al., 2017b).

Previous studies have mapped most QTL linked to fruit traits and disease resistance using dessert × citron watermelon-derived populations (Table 1.7). Very few studies are available that developed genetic linkage maps and identified QTL of important traits or disease resistance derived from citron watermelon populations (Branham et al., 2017a). Further, there exists a limited number of molecular markers linked to resistance to major diseases in citron watermelon which hinders the transfer of resistance genes into dessert watermelon. Development of such markers may facilitate rapid identification of resistant genotypes for breeding, genetic analysis and marker-assisted selection (Branham et al., 2017b).

1.4.2. Abiotic stress tolerance

Citron watermelon is relatively a drought tolerant crop (Akashi et al., 2001; Yoshimura et al., 2008; Zhang et al., 2011; Mo et al., 2015) compared with dessert watermelon. This makes it a useful source of genes for drought tolerance breeding in dessert watermelon (Mo et al., 2015; Rhee et al., 2015). Owing to its drought tolerance citron watermelon is an ideal crop for cultivation in arid and semi-arid environments.

Table 1.5 Citron watermelon genotypes identified as drought tolerant.

Genotype	Country of origin	References
PI 482246	Zimbabwe	Zhang et al. (2011)
PI 482312	Zimbabwe	Zhang et al. (2011)
PI 482311	Zimbabwe	Zhang et al. (2011)
PI 482316	Zimbabwe	Zhang et al. (2011)
PI 482283	Zimbabwe	Zhang et al. (2011)
PI 500332	Zambia	Zhang et al. (2011)
PI 482308	Zimbabwe	Zhang et al. (2011)
PI 482309	Zimbabwe	Zhang et al. (2011)
PI 500335	Zambia	Zhang et al. (2011)
PI 482303	Zimbabwe	Zhang et al. (2011)
PI 482307	Zimbabwe	Zhang et al. (2011)
PI 482302	Zimbabwe	Zhang et al. (2011)
Anguria da marmellata/mostarda	Italy	Guzzon et al. (2017)
Anguria da mostarda	Italy	Guzzon et al. (2017)
Anguria da mostarda da semi verdi	Italy	Guzzon et al. (2017)
Anguria da mostarda da semi rossi	Italy	Guzzon et al. (2017)
Kormovoy 4–73	Ukraine	Guzzon et al. (2017)
Bethulie	South Africa	Guzzon et al. (2017)
Bogarnyi 112	Uzbekistan	Guzzon et al. (2017)
Colourado green seeds	United States of America	Guzzon et al. (2017)
Saratovskyi	Russia	Guzzon et al. (2017)
Colourado Prosor	Canada	Guzzon et al. (2017)

Citron watermelon germplasm with drought tolerance have been identified (Guzzon et al., 2017) (Table 1.5). Therefore, it can serve as useful genetic material for drought tolerance breeding or commercial rootstock development for dessert watermelon or related cucurbit crops. Further, the identified genotypes are useful for investigating unique mechanisms (e.g., physiological and molecular) for abiotic stress tolerance and for identifying, mapping and pyramiding genes for abiotic stress tolerance. However, incorporating drought tolerance genes from citron to dessert watermelon has not been successfully achieved as yet. As a result, no dessert watermelon genotypes with improved drought tolerance have been bred and released for cultivation so far. Further, it is not known whether the level of drought stress tolerance between crosses of elite citron watermelon genotypes could be improved. Citron watermelon F_1 hybrid rootstocks with enhanced resistance to root-knot nematodes have been developed (Thies et al., 2015b) suggesting this possibility. These genotypes may be used as rootstocks for improving water-use efficiency of dessert watermelon grown in dry and hot environments. This necessitates further characterization on citron watermelon germplasm to identify other sources of resistance including abiotic stress tolerance (Guzzon et al., 2017).

1.4.3. Breeding progress in citron watermelon for fruit quality traits

Citron watermelon possess considerable variation for fruit quality traits than can be exploited in the breeding of sweet dessert watermelon (Mashilo et al., 2017). Flesh colour, sugar content and rind stripe patterns are important fruit quality traits in dessert watermelon improvement programmes (Gusmini and Wehner, 2005, 2006). Yellow- and orange-fleshed (Figure 1.4) citron watermelon types may have significantly higher carotenoid contents (e.g., prolycopene, phytoene, and ζ-carotene, violaxanthin, neoxanthin and β-carotene) (Bang et al., 2007, 2010; Davis et al., 2007). These attributes are important for developing unique varieties with specific colour profiles including optimal levels of carotenoids composition (Bang et al., 2007, 2010; Branham et al., 2017a). The bright yellow and orange ripe fruit flesh of citron watermelon is a characteristic of some sweet dessert watermelon cultivars (e.g. Yellow Doll, NY0016, PI 192937, H12, H13, H14, H15, H18, H20, H22, H24, H25, H31, H36 H26, H29, Amarillo, PI 221430, PI 193964, PI 189316, PI 270306, PI 270306, PI 163572, 5W3308, 5WI055, 5WI056, 5WI057, 5WI058, 5WI060, 5WI061, PI 165002, Early Moonbeam, Yellow Sunshine, Sweet Siberian-1, Sweet Siberian-2, Texas golden, Black Diamond Yellow, Luscious Golden-1, Luscious Golden-2, Golden Honey Cream, PI 183398 and PI 255137) (Tadmor et al., 2005; Bang et al., 2007, 2010; Nimmakayala et al., 2009; Solmaz and Sari, 2009; Szamosi et al., 2009; Yoo et al., 2012; Singh et al., 2017; Zhu et al., 2017). This makes orange- and yellow-fleshed citron watermelon germplasm a useful source of genes for improved carotenoids contents in sweet yellow and orange-fleshed dessert watermelon cultivars. Therefore, understanding molecular and genetic basis of carotenoid biosynthesis is fundamental for targeted breeding aimed at improving carotenoid content of yellow and orange-fleshed citron watermelon cultivars for cooking purposes or direct consumption (Kang et al., 2010; Branham et al., 2017b).

Comparative studies on watermelon fruit ripening and transcriptome analysis have mostly tended to use the non-ripening, white-green fleshed citron watermelon line PI 246341-FR (Liu et al., 2013; Guo et al., 2015; Zhou et al., 2016). There is no study that reported fruit ripening physiology using yellow or orange-fleshed citron types. Mechanisms of fruit ripening at physiological and molecular level have been shown to be completely different between the two varieties (Guo et al., 2015). There is still a lack of knowledge on genes involved in watermelon ripening due to a limited understanding of the physiology of watermelon fruit development. For example, gene expression during fruit development of yellow and orange-fleshed citron watermelon has not been previously investigated. Differences in flesh colour in citron watermelon is important for comparative genetic analysis and to identify underlying molecular and physiological mechanisms of flesh colour during fruit development in this crop. Further, extensive research on genetic control of flesh colour in sweet dessert watermelon has been carried out; however, there are very limited studies on genes controlling flesh colour in citron watermelon.

Increased fruit sugar measured as total soluble solids (TSS) content is another important breeding objective in sweet dessert watermelon improvement programmes (Davis et al., 2008; Zhang et al., 2012). Citron watermelon has low total sugar content (~3.45% TSS) (Hashizume et al., 2003; Perkins-Veazie et al., 2006). The proportions of fructose, glucose and sucrose contents are 3.95, 1.57 and 0.35 mg/g fresh weight, respectively (Ren et al., 2012). These values were lower than 11, 31.2, 18.75 and 42.7 mg/g fresh weight, respectively reported by (Ren et al., 2012). Citron watermelon may therefore be a useful candidate for breeding dessert watermelon cultivars for consumers interested in low sugar content for dietary purposes (Davis et al., 2008). Understanding sugar metabolism in citron watermelon may therefore lead to novel selection criteria or molecular genetic strategies to improve soluble sugar levels in this crop. Also, there exists extensive variation in rind stripe patterns (Mashilo et al., 2017).

Figure 1.3 present citron watermelon genotypes with unique rind stripe patterns that can be explored in the breeding of interesting stripe patterns. Further, understanding the genetic control of rind stripe patterns in citron watermelon can aid further identification of QTLs linked to this trait for rapid genetic analysis, cloning of genes and in marker-assisted breeding.

1.5. Genomic resources for citron watermelon breeding

Genomic resources and genetic linkage maps (Table 1.6) have been developed for dessert watermelon that incorporated citron watermelon genome. However, few molecular tools have been specifically developed for citron watermelon (Zhang et al., 2004, 2012, 2018; Ling et al., 2009; Guo et al., 2013; Ren et al., 2014; Shang et al., 2016,). For example, large number of integrated linkage maps to determine QTLs controlling important horticultural traits and diseases resistance (e.g., *Fusarium* wilt) have been developed using crosses derived from dessert and citron watermelon. Molecular markers can be utilized for QTL analysis in other crosses between dessert and citron watermelon (Hashimuze et al., 2003). So far, only one genetic linkage map has been developed for citron watermelon with QTLs linked to *Fusarium* wilt race 2 resistance (Branham et al., 2017a) (Table 1.6). Qualitative trait locus' (QTL's) namely: qFon2-9, qFon2-2, qFon2-5, qFon2-8 and qFon2-10 were reportedly linked to *Fusarium* wilt race 2 resistance in citron watermelon (Ren et al., 2015; Branham et al., 2017b). Other genomic resources were developed for sweet dessert watermelon such as SSR (Joobeur et al., 2006; Zhang et al., 2012; Zhu et al., 2016), insertion-deletion markers (Ren et al., 2012, 2014), structure variation markers, SNPs (Ren et al., 2012; Nimmakayala et al., 2014) and RAPD markers. These marker systems may also be useful in genetic analysis, marker-assisted selection and breeding in citron watermelon (Mujaju et al., 2010; Munnisse et al., 2013; Nantoumé et al., 2013; Mashilo et al., 2017). Further, molecular tools of cross-compatible cucurbit crops could be exploited for genetic analysis, genetic linkage map construction and marker-assisted selection in citron watermelon genetic improvement and cultivar development (Table 1.7). For example, EST-SSR and SSR markers developed for cucumber (*Cucumis sativus* L.) were shown to be transferable to watermelon (Hu et al., 2010, Watcharawongpaiboon and Chunwongse, 2008). Simple sequence repeat markers (SSR) developed for bitter gourd (*Momordica charantia* L.) were also reported to be transferable to watermelon (Ji et al., 2012).

Next-generation sequencing (NGS) technologies (e.g., Roche/454 and Illumina/Solexa) platforms offer unique opportunities to study genomics and functional genomics in crops such as watermelon (Guo et al., 2011). Next-generation sequencing (NGS) technologies generate large amounts of sequence information that aid in the development of microsatellite markers (Zhu et al., 2016). In watermelon, Ren et al. (2012) identified 13,744 putative SSR markers and 1877 unique SSRs with long repeat motifs used for genetic analysis and genetic map construction. Similarly, Zhu et al. (2016) identified a total of 39,523 SSR markers from the watermelon draft genome of which 32 SSR loci were used for genetic diversity analysis of watermelon (e.g., sweet and citron) accessions. Lambel et al. (2014) also identified a major QTL associated with *Fusarium* wilt race 1 based on genotyping-by-sequencing (GBS) technology which produced 266 SNP markers.

The construction of high-resolution intraspecific genetic maps and QTL analysis in watermelon has been made possible by NGS (Sandlin et al., 2012; Ren et al., 2014). Bang et al. (2007) using gene sequence data identified a SNP marker in lycopene b-cyclase (LCYB) for distinguishing between canary yellow and red fleshed watermelons. Thus, NGS technology will aid in marker-assisted breeding via development of efficient molecular tools for genetic improvement and cultivar development of crops such as citron watermelon.

1.6. Research focus areas to facilitate variety design, development and deployment in citron watermelon

Citron watermelon exhibits extensive phenotypic diversity comparable with sweet dessert watermelon. However, limited studies have reported the phenotypic diversity present among citron watermelon genotypes, especially in areas of the centre of diversity of the crop. There are abundant collections of citron watermelon germplasm maintained in various gene banks worldwide, which offer considerable opportunities for trait improvement and product development (Zhang et al., 2016). However, unavailability of information on phenotypic diversity hamper the successful utilization of the genetic potential of these collections for effective breeding (Zhang et al., 2016). There is a need for additional studies to be conducted to identify useful horticultural traits among citron watermelon genetic resources using genetically diverse accessions from the centers of origin for genetic improvement in this crop or dessert watermelon (Laghetti and Hammer, 2007; Zhang et al., 2016).

Table 1.6 Genetic linkage maps derived through crosses between dessert and citron watermelon.

No and type of marker	Population	Species	LG	Map length (cM)
RAPD, RFLP, Isozyme, morphological	F_1BC_1, H-7 × SA-1	*lanatus* × *citroides*	11	524
155 (RAPD), 1 (SCAR)	BC_1, PI 296341-FR × Hew Hampshire Midget	*citroides* × *lanatus*	17	1295
Isozyme, RAPD, SSR	$F_{2:3}$, Hew Hampshire Midget × PI 296341-FR	*lanatus* × *citroides*	5	112.9 (F_2), 139 (F_3)
141 (RAPD), 1 (SCAR), 21 (ISSR)	F_2, Griffin 14113 × Hew Hampshire Midget	*Citroides* × *lanatus*	25	1166.2
477 (RAPD), 53 (RLPP), 23 (ISSR), 1 (Isozyme)	F_2, H-7 × SA-1	*lanatus* × *citroides*	11	1729
87 (RAPD), 13 (ISSR), 4 (SCAR) 30 (ISSR)	RIL; F_2S_7, 97103 × PI 296341-FR	*lanatus* × *citroides*	15	1027.5
89 (SRAP), 72 (TRAP), 111 (HFO-TAG)	RIL, BC_2F_2, PI 494817× Crimson sweet	*citroides* × *lanatus*	51	2162
	RIL, BC_2F_2, Griffin 14113 × Hew Hampshire Midget × PI 386 015	*citroides* × *lanatus* × *colocynthis*	41	2760.8
168 (SSR), 219 (InDel), 36 (SV)	F_8, 97103 × PI 296341-FR	*lanatus* × *citroides*	11	800
	F_2, ZWRM50 × PI 244019	*lanatus* × *citroides*		1144
SSR, InDel, SV, SNPs	F_8, 97103 × PI 296341-FR	*lanatus* × *citroides*	-	798
	F_2, ZWRM50 × PI 244019	*lanatus* × *citroides*		
10480 (SNPs)	F_2, PI 482362 × citroides (not mentioned)	*lanatus* × *citroides*	-	1096
232 (SNPs), 50 (SSR)	F_1, PI 224018 × PI 306270	*citroides* × *lanatus*	11	924.72
3465 (SNPs)	F_2, K3 × PI 189225	*lanatus* × *citroides*	11	1099.2
33 (EST-SSR)	F_2, PI 189225 × TS	*citroides* × *lanatus*	-	1178.3
2495 (SNPs)	$F_{1:2}$, USVL246-FR2 × PI 542114	*citroides* × *citroides*	11	1798.9
186 (CAPS)	BC_1, W1−1 × PI 186490	*lanatus* × *citroides*	-	1468.09

Table 1.6 (Continued).

Traits associated with the putative gene/QTL	References
-	Hashizume et al. (1996)
fruit quality and *Fusarium* wilt races 1 & 2 resistance	Levi et al. (2001b)
Fusarium wilt races 1 & 2 resistance	Hawkins et al. (2001b)
-	Levi et al. (2002)
Rind hardness, yellow & red flesh colour, sugar content	Hashizume et al. (2003)
-	Zhang et al. (2004)
-	Levi et al. (2011)
Fruit size and fruit shape	Ren et al. (2012)
Brix, fructose, glucose and sucrose	Ren et al. (2014)
-	Reddy et al. (2014)
-	Nimmakayala et al. (2014)
Fusarium wilt race 1& 2 resistance	Ren et al. (2015)
-	Rhee et al. (2015)
Fusarium wilt race 2 resistance	Branham et al. (2017b)
Flesh colour, find stripe, flesh bitterness	Zhang et al. (2018)

RAPD = random amplified polymorphic DNA markers; RFLP = restriction fragment length polymorphism markers; SCAR = sequence characterized amplified region markers; SSR = simple sequence repeat markers; ISSR = inter-simple sequence repeats; SRAP = sequence related amplified polymorphism markers; TRAP = target region amplification polymorphism; HFO–TAG = high frequency oligonucleotides: targeting active gene markers; InDel = insertion-deletion markers; SV = structure variation markers; SNPs = single nucleotide polymorphism markers; EST–SSR = Expressed sequence tag (EST)-derived simple sequence repeats markers; CAPS = cleavage amplified polymorphic sequence markers; LG = Linkage group; QTL, quantitative trait loci; cM = centimorgans. - = unknown.

Citron watermelon is an important crop in drought-prone arid and semi-arid areas. In SSA drought is the main abiotic stress factor for sustainable crop production (Zhang et al., 2011). Citron watermelon is adapted to extreme heat and drought stress conditions providing an attractive source of useful genetic traits for improving drought tolerance of cultivated sweet dessert watermelon (Kawasaki et al., 2000; Mo et al., 2016). However, success to incorporate genes for drought tolerance will depend on information on genetic variability for drought tolerance among citron watermelon genotypes. Currently there is little information on the level of drought tolerance among citron watermelon genetic resources. Information on genotypic variability for drought tolerance will aid in the selection of superior genotypes that can be used for rootstock development to enhance drought tolerance and water-use efficiency of dessert watermelon cultivated in water-limited environments. Development of such rootstocks should not only be drought tolerant, but also disease resistance. Citron watermelon genotypes such as PI 482 311, PI 482 283 and PI 482 246 were reported to be drought tolerant and resistant to powdery mildew race 2 (Thomas, 2005; Zhang et al., 2011) which can be used in the development of citron watermelon rootstocks with enhanced level of resistance to biotic and abiotic stresses.

Citron watermelon is resistant to major economically important diseases of dessert watermelon. Understanding the genetic basis of resistance to economic diseases is not adequately investigated in citron watermelon. Such information is important to develop highly resistant citron watermelon genotypes for use as rootstocks or transfer of genes for improving disease tolerance in sweet dessert watermelon. Additionally, there have been little attempts to locate the genomic regions controlling disease resistance of economically important diseases in citron watermelon. Development of diagnostic molecular markers linked to QTL controlling biotic stress tolerance may facilitate rapid genetic differentiation among citroides genotypes for effective selection and breeding. Such markers should also be developed for important fruit qualitative traits such as fruit shape, rind stripe patterns and flesh colour. There is limited genetic variation for resistance to important diseases such as watermelon mosaic virus, fruit rot, squash vein yellowing virus, watermelon bud necrosis virus in citron watermelon (Kousik et al., 2009, 2012; Gillaspie and Wright, 2013; Holkar et al., 2018). Collection and screening of additional citron watermelon germplasm is critical to identify new sources of resistance to these diseases for breeding or direct production. There is also a limited number of citron watermelon rootstocks developed for sweet dessert watermelon cultivation. Development of citron watermelon rootstocks with multiple-disease resistance should boost sweet dessert watermelon productivity. Several accessions of citron watermelon with multiple disease resistance such as PI 189225, PI 482308, PI 482282, PI 482259, PI 244017, PI 244018, PI 271771, PI 244019, PI 500354, PI 299379, PI 482332, PI 482338 and PI 482322 (Table 1.3) may be useful for developing multiple-disease resistant rootstocks for sweet dessert watermelon production or other cucurbit crops. Citron watermelon may provide useful genetic variation for improvement of fruit qualitative traits for development of unique sweet dessert watermelon cultivars. Genetic variation of fruit quality traits such as rind stripe patterns, flesh colour and sugar and carotenoids content among citron watermelon genetic resources is important to identify and select unique/suitable genotypes for breeding. Identification of useful citron watermelon genotypes possessing biotic and abiotic stress tolerance is a pre-requisite for breeding and cultivar development including dessert watermelon. There is limited breeding progress in incorporating useful genes between elite citron and watermelon genotypes. This may be attributed to the use of a limited number of citron (e.g., PI 296341-R, PI 189225, PI 244019) and dessert (e.g., Crimson Sweet, Charleston Gray, Sugar Baby, New Hampshire Midget and Klondike Black Seeded) watermelon genotypes for developing breeding populations or F_1 hybrids. This can be mitigated using a wide gene pool of citron and dessert watermelon genotypes since the choice of cultivar and/or donor parents affect gene introgression (McGregor and Waters, 2013). There are limited genomic resources developed specifically for citron watermelon which may hinder genetic analysis and marker-assisted selection for effective breeding and cultivar development. The availability of a limited number of markers in citron watermelon necessitates development of additional and diagnostic molecular markers being specific for citron watermelon. Development of such markers should also be linked to biotic and abiotic stress resistance and for locating genes that control important traits like fruit quality traits (Hashizume et al., 1996). Molecular markers that are tightly linked to important fruit traits can be useful for marker-assisted selection for accelerated breeding (Table 1.7) (Hawkins et al., 2001a; Kim et al., 2015). Cross-species transferrable markers of related cucurbit crops should be exploited for genetic analysis, genetic linkage map construction and marker-assisted selection for citron watermelon genetic improvement and cultivar development (Table 1.7).

Table 1.7 Molecular markers linked to important diseases and fruit traits in cross compatible cucurbit species useful for genetic analysis and marker-assisted selection in citron watermelon.

Marker type	Marker name/s	marker sequence	Crop	Marker linkage	References
AFLP	ETG/M-CTC	GACTGCGTACCAATTCTG GATGAGTCCTGAGTAACTC	*Cucumis melo*	GSB	Wolukau et al. (2009)
	E-AT/M-CTG	GACTGCGTACCAATTCAT GATGAGTCCTGAGTAACTG			
	E-TC/M-CAG	GACTGCGTACCAATTCTC GATGAGTCCTGAGTAACAG			
	E-TG/MCTA	GACTGCGTACCAATTCTG GATGAGTCCTGAGTAACTA			
CAPS	CAPS-1	CCAACAGCAAGAACCGAAAG TTTGGTTCGATAACCCATCC	*Citrullus lanatus*	ZYMV	Harris et al. (2009)
	CAPS-2	AAAG CTACACCCACGGAAGA CTCCAGAACTCCTCGACAGTAG			
CAPS	NBS1-CAPS	TATTGCTAAAGCTGTTTTCAAAAGCG AACAAAAACTTTTCGATTTCCTAAGTT	*Cucumis melo*	PRSV; FW race 2	Brotman et al. (2005)
62-CAPS		GGAGAAGATGCTAGAGCCATTC AATCGGGCATCCTGTTTTGG			
SSR		-	*Cucumis melo*	WMV	Palomares-Rius et al. (2011)
CAPS & SNP		-	*Citrullus lanatus*	PM	Kim et al. (2013)
SNP	Chr1SNP_502124	F: ACACCACCCACTTTGGAGCTTCG R: TTTTAGGGTGAAAATGGGTATTGTA	*Citrullus lanatus*	FW race 1	Ren et al. (2015)
CAPS		-	*Citrullus lanatus*	Canary yellow flesh	Bang et al. (2007)
SCAR	wsbin6-11	F: GGTGAAAACTGGGATGGAGA R: CATTTTGAGGGTGCATTGTG	*Citrullus lanatus*	Rind stripe patterns	Kim et al. (2015)

Table 1.7 (Continued).

Marker type	Marker name/s	Marker sequence	Crop	Marker linkage	References
SSR	BVWS00358	F: CATTTCCGTTTCCATTTTCTTCAC R: AAGTAACATCAAGCAGTTCGCCAT	*Citrullus lanatus*	Fruit length	Reddy et al. (2014)
SSR	BVWS01708	F: GGGTTTGTTTCCATTTCCCT R: GGAAGGGTTCTGCATGTGTT	*Citrullus lanatus*	Fruit length	Reddy et al. (2014)
SSR	BVWS00711	F: TGCGACTTGAATTTTCTTGC R: CATCTGACAGGTCCAAGAATGA	*Citrullus lanatus*	fruit width and length	Reddy et al. (2014)
SSR	BVWS00681	F: TCTTGTCGTGCAATCTCTGC R: TTCAAGAAGAAAATTGGTCACCT	*Citrullus lanatus*	Rind thickness	Reddy et al. (2014)

AFLP = amplified fragment length polymorphism; CAPS = cleavage amplified polymorphic sequence; SNP = single nucleotide polymorphism; SSR = simple sequence repeat markers; GSB = gummy stem blight; ZYMV = *Zucchini* yellow mosaic virus; PRSV = *papaya* ringspot virus; FW = *Fusarium* wilt; WMV = watermelon *mosaic* virus; PM = *powdery* mildew; F = forward; R = reverse.

1.7. Conclusions

Citron watermelon serves as an important genetic resource for sweet dessert watermelon breeding to enhance yields, fruit quality, biotic and abiotic stress tolerance. However, citron watermelon has generally received little research priority aimed at cultivar development and deployment. This has resulted in a limited number of improved genotypes for cultivation. To aid cultivar development of citron watermelon, genetic resources within the variety citroides should be further studied for traits such as quality, yield potential, and biotic and abiotic stress tolerance. This will assist in identification and selection of promising genotypes which may facilitate development of genetically superior and improved citron watermelon cultivars for direct production or breeding.

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Chapter 2. Characterization of genetic diversity of citron watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.) landrace accessions of South Africa through simple sequence repeat markers

Abstract

Profiling the genetic diversity of germplasm collections is fundamental for determining the extent of genetic diversity for selection programs and strategic conservation. Citron watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.) is an under-researched cucurbit crop despite providing food and nutritional security in sub-Saharan Africa. There is limited information on genetic relationships of South African citron watermelon accessions for effective breeding. The objective of this study was to determine the extent of genetic diversity among 48 South African citron watermelon landrace accessions using selected simple sequence repeat (SSR) markers to identify genetically divergent accessions for trait integration and variety development. The SSR markers amplified a total of 24 alleles, with a mean expected heterozygosity value of 0.38, indicating moderate genetic diversity among the studied accessions. The mean polymorphic information content value of 0.21 indicated low discriminatory power of the SSR markers. Analysis of molecular variance attributed 8%, 75%, and 17% of the molecular variation between populations, among accessions and within accessions, respectively. Three distinctive genetic groups were identified based on cluster analysis, which aided the selection of genetically diverse citron watermelon accessions for breeding and cultivar development.

Keywords: *Citron watermelon, cluster analysis, genetic diversity, microsatellites*

2.1. Introduction

Citron watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.) is an important cucurbit crop grown for diverse uses in sub-Saharan Africa (SSA). Fresh and dried leaves are cooked and consumed as vegetable or relish with staple foods, such as maize, sorghum and millet (Mashilo et al., 2017; Ngwepe et al., 2019). Often, fresh bright-yellow male flowers are added to the relish to improve taste and flavour. Citron watermelon bears multiple branches and large fruits, which are used as livestock feed. The non-sweet yellow fruit and orange-fleshed fruit are consumed fresh as fruit salad, or cooked to prepare unique traditional dishes called “Mokgapu” and “Kgodu” in the local Sepedi language of South Africa (Ngwepe et al., 2019, 2021a; Nkoana et al., 2021). The red and brown-coloured seeds extracted from matured fruit are roasted and consumed as a snack or with maize porridge.

Citron watermelon originated in Africa (Dane and Lang, 2004; Dane and Liu, 2007). This assumption is supported by the extensive phenotypic diversity of the crop present in the region and attributed to long-term cultivation and conservation by smallholder farmers (Mujaju et al., 2010, 2013; Achigan-Dako et al., 2015; Mashilo et al., 2017; Ngwepe et al., 2019). Citron watermelon accessions exhibit a wide diversity of traits, including fruit attributes (i.e., fruit shape, colour, rind stripe patterns) and seed coat colours (i.e., red, brown, grey, green, maroon, black) (Mujaju et al., 2011; Achigan-Dako et al., 2015; Shaik et al., 2017; Ngwepe et al., 2021a). Despite the presence of unique genetic resources of citron watermelon in SSA, the crop has received limited research priority. Farmers in SSA use recycled seeds of unimproved and low-yielding landrace accessions developed through mass selection. As a result, improved cultivars of the crop have not been developed and commercialized in SSA. Also, sweet watermelon (*Citrullus lanatus* var. *lanatus*) breeding programs have not exploited the citron watermelon genetic resources for rootstock breeding, and for improving biotic and abiotic stress tolerance, productivity and fruit quality.

Assessing the extent of genetic diversity is a critical step to guide the effective use of citron watermelon genetic resources available in SSA for breeding. Previously, genetic diversity analysis in citron watermelon was carried out using various molecular markers such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), SSR markers and peroxidase gene markers (Jarret et al., 1997; Levi et al., 2004; Zhang et al., 2016; Mashilo et al., 2017). These marker systems detected the presence of low to moderate genetic diversity in citron watermelon genetic resources. In South Africa, citron watermelon is widely grown from landrace accessions (Mashilo et al., 2016, 2017). Previously, SSR analysis based on some 36 selected and widely cultivated landrace accessions resolved moderate genetic diversity (Mashilo et al., 2017). However, the extent of genetic diversity of local accessions remains unknown. Therefore, citron watermelon genetic resources of South Africa should be systematically chararecterized for effective use and breeding. A collection of citron watermelon accessions obtained from various districts of the Limpopo Province; South Africa was constituted. Fruit and seed samples of some widely grown accessions of the crop were provided by smallholder farmers. These accessions are maintained by the Limpopo Department of Agriculture and Rural Development (LDARD), Towoomba Agricultural Development Centre (TADC). There is a need for further studies to characterize the genetic diversity present among these collections to inform breeding and strategic conservation of the crop. Therefore, the objective of this study was to determine the extent of genetic diversity among 48 South African citron watermelon landrace accessions using selected SSR markers to identify genetically divergent accessions for trait integration and variety development.

2.2. Materials and methods

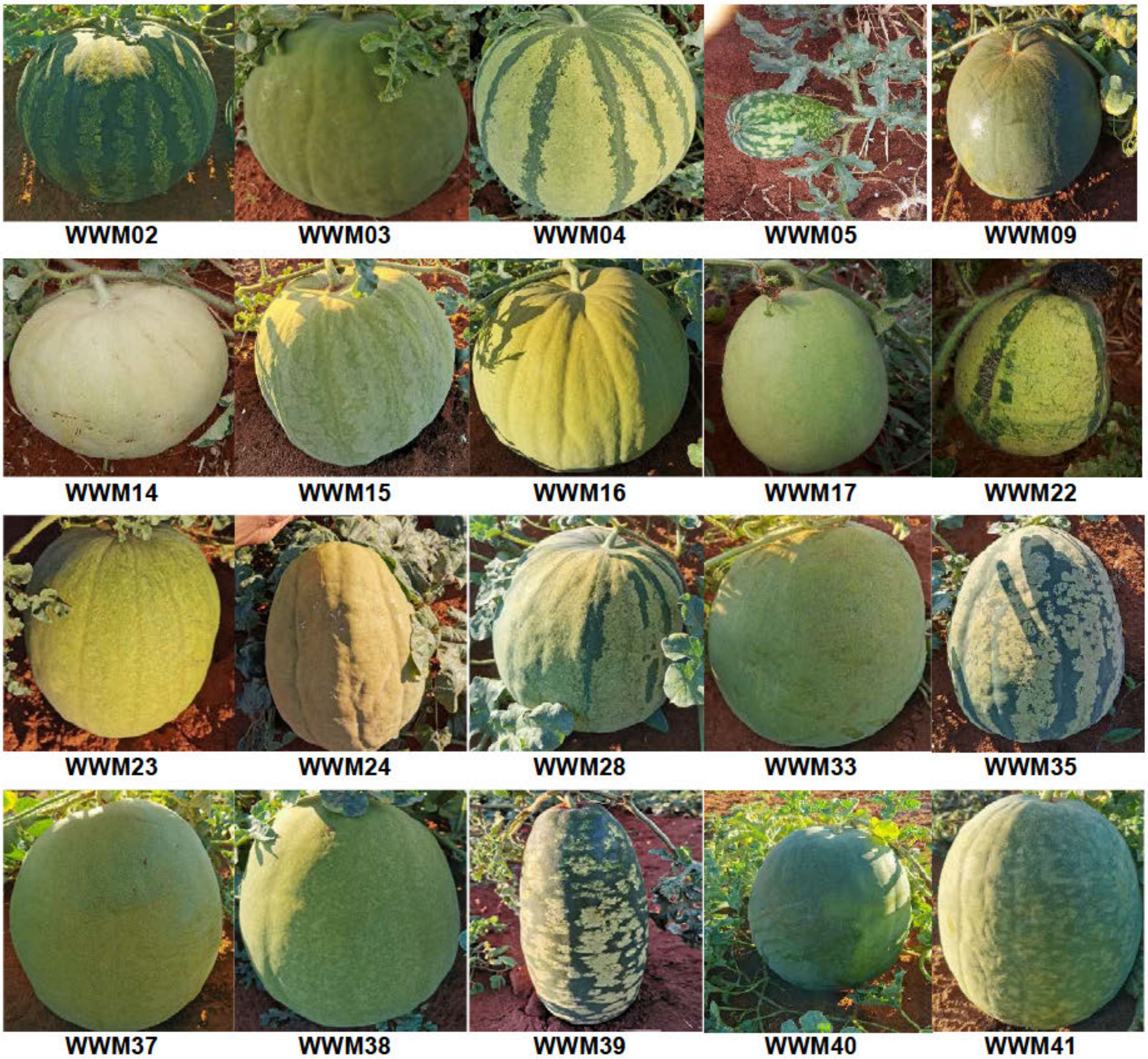
2.2.1. Plant materials and experimental design

Forty-eight citron watermelon landrace collections sampled across three districts of the Limpopo Province of South Africa were used for the study (Table 2.1). Also, Figures 2.1 and 2.2 represent variations of selected accessions for fruit and seed traits. Ten seeds per accession were grown in black polyethylene plastic bags in a net-house at TADC, Bela-Bela (28º19’28’’E, 24º53’57’’S) in South Africa. After four weeks, seedlings were sampled by Sci-Corp Laboratories in Pietermaritzburg, KwaZulu-Natal Province, South Africa, for genotyping. Young and healthy fresh leaves were sampled from 10 individual seedlings per accession for bulked DNA sampling.

Table 2.1 List of citron watermelon landrace accessions used in the study with district and village of collection in the Limpopo Province in South Africa and their corresponding fruit and seed traits.

	Origin of collection		Fruit traits		
Accession code	District	Village	Shape	Flesh colour	Seed colour
WWM02	Capricorn	Kgohloane	R	Y	RW
WWM03	Capricorn	Kgohloane	E	O	RW
WWM04	Capricorn	Kgohloane	R	O	G
WWM05	Capricorn	Kgohloane	E	W	GBrS
WWM08	Capricorn	Moletjie-Moshate	E	Y	RW
WWM09	Capricorn	Moletjie-Moshate	R	W	B
WWM14	Capricorn	Turfloop	R	O	RW
WWM15	Capricorn	Turfloop	R	O	Mr
WWM16	Waterberg	Bela-Bela	R	Y	R
WWM17	Waterberg	Styloop	R	O	BrS
WWM18	Waterberg	Styloop	-	-	CW
WWM22	Waterberg	Maeteletsa	-	-	Br
WWM23	Waterberg	Maeteletsa	E	Y	RW
WWM24	Capricorn	Westenburg	E	Y	G
WWM25	Sekhukhune	Malegane	-	-	Br
WWM26	Sekhukhune	Malegane	-	-	G
WWM28	Sekhukhune	Malegane	R	O	R
WWM33	Capricorn	Ga-Molepo	E	Y	RW
WWM34	Capricorn	Ga-Molepo	R	O	CW
WWM35	Capricorn	Ga-Molepo	E	Y	T
WWM37	Capricorn	Ga-Molepo	E	O	Mr
WWM38	Capricorn	Ga-Molepo	E	O	RW
WWM39	Capricorn	Ga-Mphela	BE	O	T
WWM40	Capricorn	Kgohloane	E	O	RW
WWM41	Sekhukhune	Nebo	E	Y	RW
WWM42	Sekhukhune	Nebo	E	O	R
WWM44	Sekhukhune	Nebo	R	O	R
WWM46	Sekhukhune	Nebo	E	O	R
WWM47	Sekhukhune	Nebo	R	O	R
WWM48	Sekhukhune	Nebo	R	O	RW
WWM50	Sekhukhune	Nebo	E	Y	R
WWM57	Capricorn	Uitkyk 3	E	O	BrS
WWM62	Capricorn	Kgohloane	-	-	R
WWM64	Capricorn	Ga-Mphela	E	O	Br
WWM65	Waterberg	Vienna	R	O	R
WWM66	Capricorn	Uitkyk 3	E	O	BrS
WWM67	Capricorn	Uitkyk 3	E	O	RW
WWM68	Capricorn	Ga-Manamela	E	Y	G
WWM76	Capricorn	Ga-Manamela	E	Y	BrS
WWM79	Capricorn	Ga-Manamela	E	O	CW
WWM80	Capricorn	Boslaagte	-	-	DB
WWM81	Capricorn	Ga-Manamela	E	Y	CW
WWM82	Waterberg	Vienna	-	-	G
WWM84	Waterberg	Vienna	-	-	RW
WWM85	Capricorn	Ga-Manamela	R	O	BrS
WWM86	Waterberg	Vienna	-	-	Br
WWM87	Waterberg	Vienna	-	-	R
WWM89	Waterberg	Vienna	-	-	CW

Fruit shape: BE = broad elliptic; E = elliptic; R = round. Flesh colour: O = orange; W = white; Y = yellow. Seed colour: B = black; Br = brown; BrS = brown with black spot; CW = cream-white; DB = dark brown; G = green; GBrs = green with brown spot; Mr = maroon; R = red; RW = red-white; T = tan. -, information not available. Accessions in bold font text are new to this study, while non-bold entries were evaluated by Mashilo et al. (2017) and included as comparative controls in the present study.



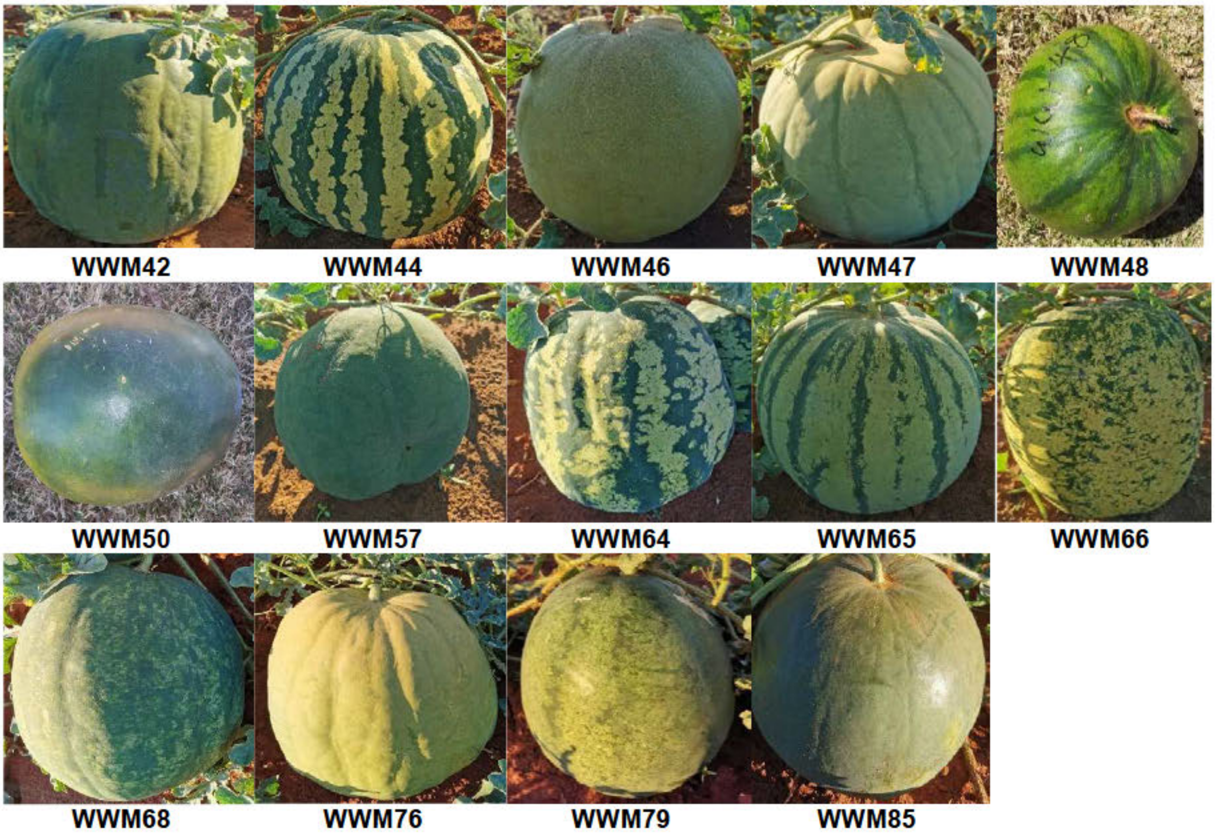
WWM02 **WWM03** **WWM04** **WWM05** **WWM09**

WWM14 **WWM15** **WWM16** **WWM17** **WWM22**

WWM23 **WWM24** **WWM28** **WWM33** **WWM35**

WWM37 **WWM38** **WWM39** **WWM40** **WWM41**

Figure 2.1: Fruit characteristics of selected citron watermelon landrace collections from three districts in the Limpopo Province of South Africa. Note the following unique accessions were resolved based on SSR markers: WWM02, WWM04, WWM09, WWM15, WWM17, WWM18, WWM22, WWM23, WWM24, WWM28, WWM33, WWM39, WWM40, WWM41, WWM64, WWM66, WWM68 from clusters I; WWM76, WWM05, WWM14, WWM37, WWM46, WWM42, WWM50 (cluster II); and WWM38, WWM47 and WWM48 (cluster III). See codes of accessions in Table 2.1.



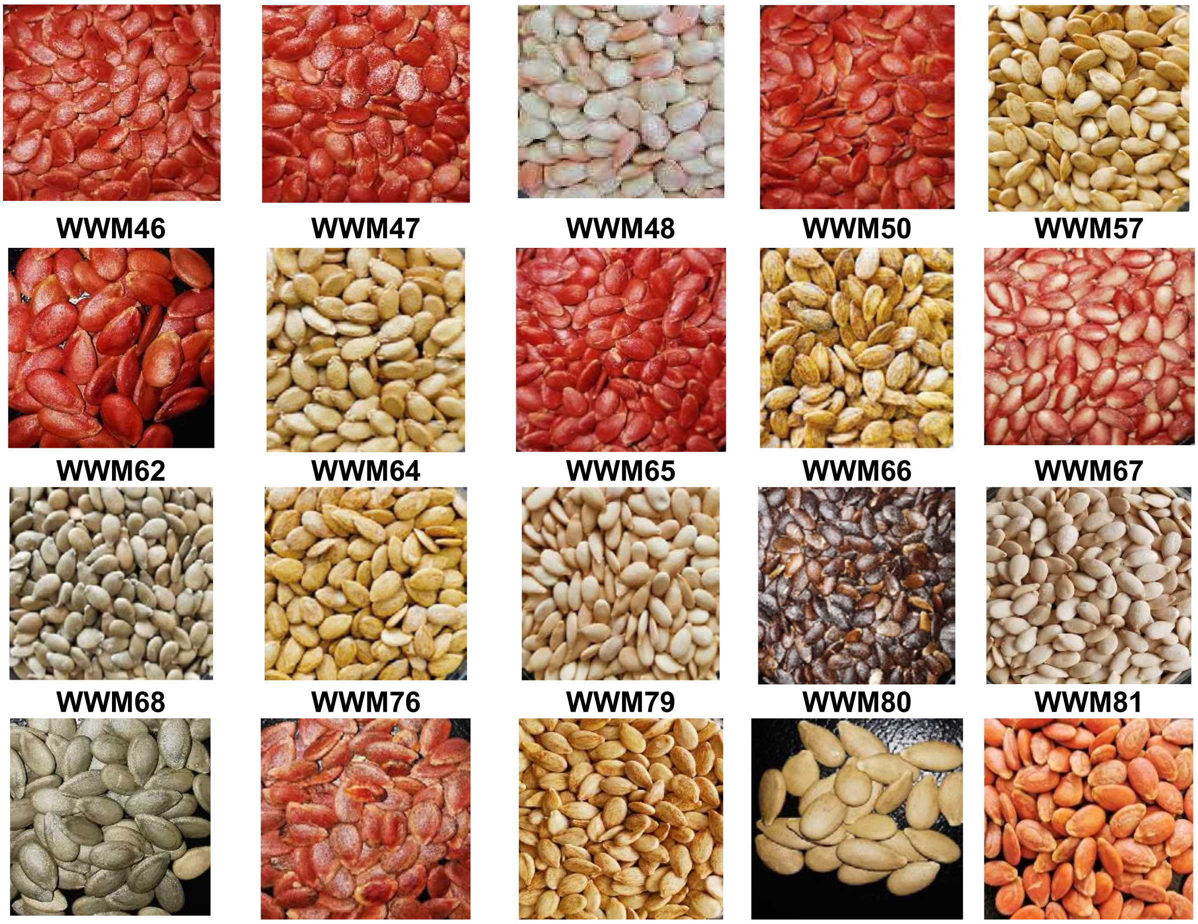
WWM42 WWM44 WWM46 WWM47 WWM48

WWM50 WWM57 WWM64 WWM65 WWM66

WWM68 WWM76 WWM79 WWM85

Figure. 2.1: (Continued). Fruit characteristics of selected citron watermelon landrace collections from three districts in the Limpopo Province of South Africa. Note the following unique accessions were resolved based on SSR markers: WWM02, WWM04, WWM09, WWM15, WWM17, WWM18, WWM22, WWM23, WWM24, WWM28, WWM33, WWM39, WWM40, WWM41, WWM64, WWM66, WWM68 from clusters I; WWM76, WWM05, WWM14, WWM37, WWM46, WWM42, WWM50 (cluster II); and WWM38, WWM47 and WWM48 (cluster III). See codes of accessions in Table 2.1.

Figure 2.2: Seed morpho-types of selected citron watermelon landrace collections from three districts in the Limpopo Province of South Africa. Note the following complementary and unique accessions were resolved using the SSR markers: WWM03, WWM04, WWM09, WWM15, WWM16, WWM17, WWM24, WWM34, WWM35, WWM64, WWM66 and WWM81 in cluster I, WWM05 (cluster II) and WWM48 (cluster III). See codes of accessions in Table 2.1.



WWM46 WWM47 WWM48 WWM50 WWM57

WWM62 WWM64 WWM65 WWM66 WWM67

WWM68 WWM76 WWM79 WWM80 WWM81

WWM82 WWM84 WWM85 WWM86 WWM87

Figure. 2.2: (Continued). Seed morpho-types of selected citron watermelon landrace collections from three districts in the Limpopo Province of South Africa. Note the following complementary and unique accessions were resolved using the SSR markers: WWM03, WWM04, WWM09, WWM15, WWM16, WWM17, WWM24, WWM34, WWM35, WWM64, WWM66 and WWM81 in cluster I, WWM05 (cluster II) and WWM48 (cluster III). See codes of accessions in Table 2.1.

2.2.2. DNA extraction and SSR analysis

The DNA was extracted following the cetyl-tetramethyl ammonium bromide (CTAB) method following the initial procedure of CIMMYT (2005). The DNA concentration was quantified using 0.7% Tris-EDTA (TE) buffer. Samples were bulked and used for SSR amplification. Simple sequences repeat (SSR) markers were amplified through polymerase chain reaction (PCR). Eleven SSR markers were used for this study (Table 2.2). The SSR markers were selected based on their high polymorphic information content determined in a study of sweet dessert watermelon (Zhang et al., 2012), a close botanical variety of citron watermelon. The PCR cycle of denaturation temperature was 60-52°C. The PCR products were fluorescently labeled and separated by capillary electrophoresis on an ABI 3130 automatic sequencer (Applied Biosystems, Johannesburg, SA).

2.2.3. Data analysis

Genetic diversity was assessed using GenAlEx version 6.5 (Peakall and Smouse, 2012). The following genetic parameters were computed using the protocol given by Nei and Li (1979): the number of alleles per locus (N_a), major allele frequency (M_{AF}), number of effective alleles per locus (N_e), observed heterozygosity (H_o) and expected heterozygosity (H_e) and unbiased gene diversity (uH_e). Further, the level of gene flow (N_m) and genetic differentiation (F_{ST}) were calculated in GenAlEx. N_m was calculated using the formula: $N_m = 0.25\ (1–F_{ST}/F_{ST})$,

where F_{ST} is the genetic differentiation.

Polymorphic information content (PIC) was calculated using the formula:

$PIC = 1\text{-}\Sigma Pij^2$,

where P_{ij} is the frequency of j^{th} allele of the i^{th} locus.

Genetic diversity was further partitioned based on the Shannon index (I). The Shannon index varies from 0 to 1, and lower genetic diversity is indicated by values closer to zero (Silva et al., 2015). Nei's unbiased genetic distance and identity were estimated to determine population differentiation among the tested accessions (Nei, 1978). Analysis of molecular variance (AMOVA) was performed to estimate the degree of genetic differentiation among populations, within and among the sources of collection of citron watermelon accessions. The genetic relationships of the 48 citron watermelon landrace accessions were revealed through cluster analysis. Genetic relatedness was estimated using the genetic dissimilarity coefficients, and a dendrogram was drawn using the unweighted pair group method with arithmetic mean (UPGMA) in DARwin 6.0 (Perrier and Jacquemoud-Collet, 2006).

Table 2.2 Descriptions of the simple sequence repeat markers used for genetic profiling of citron watermelon landrace accessions from Limpopo Province, South Africa.

SSR marker	Forward primer sequence	Reverse primer sequence	LG	PIC
BVWS00948	TCAAACCGACTGCCATATCA	AGCTTGTCTTCCTGGCCTTT	LG1	0.63
BVWS00297	ACAACTTTGATTGATTGCACGATG	AAGTGAAAGACCCTTTTCCCAAAC	LG2	0.69
BVWS00048	TCAAAAGGTTTGCCCTAAATGAAA	TGCTGATCTCCCATTCTTAACCTC	LG3	0.60
BVWS01734	AAAATTACATCTTAAATGCGCC	GGAACATTGACTTCAATCAGCA	LG4	0.74
BVWS00441	TGGTTGAAATCAATAAAAAGTGAA	TGGATGTTTTTGGCATTTGA	LG5	0.69
BVWS00233	AAACCATGATTTTACAGGGGATCA	TTTCTGTCTTCTTTTGACCAATGC	LG6	0.73
BVWS00433	TCTTTTAAGTTTTGAGGGAGAGC	TTCCCAAGCTAGCCTTTTCA	LG7	0.82
BVWS00369	TGAGAAAATGGAAGATGCAAATGA	TTCTTCTCACTCTCTCCTAAGATTTTGC	LG8	0.73
BVWS00209	TGCTTCAAAATCTATTCACAATTTGC	TTCTTGGTTTCGGGTTTCTTTACA	LG9	0.79
BVWS02048	TCTGTGTGGATGCAAATGGT	GCTAATCGAGCCCAGTTACG	LG10	0.67
BVWS00228	GGAAGAGTGAGGTGATAAATCAATATGT	AATTGGCCCAAATATCCATATGAC	LG11	0.74

Source: Zhang et al. (2012). LG = linkage group and PIC = polymorphic information content.

2.3. Results

2.3.1. Polymorphism and allelic diversity of SSR markers

Estimates of genetic diversity parameters among the studied citron watermelon accessions are presented in Table 2.3. A total of 24 alleles were amplified across the 48 samples. The number of alleles per locus (N_a) ranged from 1.00 for markers BVWS00948, BVWS00048, BVWS00288, and BVWS00106 to 7.00 for marker BVWS00369, with a mean value of three alleles per locus. Major allele frequency (M_{AF}) ranged from 0.57 for marker BVWS00433 to 1.00 for markers BVWS00948, BVWS00048, BVWS00288, and BVWS00106. The mean value of M_{AF} was 0.83. Gene diversity (H_e) ranged from 0.00 (BVWS00233) to 0.59 (BVWS00369), with a mean of 0.24, suggesting that 24% of the individuals were heterozygous at a given locus under random mating conditions. The observed heterozygosity (H_o) ranged from 0.00 to 0.18, with a mean value of 0.04 across the 11 markers. The mean PIC of the SSR primers was 0.21. BVWS00369 was the most polymorphic locus with a PIC of 0.55. The fixation index (F) ranged from 0 to 1.00, with a mean of 0.83.

Table 2.3 Genetic diversity parameters generated for 48 citron watermelon landrace accessions using simple sequence repeat markers.

	Genetic parameters					
SSR marker	N_a	MAF	H_e	H_o	PIC	F
BVWS00948	1.00	1.00	0.00	0.00	0.00	-
BVWS00297	2.00	0.67	0.44	0.00	0.34	1.00
BVWS00048	1.00	1.00	0.00	0.00	0.00	-
BVWS00433	2.00	0.57	0.49	0.00	0.37	1.00
BVWS02048	3.00	0.72	0.41	0.14	0.34	0.66
BVWS00209	5.00	0.80	0.35	0.10	0.33	0.72
BVWS01734	3.00	0.88	0.22	0.04	0.21	0.82
BVWS00233	2.00	0.92	0.15	0.00	0.14	1.00
BVWS00369	7.00	0.59	0.59	0.18	0.55	0.70
BVWS00288	1.00	1.00	0.00	0.00	0.00	-
BVWS00106	1.00	1.00	0.00	0.00	0.00	-
Mean	3.42	0.83	0.38	0.04	0.21	0.83

N_a = number of alleles; M_{AF} = major allele frequency; H_e = gene diversity; H_o = observed heterozygosity; PIC = polymorphic information content and F = fixation index.

2.3.2. Genetic divergence

Genetic diversity parameters based on the sources of collection of the citron watermelon accessions are shown in Table 2.4. The number of alleles for population 1 (collections from Capricorn district) was 1.91, whereas populations 2 (Waterberg district) and 3 (Sekhukhune district) each had 1.82 alleles. The number of effective alleles for population 2 was 1.50, followed by population 3 (1.39) and population 1 (1.32). The mean I was 0.31, 0.40, and 0.31 for populations 1, 2, and 3, respectively. The H_o and H_e values for population 1 were 0.03 and 0.18, respectively. For population 2, H_o and H_e values of 0.05 and 0.26 were recorded, respectively. Population 3 had H_o and H_e values of 0.07 and 0.18, respectively. An unbiased gene diversity value of 0.19 was recorded for population 1 and population 3, whereas population 2 had a mean value of 0.27. The F was computed for population 1 (0.80), followed by population 2 (0.77) and population 3 (0.58) (Table 2.4).

Table 2.4 Genetic diversity parameters generated for 48 citron watermelon populations using simple sequence repeat markers.

					Genetic parameters				
Population	Statistics		N_a	N_e	I	H_o	H_e	uH_e	F
1	Mean	27	1.91	1.32	0.31	0.03	0.18	0.19	0.80
	SE	0.09	0.46	0.13	0.12	0.02	0.07	0.07	0.06
2	Mean	10	1.82	1.50	0.40	0.05	0.26	0.27	0.77
	SE	0.37	0.30	0.15	0.12	0.03	0.08	0.08	0.09
3	Mean	11	1.82	1.39	0.31	0.07	0.18	0.19	0.58
	SE	0.12	0.38	0.19	0.13	0.04	0.07	0.08	0.14

Populations 1, 2 and 3 represent collections from Capricorn, Waterberg and Sekhukhune districts in South Africa, in that order. N_a = number of alleles; N_e = number of effective alleles; I = Shannon's information index; H_o = observed heterozygosity; H_e = gene diversity; uH_e = unbiased gene diversity and F = fixation index.

The Nei's genetic distance and genetic identity among citron watermelon populations are shown in Table 2.5**.** The Nei's genetic distance was 0.04 between populations 1 and 2, 0.02 for populations 1 and 3, and 0.06 for populations 2 and 3. A genetic identity of 0.92 was calculated between populations 1 and 2, whereas populations 1 and 3 recorded a genetic identity of 0.98. The genetic identity value of 0.94 was recorded for populations 2 and 3.

Table 2.5 Estimates of Nei genetic distance (below diagonal) and Nei genetic identity (above diagonal) among 48 citron watermelon landrace accessions grouped based on populations.

Population	1	2	3
1		0.96	0.98
2	0.04		0.94
3	0.02	0.06	

Populations 1, 2 and 3 represent collections from Capricorn, Waterberg and Sekhukhune districts in South Africa, in that order.

Estimates of genetic differentiation and gene flow among citron watermelon accessions based on the source of the collection are shown in Table 2.6. Low genetic differentiations of 0.13 and 0.01 were recorded for populations 1 and 2 and populations 1 and 3, in that order. Also, low genetic differentiation of 0.10 occurred between populations 2 and 3. Relatively low gene flow (1.74) was computed between populations 1 and 2, whereas a value of 2.13 was recorded for populations 2 and 3 (2.13). High gene flow (18.72) was recorded between populations 1 and 3.

Table 2.6 Estimates of genetic differentiation (F_{ST}) (above diagonal) and gene flow (N_m) (below diagonal) among 48 citron watermelon landrace accessions grouped based on population.

Population	1	2	3
1		0.13	0.01
2	1.74		0.10
3	18.72	2.13	

Populations 1, 2 and 3 represent collections from Capricorn, Waterberg and Sekhukhune in that order.

2.3.3. Analysis of molecular variance

The analysis of molecular variance (AMOVA) showed that there were significant differences among the populations, among and within individuals in the entire panel of genotypes (Table 2.7). The total variance accounted for by the differences among populations, among and within individuals was 8%, 75%, and 17% respectively.

Table 2.7. Analysis of molecular variance of 48 citron watermelon landrace accessions using simple sequence repeat markers.

Source of variation	df	SS	MS	Estimated variance	Percent variation	F-Statistics	Probability Level
Among populations	2	10.89	5.45	0.11	8.00	0.08	$p < 0.001$
Among individuals	45	104.81	2.33	1.05	75.00	0.81	$p < 0.001$
Within individuals	48	11.50	0.24	0.24	17.00	0.83	$p < 0.001$

df = degrees of freedom; SS = sum of squares and MS = mean squares.

2.3.4. Cluster analysis

Cluster analysis showing groupings of the tested citron watermelon accessions is shown in Figure 2.3. The analysis revealed three major clusters, namely: Clusters I, II, and III. Clusters I and II comprised 34 and 11 accessions, respectively. Only three accessions were grouped in Cluster III.

Genetic distance correlation matrix showing relationships among the accessions is presented in Table 2.8. Genetic distance ranged from 0.00 to 0.90 across all accessions. There were six pairs with very low (0.00) genetic distance. Also, moderate genetic distance (0.40 – 0.60) was recorded between six pairs. Thirty-nine pairs of accessions recorded the highest (> 0.70) genetic distance.

Some fruit and seed morphotypes of selected accessions are shown in Figure 2.1 and Figure 2.2, respectively. The accessions showed variations in fruit colour, shape and flesh colour and seed coat colour (Table 2.1). There was no clear and distinct clustering pattern of accessions based on either district of collection and/or fruit or seed traits (Figure 2.3).

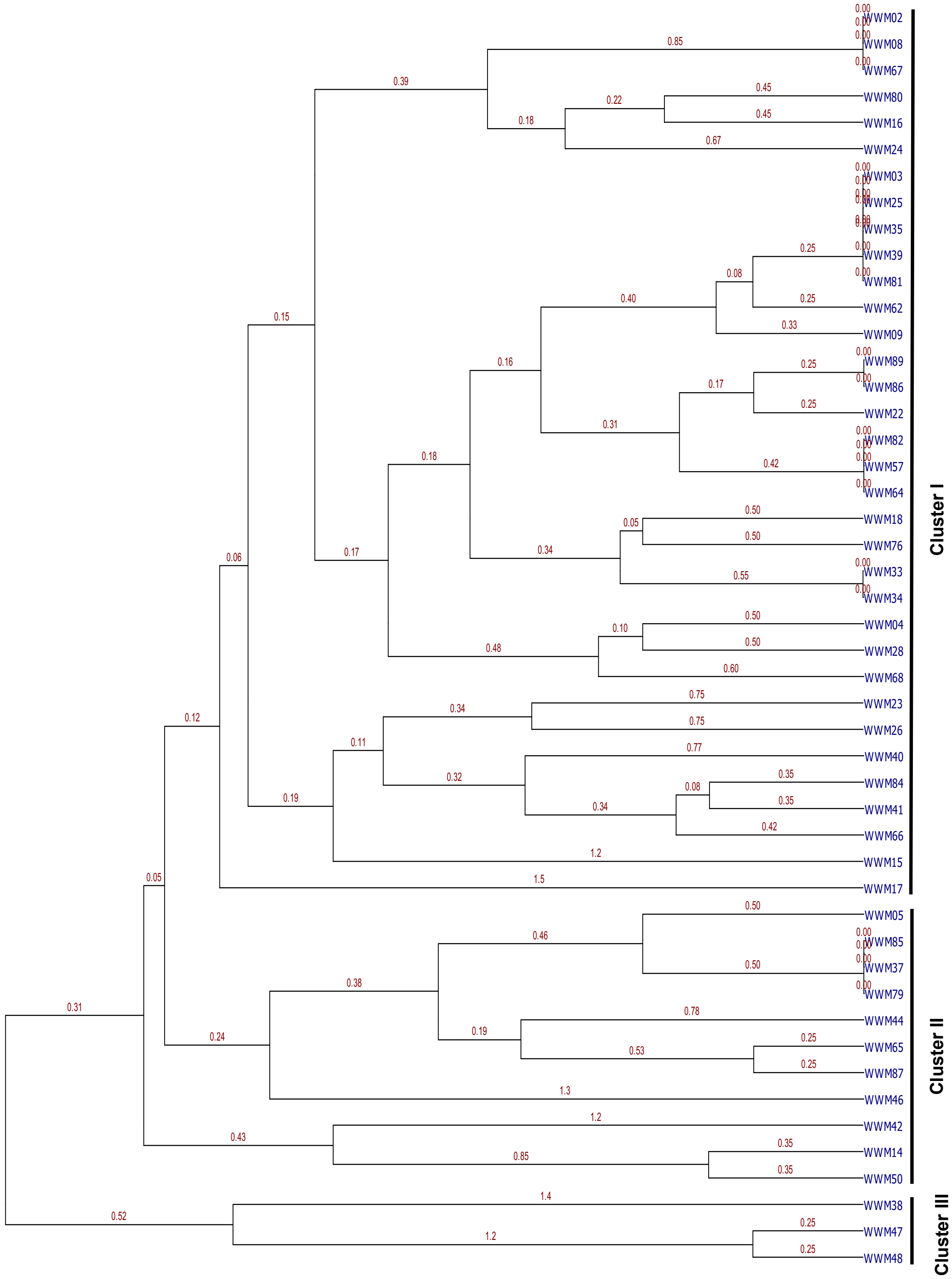


Figure 2.3: Dendogram based on neighbour-joining algorithm with the un-weighted pair group method with arithmetic mean (UPGMA) showing the genetic groupings of 48 citron watermelon landrace collections using simple sequence repeat markers. Collections were made from three districts in the Limpopo Province, South Africa. See codes of landraces in Table 2.1.

Table 2.8. Genetic distance correlation matrix showing relationships among the accessions.

Accession	WWM 02	WWM 03	WWM 04	WWM 05	WWM 08	WWM 09	WWM 14	WWM 15	WWM 16	WWM 17	WWM 18	WWM 22	WWM 23	WWM 24	WWM 25
WWM02	0.00														
WWM03	0.13	0.00													
WWM04	0.26	0.13	0.00												
WWM05	0.19	0.32	0.45	0.00											
WWM08	0.00	0.13	0.26	0.19	0.00										
WWM09	0.26	0.13	0.13	0.39	0.26	0.00									
WWM14	0.26	0.39	0.26	0.45	0.26	0.39	0.00								
WWM15	0.39	0.26	0.39	0.32	0.39	0.39	0.39	0.00							
WWM16	0.19	0.32	0.32	0.32	0.19	0.30	0.32	0.58	0.00						
WWM17	0.39	0.26	0.39	0.50	0.39	0.30	0.64	0.51	0.50	0.00					
WWM18	0.37	0.24	0.37	0.48	0.37	0.26	0.50	0.37	0.48	0.32	0.00				
WWM22	0.26	0.13	0.26	0.37	0.26	0.13	0.51	0.39	0.37	0.26	0.19	0.00			
WWM23	0.50	0.37	0.37	0.37	0.50	0.30	0.50	0.24	0.54	0.54	0.35	0.42	0.00		
WWM24	0.13	0.26	0.39	0.24	0.13	0.26	0.39	0.51	0.24	0.39	0.32	0.13	0.54	0.00	
WWM25	0.13	0.00	0.13	0.32	0.13	0.13	0.39	0.26	0.32	0.26	0.24	0.13	0.37	0.26	0.00
WWM26	0.39	0.26	0.13	0.32	0.39	0.26	0.39	0.26	0.45	0.51	0.50	0.39	0.24	0.51	0.26
WWM28	0.39	0.26	0.13	0.50	0.39	0.18	0.39	0.51	0.37	0.39	0.37	0.26	0.42	0.39	0.26
WWM33	0.19	0.06	0.19	0.39	0.19	0.19	0.32	0.19	0.39	0.32	0.22	0.19	0.35	0.32	0.06
WWM34	0.19	0.06	0.19	0.39	0.19	0.19	0.32	0.19	0.39	0.32	0.22	0.19	0.35	0.32	0.06
WWM35	0.13	0.00	0.13	0.32	0.13	0.13	0.39	0.26	0.32	0.26	0.24	0.13	0.37	0.26	0.00
WWM37	0.13	0.26	0.39	0.06	0.13	0.39	0.39	0.26	0.32	0.51	0.50	0.39	0.37	0.26	0.26
WWM38	0.26	0.39	0.51	0.37	0.26	0.43	0.51	0.64	0.37	0.39	0.45	0.39	0.67	0.26	0.39
WWM39	0.13	0.00	0.13	0.32	0.13	0.13	0.39	0.26	0.32	0.26	0.24	0.13	0.37	0.26	0.00
WWM40	0.26	0.13	0.26	0.19	0.26	0.26	0.51	0.13	0.45	0.39	0.37	0.26	0.24	0.39	0.13

Table 2.8 (continued),

Accession	WWM 02	WWM 03	WWM 04	WWM 05	WWM 08	WWM 09	WWM 14	WWM 15	WWM 16	WWM 17	WWM 18	WWM 22	WWM 23	WWM 24	WWM 25
WWM41	0.51	0.39	0.51	0.37	0.51	0.39	0.77	0.39	0.63	0.39	0.45	0.26	0.42	0.39	0.39
WWM42	0.39	0.51	0.39	0.58	0.39	0.51	0.26	0.64	0.45	0.51	0.63	0.64	0.63	0.51	0.51
WWM44	0.26	0.39	0.39	0.19	0.26	0.39	0.39	0.39	0.37	0.56	0.63	0.51	0.37	0.39	0.39
WWM46	0.32	0.45	0.58	0.26	0.32	0.58	0.58	0.45	0.51	0.63	0.69	0.58	0.56	0.45	0.45
WWM47	0.64	0.77	0.64	0.75	0.64	0.69	0.51	0.90	0.63	0.51	0.71	0.77	0.80	0.64	0.77
WWM48	0.51	0.64	0.51	0.63	0.51	0.56	0.51	0.90	0.50	0.39	0.71	0.64	0.80	0.51	0.64
WWM50	0.13	0.26	0.26	0.32	0.13	0.26	0.13	0.39	0.24	0.51	0.42	0.39	0.41	0.26	0.26
WWM57	0.26	0.13	0.26	0.37	0.26	0.18	0.51	0.39	0.37	0.13	0.19	0.13	0.42	0.26	0.13
WWM62	0.19	0.06	0.19	0.32	0.19	0.06	0.45	0.32	0.32	0.24	0.19	0.06	0.37	0.19	0.06
WWM64	0.26	0.13	0.26	0.37	0.26	0.18	0.51	0.39	0.37	0.13	0.19	0.13	0.42	0.26	0.13
WWM65	0.26	0.39	0.26	0.19	0.26	0.39	0.26	0.39	0.32	0.64	0.63	0.51	0.37	0.39	0.39
WWM66	0.39	0.26	0.39	0.24	0.39	0.30	0.64	0.26	0.45	0.39	0.37	0.26	0.29	0.39	0.26
WWM67	0.00	0.13	0.26	0.19	0.00	0.26	0.26	0.39	0.19	0.39	0.37	0.26	0.50	0.13	0.13
WWM68	0.32	0.19	0.19	0.45	0.32	0.06	0.37	0.37	0.37	0.37	0.24	0.19	0.28	0.32	0.19
WWM76	0.39	0.26	0.39	0.50	0.39	0.30	0.39	0.26	0.50	0.39	0.24	0.26	0.37	0.39	0.26
WWM79	0.13	0.26	0.39	0.06	0.13	0.39	0.39	0.26	0.32	0.51	0.50	0.39	0.37	0.26	0.26
WWM80	0.32	0.45	0.45	0.43	0.32	0.32	0.32	0.58	0.35	0.58	0.39	0.32	0.48	0.19	0.45
WWM81	0.13	0.00	0.13	0.32	0.13	0.13	0.39	0.26	0.32	0.26	0.24	0.13	0.37	0.26	0.00
WWM82	0.26	0.13	0.26	0.37	0.26	0.18	0.51	0.39	0.37	0.13	0.19	0.13	0.42	0.26	0.13
WWM84	0.39	0.26	0.39	0.24	0.39	0.30	0.64	0.26	0.50	0.26	0.32	0.26	0.29	0.39	0.26
WWM85	0.13	0.26	0.39	0.06	0.13	0.39	0.39	0.26	0.32	0.51	0.50	0.39	0.37	0.26	0.26
WWM86	0.19	0.06	0.19	0.32	0.19	0.13	0.45	0.32	0.32	0.19	0.19	0.11	0.37	0.24	0.06
WWM87	0.19	0.32	0.32	0.13	0.19	0.32	0.32	0.32	0.30	0.58	0.56	0.45	0.30	0.32	0.32
WWM89	0.19	0.06	0.19	0.32	0.19	0.13	0.45	0.32	0.32	0.19	0.19	0.11	0.37	0.24	0.06

Table 2.8 (continued),

Accession	WWM 26	WWM 28	WWM 33	WWM 34	WWM 35	WWM 37	WWM 38	WWM 39	WWM 40	WWM 41	WWM 42	WWM 44	WWM 46	WWM 47	WWM 48	WWM 50
WWM26	0.00															
WWM28	0.26	0.00														
WWM33	0.32	0.32	0.00													
WWM34	0.32	0.32	0.00	0.00												
WWM35	0.26	0.26	0.06	0.06	0.00											
WWM37	0.26	0.51	0.32	0.32	0.26	0.00										
WWM38	0.64	0.51	0.45	0.45	0.39	0.39	0.00									
WWM39	0.26	0.26	0.06	0.06	0.00	0.26	0.39	0.00								
WWM40	0.13	0.39	0.19	0.19	0.13	0.13	0.51	0.13	0.00							
WWM41	0.39	0.51	0.45	0.45	0.39	0.39	0.64	0.39	0.26	0.00						
WWM42	0.51	0.51	0.50	0.50	0.51	0.51	0.64	0.51	0.64	0.77	0.00					
WWM44	0.26	0.51	0.45	0.45	0.39	0.13	0.51	0.39	0.26	0.39	0.43	0.00				
WWM46	0.45	0.71	0.51	0.51	0.45	0.19	0.32	0.45	0.32	0.45	0.63	0.19	0.00			
WWM47	0.77	0.64	0.75	0.75	0.77	0.77	0.39	0.77	0.90	0.90	0.26	0.69	0.63	0.00		
WWM48	0.64	0.51	0.71	0.71	0.64	0.64	0.26	0.64	0.77	0.77	0.39	0.56	0.50	0.13	0.00	
WWM50	0.39	0.39	0.19	0.19	0.26	0.26	0.39	0.26	0.39	0.64	0.30	0.26	0.45	0.56	0.51	0.00
WWM57	0.39	0.26	0.19	0.19	0.13	0.39	0.26	0.13	0.26	0.39	0.64	0.51	0.58	0.64	0.51	0.39
WWM62	0.32	0.24	0.13	0.13	0.06	0.32	0.37	0.06	0.19	0.32	0.58	0.45	0.51	0.75	0.63	0.32
WWM64	0.39	0.26	0.19	0.19	0.13	0.39	0.26	0.13	0.26	0.39	0.64	0.51	0.58	0.64	0.51	0.39
WWM65	0.13	0.39	0.45	0.45	0.39	0.13	0.51	0.39	0.26	0.51	0.39	0.13	0.32	0.64	0.51	0.26
WWM66	0.26	0.39	0.32	0.32	0.26	0.26	0.51	0.26	0.13	0.26	0.77	0.39	0.45	0.90	0.77	0.51
WWM67	0.39	0.39	0.19	0.19	0.13	0.13	0.26	0.13	0.26	0.51	0.39	0.26	0.32	0.64	0.51	0.13
WWM68	0.32	0.24	0.19	0.19	0.19	0.45	0.50	0.19	0.32	0.45	0.45	0.45	0.64	0.63	0.63	0.26
WWM76	0.51	0.39	0.19	0.19	0.26	0.51	0.51	0.26	0.39	0.51	0.64	0.64	0.71	0.77	0.77	0.39
WWM79	0.26	0.51	0.32	0.32	0.26	0.00	0.39	0.26	0.13	0.39	0.51	0.13	0.19	0.77	0.64	0.26
WWM80	0.58	0.45	0.43	0.43	0.45	0.45	0.45	0.45	0.58	0.58	0.32	0.45	0.64	0.45	0.58	0.24
WWM81	0.26	0.26	0.06	0.06	0.00	0.26	0.39	0.00	0.13	0.39	0.51	0.39	0.45	0.77	0.64	0.26
WWM82	0.39	0.26	0.19	0.19	0.13	0.39	0.26	0.13	0.26	0.39	0.64	0.51	0.58	0.64	0.51	0.39
WWM84	0.26	0.39	0.32	0.32	0.26	0.26	0.39	0.26	0.13	0.26	0.77	0.39	0.45	0.77	0.64	0.51
WWM85	0.26	0.51	0.32	0.32	0.26	0.00	0.39	0.26	0.13	0.39	0.51	0.13	0.19	0.77	0.64	0.26
WWM86	0.32	0.24	0.13	0.13	0.06	0.32	0.32	0.06	0.19	0.37	0.58	0.45	0.51	0.71	0.58	0.32
WWM87	0.19	0.45	0.39	0.39	0.32	0.06	0.45	0.32	0.19	0.45	0.45	0.06	0.26	0.71	0.58	0.19
WWM89	0.32	0.24	0.13	0.13	0.06	0.32	0.32	0.06	0.19	0.37	0.58	0.45	0.51	0.71	0.58	0.32

Table 2.8 (continued).

Accession	WWM57	WWM62	WWM64	WWM65	WWM66	WWM67	WWM68	WWM76	WWM79	WWM80	WWM81	WWM82	WWM84	WWM85	WWM86	WWM87	WWM89
WWM57	0.00																
WWM62	0.11	0.00															
WWM64	0.00	0.11	0.00														
WWM65	0.51	0.45	0.51	0.00													
WWM66	0.26	0.24	0.26	0.39	0.00												
WWM67	0.26	0.19	0.26	0.26	0.39	0.00											
WWM68	0.24	0.13	0.24	0.45	0.37	0.32	0.00										
WWM76	0.26	0.24	0.26	0.64	0.39	0.39	0.29	0.00									
WWM79	0.39	0.32	0.39	0.13	0.26	0.13	0.45	0.51	0.00								
WWM80	0.45	0.39	0.45	0.45	0.58	0.32	0.26	0.45	0.45	0.00							
WWM81	0.13	0.06	0.13	0.39	0.26	0.13	0.19	0.26	0.26	0.45	0.00						
WWM82	0.00	0.11	0.00	0.51	0.26	0.26	0.24	0.26	0.39	0.45	0.13	0.00					
WWM84	0.13	0.24	0.13	0.39	0.13	0.39	0.37	0.39	0.26	0.58	0.26	0.13	0.00				
WWM85	0.39	0.32	0.39	0.13	0.26	0.13	0.45	0.51	0.00	0.45	0.26	0.39	0.26	0.00			
WWM86	0.06	0.06	0.06	0.45	0.24	0.19	0.19	0.24	0.32	0.43	0.06	0.06	0.19	0.32	0.00		
WWM87	0.45	0.39	0.45	0.06	0.32	0.19	0.39	0.58	0.06	0.39	0.32	0.45	0.32	0.06	0.39	0.00	
WWM89	0.06	0.06	0.06	0.45	0.24	0.19	0.19	0.24	0.32	0.43	0.06	0.06	0.19	0.32	0.00	0.39	0.00

2.4. Discussion

The present study assessed the genetic diversity of 48 citron watermelon landrace collections commonly grown for food and feed across various districts in the Limpopo Province of South Africa. The goal was to identify new sources of variation for trait integration or new variety design possessing farmer and consumer attributes. Previously, Mashilo et al. (2017) genotyped 34 accessions of citron watermelon, resolving moderate genetic differentiation. Ngwepe et al. (2021b) phenotyped a collection of some 36 widely grown accessions of citron watermelon using agronomic and horticultural traits. High-yielding accessions were identified and selected for breeding. Hybridization of some selected accessions resulted in relatively low hybrid vigour for important agronomic traits, including fruit yield. The reduced vigour could be attributed to the low genetic variation and recombination of the parental genotypes. To harness the genetic variation and possibly enhance hybrid vigour for desirable traits, we included 17 new promising accessions and subjected to genetic analysis (Table 2.1). Together with the previously genotyped accessions, 11 SSR markers developed specifically for sweet dessert watermelon were used for genetic diversity analysis of the 48 accessions for genotype selection. The SSR markers amplified a total of 24 alleles. The number of alleles detected in the present populations was higher than the number of alleles reported by Zhang et al. (2012) and Guerra-Sanz (2002) using the same set of SSR markers. Four of the eleven markers, namely: BVWS00948, BVWS00048, BVWS00288, and BVWS00106, were monomorphic in the present study. However, the monomorphic loci were found to be polymorphic when analyzing for genetic diversity of dessert watermelon genotypes (Zhang et al., 2012). Citron and dessert watermelon are genetically distinct species (Levi et al., 2001, 2013). Previous reports indicated that SSR markers can be used interchangeably among the *Citrullu*s species, given syntenic genes (Jarret et al., 1997; Mashilo et al., 2017). The poor discriminatory power of some of the SSR loci (i.e., BVWS00948, BVWS00048, BVWS00288, and BVWS00106) suggests the need to develop SSR markers specific for the current citron watermelon populations for effective germplasm characterization, marker assisted selection and breeding.

The mean of the expected heterozygosity value was 0.38, indicating moderate genetic differentiation among the citron watermelon accessions. The tested citron watermelon landrace accessions were collected from within three districts (i.e., Capricorn, Waterberg, and Sekhukhune) in the Limpopo Province of South Africa. Farmers across different villages and districts often share/exchange citron watermelon seed for production, contributing to a high level of gene flow observed in the present study. Also, outcrossing between different accessions in farmers' fields may cause gene exchange in citron watermelon (Dane and Liu, 2007). The current findings suggest that successive artificial selection and domestication may have reduced the genetic diversity within and among citron watermelon genetic resources. This suggests the low molecular variation observed among populations.

The selection of distantly related accessions of citron watermelon can maximize the success in developing high-yielding hybrids. In the current study, several accessions were found to be genetically related. WWM02, WWM08, and WWM67 were collected from villages Kgohloane, Moletjie-Moshate, and Uitkyk respectively, located within the Capricorn district. In addition, WWM02 and WWM08 with yellow flesh colour were sourced from Kgohloane and Moletjie-Moshate, respectively, whereas WWM67 from Uitkyk had an orange flesh colour (Figure 2.1). Furthermore, accessions WWM03, WWM25, WWM35, WWM39 and WWM81 were genetically related but were collected from villages Kgohloane, Malegane, Ga-Molepo, Ga-Mphela and Ga-Manamela, respectively (Table 2.1; Figure 2.1). Accessions WWM34 and WWM33 were both collected from Ga-Molepo village in Capricorn district and had different flesh and seed coat colours but were genetically similar. These observations indicated that variations in morphological traits and collection sites were not useful indicators to measure the extent of genetic diversity in the presently assayed population of citron watermelon. This is because accessions collected from the different villages and districts might have similar parentage. Contrary to the present study, Mujaju et al. (2011) indicated that on-farm conservation of landraces through farmers' cultivation practices resulted in genetic variation. Extensive phenotypic variation occurs in citron watermelon genetic resources for fruit and seed traits (Ngwepe et al., 2021a), most likely through uncontrolled crosses of accessions planted in close proximity. As evidenced in the present study, the detected phenotypic variations have not translated into the desired genetic profiles between accessions using the current set of SSR markers. For example, accessions WWM15 and WWM47 had light green fruit colour and orange flesh but their seed coat colours were maroon and red, respectively.

Accessions WWM23, WWM40, WWM41 and WWM66 were characterized by elliptic fruit shape and red seed coat colour, except for WWM66, which had brown seed with black spots (Figure 2.1 and Figure 2.2). Accessions WWM15, WWM23, WWM40, WWM41 and WWM66 were collected from Turfloop (Capricorn district), Maeteletsa (Waterburg), Kgohloane (Capricorn district), and Uitkyk (Capricorn district), respectively, whereas WWM47 and WWM48 were collected from Nebo (Sekhukhune district). The results indicated that farmers in the respective districts maintained and cultivated diverse citron watermelon genetic resources. Therefore, the citron watermelon accessions with low genetic distances are not recommended for hybrid breeding. Mashilo et al. (2017) also found accessions WWM02, WWM03, WWM08, WWM14, WWM15, WWM16, WWM34, WWM35, WWM37, WWM39, WWM44 and WWM48 to be genetically related, agreeing with the present study. Of the 17 newly included accessions, WWM86 and WWM89, and WWM64, WWM82 and WWM57 were closely related (Figure 2.3). Also, accessions WWM85 and WWM57 were highly similar (Figure 2.3). However, the newly included accessions were found to be genetically unrelated comapred with the previously genotyped accessions WWM02, WWM03, WWM05, WWM08, WWM14, WWM15, WWM16, WWM22, WWM23, WWM24, WWM33, WWM34, WWM37, WWM38, WWM39 and WWM40. This was discerned based on Euclidean genetic distance matrix (Table 2.8). Among the 17 newly studied accessions, most genotype pairs were unrelated, including WWM62 and WWM57, WWM81 and WWM57, WWM85 and WWM66, WWM84 and WWM80 and WWM84 and WWM81. Therefore, the development of superior hybrids from the stated pairs of accessions would ensure better integration of desirable agronomic traits and yield gains. Also, new variety development for leafy vegetable, fodder, fruit and seed yields could be possible through crosses between distantly related genotypes selected from different clusters.

2.5. Conclusions

In conclusion, the following distantly related genotypes are recommended as breeding parents, WWM03, WWM04, WWM15, WWM16, WWM18, WWM22, WWM23, WWM24, WWM25, WWM26, WWM28, WWM33, WWM34, WWM35, WWM38, WWM39, WWM41, WWM66, WWM76, WWM78, WWM81, WWM84, WWM86 and WWM89 (selections from Cluster I), WWM14, WWM37, WWM42, WWM44, WWM46, WWM65, WWM79, WWM85 and WWM87 (Cluster II), and WWM38, WWM47 and WWM48 (Cluster III). These are useful parental lines for pre-breeding to develop and release new varieties with multiple uses.

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Chapter 3. Variation in South African citron watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.) landrace accessions assessed through qualitative and quantitative phenotypic traits

Abstract

Citron watermelon is an important genetic resource serving for food, feed, and breeding dessert watermelon (*C. lanatus* var. *lanatus*) and other cross-compatible species. Wide phenotypic variation exists among South African citron watermelon landraces which are yet to be systematicaly assessed for diverse uses and cultivar design. The objective of this study was to assess phenotypic diversity of citron watermelon landrace accessions of South Africa and to select desirable genotypes with suitable agronomic and horticultual traits for direct production, breeding and conservation. Thirty six citron watermelon landrace accessions were evaluated under field conditions in two seasons using a 6 × 6 lattice design with three replicates. Data on key qualitative and quantitative traits were collected and subjected to non-parametric and parametric statistical analyses. The accessions showed wide phenotypic variation and unique traits for genetic improvement. Positive and significant correlations ($p < 0.001$) were recorded between total fruit yield per plant with plant height ($r = 0.64$), number of harvestable fruits ($r = 0.70$), number of marketable fruits ($r = 0.73$) and marketable fruit yield ($r = 0.96$). Seed yield per plant positively and significantly ($p < 0.001$) correlated with number of male flowers ($r = 0.68$), plant height ($r = 0.61$) and total fruit yield ($r = 0.79$). Prinicipal component analysis identified nine components which accounted for 86.38% of total variation amongst accessions for assessed phenotypic traits. Citron watermelon accessions WWM16, WWM67 and WWM79 were high-yielding suitable for fruit production for food or livestock feed. Accessions namely WWM03, WWM09, WWM16, WWM39, WM64 and WWM76 produced high fruit number and marketable yield per plant which are recommended as parental genotypes for breeding. Furthermore, accessions WWM04, WWM15, WWM24, WWM28, WWM46, WWM66, WWM68, WWM76 and WWM79 were selected with higher seed production for the food or feed industry. The study recommends citron watermelon accessions such as WWM14, WWM16, WWM39, WWM41, WWM67 and WWM79 for use as leafy vegetables owing to their profuse branching ability and longer vine production, whereas accessions including WWM03, WWM17, WWM35, WWM40, WWM50, WWM67, WWM79 and WWM85 are selected with larger fruit size. Accessions WWM05 and WWM09 are sour-flesh types which are suitable genetic stocks for breeding sweet-and-sour dessert watermelons. Orange-fleshed accessions such as WWM03, WWM04, WWM46, WWM64, WWM66 and WWM67 are recommended for fresh consumption, cooking, processing or variety design. Accessions WWM02, WWM03, WWM08, WWM14, WWM16, WWM23, WWM38, WWM40, WWM41 and WWM67 have red and white seed coat colour which are superior selections to prepare roasted citron watermelon seed snack.

Keywords: *Citron watermelon, genetic resources, horticultural traits, principal component analysis, quantitative traits*

3.1. Introduction

Citron watermelon [*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb., 2n=2x=22] is an important cucurbit species serving for human consumption, animal feed and for breeding of sweet watermelon. According to Chomicki and Renner (2015) seven species of *Citrullus* are recognized. *Citrullus lanatus* (Thunb.) Matsum. et Nakai includes the sweet dessert watermelon (*C. lanatus* var. *lanatus*) and citron watermelon [*C. lanatus* var. *citroides* (Bailey) Mansf. ex Greb.] (Laghetti and Hammer, 2007). Sweet and citron watermelon are thought to have originated from *C. ecirrhosus* (Dane and Liu, 2007). *Citrullus lanatus* var. *citroides* is also referred to as *C. amarus* (Chomicki and Renner, 2015). In sub-Saharan Africa (SSA), citron watermelon is domesticated and cultivated for its fruit and succulent leaves which are consumed as cooked vegetable. The leaves possess bioactive compounds such cucurbitacins with anti-cancer, anti-biotic, anti-tumor and other therapeutic properties (Davidovich-Rikanati et al., 2015; Kim et al., 2018). The large yellow or orange-fleshed fruits referred to as “Lerotse” in the local South African Sepedi language are used for cooking traditional meals referred to as “Kgodu” and “Mokgapu” (Ngwepe et al., 2019). Kgodu is prepared by boiling the flesh until is soft and then mixing with maize meal to make porridge, whereas Mokgapu is prepared by boiling the flesh and consumed as soup by adding sugar for taste. The non-sweet yellow and orange-fleshed types possess various carotenoid profiles (i.e., prolycopene, phytoene, and ζ-carotene, violaxanthin, neoxanthin and β-carotene) (Tadmor et al., 2005; Davis et al., 2007; Bang et al., 2007, 2010; Maragal et al., 2019). Orange-fleshed types serve as a cheap alternative source for combating vitamin A deficiencies and other health-related illnesses prevalent in SSA.

Citron watermelon fruits are an important source of water and bioactive compounds and are commonly used as livestock (i.e., cattle, goats and pigs) feed in the region (Laghetti and Hammer, 2007; Nantoumé et al., 2013; Ngwepe et al., 2019). Seed extracted from mature fruit are eaten as snack after roasting. Roasted seed is rich in protein (≥ 23%), minerals, essential vitamins and essential amino acids (i.e., arginine, isoleucine, eucine, phenylalanine, glutamic acid and aspartic acid (Singh and Matta, 2010; Achu et al., 2013; Yoo et al., 2013). Therefore, underutilized and neglected crops such as citron watermelon have diverse dietary benefits useful to combat food insecurity and nutrition deficiency.

Citron watermelon exhibits extensive phenotypic variation for fruit shape, fruit size, fruit skin colour patterns and seed coat colours (Dane and Liu 2007; Choudhary et al., 2012; Mujaju et al., 2013). The wide phenotypic variation present in the species is useful for selecting promising genotypes for diverse uses (Munisse et al*.,* 2013; Mashilo et al., 2017; Singh et al., 2017). Genetically diverse landrace accessions of citron watermelon are widely grown by small-holder farmers in South Africa under low-input agricultural systems (Mashilo et al., 2017). Citron watermelon genetic resources from the country were previously identified as a source of resistance to several diseases, and thus serve as useful source of genes for disease resistance breeding for sweet dessert watermelon (Ma and Wehner, 2015; Tetteh et al*.,* 2010; Thies et al*.,* 2015). Citron watermelon could also be a useful source of variation for agronomic and horticultural traits for breeding. The crop is used as a rootstock for commercial production of sweet dessert watermelon owing to its resistance to biotic (e.g., root-knot nematodes and *Fusarium* wilt) and abiotic stress (e.g., drought and heat) factors (Cohen et al., 2014; Edelstein et al., 2014; Thies et al., 2015). The phenotypic variation present among South African citron watermelon landraces is yet to be systematicaly assessed for diverse uses and socio-economic benefits.

Characterization of crop genetic resources is an essential step to develop conservation and breeding strategies in crop improvement programmes. Germplasm characterization usually comprises of a detailed description of accessions for morphological and agronomic traits, and other useful attributes. Quantitative and qualitative phenotypic traits aid in identifying differences between individuals of a species (Solmaz and Sarı, 2009; Szamosi et al., 2009; Achigan-Dako et al., 2015). Quantitative traits are measurable characteristics and generally controlled by multiple genes and highly influenced by the environment. Conversely, qualitative traits have categorical values and categorized as binary or multi-categorical (defined by several categories or classes) and are controlled by single dominant or recessive genes and less influenced by the environment. Watermelon exhibits extensive variation for fruit qualitative traits (e.g.

fruit shape, fruit colour, fruit rind stripe patterns) and seed coat traits (i.e., Seed shape, seed colour) which are key attributes for germplasm classification (Gusmini and Wehner, 2006; Solmaz and Sarı, 2009; Lou and Wehner, 2016). Also, watermelon shows variation for quantitative traits such the number of male flowers per plant, plant height, number of fruit per plant, fruit and seed yield (Solmaz and Sarı, 2009; Szamosi et al., 2009). Consequently, both qualitative and quantitative traits are widely used for characterization of watermelon genetic resources for genetic analysis and breeding (Lima et al., 2017). Currently, there is limited information on phenotypic variation of citron watermelon crop for economic traits. This has partly resulted in limited breeding opportunity for cultivar development and release to serve the food and feed sectors and their value chains nationally and internationally. The genetic value of citron watermelon crop has not been fully utilized for sweet dessert watermelon breeding and production. Therefore, the objective of this study was to assess the phenotypic diversity of citron watermelon landrace accessions of South Africa to select desirable genotypes with suitable agronomic and horticultual traits for direct production, breeding and conservation.

3.2. Materials and methods

3.2.1. Study site

Field experiments were conducted in 2018/19 and 2019/20 growing seasons under dry-land conditions at Towoomba Agricultural Development Centre (TADC), Bela-Bela, South Africa (28º19'28''E, 24º53'57''S; 1 184 m above sea level). The soil type is of the Hutton soil form (Botha et al., 2014). The area usually receives mean annual rainfall of 627 mm with erratic distribution. The daily mean minimum and maximum temperatures ranged from 16.5°C to 29.7°C during the growing season (South African Weather Services (SAWS), www.weathersa.co.za).

3.2.2. Plant material and experimental layout

Thirty-six citron watermelon landrace accessions were used for the study. The accessions were collected from three districts in the Limpopo Province (i.e., Capricorn, Waterberg and Sekhukhune), known for domesticated and wild forms of citron watermelon. The collections are maintained at TADC of the Limpopo Department of Agriculture and Rural

Development, South Africa. The accessions used in the present study were confirmed to be *Citrullus lanatus* var. *citroides* when compared with the herbarium specimens maintained at the Agricultural Research Council (ARC) Roodeplaat, Herbarium Unit, Pretoria, South Africa. Details of the accessions and districts of collection is provided in Table 3.1. The 36 landrace accessions were evaluated using a 6 × 6 lattice design in three replicates. Three seeds were directly sown per planting hole in the field at 7 m inter-row and 6 m intra-row spacing. Three weeks after emergence (at 3–4 true leaf stage) seedlings were thinned to one plant per hill. Weeding was done manually, and no fertilizer and pesticides were applied to mimic low-input agricultural and cultural practices under which the crop is mostly grown in South Africa.

3.2.3. Data collection

Data for the following qualitative traits were collected: leaf shape, primary fruit colour, secondary fruit colour, fruit shape and size, presence of rind stripes, rind stripes colour, rind stripe size, rind stripe patterns, fruit grooves, rind hardness, flesh colour, flesh firmness, seed coat colour, presence of seed eyes and seed size. Qualitative data were collected following descriptor list of watermelon (IPGRI, 2003; UPOV, 2004) at seedling stage, flowering, and at mature fruit stages (i.e., approximately 120 days after planting). Also, additional descriptors were used to classify other useful qualitative traits to reveal the pattern of variation among the accessions in addition to the IBPGR and UPOV descriptors. These included qualitative traits such as rind stripe colour and size, rind stripe uniformity, and presence of fruit grooves. The qualitative traits and their respective categories are summarized in Table 3.2. Quantitative data was collected for leaf, plant, fruit and seed traits. Leaf traits were measured during the vegetative growth stages, whereas plant, fruit and seed traits were measured at harvest. Details of the measured quantitative traits are provided in Table 3.3.

Table 3.1 Names, district and village of collection in Limpopo Province of South Africa, fruit and seed traits of 36 citron watermelon landrace accessions used in the study.

		Origin/source		Fruit traits		Seed traits	
Sr. No	Accession code	District	Village	FrShp	FCol	Sdcol	Sdsz
1	WWM02	Capricorn	Kgohloane	R	Y	RW	M
2	WWM03	Capricorn	Kgohloane	E	O	RW	S
3	WWM04	Capricorn	Kgohloane	R	O	G	M
4	WWM05	Capricorn	Kgohloane	E	W	GBrS	M
5	WWM08	Capricorn	Moletjie-Moshate	E	Y	RW	L
6	WWM09	Capricorn	Moletjie-Moshate	R	W	B	M
7	WWM14	Capricorn	Turfloop	R	O	RW	L
8	WWM15	Capricorn	Turfloop	R	O	Mr	L
9	WWM16	Waterberg	Bela-Bela	R	Y	R	L
10	WWM17	Waterberg	Styloop	R	O	BrS	M
11	WWM23	Waterberg	Maeteletsa	E	Y	RW	M
12	WWM24	Capricorn	Westenburg	E	Y	G	L
13	WWM28	Sekhukhune	Malegane	R	O	R	L
14	WWM33	Capricorn	Ga-Molepo	E	Y	RW	M
15	WWM34	Capricorn	Ga-Molepo	R	O	CW	S
16	WWM35	Capricorn	Ga-Molepo	E	Y	T	M
17	WWM37	Capricorn	Ga-Molepo	E	O	Mr	M
18	WWM38	Capricorn	Ga-Molepo	E	O	RW	S
19	WWM39	Capricorn	Ga-Mphela	BE	O	T	M
20	WWM40	Capricorn	Kgohloane	E	O	RW	S
21	WWM41	Sekhukhune	Nebo	E	Y	RW	S
22	WWM42	Sekhukhune	Nebo	E	O	R	M
23	WWM44	Sekhukhune	Nebo	R	O	R	M
24	WWM46	Sekhukhune	Nebo	E	O	R	M
25	WWM47	Sekhukhune	Nebo	R	O	R	M
26	WWM50	Sekhukhune	Nebo	E	Y	R	M
27	WWM57	Capricorn	Uitkyk 3	E	O	BrS	M
28	WWM64	Capricorn	Ga-Mphela	E	O	Br	S
29	WWM65	Waterberg	Vienna	R	O	R	M
30	WWM66	Capricorn	Uitkyk 3	E	O	BrS	M
31	WWM67	Capricorn	Uitkyk 3	E	O	RW	S
32	WWM68	Capricorn	Ga-Manamela	E	Y	G	S
33	WWM76	Capricorn	Ga-Manamela	E	Y	BrS	M
34	WWM79	Capricorn	Ga-Manamela	E	O	CW	M
35	WWM81	Capricorn	Ga-Manamela	E	Y	CW	S
36	WWM85	Capricorn	Ga-Manamela	R	O	BrS	M

Sr. no = Serial number; FrShp = fruit shape; R = round; E = elliptic; B = broad elliptic; FCol = flesh colour; O = orange; Y = yellow; W white; Sdcol = seed colour; B = black; Br = brown; BrS = brown with black spot; CW = cream white; G = green; GBrs = green with brown spot; Mr = marron; R = red; RW = red-white; T = tan; Ssz = seed size; L = large; M = medium; S = small.

Table 3.2 Description of qualitative traits used for phenotypic assessment of citron watermelon accessions.

Qualitative traits	Trait abbreviation	Description of traits
Leaf shape	LShp	1=lobed, 2 = non-lobed
Fruit size	Frsz	1=small, 2=medium, 3=large
Main fruit colour	MFrC	1=green, 2=light green, 3=dark green
Secondary fruit colour	SFrC	None = 0, 1=green, 2=light green, 3=dark green
Fruit shape	FrShp	1=round, 2=elliptic, 3=broad elliptic
Rind stripe presence	RStrp	0=no, 1=yes
Rind stripe colour	RStrC	None = 0, 1=green, 2=light green, 3=dark green
Rind stripe size	RStrsz	None = 0, 1=thin, 2=Medium, 3=Thick, 4=Patchy
Rind uniformity	RStrU	None = 0, 1=Uniform, 2 = non-uniform
Presence of fruit grooves	FrGr	0=No, 1=yes
Flesh colour	FCol	1=orange, 2=white, 3=yellow
Seed colour	SdCol	1=black, 2=brown, 3=brown with black spots, 4=green with brown spots, 5=cream white, 6=green, 7=Maroon, 8=red, 9=red & white, 10=tan
Presence of seed eyes	SdEy	0=no, 1=yes
Seed size	Sdsz	1=small, 2=medium, 3=large

Table 3.3 Descriptions of measurement of quantitative traits used for phenotypic assessment of citron watermelon accessions.

Quantitative traits	Abbreviations	Details of measurement
Leaf length	LL	Measured in mm using a digital Vernier caliper (Metr.-ISO)
Leaf width	LW	Measured in mm using a digital Vernier caliper (Metr.-ISO)
Number of days to first male flower appearance	NdMFl	Counted as number of days from date of planting to when the first male flower appeared
Number of days to first female flower appearance	NdFFl	Counted as number of days from planting to when a first female flower appeared
Total number of male flowers	NMFl	were regularly counted and added as flower peduncles attached to the plant without the ovary
Total female flowers	TNFfl	were regularly counted and added based on flower peduncles showing well-developed ovary
Sex ratio	SexR	Computed as the ratio of total female to male flowers
Plant height	PH	Measured in m from the base to the tip of the main vine/stem at harvest
Number of primary branches	NPBr	Counted as branches from the main stem
Number of secondary branches	NSBr	Counted as branches from primary branches
Total number of branches	TNBr	Computed as the sum of primary and secondary branches
Total number of fruits	TNFr	Counted as number fruits per plant at harvest
Ratio of total female flowers to total number of fruits per plant	RFflTFr	Computed
Total fully grown fruits	FGFr	Weight in kg of total fully grown fruits
Number of marketable fruits	NMFr	Counted as medium to large fruits without defects
Number of unmarketable fruits	NUFr	Counted as small sized fruits and/or fruits with defects
Number of aborted fruits	NAFr	Counted
Marketable yield	MYld	Weight in kg of medium to large-sized fruits per plant
Total fruit yield	TFYld	Weight in kg of total number of fruits per plant
Fruit weight	FrW	Weight in kg of one fully grown fruit
Fruit length	FrL	Measured in cm
Fruit diameter	FrD	Measured in cm
Rind diameter	RD	Measured in mm using a Vernier caliper (Metr.-ISO)
Total soluble solids	TSS	Measure using a portable refractometer ATAGO Brix (0~53)
Number of seed per fruit	NSPFr	Counted
Seed yield per fruit	SYPFr	Weight in g of total number of seeds per fruit
Total seed yield	TSY	Computed as the product of total number of fruits per plant by seed yield per fruit
Seed diameter	SL	Measured in mm using a digital Vernier caliper (Metr.-ISO)
Seed length	SD	Measured in mm using a digital Vernier caliper (Metr.-ISO)
Hundred seed weight	HSW	Weight in g using weighing scale

3.2.4. Data analysis

Data collected on qualitative traits were subjected to non-parametric test to summarise frequency analysis using the cross tabulation procedure of SPSS version 26.0 (IBM Crop. Armonk, NY, 2019). Data collected for quantitative traits were subjected to analysis of variance (ANOVA) using Genstat (Payne et al., 2017). Accession means were separated using the Least Significant Test (LSD) at 5% level of significance. Correlation analysis was performed to determine the level of associations for the studied quantitative traits using R version 4.0 (Integrated Development for R 2020). Significance of the correlations of the test variables was assessed using a t-test at 5% significance level in R. Principal component analysis (PCA) was performed using SPSS version 26.0. Principal components (PC) with eigenvalues greater than unity were considered important in explaining the variability for studied quantitative traits. A PC biplot using principal component 1 (PC1) and principal component 2 (PC2) was constructed to identify relationships between citron watermelon accessions and evaluated quantitative traits.

3.3. Results

3.3.1. Variation for qualitative traits among citron watermelon accessions

The frequency distribution of fourteen qualitative traits for 36 citron watermelon accessions is presented in Table 3.4. Cross tabulation analysis revealed significant associations between accessions and assessed qualitative traits. Most accessions (97%) had lobed leaf shape except for WWM24 which exhibited a non-lobed leaf shape. For fruit size, thirteen accessions had large fruit, twelve accessions had medium-sized fruit and eleven accessions had small fruits. The main fruit colour was light green recorded for 27 accessions, followed by dark green (five accessions). The dominant secondary fruit colour was dark-green recorded for 14 accessions, whereas 18 accessions had no secondary fruit colour. Twenty-two of the studied citron watermelon accessions possessed elliptic fruit shape, whereas 13 accessions had round fruit shape, and only one accession (i.e., WWM39) exhibited a broad-elliptic fruit shape. As depicted in Figure 3.1, the assessed citron watermelon accessions showed extensive variation for fruit shapes, rind stripe patterns and fruit colour.

For rind stripes, 33.3% (12 accessions) possessed rind stripes, whereas 24 accessions had no fruit rind stripes. Rind stripes colour varied between green (8.3% of the accessions) and dark green (91.7%). Four accessions had medium stripes, followed by three accessions which had patchy and wide stripes, two accessions had thin rind stripes. Rind stripe uniformity was categorized as uniform (stripes which are distinct and follow a clear pattern from the stem end to the blossom end) and non-uniform (stripes which do not follow a distinct pattern). Eight accessions had non-uniform rind stripes, whereas six accessions had uniform rind stripes and were most round in shape. The fruits of 18 accessions were without grooves, whereas 18 of the remaining accessions had groove fruits (Figure 3.1).

Figure 3.1: Variation in fruit shape and colour, rind stipe patterns and flesh colour of 36 citron watermelon accessions collected from three districts of the Limpopo Province, South Africa.

Figure. 3.1: (Continued)**.** Variation in fruit shape and colour, rind stipe patterns and flesh colour of 36 citron watermelon accessions collected from three districts of the Limpopo Province, South Africa.

Table 3.4 Frequency distribution and significance tests comparing 36 citron watermelon accessions based on qualitative traits.

Trait	Description	Accessions possessing trait	Frequency (%)	df	Chi-square	*P*-value
Leaf shape	Lobed	35	97	35	36	0.42
	Non-lobed	1	3			
Fruit size	Small	11	31	70	72	0.41
	Medium	12	33			
	Large	13	36			
Main fruit colour	Green	4	11	70	72	0.41
	Light green	27	75			
	Dark green	5	14			
Secondary fruit colour	None	18	50	70	72	0.41
	Green	-	-			
	Light green	4	11			
	Dark green	14	39			
Fruit shape	Round	13	36	70	72	0.41
	Elliptic	22	61			
	Broad-elliptic	1	3			
Rind stripe presence	No	24	67	35	36	0.42
	Yes	12	33			
Rind stripe colour	None	24	67	70	72	0.41
	Green	1	3			
	Light green	-	-			
	Dark green	11	31			
Rind stripe size	None	24	67	140	144	0.39
	Thin	2	6			
	Medium	4	11			
	Thick	3	8			
	Patchy	3	8			
Rind uniformity	None	22	61	70	72	
	Uniform	6	17			
	Non-uniform	8	22			
Presence of fruit grooves	No	18	50	35	36	0.42
	Yes	18	50			
Flesh colour	Orange	22	61	70	72	0.41
	White	2	6			
	Yellow	12	33			

Table 3.4 (Continued).

Trait	Description	Accessions possessing trait	Frequency (%)	df	Chi-square	*P*-value
Seed colour	Black	1	3	315	324	0.36
	Brown	1	3			
	Brown with black spots	5	14			
	Green with brown spots	2	6			
	Cream white	3	8			
	Green	3	6			
	Maroon	2	6			
	Red	8	22			
	Red & white	10	28			
	Tan	2	6			
Presence of seed eyes	No	17	47	35	36	0.42
	Yes	19	53			
Seed size	Small	9	25	70	72	0.41
	Medium	21	58			
	Large	6	17			

df = degrees of freedom.

Fruit flesh colours were orange, white or yellow. Of the thirty-six accessions, 22 accessions had orange flesh colour, followed by 12 accessions (33.3%) with yellow flesh colour and two accessions namely WWM05 and WWM09 had white flesh colour. Seed coat colours were black, light brown, brown with spots, cream white, green, green with brown spots, maroon, red, red-white and tan (Figure 3.2). Ten accessions had a red-white seed coat colour, followed by red for eight accessions, and five accessions had brown with black spots. Cream white and green seed coat colours were observed for accessions. Maroon and tan were observed for two accessions, whereas black, brown and green with brown spots seed coat colours were observed each for one accession. Nineteen accessions had seed eyes, whereas 17 accessions had no seed eyes. Twenty-one accessions exhibited medium seed size, nine accessions had smaller seed, and six accessions had large seed. Leaf, fruit and seed qualitative traits for all 36 citron watermelon accessions is presented in Table 3.5.

Figure 3.2: Variation of seed coat colours among 36 citron watermelon accessions collected from three districts of the Limpopo Province, South Africa.

Figure. 3.2: (Continued). Variation of seed coat colours among 36 citron watermelon accessions collected from three districts of the Limpopo Province, South Africa.

Table 3.5 Variation for qualitative traits among 36 citron watermelon landrace accessions collected from three districts of the Limpopo Province of South Africa.

	Leaf trait	Fruit traits										Seed traits		
Accession code	LShp	Frsz	MFrC	SFrC	FrShp	RStrp	RStrC	RStrsz	RStrU	FrGr	FCol	Sdcol	SdEy	Sdsz
WWM02	1	3	2	3	1	1	3	3	1	1	3	9	0	2
WWM03	1	3	3	0	2	0	0	0	0	0	1	9	0	1
WWM04	1	3	1	3	1	1	3	2	1	1	1	6	1	2
WWM05	1	1	2	3	2	0	0	0	0	0	2	4	1	2
WWM08	1	3	2	0	2	0	0	0	0	0	3	9	0	3
WWM09	1	1	2	0	1	0	0	0	0	1	2	1	1	2
WWM14	1	2	2	0	1	0	0	0	0	1	1	9	0	3
WWM15	1	1	2	0	1	0	0	0	0	1	1	7	1	3
WWM16	1	1	2	0	1	0	0	0	0	1	3	8	1	3
WWM17	1	2	2	0	1	0	0	0	0	1	1	3	0	2
WWM23	1	2	2	0	2	0	0	0	0	1	3	8	0	2
WWM24	2	1	2	0	2	0	0	0	0	1	3	6	1	3
WWM28	1	2	2	3	1	1	3	2	2	1	1	8	1	3
WWM33	1	1	2	0	2	0	0	0	0	1	3	9	0	2
WWM34	1	2	1	3	1	1	3	2	1	1	1	5	1	1
WWM35	1	2	2	3	2	1	3	3	2	0	3	10	1	2
WWM37	1	2	2	0	2	0	0	0	0	0	1	7	1	2
WWM38	1	1	2	0	2	0	0	0	0	0	1	8	0	1
WWM39	1	3	2	3	3	0	0	0	0	0	1	10	1	2
WWM40	1	3	3	0	2	0	0	0	0	0	1	9	0	1
WWM41	1	2	2	1	2	0	0	0	0	1	3	9	0	1

Table 3.5 (Continued).

	Leaf trait	Fruit traits										Seed traits		
Accession code	LShp	Frsz	MFrC	SFrC	FrShp	RStrp	RStrC	RStrsz	RStrU	FrGr	FCol	Sdcol	SdEy	Sdsz
WWM42	1	1	1	3	2	0	0	0	2	0	1	8	1	2
WWM44	1	3	2	3	1	1	3	1	1	1	1	8	1	2
WWM46	1	1	2	0	2	0	0	0	0	0	1	8	1	2
WWM47	1	1	2	1	1	1	1	1	1	1	1	8	1	2
WWM50	1	2	3	0	2	0	0	0	0	1	3	8	1	2
WWM57	1	2	2	0	2	0	0	0	0	0	1	3	0	2
WWM64	1	3	2	3	2	1	3	3	2	1	1	2	1	1
WWM65	1	1	2	3	1	1	3	2	1	0	1	8	1	2
WWM66	1	3	2	3	2	1	3	4	2	0	1	3	1	2
WWM67	1	3	2	3	2	1	3	4	2	0	1	9	0	1
WWM68	1	2	3	1	2	1	3	4	2	0	3	4	0	1
WWM76	1	3	2	1	2	0	0	0	2	0	3	3	0	2
WWM79	1	3	1	3	2	0	0	0	0	0	1	5	0	2
WWM81	1	3	2	0	2	0	0	0	0	0	3	5	0	1
WWM85	1	2	3	0	1	0	0	0	0	1	1	3	0	2

See Table 3.3 for trait abbreviation.

3.3.2. Variation for quantitative traits among citron watermelon accessions

Combined analysis of variance revealed a presence of significant accession × season interaction effect for most traits, except for leaf length, leaf width, plant height and seed length (Table 3.6). Mean values of phenotypic quantitative traits among the studied citron watermelon landrace accessions are presented in Table 3.7. Significant differences *($p < 0.001$)* were detected among the landrace accessions for the studied quantitative traits. Leaf length ranged from 56.1 to 76.45 mm (mean = 66.84 mm), whereas leaf width ranged from 59.43 to 86.91 mm (mean = 74.90 mm). The number days to first male and female flower appearances ranged from 41 to 50 days (mean = 44 days), and 48 to 58 days (mean = 52 days), respectively. Accessions WWM15, WWM50 and WWM64 recorded the first male flower that appeared on 41 days after planting, whereas accessions WWM05, WWM33, WWM34 and WWM44 recorded the first male flower 47 days after planting. For appearance of first female flower, accessions WWM02, WWM40 and WWM41 recorded first female flower in 48 days, whereas accessions WWM05, WWM34 and WWM79 recorded first female flower after 56 days.

Total number of male flowers per plant varied from 1065 to 3152 with a mean of 1950. Accessions WWM14, WWM35 and WWM67, recorded the highest number of male flowers of 3152, 2786 and 2599, respectively. The lowest number of male flowers of < 1200 were recorded for accessions WWM23, WWM28 and WWM57. Number of female flowers per plant varied from 26 to 102 with a mean of 49 flowers per plant. Accessions WWM02, WWM14 and WWM79 recorded the highest number of female flowers (≥ 96 flowers), whereas accessions WWM05, WWM23, and WWM33 recorded the lowest number of female flowers (≤ 30 flowers). Sex ratio ranged from 26:1 to 65:1, with a mean of 37:1. Accessions WWM08, WWM05, WWM42 and WWM35 recorded the highest sex ratio of 62:1, 65:1, 65:1 and 75:1, respectively, whereas accessions WWM04, WWM15, WWM28 and WWM33 had the lowest sex ratio 30:1 (Table 3.7).

Table 3.6 Mean squares and significant tests for 30 quantitative phenotypic traits among 36 citron watermelon accessions assessed across two seasons.

Trait	Accession (A) df = 35	Season (S) df = 1	A × S df = 35	Residual df = 142
LL	155.15*	121.25ns	119.07ns	85.56
LW	229.90*	21040.70*	240.90ns	126.70
NdMfl	28.71**	1266.34**	32.15**	7.17
NdFfl	45.63**	3174**	37.20**	9.95
NMfl	1794263**	8932616**	1569882**	585162
TNFfl	2296.9**	93500.20**	1895.10**	752.90
SexR	976.80**	39962.20**	1060.30**	259.90
PH	1.81*	0.14ns	0.79ns	0.57
NPBr	3.61ns	23.34*	5.29*	2.83
NSBr	28.71**	1266.34**	32.15**	7.17
TNBr	2475.20**	13744.10**	2689.80**	554.30
TNFr	247.62**	14032.78**	211.08**	93.44
RFflTFr	12.93**	48.38**	10.86**	4.64
FGFr	75.02**	1472.67**	92.19**	21.81
NMFr	30.06**	8.96ns	24.61**	7.83
NUFr	201.79**	15504.17**	205.83**	65.95
NAFr	1867.70**	36192.70**	1457.30**	496.60
MYld	1857.30**	14231.00**	1305.20**	364.40
TFYld	2238.20**	22268.50**	1959.70**	581.80
FrW	40.15**	4000.02**	16.31**	4.09
FrL	141.45**	4040.33**	25.14**	6.61
FrD	51.39**	1879.39**	22.09**	3.97
RD	73.14**	1402.40**	60.00**	16.99
TSS	0.54**	3.20**	0.22**	0.10
NSPFr	88960**	2061744**	56680**	25138
SYPFr	1619.50**	80118.50**	1691**	420.80
TSY	1351456**	25694222**	1435286**	383571
SL	5306ns	5411ns	5289ns	5373
SD	1.43**	7.71**	0.76**	0.24
HSW	40.37**	355.23**	14.82**	2.31

See Table 3.3 for trait abbreviation. df = degrees of freedom. * and ** = significant at $p < 0.05$ and $p < 0.001$, respectively; ns = non-significant.

Table 3.7 Mean values for quantitative traits of 36 citron watermelon landrace accessions collected from three districts of the Limpopo Province of South Africa.

Accession	Leaf traits		Flower traits					Plant traits				Fruits traits		
code	LL	LW	NdMfl	NdFfl	NMfl	TNFfl	SexR	PH	NPBr	NSBr	TNBr	TNFr	RFflTFr	FGFr
WWM02	68.35	69.98	43	48	1653	98	41	5.78	8	44	52	12	7	9
WWM03	67.95	74.37	44	54	2164	65	37	5.27	8	63	71	25	4	13
WWM04	73.60	81.25	44	52	1723	58	28	5.47	7	40	47	18	5	8
WWM05	56.10	59.43	48	57	1855	30	65	4.98	8	77	84	12	3	5
WWM08	71.53	77.67	44	53	1707	35	62	5.59	8	54	62	12	3	9
WWM09	69.57	70.03	45	51	2736	73	41	5.40	8	62	69	36	2	6
WWM14	65.76	76.26	43	50	3152	102	50	6.29	8	108	116	20	7	18
WWM15	69.15	73.78	41	49	1326	61	27	5.59	8	41	49	13	6	6
WWM16	64.22	72.02	42	51	2576	87	31	6.86	8	62	70	24	4	17
WWM17	68.17	79.61	46	53	1539	34	55	5.24	7	34	41	12	3	7
WWM23	76.45	76.68	45	51	1065	26	60	4.27	6	26	33	9	3	5
WWM24	68.00	83.68	43	52	1801	46	41	4.77	8	38	46	10	6	3
WWM28	70.75	80.28	44	50	1123	56	26	4.92	7	39	46	15	4	9
WWM33	64.57	69.74	47	55	1311	26	30	4.88	7	38	44	11	4	8
WWM34	63.10	76.01	50	58	1527	31	53	4.42	7	45	52	11	3	6
WWM35	74.49	83.56	42	49	2786	64	75	4.65	8	70	78	18	4	14
WWM37	67.73	76.39	45	51	1726	55	33	5.26	7	51	57	11	5	6
WWM38	66.90	86.91	42	49	2199	49	52	5.67	8	50	57	22	2	12
WWM39	72.19	82.08	45	54	2456	73	53	5.55	9	74	83	33	3	8
WWM40	69.61	77.24	44	48	1890	48	44	4.99	8	51	59	11	5	8

Table 3.7 (Continued),

Accession	Fruit traits									Seed traits					
code	NMFr	NUFr	NAFr	Myld	TFYld	FrW	FrL	FrD	RD	TSS	NSPFr	SYPFr	TSY	SL	SD	HSW
WWM02	8	5	86	44.00	65.47	13.40	26.85	27.22	24.53	3.72	675	79.00	882.17	11.66	7.10	11.67
WWM03	5	21	40	48.32	76.91	11.57	30.47	25.48	20.92	3.92	600	70.17	1105.50	12.10	6.26	10.83
WWM04	6	11	40	33.20	51.89	11.34	24.97	26.58	18.94	3.45	797	116.50	1427.67	12.81	7.28	11.83
WWM05	0	12	22	0.00	15.83	2.40	18.85	14.83	13.44	2.75	593	73.50	896.33	13.80	7.43	12.33
WWM08	6	7	23	36.36	58.47	9.78	27.33	24.18	25.01	3.32	554	72.83	923.50	12.84	7.55	13.50
WWM09	6	30	37	0.00	40.74	2.91	14.63	18.62	19.11	2.75	475	71.17	2083.67	12.62	7.62	14.83
WWM14	10	10	82	55.31	82.76	8.84	21.37	26.55	24.12	3.30	626	88.50	1826.83	13.34	7.90	14.00
WWM15	4	9	50	33.98	50.65	7.99	18.87	24.56	18.09	3.13	597	96.33	1106.33	12.17	7.57	16.67
WWM16	13	12	67	93.50	123.41	4.92	18.83	20.77	19.64	2.83	602	97.33	2380.67	13.33	7.61	15.83
WWM17	7	5	23	40.67	63.47	11.63	25.73	26.78	24.5	3.53	490	60.33	712.67	11.71	6.97	12.00
WWM23	4	5	17	23.06	40.01	7.28	23.85	22.62	19.49	3.12	676	70.17	662.17	10.99	6.37	10.00
WWM24	2	9	39	10.34	26.87	8.10	28.13	21.38	21.01	3.78	479	94.00	705.33	15.25	8.64	20.83
WWM28	5	10	41	34.40	56.60	11.12	25.93	23.98	19.01	3.47	551	99.50	1416.17	13.10	7.88	19.33
WWM33	4	8	14	26.82	45.41	10.66	26.05	23.72	19.92	3.27	615	80.33	694.33	12.10	7.32	13.00
WWM34	6	5	20	37.57	55.99	11.15	21.48	27.83	15.47	3.95	487	59.17	590.50	12.73	7.02	11.50
WWM35	10	12	43	32.36	50.86	9.90	32.22	23.05	21.43	3.55	756	107.17	1406.17	12.20	7.21	13.33
WWM37	6	5	44	40.53	62.40	10.60	29.45	24.25	21.17	3.52	706	93.00	933.50	12.51	6.96	14.00
WWM38	5	17	26	40.54	72.51	7.85	25.47	22.45	19.63	3.50	614	70.33	1190.17	11.99	7.01	10.67
WWM39	6	26	40	51.27	81.69	8.99	40.37	19.1	22.52	3.87	372	57.67	1859.67	13.29	7.82	15.83
WWM40	5	6	37	40.18	64.74	13.23	31.6	25.47	26.67	3.62	755	80.33	825.50	11.31	6.53	10.50

Table 3.7 (Continued),

Accession	Leaf traits		Flower traits					Plant traits				Fruits traits		
code	LL	LW	NdMfl	NdFfl	NMfl	TNFfl	SexR	PH	NPBr	NSBr	TNBr	TNFr	RFflTFr	FGFr
WWM41	61.25	70.42	43	48	2389	51	54	5.61	8	49	56	25	2	9
WWM42	62.51	78.83	44	49	1925	46	65	5.15	6	51	57	16	2	9
WWM44	67.58	76.56	49	55	1706	79	28	4.96	10	43	53	10	8	5
WWM46	57.68	60.70	42	49	1346	43	44	4.46	8	24	32	13	4	11
WWM47	65.85	73.77	43	50	2058	60	37	4.93	8	62	71	19	5	10
WWM50	69.25	73.57	41	54	2663	52	36	5.37	8	76	84	18	4	13
WWM57	64.12	73.47	46	51	1090	47	38	4.59	7	27	33	12	4	6
WWM64	61.90	74.94	41	51	2447	54	56	5.76	7	53	60	17	3	12
WWM65	73.90	81.03	44	49	2295	74	33	4.98	8	55	63	24	4	9
WWM66	58.03	71.06	46	52	1645	45	37	4.53	7	41	48	13	4	6
WWM67	67.32	75.91	42	52	2759	63	61	5.59	8	102	110	22	3	15
WWM68	62.57	65.87	45	54	1601	47	39	5.56	8	36	45	23	2	11
WWM76	62.71	70.54	43	56	1559	34	56	5.18	8	26	34	15	2	9
WWM79	70.82	81.74	46	56	2759	96	33	5.61	8	88	96	20	6	13
WWM81	58.53	64.52	43	54	1600	53	34	4.90	8	38	46	15	4	9
WWM85	74.00	76.57	45	56	2054	55	39	4.37	8	51	59	16	4	7
Mean	66.84	74.90	44	52	1950	56	44	5.20	8	52	60	17	4	9
SE	5.34	6.50	2	2	442	16	9	0.44	1	13	14	6	1	2.7
P-value	*	*	**	**	**	**	**	*	ns	*	*	*	*	**
CV (%)	13.80	15.00	6	6	39	49	37	14	22	44	39	5.7	5.4	5.1
LSD	10.56	12.85	3	4	873	31	18	0.86	2	27	27	11	3	5.33

Table 3.7 (Continued).

Accession	Fruit traits										Seed traits					
code	NMFr	NUFr	NAFr	Myld	TFYld	FrW	FrL	FrD	RD	TSS	NSPFr	SYPFr	TSY	SL	SD	HSW
WWM41	7	18	26	35.93	61.41	8.32	25.35	23.83	29.67	3.52	773	100.67	1750.50	11.63	7.14	13.67
WWM42	6	14	31	39.12	61.54	9.45	27.25	22.22	19.49	3.68	657	76.33	1107.50	11.31	7.02	13.17
WWM44	5	5	69	34.07	51.59	13.17	21.68	28.55	17.41	3.45	572	68.67	585.67	11.73	7.34	11.50
WWM46	4	11	29	17.62	44.61	6.81	23.5	21.95	16.36	3.42	532	83.67	840.83	12.60	7.51	16.17
WWM47	7	12	43	34.95	50.80	6.73	21.28	19.57	16.2	3.40	456	70.50	1073.83	12.56	7.86	14.50
WWM50	7	10	34	57.84	77.53	9.75	27.43	24.32	18.15	3.38	742	100.83	1556.33	12.97	7.37	13.50
WWM57	4	7	35	35.20	61.52	10.44	26.82	24.53	19.9	3.72	717	90.50	1063.50	10.53	6.48	13.00
WWM64	6	11	36	60.49	86.71	12.67	31.95	26.65	25.05	3.72	795	87.67	1207.83	13.14	7.56	10.67
WWM65	9	16	50	54.07	77.08	8.39	24.03	21.28	22.11	3.17	640	84.17	1611.50	12.21	7.24	12.50
WWM66	5	8	32	43.97	62.26	11.14	28.4	25.93	15.31	3.52	768	112.17	1400.17	12.93	7.34	14.33
WWM67	7	15	41	59.28	84.22	10.72	27.42	26.22	19.61	3.45	781	105.33	2259.67	12.32	6.96	13.67
WWM68	7	15	23	31.21	56.59	7.32	29.6	20.87	23.14	3.27	826	73.33	1588.17	9.13	6.95	9.00
WWM76	7	8	21	60.04	80.44	11.42	28.38	25.27	24.27	3.05	757	114.50	1577.67	13.68	7.80	15.00
WWM79	7	13	77	58.32	86.81	11.02	30.63	25.53	24.59	3.70	908	97.83	1689.33	12.44	6.83	11.67
WWM81	7	7	38	45.26	57.95	9.15	27.03	23.65	21.43	3.57	629	61.50	914.50	10.95	7.14	8.67
WWM85	6	10	39	34.34	61.75	10.18	19.72	26.45	22.64	3.65	689	73.33	1131.50	11.55	6.72	11.00
Mean	6	11	39	39.56	62.49	9.45	25.91	23.78	20.83	3.44	646	84.40	1249.65	17.30	7.26	13.18
SE	1.62	5	13	11.02	13.93	1.17	1.48	1.15	2.38	0.18	92	11.84	357.60	0.43	0.28	0.88
P-value	*	**	**	**	**	**	**	**	**	**	**	**	**	ns	**	**
CV (%)	46.9	43	5.7	48.30	38.60	21.40	9.9	8.4	19.8	9.00	25	24.30	49.60	6.10	6.70	11.50
LSD	3.19	9	25	21.79	27.53	2.31	2.93	2.27	4.7	0.35	181	23.41	706.90	0.85	0.56	1.74

* and ** = significant at $p < 0.05$ and $p < 0.001$, respectively; ns = non-significant, SE = standard error; CV = coefficient of variables; LSD = Least significant difference. See Table 3.3 for trait abbreviation.

Plant height varied from 4.27 to 6.86 m, with a mean of 5.20 m across accessions. The longest plants measuring > 5.75 m were recorded for accessions WWM02, WWM14, WWM16, and WWM64, whereas the shortest plants (< 4.5 m) were recorded for accessions WWM23, WWM34 WWM46 and WWM85. Number of primary and secondary branches per plant varied from 6 to 10 (mean = 7) and 24 to 108 (mean = 52), respectively. Two accessions namely: WWM39 and WWM44 recorded the highest number of primary branches per plant of ≥ 9, whereas accessions WWM23 and WWM42 recorded the lowest number of primary branches per plant of ≤ 6. Accessions WWM23, WWM46 and WWM76 recorded the lowest number of secondary branches per plant of < 27, whereas WWM14 and WWM67 had the highest number of secondary branches per plant of > 100. Total number of branches per plant varied from 32 to 116 (mean = 60). Two accessions namely WWM14 and WWM67 had the highest total number of branches per plant (≥ 100), whereas accessions WWM23, WWM46, WWM57 and WWM76 had the lowest total number of branches per plant (≤ 35) (Table 3.7).

Total number of fruits per plant varied from 9 to 36, with a mean of 17. Accessions WWM03, WWM09 and WWM39 recorded the highest number of fruits per plant (25, 36 and 33 fruits), respectively. The lowest number of fruits per plant (≤ 10 fruits) were recorded for accessions WWM23, WWM24 and WWM24. The ratio of total female flowers per plant to total number of fruit per plant ranged from 2:1 to 8:1 with a mean of 4:1. The highest ratio of total female flowers per plant to total number of fruits per plant were recorded for accessions WWM02, WWM14 and WWM44. Total number of fully grown fruits varied from 0 to 30 fruits per plant, with a mean of 12. Four accessions WWM05, WWM23, WWM24 and WWM44 had the lowest fully developed number of fruits (< 5 fruits per plant), whereas WWM14, WWM16 and WWM67 recorded the highest number (≥ 15 fruits per plant).

Number of marketable fruits varied from 0 to 13 (mean = 6), whereas the number of unmarketable fruits ranged from 5 to 30 (mean = 11). Accessions WWM02, WWM14, WWM16, WWM35 and WWM65 had the highest number of marketable fruits (8, 10, 13, 10 and 9 fruits respectively), whereas accession WWM05 and WWM09 had no marketable fruits. Accessions WWM03, WWM09 and WWM39 had the highest number of unmarketable fruits (≥ 21). Number of aborted fruits varied from 14 to 86, with a mean of 39 aborted fruits across accessions. Accessions WWM23, WWM33 and WWM34 recorded the lowest number of aborted fruits per plant (≤ 20), whereas WWM02 and WWM14 recorded the highest number of aborted fruits of 86 and 82, respectively.

Marketable fruit yield ranged from 0 to 93.50 kg per plant with a mean of 39.56 kg recorded across accessions. The low-yielding accessions in terms of marketable yield (< 20 kg) were WWM05, WWM09, WWM24 and WWM46, whereas the highest marketable yields (> 60 kg) were recorded for accessions WWM16, WWM64 and WWM76. Total fruit yield ranged from 15.83 to 123.41 kg with a mean of 62.49 kg across accessions. Accessions WWM16, WWM67 and WWM79 recorded the highest total fruit yield per plant (> 85 kg), whereas accessions WWM05, WWM09, WWM23 and WWM24 recorded the lowest total fruit yield per plant of < 41 kg (Table 3.7).

Fruit weight ranged from 2.40 to 13.40 kg per fruit (mean = 9.45). Accessions WWM02, WWM40, WWM44, and WWM64 were high-yielding (≥ 12 kg per plant), compared to low-yielding accessions (< 3 kg per plant) such as WWM05 and WWM09. Fruit length and diameter ranged from 14.63 to 40.37 cm (mean = 25.91 cm), and 14.83 to 28.55 cm (mean = 23.78 cm), respectively. Accession WWM39 bore the longest fruit measuring 40.37 cm, followed by WWM35 (32.22 cm), whereas WWM09 recorded the shortest fruit length of 14.63 cm. Accessions WWM02, WWM34 and WWM44 recorded the largest fruit diameter (> 27 cm), whereas WWM05 and WWM09 recorded the lowest fruit diameter (< 19 cm). Rind diameter varied from 13.44 to 29.67 mm with a mean of 20.83 mm. Accessions WWM08, WWM40, WWM41 and WWM64 had thicker rind diameter of > 25.50 mm, compared to accessions WWM05, WWM34 and WWM66 which had thinner rind of < 15.50 mm. Total soluble solids (TSS) varied from 2.75 and 3.95 with a mean of 3.45 across all accessions. Accessions WWM03, WWM34 and WWM39 recorded highest TSS of > 3.80, whereas WWM05 and WWM09 had the lowest TSS content (< 3.0) (Table 3.7).

Total number of seeds per fruit varied from 479 to 908 (mean = 646). Two accessions namely: WWM68, and WWM79 recorded the highest number of seeds per fruit (> 800), whereas accessions WWM39 and WWM47 had the lowest number of seeds per fruit (< 500). Seed yield per fruit varied from 61.50 to 116.50 g with a mean of 84.40 g. Three accessions namely: WWM04, WWM66, and WWM76 had the highest seed yield per fruit (> 110 g) compared to WWM17, WWM34 and WWM39 which had the lowest seed yield per fruit (< 61.00 g). Total seed yield per plant varied from 585.67 to 2380.67 g with a mean of 1249.65 g. The highest total seed yield per plant (≥ 2000) was recorded for accessions WWM09, WWM16, and WWM67, whereas lowest total seed yield per plant (≤ 700) was recorded for accessions WWM23, WWM33, WWM34 and WWM44. Seed length and seed diameter varied from 9.13 to 15.25 mm (mean = 12.32 mm), and 6.26 to 8.60 mm (mean = 7.26 mm), respectively. Accessions WWM23, WWM57, WWM68 and WWM81 had the lowest seed length (< 11 mm) compared to accessions WWM05, WWM24 and WWM76 with the longest seed length (> 13.50 mm). Several accessions such as WWM03, WWM23 and WWM57 had the lowest seed diameter (< 6.5 mm) compared to accessions WWM14 and WWM24 which had the highest seed diameter (> 7.9 mm). Hundred seed weight varied from 8.67 to 20.83 g with a mean of 13.18 g. Accessions WWM15, WWM24, WWM28 and WWM46 recorded the highest hundred seed weight (> 15 g), whereas accessions WWM33, WWM68, and WWM81 had the lowest hundred seed weight (< 10 g) (Table 3.7).

3.3.3. Associations of quantitative traits among citron watermelon accessions

A correlogram based on Pearson's correlation coefficients showing the level of associations for assessed traits is shown in Figure 3.3. Plant height positively and moderately ($p < 0.001$) correlated with number of male flowers per plant ($r = 0.54$) and total number of female flowers per plant ($r = 0.58$). Total number of fruits per plant strongly and positively correlated ($p < 0.001$) with number of male flowers ($r = 0.67$) and number of unmarketable fruits ($r = 0.93$).

Total fruit yield per plant and marketable yield per plant both positively and significantly ($p < 0.001$) correlated with plant height ($r = 0.57$ and $r = 0.64$, respectively), number of fully grown fruits per plant ($r = 0.66$ and $r = 0.70$, respectively) and number of marketable fruits per plant ($r = 0.72$ and $r = 0.96$; respectively). Fruit weight per plant positively and significantly ($p < 0.001$) correlated with fruit length ($r = 0.52$) and diameter

(r = 0.86) and total soluble solids (r = 0.67). Total seed yield per fruit positively and strongly correlated *($p < 0.001$)* with total number male flowers (r = 0.68), plant height (r = 0.56, total number fruits per plant (r = 0.79), fully grown fruits per plant (r = 0.60), and total fruit yield per fruit (r = 0.60). Hundred seed weight strongly and positively correlated *($p < 0.001$)* with both seed length (r = 0.67) and seed diameter (r = 0.74).

3.3.4. Principal component analysis

Principal component analysis identified nine principal components (PCs) with eigenvalues > 1 which accounted for 86.38% of total variation for assessed phenotypic traits (Table 3.8). The nine principal components (PCs) and corresponding correlation coefficients (eigenvectors) for quantitative traits are presented in Table 3.8. The first principal component (PC1) accounted for 26.4% of the total variation and positively correlated with plant height, total fully grown fruits per plant, number of marketable fruits per plant, marketable fruit yield and total fruit yield per plant. Total number of fruits, number of unmarketable fruits per plant, and total seed yield per plant positively correlated with PC2, whereas fruit diameter negatively correlated with PC2 which accounted for 15.4% of the total variation (Table 3.8).

Number of male flowers per plant, number of secondary branches and total number of branches per plant positively correlated with PC3 which accounted for 10.62% of the total variation. Principal component (PC4) which accounted for 8.03% of the total variation was positively correlated with total number of female flowers per plant, number of primary branches per plant, ratio of female flowers to total number of fruits, number of aborted flowers but negatively correlated with the sex ratio. Seed length and diameter, and hundred seed weight positively correlated with PC5 which accounted for 7.43% of the total variation. Fruit weight and length, and total soluble solids positively correlated with PC6 accounting for 5.91% of the total variation. Number of days to male and female flowering negatively correlated with PC7 which accounts to 4.72% of the total variation, whereas number of seeds per fruit and seed yield per fruit positively correlated with PC8 accounting for 4.01% of the total variation. Leaf length and width positively correlated with PC9 which accounted to 3.83% of the total variation (Table 3.8).

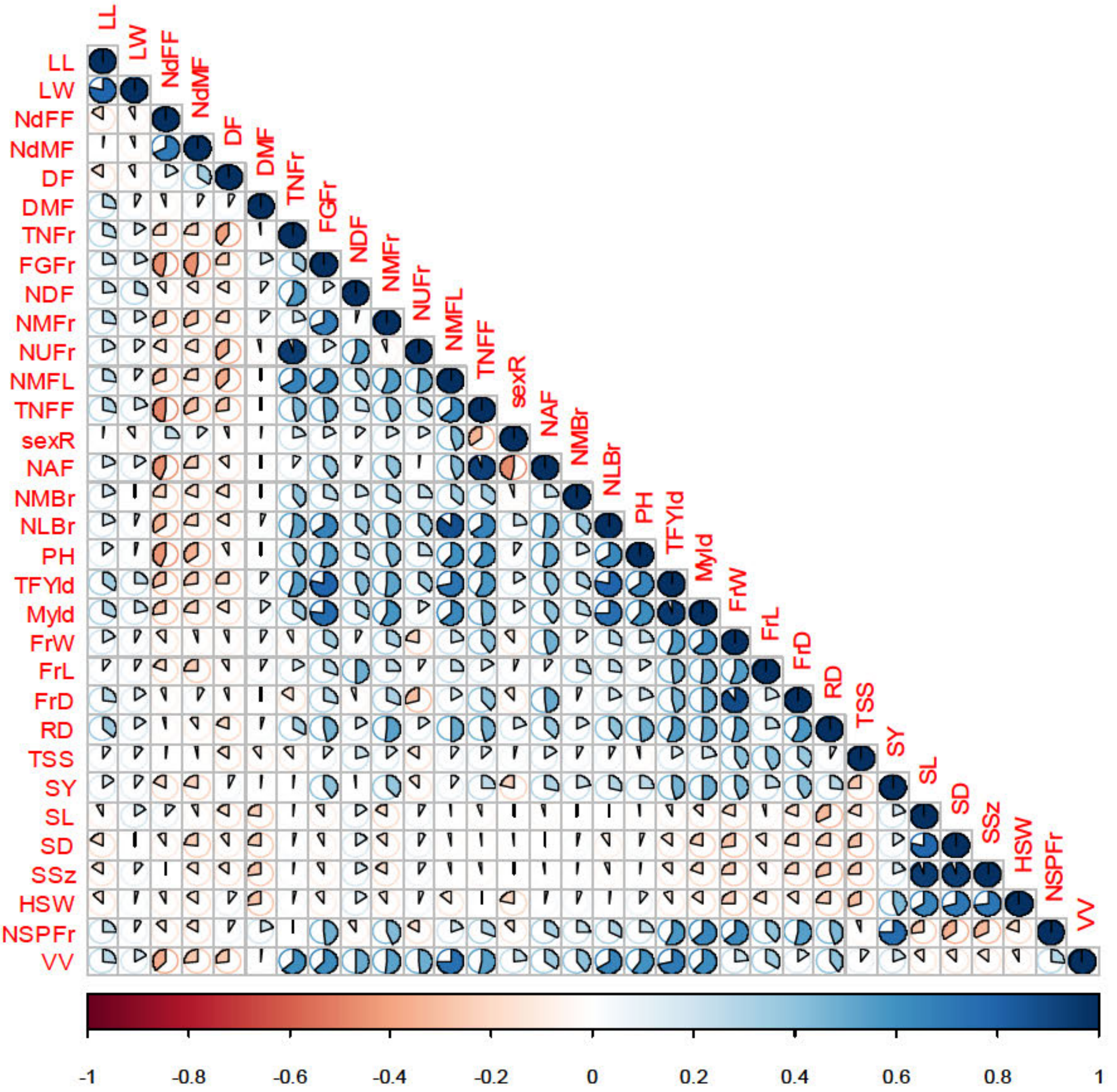

Figure 3.3: Pearson correlation coefficients showing associations of quantitative traits among 36 citron watermelon landrace accessions of South Africa. Colour intensity and the size of the pie are proportional to the correlation coefficients. Positive correlations are displayed in blue and negative correlations in red colour. See codes of abbreviations in Table 3.3.

Table 3.8 Principal components analysis showing eigenvectors, eigenvalues and percent variable explained by nine principal components (PCs) for 30 quantitative traits of 36 citron watermelon landrace accessions.

	Principal components								
Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
LL	-0.01	0.08	0.05	0.15	-0.09	0.05	0.09	0.00	**0.89**
LW	0.12	-0.03	0.12	-0.01	0.15	0.34	0.06	-0.03	**0.85**
NdMfl	-0.34	-0.12	-0.08	0.04	-0.23	-0.06	**-0.73**	-0.22	0.05
NdFfl	-0.04	-0.03	0.08	-0.09	-0.01	0.05	**-0.90**	-0.06	-0.17
NMfl	0.39	0.39	**0.75**	0.15	0.06	0.03	0.09	0.07	0.10
TNFfl	0.41	0.21	0.31	**0.76**	0.00	0.00	0.16	0.02	0.16
SexR	-0.09	-0.07	0.54	**-0.66**	-0.04	0.14	0.21	-0.18	-0.05
PH	**0.68**	0.22	0.25	0.23	0.11	-0.07	0.23	-0.01	-0.11
NPBr	0.04	0.32	0.22	**0.60**	0.13	0.13	-0.09	-0.23	-0.22
NSBr	0.25	0.21	**0.89**	0.19	0.07	-0.04	-0.08	0.06	0.08
TNBr	0.25	0.21	**0.88**	0.21	0.08	-0.03	-0.08	0.05	0.08
TNFr	0.33	**0.89**	0.22	0.06	-0.01	-0.04	0.05	-0.06	0.09
RFflTFr	-0.08	-0.44	0.08	**0.85**	0.13	0.04	0.03	0.03	0.12
FGFr	**0.70**	0.10	0.47	0.05	-0.06	0.02	0.23	0.14	-0.10
NMFr	**0.82**	0.06	0.16	0.14	-0.11	-0.07	0.21	0.00	0.16
NUFr	0.03	**0.93**	0.22	-0.03	0.05	-0.02	0.05	-0.08	0.06
NAFr	0.34	-0.10	0.27	**0.83**	0.04	-0.02	0.15	0.03	0.13
Myld	**0.92**	-0.08	0.07	0.08	0.00	0.22	-0.05	0.14	0.03
TFYld	**0.93**	0.11	0.10	0.08	-0.04	0.20	-0.02	0.10	0.06
FrW	0.17	-0.47	-0.15	0.17	-0.17	**0.71**	-0.12	0.18	0.20
FrL	0.06	0.11	0.06	-0.16	0.00	**0.87**	0.06	0.12	0.03
FrD	0.28	**-0.56**	-0.11	0.23	-0.22	0.42	-0.14	0.21	0.20
RD	0.32	0.09	0.03	0.02	-0.23	**0.51**	0.39	0.01	0.07
TSS	-0.05	-0.18	-0.01	0.08	-0.09	**0.82**	-0.07	-0.12	0.18
NSPFr	0.17	-0.11	0.13	-0.02	-0.46	0.18	0.09	**0.80**	-0.10
SYPFr	0.16	-0.07	0.01	0.03	0.33	-0.01	0.18	**0.89**	0.04
TSY	0.55	**0.62**	0.28	0.05	0.13	-0.16	0.07	0.36	0.04
SL	0.02	-0.05	0.27	0.00	**0.89**	-0.03	-0.14	0.05	0.04
SD	0.01	0.09	0.03	0.13	**0.88**	-0.11	0.12	-0.10	-0.13
HSW	-0.08	0.11	-0.12	0.08	**0.88**	-0.10	0.16	0.10	0.11
Explained variance (eigenvalues)	7.92	4.63	3.18	2.41	2.23	1.77	1.42	1.20	1.15
Proportion of variance (%)	26.40	15.42	10.62	8.03	7.43	5.91	4.72	4.01	3.82
Cumulative (%)	26.40	41.83	52.44	60.47	67.90	73.82	78.54	82.55	86.38

Bold values (loadings ≥ 0.50) (positive or negative) indicate high correlation (eigenvectors) between the principal components and the corresponding quantitative traits. See Table 3.3 for trait abbreviation.

3.3.5. Groupings of accessions based on quantitative phenotypic traits

The relationship between citron watermelon landrace accessions and studied phenotypic traits are shown in Figure 3.4. Accessions WWM05, WWM08, WWM15, WWM24, WWM28, WWM46 and WWM68 located on the top left quadrant were grouped together based number of days to first female flower appearance and sex ratio. Accession WWM05 was distantly positioned from other accessions. Accessions WWM09, WWM14, WWM16, WWM38, WWM39, WWM41 and WWM47 located on the top right quadrant were grouped based on number of male flowers per plant, number of primary and secondary branches per plant, plant height, total number of fruits per plant, number of unmarketable fruits per plant, total seed yield per plant, seed length and diameter, and hundred seed weight.

Number of days to male flowering, fruit weight and total soluble solids were associated with accessions WWM17, WWM23, WWM33, WWM34, WWM37, WWM42, WWM44, WWM57, WWM66, WWM76 and WWM81. Accessions WWM02, WWM35, WWM50, WWM64, WWM67 and WWM76 were grouped in the bottom right hand of the bi-plot based on high performance for leaf length, leaf width, total number of female flowers per plant, number of fully grown fruits per plant, number of marketable and aborted fruits per plant, fruit length fruit diameter, marketable yield and total fruit yield per plant.

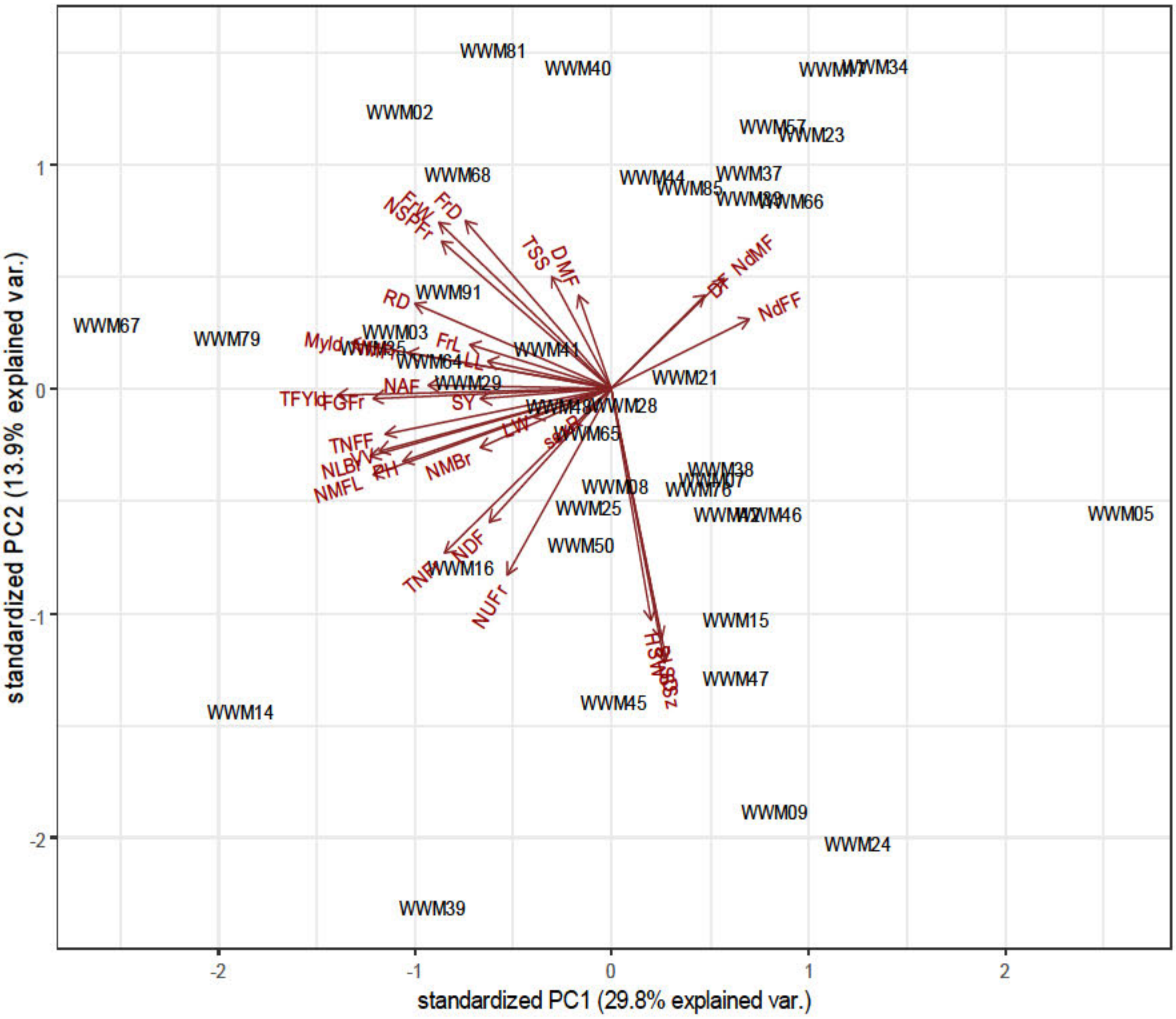

Figure 3.4: Principal component bi-plot of PC1 and PC2 showing relationships of 36 citron watermelon landrace accessions based on quantitative phenotypic traits. See Table 3.3 for trait abbreviation.

3.4. Discussion

Assessment of the phenotypic variation present in diverse citron watermelon genotypes is important for genetic improvement and cultivar development. This will allow selection of novel genotypes for breeding and trait transfer to other cross-compatible cucurbits especially dessert watermelon. The present study assessed phenotypic diversity of 36 diverse citron watermelon landrace accessions collected from various districts of the Limpopo Province, South Africa. Wide variations were observed in the present accessions for leaf shape, fruit shape and colour, rind stripe patterns, flesh colour and seed coat colours (Tables 3.4 and 3.5). Results of the present study corroborate findings of Solmaz and Sari (2009) and Szamosi et al. (2009) who reported a wide range of variation for several fruit and seed traits in sweet dessert watermelon. This suggested that fruit shape and colour, presence or absence of rind stripe patterns, flesh colour and seed coat colour may be efficient descriptors for grouping of citron watermelon germplasm if molecular markers are not available for genetic profiling. Genetic analysis of citron watermelon accessions using simple sequence repeat markers revealed the presence of marked genetic variation (Mashilo et al., 2017). The genetic distinctness revealed in the presently assessed accessions including WWM-03, WWM-04, WWM-08, WWM-14, WWM-15, WWM-16, WWM-24, WWM-34, WWM-37, WWM-39, WWM-44 and WWM-48 was resolved through genetic analysis using simple sequence repeat markers (Mashilo et al., 2017). Citron watermelon is a monoecious plant and bears relatively large number of male and female flowers on the same plant allowing for cross-pollination leading to extensive phenotypic and genetic diversity. Farmers in the Limpopo Province of South Africa, grow different accessions of citron watermelon in the same field indicating spontaneous cross pollination which is the principal cause of the observed variation for qualitative and quantitative traits of the accessions used in the present study. In the present study, some unique traits were observed including non-lobed leaf shape (e.g. accession WWM24), elliptic fruit shape (WWM39) and fruit grooves (WWM17, WWM23, WWM33, WWM57 and WWM85) which could serve as novel phenotypic markers for understanding the genetic control governing these traits in citron watermelon.

Rind stripe patterns of watermelon fruits improves visual appearance and enhance fruit marketability. Citron watermelon accessions with unique rind stripe patterns such as WWM04, WWM28, WWM35 and WWM44 (Figure 3.1) can be exploited in cultivar design with appealing visual appearance. Also, the observed variation in fruit shapes provides opportunities for cultivar development and deployment based on consumer and market needs and preferences. Flesh colour is an important fruit quality trait providing visual attractiveness. This trait is attributable to carotenoid composition in citron watermelon (Bang et al., 2010). Among the studied accessions, only two were white-fleshed. White-fleshed types are seldom cultivated for use as leafy vegetable in the Limpopo Province and largely preferred by livestock. White-fleshed types are referred to as “Kiti” in the South

African Sepedi language and have undesirable traits for consumption such as small round fruit, green-white flesh, small brown and black seed which are hard to chew and sour flesh. Similar traits of white-fleshed types were reported by Laghetti and Hammer (2007) and Achigan-Dako et al. (2015). Interestingly, white-fleshed types have been reported in Australia where the crop is an invasive weed species (Shaik et al., 2017) identical to African wild and semi-wild types (Shaik et al., 2015, 2016). In addition, morphological features of Australian and African types are similar including yellow male and female floral buds; mature dark green fruit with discontinuous longitudinal rind stripes, light-yellow, green or white-fleshed fruit, and black or dark-brown seeds (Shaik et al., 2017; Achigan-Dako et al., 2015; Ngwepe et al., 2019). Farmers in the Limpopo Province of South Africa consider “Kiti” as a wild-weedy citron watermelon type and they have limited interest cultivating and conserving this ecotype.

The orange and yellow -fleshed citron watermelon genotypes were the pre-dominant types in the present study (Table 3.4). Achigan-Dako et al. (2015) reported that yellow-fleshed citron watermelon constituted the second largest group after white-fleshed types. Outside their region, yellow and orange-fleshed accessions (e.g., PI 271773, PI 271775 and PI 271779) are maintained by the USDA and were collected from South Africa (Levi et al., 2013; Zhang et al., 2016). Also, several yellow and orange-fleshed accessions (i.e., PI 482 273, PI 482 252, PI 485 583 and PI 542 119) collected from Botswana and Zimbabwe (Levi et al., 2013) are maintained by the USDA indicating that the Southern African region is the center of genetic diversity of yellow and orange-fleshed citron watermelon types. Accessions USVL246-FR2, USVL252-FR2 and “USVL-360 (i.e., previously PI 482246, PI 482252 and PI 299379) collected from Zimbabwe and South Africa and used outside their traditional range of occurrence (e.g., USA) possess similar traits to the 34 accessions evaluated in the present study. These traits include bright yellow male and female flowers, lobed leaf shape with finely serrated edges, coiled tendrils, round and oblong fruit shape, fruit colour and rind stripe patterns, yellow and orange fruit flesh and red seeds (Levi et al., 2014; Wechter et al., 2016). Specifically, mature fruit of USVL246-FR2 is light green in colour with dark green rind stripe patterns similar several accessions including WWM04, WWM28, WWM34, WWM44 and WWM65 (Figure 3.1). The red seed coat colour of USVL246-FR2 and USVL252-FR2 is similar to accessions WWM14, WWM16, WWM28, WWM39, WWM44, WWM47, WWM50 and WWM65 (Figure 3.2). Citron watermelon accessions sourced from the Southern African region are currently used as sources of genetic variation for disease resistance breeding and as rootstock for sweet dessert watermelon improvement in the USA (Wechter et al., 2012, 2016).

A large collection of citron watermelon genetic resources including white-fleshed types mostly collected from Southern African countries (e.g., South Africa, Zimbabwe, Swaziland, Nigeria, Botswana and Zaire) are conserved by the United States Department of Agriculture (USDA) Plant Genetic Resources Conservation Unit (PGRCU) (Levi et al., 2013). For example, accessions PI 189225, PI 244017, PI 244018, PI 244019, PI 248774, PI 295850 and PI 296341 maintained by the USDA PGRCU are white-fleshed and round in fruit shape (Levi et al., 2013) similar to WWM05 and WWM09 evaluated in the present study. The white-fleshed PI 296341 collected from South Africa is resistant to races 0, 1, and 2 of *Fusarium* wilt (Netzer and Martyn, 1989; Martyn and Netzer, 1991) and used as breeding parents for developing mapping populations to study genetic control of various traits (Levi et al., 2002; Zhang et al., 2004). Additionally, white-fleshed citron watermelon accessions PI 244018 and PI 244019 are reported to be resistant to watermelon virus disease, root-knot nematodes and watermelon gummy stem blight and were collected from the Republic of South Africa (Gusmini et al., 2005; Thies et al., 2016). The various reports indicate the value of white-fleshed citron types for biotic stress tolerance breeding and their continuous collection and conservation efforts are vital especially in their region of origin where natural populations of the species are still prevalent.

In the Limpopo Province, small-holder farmers across different villages grow various types of citron watermelon landrace accessions in small fields annually during the summer season mainly for food and animal feed. To prepare animal feed, the fully grown fruits are chopped into medium-large pieces and fed to small-and large-livestock including pigs, goats, sheep, donkeys and cattle especially during dry winter months when natural grazing pastures are scarce. In the Sekhukhune district, the yellow- and orange-fleshed types are occasionally sold for cash by street vendors at costs ranging from 1 to 3 US dollars per fruit depending on fruit size. Medium-sized fruit (10-15 kg) are sold between 1 and 2 US dollars, whereas large-fruit (> 20 kg) cost around 3 US dollars. In the Capricorn district, local farmers grow and supply yellow and orange-fleshed citron watermelon types to retail outlets which are then re-sold at ~ 2 US dollars. The diverse uses of the crop in the Limpopo Province offer niche market opportunities. The yellow and orange-fleshed citron watermelons are rich sources of phytochemical compounds including carotenoids and organic acids (Yoo et al., 2013; Gao et al., 2018; Jawad et al., 2020). β-carotene content varying from 6.5 to 134 mg/kg were reported among orange-fleshed dessert watermelon types (Jin et al., 2019). Also, β-carotene content of 860 $\mu g\ g^{-1}$ was reported in the orange-yellow fleshed sweet dessert watermelon (Liu et al., 2012). Yoo et al. (2012) reported β-carotene content of 0.2 $\mu g\ g^{-1}$ and pro-lycopene, lutein and lycopene contents of 0.1 $\mu g\ g^{-1}$ in yellow-fleshed citron watermelon accession PI 255137. These germplasm (i.e., yellow and orange-fleshed types) are useful as breeding parents to design new cultivars with varied carotenoid compositions for combating vitamin A deficiencies prevalent in sub-Saharan African region including South Africa (Visser et al., 2019). Crosses derived between citron watermelon “IIHR-82” and sweet watermelon “Arka Manik” resulted in development of inbred with intermediate flesh colours combining shades of red and orange (Maragal et al., 2019). Accessions WWM03, WWM46, WWM47, WWM50, WWM64 and WWM66 with deep-orange flesh colour and accessions WWM24, WWM28, WWM41, WWM42 and WWM76 with a bright yellow-flesh colour (Figure 3.1) may serve as parental genotypes in improvement programmes for developing β-carotene enriched genotypes.

Seed coat colour is another important trait that enhances visual attractiveness of watermelon flesh. A range of seed coat colours were observed among the assessed citron watermelon genotypes (Figure 3.2) which could serve as selection markers in investigating the genetic control of seed coat colour in this crop. Red and red-white seed coat colours were predominant among the studied citron watermelon accessions. Red-white and red seeds are preferred types as roasted snack possessing medium-hard seed coats, being easy to chew, aromatic and flavourful. On the contrary, black, brown, green and maroon seeds are uneasy to chew when roasted and partly explain their low occurrences among the studied germplasm. Phenotypic variation observed for seed coat colours has implications for developing marker-desired genotypes and products.

The present study revealed extensive phenotypic variation for diverse agronomic and horticultural traits to select suitable genotypes for direct production or cultivar development with desired product profiles. In this study higher coefficient of variation were recorded for fruit traits including total number of fruits per plant, marketable yield, and total fruit yield (Table 3.7). This suggests that the tested citron watermelon landrace accessions were genetically variable to develop ideotypes with desirable agronomic attributes. Fruit and seed yields are important traits in citron watermelon production and marketing. Citron watermelon accessions WWM02, WWM14, WWM16, WWM35, WWM44, WWM64, WWM67 and WWM76 had the highest number of total fully-grown fruits per plant, marketable and total fruit yields, and seed yield. Presently there are no genetically improved cultivars of citron watermelon in South Africa for commercial production, processing and marketing along the value chains. The identified accessions are recommended for various uses including for direct production, commercialization, processing, and product-development and for future breeding programmes.

Lower total soluble solid is an important breeding objective in sweet dessert watermelon improvement programmes (Davis et al., 2008). The low sugar content of the presently assessed citron watermelon accessions (Table 3.7) make them ideal candidates for cultivar development of dessert watermelon with low sugar content. This will enable to develop novel dessert watermelon cultivars with low to medium sweetness required by consumers based on special dietary requirements (Davis et al., 2008). Accessions WWM05 and WWM09 had small-size fruits with low TSS content (< 3 ° Brix). The flesh colour of the two accessions is white, very firm texture and possess slightly sour taste attributable to the presence of malic and citric acids (Gao et al., 2018). A non-sweet and sour citron watermelon (i.e., PI271769) was recently used to develop a sour and sweet dessert watermelon (Gao et al., 2018). The non-sweet and sour citron types identified in the present study could be useful for breeding watermelon for consumers interested in low and medium sweetness, and sour flavour (Gao et al., 2018). Also, the white-fleshed citron watermelon PI 296341 originating from South Africa was identified to be a promising rootstock for production of sweet dessert watermelon to improve yield, quality, and *Fusarium* resistance (Edelstein et al., 2014). Some citron watermelon rootstocks USVL246-FR2 (PI 482246) and USVL252-FR2 (PI 482252), which originated from Zimbabwe, are used for commercial production of grafted sweet watermelon in the USA owing to their natural resistance to *Fusarium* wilt and nematodes (Wechter et al., 2016; Smith et al., 2019). In South Africa, citron watermelon germplasm has not been used as rootstock for sweet dessert watermelon to improve yield and fruit quality, and for biotic and abiotic stress tolerance breeding. The tested accessions with high yield require rigorous testing to identify suitable rootstocks for recommendation to growers. Analyses are yet to be made on biochemical compositions of the presently assessed accessions. However, the study recommends a detailed analysis and record of bioactive compounds including mineral elements, sugar, organic acids and carotenoid compositions using a relatively large set and genetically diverse citron watermelon germplasm to develop market preferred varieties.

Correlation analysis which estimates trait association is useful to aid selection of suitable traits. In the present study, positive and strong correlations were observed between fruit yield and seed yield with various traits including plant height, number of fully grown fruits per plant and number of marketable fruits per plant (Figure 3.3). Fruit traits were highly variable and explained most of the phenotypic variation among studied citron watermelon accessions (Table 3.8; Figure 3.4). Seed traits were also the largest contributors of phenotyic variation among the studied citron watermelon accessoins. Therefore, targeted selection for fruit and seed traits will ensure sucessful cultivar development and release in citron watermelon.

3.5. Conclusions

The current study assessed the phenotypic diversity present among 36 citron watermelon landrace collections from the Limpopo province of South Africa. A wide range of variations were observed for qualitative and quantitative phenotypic traits which could be used to develop new cultivars and diverse value-added products based on market needs and consumer preferences. Citron watermelon accessions WWM16, WWM67 and WWM79 were high-yielding and hence suitable for fruit production for food or livestock feed. Accessions WWM03, WWM09, WWM16, WWM39, WM64 and WWM76 produced high fruit number and marketable yield per plant; these accessions could be recommended as parental genotypes for breeding. Furthermore, accessions WWM04, WWM15, WWM24, WWM28, WWM46, WWM66, WWM68, WWM76 and WWM79 were selected with higher seed production for the food or feed industry. The study recommends citron watermelon accessions such as WWM14, WWM16, WWM39, WWM41, WWM67 and WWM79 for use as leafy vegetables owing to their profuse branching ability and longer vine production. On the other hand, accessions WWM03, WWM17, WWM35, WWM40, WWM50, WWM67, WWM79 and WWM85 were selected with larger fruit size. Accessions WWM05 and WWM09 are sour-flesh types which are suitable genetic stocks for breeding sweet-and-sour flavour watermelons. Orange-fleshed accessions such as WWM03, WWM04, WWM46, WWM64, WWM66 and WWM67 are recommended for fresh consumption, cooking, processing or variety design. Accessions WWM02, WWM03, WWM08, WWM14, WWM16, WWM23, WWM38, WWM40, WWM41 and WWM67 have red and white seed coat colour which are superior selections to prepare roasted citron watermelon seed snack.

3.6. References

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Chapter 4. Estimates of the variance components, heritability and genetic gains of phenotypic traits in citron watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.)

Abstract

The present study estimated variance components, heritability and genetic advance of phenotypic traits in citron watermelon to guide selection of superior genotypes for direct production and breeding. Thirty-six citron watermelon landrace accessions were evaluated across two seasons using a 6 × 6 lattice design with three replications. High broad-sense heritability and genetic advance as percent of the mean were recorded for fruit length at 83.86 and 4730.45%, seed length (77.73 and 1731.27%), hundred seed weight (73.73 and 4027,36%), fruit diameter (70.44 and 2949.64%) and fruit weight (70.39 and 8490.05%), respectively. Step-wise regression analysis revealed marketable fruit yield (Myld) and total number of fruits per plant (TNFr) explaining 89% (R^2 = 0.89) of total variation for total fruit yield per plant (TFYld), whereas number of seed per fruit (NSPFr) and hundred seed weight (HSW) explained 92% (R^2 = 0.92) of total variation for seed yield per fruit (SYPFr). Citron watemelon landraces accessions WWM03, WWM14, WWM16, WWM39, WWM65, WWM67 and WWM79 with high TFYld and SYPFr were selected for production or breeding.

Keywords: *Agronomic traits, Cucurbitaceae, genotypic variation, horticultural traits, phenotypic variation*

4.1. Introduction

Citron watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.) belonging to the Cucurbitaceae family is widely cultivated in sub-Saharan Africa (SSA) for diverse purposes mainly for food and feed (Van Wyk, 2011; Mokganya and Tshisikhawe, 2019). In Southern Africa fresh succulent leaves of citron watermelon are cooked as leafy vegetable and referred to as “Morogo”, “Talane”, “Motshatsha” or Mokhusa in the South African indigenous Sepedi language. Morogo is consumed with maize, sorghum or millet porridge (Franz, 1971; Fox and Norwood, 1982; Maggs-Kolling and Christiansen, 2003; Schippers, 2002, Mashilo et al., 2017; Ngwepe et al., 2019). The leaves are rich in protein (23%), minerals (i.e., N, P, K, Ca, Mg, Na, Zn and Fe) and flavonoids contents (Jarret and Levy, 2012; Ogbuji et al., 2012). Seeds are eaten as roasted snacks and valued as a source of unsaturated fatty acids including palmitic acid (10.6%), stearic acid (8.05%), oleic acid (16.42%) and linoleic acid (63.37%). The seed oil content is about 22.6% (Jarret and Levi, 2012).

The non-sweet citron watermelon types with yellow and orange-flesh are favoured for cooking various traditional dishes in SSA (Nantoumé et al., 2012, 2013; Mashilo et al., 2016; Ngwepe et al., 2019, 2021). Orange-fleshed sweet dessert watermelon (*C. lanatus* var. *lanatus*) possesses various carotenoids such as prolycopene, phytoene, and ζ-carotene, violaxanthin, neoxanthin and β-carotene (Tadmor et al., 2005; Davis et al., 2007; Bang et al., 2007, 2010; Maragal et al., 2019; Perkins-Veazie et al., 2006). Yoo et al. (2012) reported β-carotene content ranging from 1 to 8.5 µg/g, whereas Jin et al. (2019) reported β-carotene ranging from 6.5 to 134 mg/kg in orange-fleshed dessert watermelon. This suggests that the orange-fleshed citron watermelon may be a rich source of β-carotene important for reducing vitamin-A deficiency and related-illnesses prevalent in SSA (Black et al., 2013). However, the composition of carotenoids may be influenced by the genotype, environment and genotype-by-environment interactions. Also, citron watermelon is currently valued as a suitable rootstock for improving fruit yield and quality of grafted sweet dessert watermelon (Kombo and Sari, 2019;Yan et al., 2018; Zhong et al., 2018; Yavuz et al., 2020). Other production attributes of the crop include tolerance to biotic (Thies et al. 2015a, 2005b, 2016) and abiotic stresses (Mo et al., 2016;

Yavuz et al., 2020). Therefore, citron watermelon is useful genetic resource for breeding and cultivar development of *C. lanatus* var. *lanatus*.

Despite the nutritional, socio-economic importance and breeding value of citron watermelon, the crop is underutilized, and its economic value is not fully realized and exploited for food, medicine and commercial applications in SSA (Mashilo et al., 2016, 2017; Ngwepe et al., 2019). As a result, there are no improved varieties released and commercialized for production, processing or value-adding (Nkoana et al., 2021; Ngwepe et al., 2021). Extensive genetic variation of citron watermelon is available in SSA to breed and release high-yielding genotypes for production and commercialization. Also, small-scale farmers use low yielding local landraces of sweet dessert watermelon for production. Hence, there is a need to unlock the genetic potential of citron watermelon genetic resources for developing high-yielding, biotic and abiotic stress tolerant dessert watermelon genotypes adapted to the changing growing conditions in SSA or elsewhere.

Various genetic diversity and analyses studies were conducted on citron watermelon using molecular markers such as reverse transcriptase reaction-random fragment length polymorphism (PCR-RFLP), simple sequence repeat (SSR), random amplified polymorphic DNA (RAPD), high frequency oligonucleotides (HFO) and peroxidase gene. These studies revealed the presence of high genetic differentiation among and within genotypes (Levi et al., 2001, 2013; Dane and Lang, 2004; Dane and Liu, 2007; Hwang et al., 2011; Solmaz et al., 2010; Ocal et al., 2014; Zhang et al., 2016; Mashilo et al., 2017). Based on SSR genotyping, Nantoumé et al. (2013) and Mashilo et al. (2016, 2017) reported wide genetic differences amongst citron watermelon landrace accessions in Mali and South Africa, respectively. Extensive genetic differences were detected among Zimbabwean citron watermelon accessions revealed through RAPD and SSR markers (Mujaju et al., 2010).

Also, various reports indicated that citron watermelon exhibits extensive phenotypic variation for economic traits which can be targeted for variety design and commercialization (Maggs-Kölling et al., 2000; Achigan-Dako et al., 2015; Mashilo et al. 2016, 2017). This will allow watermelon breeders to develop best performing varieties that meet customer requirements and market demand. This can be achieved through exploring the genetic resources of the crop adapted and cultivated in SSA. However, the magnitude of genetic variation, heritability and genetic gains of phenotypic traits in citron watermelon is yet to be explored for variety design and development according to the needs of clients and marketplace in SSA and similar agro-ecological zones. Considering the above background, the objective of this study was to estimate variance components, heritability and genetic advance of phenotypic traits in citron watermelon genetic resources to guide selection of superior genotypes for direct production and breeding.

4.2. Materials and methods

4.2.1. Study site

The study was conducted at Towoomba Agricultural Development Centre (TADC), Bela-Bela, South Africa (28º19’28’’E, 24º53’57’’S; 1184 m above sea level) under field conditions during the 2018/19 and 2019/20 summer growing seasons. Total rainfall received during 2018/19 and 2019/20 growing seasons was 231 mm and 237 mm, respectively. Average maximum and minimum temperatures ranged between 17.5°C and 29.7°C during the 2018/19 season, and 19.5°C and 31.7°C in the 2019/20 cropping season. Average relative humidity was 55 and 58% in 2018/19 and 2019/20 growing seasons, respectively (South African Weather Services (SAWS), www.weathersa.co.za).

4.2.2. Plant materials and experimental design

Thirty-six genetically diverse citron watermelon landrace accessions were used in the study. Details including accession code, districts and village of collection, and the description of fruit and seed traits are provided in Table 4.1. The accessions were evaluated using a 6 × 6 lattice design with three replications. The trial was planted in a single row constituting of 6 plants per accession using intra- and inter-row spacing of 6 × 7 m, respectively. Six accessions were randomly allocated in an incomplete block per replication. Weeding was done manually, and no fertilizers and pesticides were applied.

Table 4.1 Names, district and village of collection in Limpopo Province of South Africa, fruit and seed traits of 36 citron watermelon landrace accessions used in the study.

		Origin/source		Fruit traits		Seed traits	
Sr. No	Accession code	District	Village	FrShp	FCol	Sdcol	Sdsz
1	WWM02	Capricorn	Kgohloane	R	Y	RW	M
2	WWM03	Capricorn	Kgohloane	E	O	RW	S
3	WWM04	Capricorn	Kgohloane	R	O	G	M
4	WWM05	Capricorn	Kgohloane	E	W	GBrS	M
5	WWM08	Capricorn	Moletjie-Moshate	E	Y	RW	L
6	WWM09	Capricorn	Moletjie-Moshate	R	W	B	M
7	WWM14	Capricorn	Turfloop	R	O	RW	L
8	WWM15	Capricorn	Turfloop	R	O	Mr	L
9	WWM16	Waterberg	Bela-Bela	R	Y	R	L
10	WWM17	Waterberg	Styloop	R	O	BrS	M
11	WWM23	Waterberg	Maeteletsa	E	Y	RW	M
12	WWM24	Capricorn	Westenburg	E	Y	G	L
13	WWM28	Sekhukhune	Malegane	R	O	R	L
14	WWM33	Capricorn	Ga-Molepo	E	Y	RW	M
15	WWM34	Capricorn	Ga-Molepo	R	O	CW	S
16	WWM35	Capricorn	Ga-Molepo	E	Y	T	M
17	WWM37	Capricorn	Ga-Molepo	E	O	Mr	M
18	WWM38	Capricorn	Ga-Molepo	E	O	RW	S
19	WWM39	Capricorn	Ga-Mphela	BE	O	T	M
20	WWM40	Capricorn	Kgohloane	E	O	RW	S
21	WWM41	Sekhukhune	Nebo	E	Y	RW	S
22	WWM42	Sekhukhune	Nebo	E	O	R	M
23	WWM44	Sekhukhune	Nebo	R	O	R	M
24	WWM46	Sekhukhune	Nebo	E	O	R	M
25	WWM47	Sekhukhune	Nebo	R	O	R	M
26	WWM50	Sekhukhune	Nebo	E	Y	R	M
27	WWM57	Capricorn	Uitkyk 3	E	O	BrS	M
28	WWM64	Capricorn	Ga-Mphela	E	O	Br	S
29	WWM65	Waterberg	Vienna	R	O	R	M
30	WWM66	Capricorn	Uitkyk 3	E	O	BrS	M
31	WWM67	Capricorn	Uitkyk 3	E	O	RW	S
32	WWM68	Capricorn	Ga-Manamela	E	Y	G	S
33	WWM76	Capricorn	Ga-Manamela	E	Y	BrS	M
34	WWM79	Capricorn	Ga-Manamela	E	O	CW	M
35	WWM81	Capricorn	Ga-Manamela	E	Y	CW	S
36	WWM85	Capricorn	Ga-Manamela	R	O	BrS	M

Sr. no = Serial number; FrShp = fruit shape; R = round; E = elliptic; B = broad elliptic; FCol = flesh colour; O = orange; Y = yellow; W white; Sdcol = seed colour; B = black; Br = brown; BrS = brown with black spot; CW = cream white; G = green; GBrs = green with brown spot; Mr = marron; R = red; RW = red-white; T = tan; Ssz = seed size; L = large; M = medium; S = small.

4.2.3. Data collection

Data on leaf, flower, plant, fruit and seed attributes were collected for 26 phenotypic traits from three selected plants. The crop has long vines (some genotypes reaching 9 meter) and profuse branches, and three plants that showed maximum growth with the main vine reaching at least 5m long were selected and tagged for data collection.

Data for leaf parameters were measured six weeks after planting, a stage when succulent leaves are harvested as leaf vegetable. Data were recorded from the third fully expanded leaf situated below the tip of the plant as described by Achigan-Dako et al. (2015). Leaf length was measured as the vertical distance from the base to the tip of the second lobe. Leaf width was measured as the horizontal distance across the first and third lobes. Data for flower traits were measured during crop growth and at maturity. At maturity, the number of male flowers were counted on the primary and secondary branches. The number of female flowers were counted as the sum of aborted and developed fruits at maturity on primary and secondary branches. Data on plant, fruit and seed traits were collected at physiological maturity when the stem attached to the blossom end of the fruit had dried up. Fruit and seed traits were measured from three randomly selected mature fruits for each accession from each block at maturity. Details of the assessed traits and description of their measurements are presented in Table 4.2.

Table 4.2 Traits descriptions and measurements for evaluation of citron watermelon accessions.

Trait category	Description/measurement
Leaf	Leaf length (LL) and Leaf width (LW) were measured using a digital Vernier caliper (Metr-ISO) and expressed in millimeter (mm).
Flower	Number of days to first male flower appearance (NdMFl) counted as number of days from date of planting to when the first male flower appeared, Number of days to first female flower appearance (NdFFl) counted as number of days from planting to when a first female flower appeared, Total number of male flowers (NMFl) were regularly counted and added as flower peduncles attached to the plant without the ovary, Total female flowers (TNFfl) were regularly counted and added based on flower peduncles showing well-developed ovary and Sex ratio (SexR) computed as the ratio of total female to male flowers.
Plant	Plant height (PH) measured from the base to the tip of the main vine/stem at harvest and expressed in metre (m), Number of primary branches (NPBr) counted as branches emerged from the main stem, Number of secondary branches (NSBr) counted as branches from primary branches.
Fruit	Total number of fruits (TNFr) counted as number fruits per plant at harvest, Total fully grown fruits (FGFr) weight expressed in kilograms (kg) of total fully grown fruits , Number of marketable fruits (NMFr) counted as medium to large fruits without defects, Number of unmarketable fruits (NUFr) counted as small sized fruits and/or fruits with defects, Marketable yield (Myld, kg) weight of medium to large-sized fruits per plant, Total fruit yield (TFYld, kg) weight of total number of fruits per plant, Fruit weight (FrW, kg) weight of one fully grown fruit, Fruit length (FrL, cm) and fruit diameter (FrD, cm) were measured with ruler, Rind diameter (RD, mm) measured with a Vernier caliper (Metr-ISO), and Total soluble solids (TSS) measured using a portable refractometer ATAGO Brix (0~53).
Seed	Number of seed per fruit (NSPFr) counted, Seed yield per fruit (SYPFr, g) weight of total number of seeds per fruit expressed in gram, Seed diameter (SD, mm) and seed length (SL, mm) were measured with a digital Vernier caliper (Metr-ISO), And Hundred seed weight (HSW, g) weight of hundred seeds.

Table 4.3 Partial analysis of variance and expected mean squares for variance component estimates of phenotypic traits among 36 citron watermelon landrace accessions evaluated across two seasons in Limpopo Province, South Africa.

Source of variation	df	Mean square	Expected mean squares
Reps/season	r/s	$MS_{r/s}$	-
Iblocks/season/reps	$Ib/s/r$	$MS_{Ib/s/r}$	-
Accession (g)	$g-1$	MS_g	$\delta^2 e + r\,\delta^2 gs + y{*}r\,\delta^2 g$
Season (s)	$s-1$	MS_s	-
$g \times s$	$(g-1)(s-1)$	MS_{gs}	$\delta^2 e + r\,\delta^2 gs$
Residual	$s(g-1)(r-1)$	MS_e	$\delta^2 e$
Total	$gs-1$		-

df = degrees of freedom; $MS_{r/s}$ = mean square replications in season; $MS_{Ib/s/r}$ = mean square incomplete blocks in replications and season; MS_g = mean square genotype; MS_s = mean square season; MS_{gs} = mean square interaction; MSe = mean square error; $\delta^2 g$ = genotypic variance; $\delta^2 e$ = residual/error variance; $\delta^2 gs$ = genotype-by-season interaction variance; r = number of replications; s = season.

4.2.5. Estimates of variance components

Variance components for each trait were calculated using the expected mean squares (EMS) from combined analysis of variance as shown in Table 4.3. Phenotypic coefficient of variance (*PCV*) and genotypic coefficient of variance (*GCV*) were calculated using values derived from the respective variance components. Broad-sense heritability (H^2b), genetic advance (*GA*) and genetic advance as percent of the mean (*GAM%*) were calculated according to Johnson et al. (1955) and described in the following equations:

Genotypic variance $(\delta^2_g) = (MS_g - \delta^2_e)/rs$

Where, MS_g is the mean square due to genotypes, (δ^2_e) = residual or error variance = Error mean square = MS_e, r = replicates, s = season

Phenotypic variance $(\delta^2_p) = \delta^2_g + \delta^2_{gs}/r + \delta^2_e/rs$

Where δ^2_{gs} = Variance of genotype-by-season interaction, δ^2_e = residual or error variance, r = replicates, s = season

Phenotypic coefficient of variance (*PCV*) = $\frac{\sqrt{\delta^2 p}}{Grand\ mean} x\ 100$

Genotypic coefficient of variance (*GCV*) = $\frac{\sqrt{\delta^2 g}}{Grand\ mean} x\ 100$

The broad-sense heritabilities (H^2b) were estimated as the ratio of the genotypic (δ^2_g) to phenotypic variance (δ^2_p) and expressed in percentage. H^2b was classified as low (< 30%), medium (30–60%) and high (≥ 60%), according to Johnson et al. (1955).

Genetic advance were estimated using the following formulae:

$GA = k\delta pH^2b$

Where, GA = genetic advance, k is the standard selection differential at 5% selection *intensity* ($k = 2.06$), δp is phenotypic standard deviation on mean basis and H^2b is the heritability in the broad sense.

Genetic advance as percent of the mean (GAM) was calculated as described by Johnson et al. (1955) as follows:

GAM = $\frac{\mathrm{GA}}{Grand\ mean} x\ 100$

Where, *GA* = genetic advance, grand mean is the mean of the population where selection was derived.

4.2.6. Multiple and step-wise regression analyses

Multiple and step-wise regression analyses were performed using SPSS version 26.0 (IBM Crop. Armonk, NY, 2017). Total fruit yield per plant and seed yield per fruit were the dependant variables, whereas other traits were treated as independant variables for the regression analyses. Normal distribution curves and standardized residuals for total fruit yield per plant and seed yield per fruit are shown in Figures 4.1 and 4.2, respectively validating the parametric data analyses.

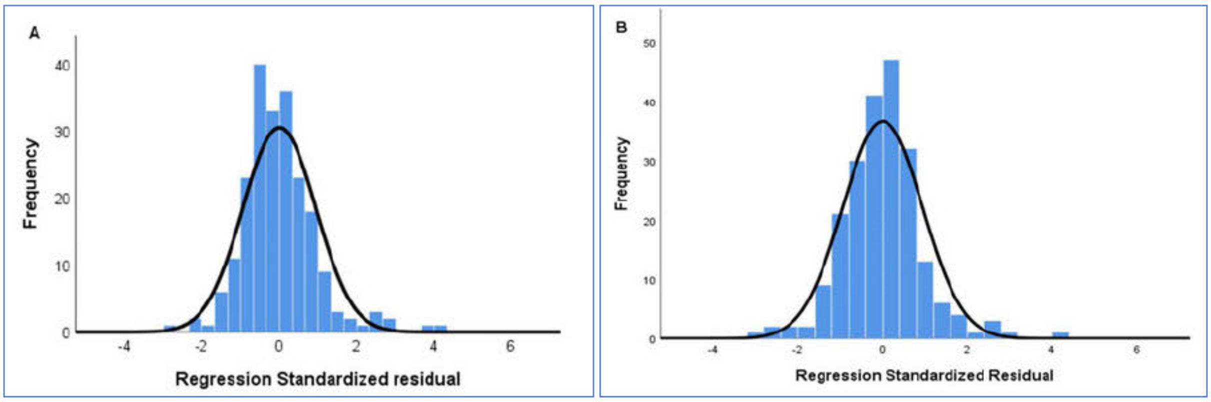

Figure 4.1: Distribution and frequency of standardized residuals for total fruit yield per plant (A) and seed yield per fruit (B) among 36 citron watermelon landrace accessions evaluated in two seasons in South Africa

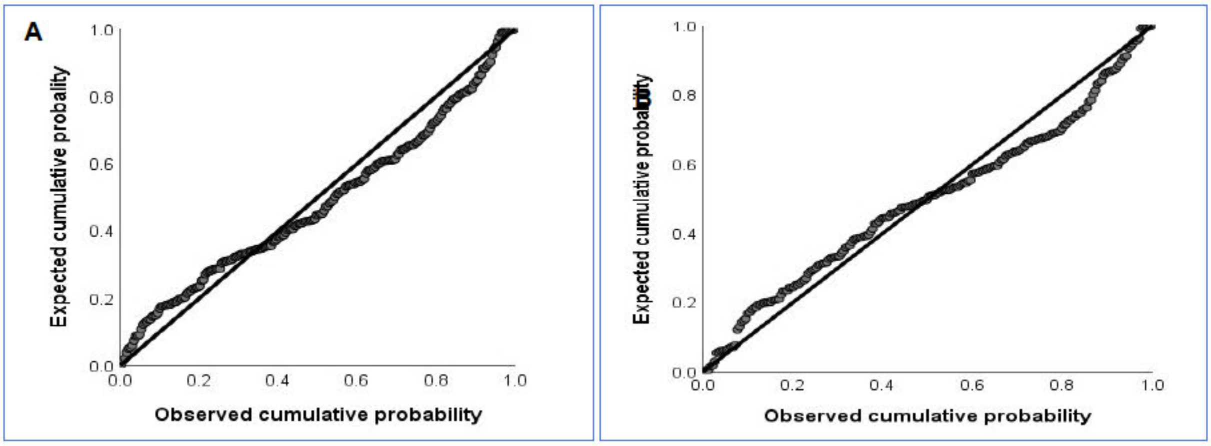

Figure 4.2: Normal observed cumulative probability of total fruit yield per plant (A) and seed yield per fruit (B) among 36 citron watermelon landrace accessions evaluated in two seasons in South Africa.

4.2.7. Path coefficient analysis

Path coefficient analysis was done based on mean data across the two seasons using a genotypic correlation matrix set up as A = B × C for total fruit yield per plant and seed yield per fruit. In this matrix, vector 'A' represents the genotypic correlation coefficients (total fruit yield per plant or seed yield per fruit) vs. other quantitative traits. In the same vector 'B' is the value of genotypic correlation for all possible combinations among the traits and vector 'C', the path coefficients. The inverse of matrix B was calculated using the Matrix Inverse function (MINVERSE) of Microsoft Excel 2016. The path coefficients were calculated as the product of vector A and each row of B^{-1} using the matrix multiplication (MMULT) function of Microsoft Excel. Direct and indirect path coefficients were calculated according to Dewey and Lu (1959). Total fruit yield per plant and seed yield per fruit were considered response variables, whereas other traits as causal/independent variables.

4.3. Results

4.3.1. Effect of genotype, season and genotype-by-season effect on measured traits

Combined analysis of variance revealed significant differences $(p < 0.001)$ among accessions for all evaluated traits except for number of primary branches (NPBr) and seed length (SL) (Table 4.4). Season effect was significant $(p < 0.05)$ for all traits except leaf length (LL), plant height (PH), number of marketable fruits per plant (NMFr) and seed length (SL). Significant $(p < 0.05)$ genotype-by-season (G × S) interaction effect was observed for all studied traits except for LL and leaf width (LW), PH and SL. Significant G × S interaction effect indicated citron watermelon accessions had variable performance across the two seasons.

4.3.2. Genetic variability, inheritance and genetic advance of phenotypic traits

Estimates of genotypic (δ^2g), phenotypic (δ^2p) and genotype by season interaction (δ^2gy) variance components, phenotypic coefficient of variance (PCV) and genotypic coefficient of variance (GCV), broad-sense heritability (H^2b), expected genetic advance (GA) and genetic advance as percent of the mean (GAM%) are presented in Table 4.5. Genotypic coefficient of variance (GCV) and phenotypic coefficient of variance (PCV) values varied from 0.08 to 43.26%, and 0.11 to 91.92%, respectively (Table 4.5). Broad-sense heritability (H^2b) varied from 23.45 to 83.86%. The highest H^2b estimates (≥ 60%) were recorded for PH, seed diameter (SD), total soluble solids (TSS), fruit weight (FrW), fruit diameter (FD), hundred seed weight (HSW), SL and flower length (FL), whereas the lowest H^2b (≤ 30%) were recorded for NPBr. Expected genetic advance (GA) varied from 62.38 to 3659.17% (Table 4.5).

Table 4.4 Mean squares and significance tests for phenotypic traits when evaluating 36 citron watermelon landrace accessions in two seasons in Limpopo Province, South Africa.

		Traits												
Source of variation	df	LL	LW	NdMfl	NdFfl	NMfl	TNFfl	SexR	PH	NPBr	NSBr	TNFr	FGFr	NMFr
Replications	2	84.47	178.10	231.13	145.48	1165571	1121.60	1025.70	0.46	2.57	1038	96.06	0.56	128.07
Genotype (G)	35	155.15*	229.90*	28.71**	45.63**	1794263**	2296.90**	976.80**	1.81*	3.61^{ns}	28.71**	247.62**	75.02**	30.06**
Season (S)	1	121.25^{ns}	21040.70**	1266.34**	3174**	8932616**	93500.20**	39962.20**	0.14^{ns}	23.34*	1266.34**	14032.78**	1472.67**	8.96^{ns}
G × S	35	119.07^{ns}	240.90^{ns}	32.15**	37.20**	1569882**	1895.10**	1060.30**	0.79^{ns}	5.29*	32.15**	211.08**	92.19**	24.61**
Residual	142	85.56	126.70	7.17	9.95	558162	752.90	259.90	0.57	2.83	540.90	93.44	21.81	7.83

		Traits												
Source of variation	df	NUFr	MyId	TFYld	FrW	FrL	FrD	RD	TSS	NSPFr	SYPFr	SL	SD	HSW
Replications	2	77.8	12.85	6.82	4.47	35.81	0.35	35.81	0.35	52.00	153.70	5.40	0.15	5.38
Genotype (G)	35	201.79**	1857.30**	2238.20**	40.15**	141.45**	51.39**	73.14**	0.54**	88960**	1619.5**	5306^{ns}	1.43**	40.37**
Season (S)	1	15504.17**	14231**	22268.5**	4000.02**	4040.33**	1879.39**	1402.4**	3.20**	2061744**	80118.5**	5411^{ns}	7.71**	355.23**
G × S	35	205.83**	1305.20**	1959.70**	16.31**	25.14**	22.09**	60**	0.22**	56680**	1691**	5289^{ns}	0.76**	14.82**
Residual	142	6595	364.40	581.80	4.09	6.61	5.97	16.99	0.11	251.38	420.80	537.30	0.24	2.31

See Table 4.2 for expanded trait abbreviations. * and ** = Significant at $p < 0.05$ and $p < 0.001$, respectively; ns = denotes non-significant; df = degrees of freedom.

The lowest GA values were recorded for TSS, NPBr and SD. Conversely, high GA estimates were recorded for total number of female flowers per plant (TNFfl) (4181.99) and seed yield per fruit (SYPFr) (3480.59). The highest GAM value was observed for number of unmarketable fruits per plant (NUFr) (8632.86%), marketable yield per plant (Myld) (5855.6%), FrW (8490.05%) and total number of fruits per plant (TNFr) (8274.53%). Low GAM values were recorded for number of days to male flowering (NdMfl) (472.09%), NPBr (1121.43%) and number of days to female flowering (NdFfl) (1298.94%) (Table 4.5).

4.3.3. Multiple regression analysis

Multiple regression analysis for TFYld and SYPFr as predictor variables is presented in Table 4.6. The model was highly significant ($p < 0.001$) and explained 93% ($R^2 = 0.93$) and 94% ($R^2 = 0.94$) of total variation for TFYld and SYPFr, respectively. This indicated both multiple regression models had high goodness of fit for both predictor variables. Standardized and unstandardized regression coefficients for predictor variables are shown in Tables 4.7 and 4.8. Positive unstandardized regression coefficients were recorded for TNFr ($\beta = 0.95$; $p < 0.001$) and Myld ($\beta = 1.04$; $p < 0.001$). Contrastingly, TFYld was negatively and significantly associated with NPBr ($\beta = -0.82$; $p < 0.05$). For SYPFr, positive and significant unstandardized regression coefficients were recorded for Myld ($\beta = 0.15$; $p < 0.05$), FrD ($\beta = 0.77$; $p < 0.05$), NSPFr ($\beta = 0.12$; $p < 0.001$), SL ($\beta = 2.51$; $p < 0.001$) and HSW ($\beta = 4.12$; $p < 0.001$), whereas negative and significant unstandardized coefficients were recorded for NdFfl ($\beta = -0.38$; $p < 0.05$) and TSS ($\beta = -4.2$; $p < 0.05$).

Table 4.5 Estimates of genetic parameters for phenotypic traits evaluated among 36 citron watermelon landrace accessions across two seasons in Limpopo Province, South Africa.

Trait Category			Variance components estimates			Coefficients of variation				Genetic advance	
	Traits	Grand Mean	$\delta^2 e$	$\delta^2 g$	$\delta^2 p$	*GCV (%)*	*PCV (%)*	*RCV (%)*	$H^2 b$ *(%)*	*GA*	*GAM (%)*
Leaf	LL	66.84	85.56	11.60	65.55	5.10	12.11	13.84	42.06	877.18	1312.36
	LW	74.9	126.7	17.20	118.62	5.54	14.54	15.03	38.08	1264.44	1688.17
Flower	NdMfl	44	7.17	3.59	15.50	4.31	8.95	6.09	48.12	472.09	1072.93
	NdFfl	52	9.95	5.95	20.01	4.69	8.60	6.07	54.52	675.45	1298.94
	NMfl	1950	585162	201516	822337	23.02	46.5	39.23	49.50	101280	5193.87
	TNFfl	56	752.9	257.33	1014.52	28.65	56.88	49.00	50.36	4181.99	7467.83
	SexR	44	259.9	119.48	516.23	24.84	51.64	36.64	48.11	2619.66	5953.76
Plant	PH	5.2	0.57	0.21	0.57	8.74	14.46	14.52	60.48	111.91	2152.06
	NPBr	8	2.83	0.13	2.37	4.51	19.22	21.03	23.45	89.71	1121.43
	NSBr	52	7.17	3.59	15.50	3.64	7.57	5.15	48.12	3493.22	6717.72
Fruit	TNFr	17	93.44	25.7	111.63	29.82	62.15	56.86	47.98	1406.67	8274.53
	FGFr	9	21.81	8.87	43.23	33.09	73.06	51.89	45.29	650.16	7223.97
	NMFr	6	7.83	3.71	13.21	32.08	60.58	46.64	52.95	409.88	6831.27
	NUFr	11	65.95	22.64	102.24	43.26	91.92	73.83	47.06	1312.21	11929.2
	Myld	39.56	364.4	248.82	744.62	39.87	68.98	48.25	57.81	3415.16	8632.86
	TFYld	62.49	581.8	276.07	1026.27	26.59	51.26	38.60	51.87	3659.17	5855.6
	FrW	9.45	4.09	6.01	12.13	25.94	36.85	21.40	70.39	802.31	8490.05
	FrL	25.91	6.61	22.47	31.96	18.30	21.82	9.92	83.86	1225.66	4730.45
	FrD	23.78	3.97	7.90	15.93	11.82	16.78	8.38	70.44	701.43	2949.64
	RD	20.83	16.99	9.36	32.19	14.69	27.24	19.79	53.92	700.29	3361.93
	TSS	3.44	0.10	0.07	0.16	7.87	11.75	9.19	67.01	62.38	1813.52
Seed	NSPFr	646	25138	10637	33720	15.97	28.43	24.54	56.17	25848.1	4001.26
	SYPFr	84.4	420.8	199.78	833.58	16.75	34.21	24.30	48.96	3480.59	4123.93
	SL	12.33	0.61	1.03	1.73	0.08	0.11	6.33	77.15	213.47	1731.27
	SD	7.26	0.24	0.20	0.49	6.13	9.66	6.75	63.51	96.88	1334.44
	HSW	13.18	2.31	6.34	11.67	19.11	25.92	11.53	73.73	530.81	4027.36

See Table 4.2 for expanded trait abbreviations. $\delta^2 e$ = environmental variance; $\delta^2 g$ = genotypic variance; $\delta^2 p$ = phenotypic variance; *GCV* = genotypic coefficient of variance; *PCV* = phenotypic coefficient of variance; RCV = Residual coefficient of variance; $H^2 b$ = heritability in broad sense*; GA* = genetic advance*; GAM* = genetic advance as a percent of the mean.

Table 4.6 Multiple regression analysis of variance for total fruit yield per plant and seed yield per fruit as predictor variables among citron watermelon accessions evaluated across two seasons in Limpopo Province, South Africa.

Predictor variable	Source of variation	df	SS	MS	F-ratio	*P*-value	R^2	SE
Total fruit yield per plant	Regression	25	234605.62	9384.23	101.71	< 0.001	0.93	9.61
	Residual	190	17530.80	92.27				
	Total	215	252136.40					
Seed yield per fruit	Regression	25	241146.78	9645.87	122.96	< 0.001	0.94	8.84
	Residual	190	14904.98	78.45				
	Total	215	256051.76					

df = degrees of freedom; SS = sum of squares; MS = mean square; R^2 = Coefficient of determination; SE = standard error.

Table 4.7 Multiple regression analysis showing (un) standardized coefficients for total fruit yield per plant as a predictor variable among citron watermelon accessions evaluated across two seasons in Limpopo Province, South Africa.

Trait category	Variables	Coefficients				
		Unstandardized		Standardized		
		β	SE	β	t-test	t-value
	(Constant)	13.72	18.57	-	0.74	ns
Leaf	LL	0.16	0.10	0.05	1.69	ns
	LW	-0.05	0.07	-0.02	-0.63	ns
Flower	NdMfl	-0.18	0.22	-0.03	-0.83	ns
	NdFfl	-0.34	0.19	-0.06	-1.81	ns
	NMfl	0.00	0.00	0.02	0.40	ns
	TNFfl	-0.01	0.03	-0.01	-0.17	ns
	SexR	0.01	0.04	0.00	0.14	ns
Plant	PH	-1.30	1.05	-0.03	-1.23	ns
	NPBr	-0.82	0.40	-0.04	-2.07	*
	NSBr	0.02	0.04	0.02	0.52	ns
Fruit	TNFr	0.95	0.28	0.40	3.38	**
	FGFr	0.24	0.16	0.05	1.50	ns
	NMFr	-0.79	0.40	-0.09	-1.97	ns
	NUFr	-0.49	0.30	-0.19	-1.61	ns
	Myld	1.04	0.03	0.87	33.63	**
	FrW	0.16	0.37	0.03	0.43	ns
	FrL	0.01	0.18	0.00	0.05	ns
	FrD	0.51	0.32	0.07	1.60	ns
	RD	0.16	0.13	0.03	1.22	ns
	TSS	-2.03	1.74	-0.03	-1.17	ns
Seed	NSPFr	0.02	0.01	0.12	1.82	ns
	SYPFr	-0.14	0.08	-0.14	-1.76	ns
	SL	-0.29	0.70	-0.01	-0.41	ns
	SD	1.92	1.21	0.04	1.58	ns
	HSW	0.59	0.42	0.06	1.42	ns

See Table 4.2 for expanded trait abbreviations. * and ** = Significant at $p < 0.05$ and $p < 0.001$, respectively; ns = non-significant; β = regression coefficient; SE = standard error.

Table 4.8 Multiple regression analysis showing (un)standardized coefficients for seed yield per fruit as a predictor variable among citron watermelon accessions evaluated across two seasons in Limpopo Province, South Africa.

		Coefficients				
		Unstandardized		Standardized		
Trait category	Variables	β	SE	β	t-test	t-value
	(Constant)	-72.72	16.32			
Leaf	LL	0.14	0.09	0.04	1.63	ns
	LW	-0.06	0.07	-0.03	-0.86	ns
Flower	NdMfl	0.37	0.21	0.05	1.79	ns
	NdFfl	-0.38	0.17	-0.07	-2.21	*
	NMfl	0.00	0.00	0.05	1.10	ns
	TNFfl	-0.04	0.03	-0.04	-1.21	ns
	SexR	-0.05	0.04	-0.04	-1.35	ns
	PH	-0.71	0.97	-0.02	-0.73	ns
Plant	NPBr	-0.58	0.37	-0.03	-1.57	ns
	NSBr	-0.04	0.03	-0.04	-1.08	ns
	TNFr	-0.14	0.27	-0.06	-0.54	ns
Fruit	FGFr	0.08	0.15	0.02	0.54	ns
	NMFr	0.31	0.37	0.03	0.83	ns
	NUFr	0.23	0.28	0.09	0.81	ns
	MYld	0.15	0.08	0.13	2.02	*
	TFYld	-0.12	0.07	-0.12	-1.76	ns
	FrW	-0.16	0.34	-0.03	-0.49	ns
	FrL	0.03	0.17	0.01	0.15	ns
	FrD	0.77	0.29	0.11	2.66	*
	RD	-0.19	0.12	-0.03	-1.55	ns
	TSS	-4.21	1.58	-0.06	-2.66	*
	NSPFr	0.12	0.00	0.78	35.45	**
Seed	SL	2.51	0.62	0.10	4.08	**
	SD	0.35	1.13	0.01	0.31	ns
	HSW	4.12	0.24	0.42	16.87	**

See Table 4.2 for expanded trait abbreviations. * and ** = Significant at $p < 0.05$ and $p < 0.001$, respectively; ns = non-significant; β = regression coefficient; SE = standard error.

4.3.4. Step-wise regression analysis

Step-wise regression analysis showing various models predicting variation for dependent variables are presented in Tables 4.9 and 4.10. For TFYld, the first variable entered in the model was MYld which explained 88% (R^2 = 0.88) of total variation. The fifth model comprised five variables entered into the model namely MYld, TNFr, NdFfl, FrD and RD and explained 92% (R^2 = 0.92) of variation for TFYld. Step-wise regression model involving Myld and TNFr was selected the best predictive model for TFYld (R^2 = 0.89). For SYPFr, the first explanatory variable entered in the model was number of seed per fruit (NSPFr) which explained 69% (R^2 = 0.69) of total variation in the predictor variable. In the second model, NSPFr and HSW were assigned which explained 92% (R^2 = 0.92) of variation for SYPFr. The 3rd, 4th, 5th and 6th step-wise regression models all explained 93% (R^2 = 0.93) variation and showed no model improvement in SYPFr. As a result, step-wise regression model involving NSPFr and HSW was the most predictive of NSPFr (R^2 = 0.92).

4.3.5. Path coefficient analysis

Path coefficient analysis showing direct effects of assessed phenotypic traits on TFYld and SYPFr as response variables are presented in Table 4.11. Marketable yield (Myld) had the highest significant direct effect (0.79) on TFYld, followed by TNFr (0.70). Total fully grown fruits (FGFr) followed by Myldhad high indirect positive effect of 0.52 and 0.57 on TFYld via Myld, respectively. In addition, NUFr had the highest indirect positive effect (0.65) on TFYld via total TNFr. Number of seeds per fruits (NSPFr) had a high significant direct positive effect (0.92) on SYPFr, followed by HSW (0.69). Seed diameter (SD) and SL had indirect positive effect of 0.51 and 0.47 on seed yield per fruit via HSW, respectively (Table 4.11).

Table 4.9 Step-wise regression analysis for total fruit yield per plant as dependent variable among 36 citron watermelon landrace accessions evaluated across two seasons in Limpopo Province, South Africa.

Entered variable/s	Variable in model	Intercept	β1	β2	β3	β4	β5	R^2	R^2 change	SE	P-value
Myld	Myld	18.06	1.12					0.88	0.884	11.67	**
TNFr	Myld, TNFr	13.35	1.12	0.29				0.89	0.015	10.94	**
NdFfl	Myld, TNFr, NdFfl	53.30	1.07	0.38	-0.77			0.91	0.015	10.09	**
FrD	Myld, TNFr, NdFfl, FrD	29.44	1.05	0.47	-0.63	0.70		0.92	0.006	9.73	**
RD	Myld, TNFr, NdFfl, FrD, RD	25.10	1.04	0.47	-0.59	0.58	0.25	0.92	0.002	9.65	*

See Table 4.2 for trait descriptions. Myld = marketable yield; TNFr = total number of fruits; NdFfl = number of days to female flowering; FrD = fruit diameter; RD = rind diameter; β = regression coefficient; SE = standard error; R^2 = Coefficient of determination; * and ** = Significant at $p < 0.05$ and $p < 0.001$, respectively.

Table 4.10 Step-wise regression analysis for seed yield per fruit as dependent variable among citron watermelon landrace accessions evaluated across two seasons in Limpopo Province, South Africa.

Entered variable	Variable in model	Intercept	β1	β2	β3	β4	β5	β5	R^2	R^2 change	SE	P-value
NSPFr	NSPFr	1.46	0.13						0.69	0.690	19.25	**
HSW	NSPFr, HSW	-58.88	0.13	4.76					0.92	0.232	9.68	**
SL	NSPFr, HSW, SL	-80.96	0.13	4.36	2.11				0.93	0.005	9.39	**
FrD	NSPFr, HSW, SL, FrD	-90.81	0.12	4.21	2.34	0.51			0.93	0.004	9.16	**
TSS	NSPFr, HSW, SL, FrD, TSS	-75.10	0.12	4.15	2.31	0.56	-4.19		0.93	0.003	8.99	**
NPBr	NSPFr, HSW, SL, FrD, TSS, NPBr	-69.44	0.12	4.13	2.34	0.54	-4.29	-0.66	0.93	0.001	8.93	*

See Table 4.2 for trait descriptions. NSPFr = number of seeds per fruit; HSW = hundred seed weight; SL = seed length; FrD = fruit diameter; TSS = total soluble solids; NPBr = number of primary branches; SE = standard error; β = regression coefficient; R^2 = coefficient of determination; * and ** = Significant at $p < 0.05$ and $p < 0.001$, respectively.

Table 4.11 Path coefficient analysis showing direct effects for total fruit yield per plant and seed yield per fruit of citron watermelon landrace accessions evaluated across two seasons in Limpopo Province, South Africa.

		Total fruit yield per plant		Seed yield per fruit	
Trait category	Traits	Direct effects	r_g	Direct effects	r_g
Leaf	LL	-0.03	0.08^{ns}	-0.10	0.02*
	LW	0.03	0.24^{ns}	0.09	0.09^{ns}
Flower	NdMfl	0.07	-0.35*	-0.03	-0.41*
	NdFfl	-0.04	-0.07^{ns}	-0.12	-0.23^{ns}
	NMfl	0.21	0.49**	-0.12	0.16^{ns}
	TNFfl	0.05	0.50**	-0.08	0.13^{ns}
	SexR	0.05	-0.09^{ns}	0.02	-0.12^{ns}
Plant	PH	0.05	0.64**	0.04	0.16^{ns}
	NPBr	-0.01	0.12^{ns}	0.08	-0.12^{ns}
	NSBr	-0.29	0.37*	-0.11	0.09^{ns}
Fruit	TNFr	**0.70**	0.40*	0.83	-0.04^{ns}
	FGFr	0.19	0.70**	0.10	0.25^{ns}
	NMFr	-0.20	0.73**	0.12	0.19^{ns}
	NUFr	-0.46	0.14^{ns}	-0.49	-0.11^{ns}
	TFYld	-	-	-0.73	0.21^{ns}
	MYld	**0.79**	0.96**	0.38	0.26^{ns}
	FrW	0.13	0.27^{ns}	0.18	0.16^{ns}
	FrL	-0.03	0.26^{ns}	-0.01	0.09^{ns}
	FrD	0.03	0.31^{ns}	0.11	0.20^{ns}
	RD	-0.04	0.38*	-0.17	0.05^{ns}
	TSS	-0.11	0.12^{ns}	-0.16	-0.15^{ns}
Seed	NSPFr	0.33	0.27^{ns}	**0.92**	0.60**
	SYPFr	-0.43	0.21^{ns}	-	-
	SL	0.15	0.02^{ns}	0.45	0.32^{ns}
	SD	-0.24	-0.10^{ns}	-0.31	0.21^{ns}
	HSW	0.43	-0.09^{ns}	**0.69**	0.40*

See Table 4.2 for expanded trait abbreviations. High path coefficient values are indicated in bold. *, ** = Significant at $p < 0.05$ and $p < 0.001$, respectively; ns = denotes non-significant; r_g = denotes genotypic correlation.

4.4. Discussion

Understanding the magnitute of genetic variation for traits of interest in citron watermelon is useful for cultivar design and product development. Citron watermelon is a multi-purpose crop with various plant parts of the crop (e.g., leaf, fruit and seed) used for food and feed. For instance, fresh succulent leaves of citron watermelon are cooked and consumed as leafy vegetable in Africa. The fully developed yellow- and orange-fleshed fruit are consumed un/cooked, while the seed are roasted for human consumption or processed for use as chicken feed (Ngwepe et al., 2019; Nkoana et al., 2021). Therefore, leaf, plant, fruit and seed attributes are important selection criteria for citron watermelon breeding and breeding programs.

There existed extensive genetic variation for phenotypic traits (Table 4.4) among the 36 citron watermelon landraces accessions. The observed variation for phenotypic traits especially fruit and seed yields will allow identification and selection of suitable genotypes for direct production, value-adding, rootstock development and commercialization. Genetic analysis of 36 citron watermelon accessions using SSR markers revealed wide genetic distances and three distinct genetic groups (Mashilo et al., 2017). Some of the accessions evaluated in the present study such as WWM03, WWM04, WWM08, WWM14, WWM15, WWM16, WWM24, WWM34, WWM37, WWM39, WWM44 and WWM48 were found to be genetically unique and unrelated (Mashilo et al., 2017). In the Limpopo Province of South Africa where citron watermelon is widely cultivated, farmers grow several accessions of the crop on the same plot for multiple uses. This causes random cross-pollinations between accessions leading to genetic recombination and variation on various phenotypic traits as observed in the present study. In citron watermelon, male and female flowers are borne on the primary and secondary branches of the same plant allowing for cross-pollination especially amongst closely planted accessions. Therefore, both events are the factors contributing to the wide phenotypic and genetic diversity present in citron watermelon.

Fresh succulent leaves are widely used as leafy vegetable in many parts of Africa, including South Africa. Traits such as leaf length and width serve as key phenotypic markers for selection of citron watermelon genotypes. Significant genotypic variations were observed for leaf length and width which allowed selection of unique accessions. Accessions with larger leaf size (i.e., leaf length and width ≥ 70 mm) including WWM04, WWM23, WWM28, WWM35, WWM37, WWM39, WWM65, WWM79 and WWM84 were selected as leaf vegetable types. In some African countries bright yellow male flowers of citron watermelon are harvested and cooked with fresh green leaves to improve taste, aroma and visual appearance of the relish. This makes male flowering rate as an important attribute to enhancing the use of citron watermelon as a functional leaf vegetable crop in sub-Saharan Africa. Accessions such as WWM03, WWM09, WWM14, WWM16, WWM35, WWM38, WWM39, WWM41, WWM47, WWM50, WWM64, WWM65, WWM67, WWM79 and WWM85 exhibited profuse male flowers (> 2000 male flowers per plant) making them ideal selections.

Vines of citron watermelon are valuable feedstock for livestock during dry spells in several sub-Saharan African countries. Citron watermelon produces multiple vines from its primary and secondary branches. Seven to 10 primary branches, and some 100 secondary branches are present per plant depending on the genotype (Ngwepe et al., 2021). Therefore, branching ability is an important trait in citron watermelon for high biomass production for feed. Accessions WWM03, WWM05, WWM14, WWM35, WWM39, WWM50, WWM67 and WWM79 had longer vines and higher number of total branches per plant (> 70) which are ideal for fodder production.

Fruit yield is economically important trait for growers. In sub-Saharan Africa, yellow and orange-fleshed fruit types are preferred to prepare traditional dishes and to deriving cash income. Yellow and orange-fleshed types are used to develop value-added products such as jam and pickles (van Wyk, 2011), traditional dishes such as “Kgodu” and “Mokgapu” (Ngwepe et al., 2019; Nkoana et al., 2021). Accessions such as WWM14, WWM16, WWM39, WWM64, WWM67, WWM76, and WWM79 identified in the present study were high-yielding and recorded total fruit yield per plant of > 80 kg which translates to total fruit yield of 331, 493, 326.26, 346.84, 336.88, 321,76, 347.24 tons/ha (extrapolated from a total of 4000 plants per hectare), respectively. In addition, actual and extrapolated (i.e., based on 4000 plants per hectare) marketable fruit yields were 55.31 kg and 221 tons/ha for WWM14, 93.50 kg and 374 tons/ha for WWM16, 51.27 kg and

205.08 tons/ha for WWM39, 60.49 kg and 241.96 tons/ha for WWM64, 59.28 kg and 237.12 tons/ha for WWM67, 60.04 kg and 240.16 tons/ha for WWM76 and 58.32 kg and 233.28 tons/ha for WWM79, in that order. Maggs-Kölling and Christiansen (2003) reported marketable fruit yields of 43.07, 63.11 and 118.84 tons/ha (i.e., based on plant population of 4000 plants) for citron watermelon genotypes NAM1632, NAM1636 and NAM1765, which is much lower compared to current findings. This suggests the assessed accessions in the present study are high yielding useful to improve overall fruit production in their region of origin or elsewhere. The high total and marketable fruit yields of the identified accessions is attributed to the large fruit weight (mean = 9.45 kg) and total number of fruit produced per plant (mean = 17). Fruit weight of the assessed accessions is higher than the mean value 5.23 and 1.20 kg reported by Maggs-Kölling and Christiansen (2003) and Achigan-Dako et al. (2015) among citron watermelon accessions, respectively. Conversely, Maggs-Kölling and Christiansen (2003) reported higher number of fruits per plant of 35 Namibian citron watermelons. The present study revealed total number of fruits per plant and marketable fruit yield per plant as key economic traits for targeted selection to improve high-yield potential (Tables 4.9 and 4.11). Accessions such as WWM14, WWM16, WWM39, WWM64, WWM67, WWM76 and WWM79 produced high number of fruits per plant (> 17) and high marketable yield per plant (> 50 kg) making them suitable parental genotypes for direct production and for household consumption. However, genotype, environment (i.e., Soil type, nutrient status and water availability) and genotype-by-environment interactions may influence yield potential of citron watermelon.

Seed of citron watermelon serve as food and feed (Ngwepe et al., 2019). Therefore, breeding for high seed yield potential is an important goal to deliver high-performing genotypes for production and value adding. Accessions such as WWM04, WWM66, and WWM76 identified in the present study were high-yielders recording seed yield per fruit of >110 g. Seed yield-related traits including number of seeds per fruit and hundred seed weight were shown to be useful traits for direct selection to improve overall seed yield (Tables 4.10 and 4.11). Accessions such as WWM04, WWM14, WWM15, WWM16, WWM24, WWM28, WWM37, WWM46, WWM66, WWM68 and WWM79 which produced higher seed number per fruit (>650 seeds per fruit), heavier seeds (> 13 g) and seed yield per fruit (> 80 g) are recommended for seed production for developing value-added products such as roasted seed snack for food and seed cake for livestock feed. The mean hundred seed weight of 13.18 g of the studied accessions was lower compared to 18.26 g reported by Maggs-Kölling and Christiansen (2003), but higher than 9.70 g reported by Achigan-Dako et al. (2015). In addition, seed length (mean = 17.30 mm) and diameter (mean = 72.6 mm) of the studied accessions were higher than 10.4 (i.e., seed length) and 6.5 (i.e., seed width) mm reported by Achigan-Dako et al. (2015) among a diverse set of citron watermelon genotypes. The current study found marked variation for seed-related traits which can be explored for breeding.

Total soluble solids is an important sensory attribute that contributes to the eating quality of watermelon. The mean total soluble solids were 3.44° Brix and values ranged from 2.75 to 3.87. These records were higher than a mean value of 1.6° Brix reported by Achigan-Dako et al. (2015), but lower than a value of 3.71° Brix reported by Maggs-Kölling and Christiansen (2003). The low total soluble solids of citron watermelon offer opportunities to develop sweet dessert watermelon genotypes with low sugar content for specific market segment and consumers.

Selection of desirable secondary traits can improve genetic gains for fruit and seed yield. Phenotypic selection depends on genetic diversity present in the population to select the best genotypes for breeding and cultivar development (Gupta et al., 2016). High broad-sense heritability is a useful measure of assessing the breeding value of phenotypic traits in crop improvement programmes. In the present study, phenotypic traits including plant height, fruit weight, fruit length and diameter, total soluble solids, seed length and diameter, and hundred seed weight exhibited high H^2b (> 60%). Comparative heritability estimates were reported for plant height (79%), fruit yield per plant (79.7%), number of seed per fruit (91.2%) and total soluble solid (89.5%) in sweet dessert watermelon (Choudhary et al., 2012). In other cucurbit crops including bottle gourd and bitter gourd, high heritability estimates were also reported for fruit weight (94.20%), fruit length (96.50%), fruit diameter (83.50%), fruit per plant (> 85.90%) and plant height (> 62%) (Gupta et al., 2016). Hence, direct selection for traits with moderate to high heritability warrants higher selection response.

Burton and De Vane (1955) suggested estimates of heritability alone are not sufficient for predicting selection gains. Genetic advance which is directly related to yield gains is useful for achieving selection gains. In this study, high heritability and genetic advance were recorded for fruit length (83.86 and 4730.45%), seed length (77.73 and 1731.27%), hundred seed weight (73.73 and 4027,36%), fruit diameter (70.44 and 2949.64%) and fruit weight (70.39 and 8490.05%). This suggest that these characters have high degree of heritable genes for effective selection (Johnson et al., 1955, Gupta, et al., 2016). Step-wise regression and path analyses revealed that total number of fruits per plant and marketable fruit yield per plant are key traits to improve fruit yield. Furthemore, selection based on higher number of seeds per fruit and heavier hundred seed weight was found to be effective for enhanced seed yield.

The findings of the present study further indicate that “new” citron watermelon varieties could be designed to improve high fruit and seed yield potential. Therefore, the following complementary parental genotypes: WWM03, WWM16, WWM39, WM64 and WWM76 (i.e. with high fruit count per plant, total and marketable fruit yield per plant), WWM04, WWM15, WWM24, WWM28, WWM46, WWM66, WWM68, WWM76 and WWM79 (i.e., high seed yield per fruit), and WWM03, WWM17, WWM35, WWM40, WWM50, WWM67, WWM79 and WWM85 (i.e., large fruit size) are recommended for watermelon improvement programmes. These accessions are useful to design new cultivars with better fruit and seed yields or for direct production and processing with value-addition.

4.5. Conclusions

The study assessed genetic variation and estimated genetic parameters among citron watermelon landrace accessions based on phenotypic traits to facilitate the selection of desirable genotypes. Marked genetic variations were observed for total and marketable fruit yields per plant, total number of fruits per plant, number of seeds per fruit, hundred seed weight and seed yield per fruit. These are useful attributes for citron watermelon for variety design and release incorporating consumers and market needs and preferences. The study recommends accessions such as WWM14, WWM16, WWM39, WWM64, WWM67, WWM76 and WWM79 with high fruit yield per plant, and WWM03, WWM04,

WWM14, WWM15, WWM16, WWM24, WWM28, WWM37, WWM46, WWM66 and WWM68 exhibiting high seed yield and number for breeding or direct production. Crosses involving the selected complementary parents will enhance citron watermelon pre-breeding and breeding programs and product development.

4.6 References

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Chapter 5. Combining ability and hybrid performance in citron watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.) for agronomic traits

Abstract

Yield gains in crop plants, such as citron watermelon can be realised through combining ability tests and hybrid breeding. The objective of the study was to determine the combining ability and hybrid performance of citron watermelon genotypes for agronomic traits. Five contrasting and relatively high-yielding citron watermelon genotypes were crossed in a 5 × 5 half-diallel mating design and 10 F_1 hybrids were developed. The 15 families (five parents and 10 F_1 hybrids) were evaluated across two environments using a randomized complete block design with three replications. Data was collected on various agronomic traits and subjected to analysis of variance (ANOVA) and combining ability analysis. Significant ($p < 0.001$) genotype × environment interaction effects were observed for most assessed traits. General combining ability (GCA) and specific combining ability (SCA) effects were significant ($p < 0.001$) for most traits. Environment × GCA was non-significant, whereas Environment × SCA effects were significant ($p < 0.001$) for most traits. The ratios of GCA/SCA variances were less than unity for most traits, indicating non-additive gene action of the traits. Broad-sense heritability varied from low to moderate, implying variable selection response of the assessed traits among the F1 hybrids. The parental genotypes, WWM16 with positive GCA effects for fruit and seed yields and WWM66, with positive GCA effects for the number of seeds per fruit and seed yield, were identified for hybrid breeding. The following F1 hybrids: WWM04 × WWM16, WWWM03 × WWM66 and WWM16 × WWM50, with positive SCA effects on total fruit yield per plant and marketable fruit yield per plant, and WWM04 × WWM50, WWM03 × WWM16 and WWM03 × WWM66, with positive SCA effects for number of seeds per fruit and total seed yield were identified. The study identified novel and best-performing F1 hybrids of citron watermelon for economic traits and are recommended for multi-environmental evaluations, variety registration and commercialization.

Keywords: *Citron watermelon, half diallel analysis, hybrid breeding, general combining ability, specific combining ability*

5.1. Introduction

Citron watermelon (*Citrullus lanatus* var. *citroides* [L.H. Bailey] Mansf. ex Greb.) is a diploid trailing/creeping monoecious crop belonging to the Cucurbitaceae family. It bears a high number of male flowers (i.e., up to 2000 male flowers per plant) and relatively few female flowers ranging from 26 to 102 flowers per plant (Ngwepe et al., 2021a). It is an annual crop mainly grown during the warm summer seasons in many parts of sub-Saharan Africa (SSA), including South Africa, Botswana, Zimbabwe and Swaziland (Levi et al. 2013; Wechter et al., 2012). In SSA, it is mainly grown for diverse uses, including as a leaf vegetable. The ripened fruits have yellow and orange flesh colour and are used to derive various food products such as a soup referred to as "Mokgapu" and porridge called "Kgodu" or "Semphemphe" which are native names referred to these products by the Bapedi tribe of the Limpopo Province of South Africa (Ngwepe et al., 2021a, 2023). The fruit flesh contains health-promoting carotenoids (e.g., prolycopene, phytoene, and ζ-carotene, violaxanthin, neoxanthin and β-carotene) (Tadmor et al., 2005; Bang et al., 2010, 2007; Davis et al., 2007b), Vitamins A, B6, C, lycopene, and antioxidants (Jensen, 2012).

The seeds are rich in micro- and macro-nutrients and are mainly prepared and consumed as roasted snacks (Mashilo et al., 2016; Ngwepe et al., 2021a, 2021b; Mandizvo et al., 2022). Red seeds are preferred over dark seeds for roasting due to their excellent eating attributes (Mandizvo et al., 2022). The flesh and seeds possess antibacterial activities (Masoko et al., 2022). The root extracts of the crop are used to treat various ailments such as tuberculosis, by traditional healers in South Africa (Semenya and Maroyi, 2019; Nkoana et al., 2021). The nutritive and medicinal properties of citron watermelon have rendered it a super-food in SSA, though the crop is largely under-utilized and under-researched compared to other cucurbit crops, including sweet watermelon (*C. lanatus* var. *lanatus*). Also, citron watermelon is tolerant to biotic (e.g., root-knot nematodes and *Fusarium* wilt) and abiotic stress (e.g., drought and heat) (Thies et al., 2003, 2007; Mo et al., 2016; Guzzon et al., 2017) allowing for successful cultivation in marginal growing conditions requiring limited agricultural inputs (i.e., water, fertilizer and pesticides). These attributes have rendered it an alternative rootstock to derive high-yield and quality grafted watermelon (Thies et al., 2015a, b, c; Fallik et al., 2019; Bikdeloo et al., 2021).

Citron watermelon shows extensive variation for agronomic traits, including fruit yield, and yield-related agronomic traits (Achigan-Dako et al., 2015; Ngwepe et al., 2019, 2021a, 2021b). Also, variation occurs for qualitative traits, including fruit (i.e., colour, rind stripes and flesh colour) and seed attributes (i.e., seed coat colour) (Achigan-Dako et al., 2015; Ngwepe et al., 2021a). Phenotypic variation in the crop can be explored for cross-breeding in sweet watermelon to derive consumer and market-preferred varieties. For example, a sweet-sour fleshed watermelon variety “SW” was developed by crossing citron watermelon accession “PI PI271769” and inbred line ‘203Z’ (Gao et al., 2018). The rootstocks IIHR-617 × Arka Manik and IIHR-82 × Arka Manik with resistance to gummy stem blight were derived between sweet and citron watermelon (Mahapatra et al., 2023). Gummy stem blight resistant watermelon varieties (i.e., NC-GSB-524W, NC-GSB-527W, NC-GSB-528W, NC-GSB-530W, NC-GSB-531W and NC-GSB-532W) were developed using citron watermelon parents (Rivera-Burgos et al., 2021). A sweet watermelon cultivar “MSW-28” exhibiting high lycopene content, was derived from half-sib families of different varieties of commercial sweet watermelon and citron watermelon lines (Davis and King, 2007a). Also, watermelon cultivars “LSW-177” and “LSW-194” with low fruit total soluble solids (TSS) content and firm texture and intense yellow flesh colour were selected from the crosses of *C. lanatus* var. *lanatus and C. lanatus* var. *citroides* accessions (Davis et al., 2007b). These reports indicated the genetic values of citron watermelon for breeding in cross-compatible cucurbit species, mainly sweet watermelon.

Citron watermelon germplasm also possesses wide genetic differentiation, which offers considerable opportunities for trait improvement through breeding (Levi et al., 2013; Nantoumé et al., 2013; Zhang et al., 2016; Mashilo et al., 2017). Therefore, the genetic resources of citron watermelon should aid in breeding future varieties that possess desirable attributes based on the requirement of farmers, consumers and markets. For instance, the citron watermelon hybrid rootstock IIHR-82 × IIHR-617 resistant to gummy stem blight when used as rootstock improved the fruit yield and quality of grafted watermelon (Mahapatra et al., 2023). Citron watermelon F_1 hybrid rootstocks (i.e., RKVL

301 × 316, RKVL 301 × 318, RKVL 318 × 301 and RKVL 318 × 317) improved fruit yield of sweet watermelon and possess higher tolerance to root-knot nematodes (Thies et al., 2015b). However, the genetic variation in citron watermelon is yet to be explored for breeding and yield gains, including fruit and seed yields.

Breeding for high-yield potential crops requires understanding the combining ability of the available parents and hybrid performance. Combining ability analysis is useful in crop improvement programs to identify superior parents for breeding, and subsequent selection of best-performing hybrids or families (Acquaah, 2007). General combining ability (GCA) effect is predominantly conditioned by additive gene action, whereas specific combining ability (SCA) provides information about intra- and inter-allelic gene interactions (Acquaah, 2007).

Citron watermelon is an important crop in SSA, currently offering niche market potential for growers. Its unique genetic profiles have been explored for breeding in sweet watermelon, mainly for disease resistance breeding and fruit yield and quality enhancement. However, there is a need to explore the possibility of creating new genetic recombination with desired attributes and traits in this crop. Exploiting hybrid vigour among the genetically divergent citron watermelon accessions can aid in developing new varieties with excellent agronomic attributes. The objective of the study was to determine the combining ability and hybrid performance of citron watermelon genotypes for agronomic traits for breeding.

5.2. Materials and methods

5.2.1. Plant material

Five selected citron watermelon accessions were used as parents in the current study. These included accessions WWM03, WWM04, WWM16, WWM50 and WWM66, which were originally sourced from different villages and districts in the Limpopo Province of South Africa (Mashilo et al., 2017). The parents were selected based on contrasting traits including better fruit and seed yield (Ngwepe et al., 2021a, b). Fruit and seeds attributes of the selected parental accessions are presented in Table 5.1.

Table 5.1 Names of citron watermelon parental accessions used in the study with their attributes.

Parental accessions	Fruit shape	Main rind colour	Secondary rind colour	Stripe colour	Flesh colour	Seed colour
WWM4	R	LG	G	G	O	Green
WWM3	E	DG	N	N	O	Red & white
WWM16	R	LG	N	N	Y	Red
WWM50	E	DG	N	N	Y	Red
WWM66	E	LG	DG	N	O	Brown

Fruit shape: R = round; E = elliptic; Main fruit colour: G = green; LG = light green; DG = dark green. Stripe colour: G= green; N: None. Flesh colour: O = orange; Y = yellow.

5.2.2. Crossing block and development of experimental hybrids

A crossing block was established under field conditions at Towoomba Agricultural Development Centre (TADC), Bela-Bela (28º19’28’’E, 24º53’57’’S; 1 184 m above sea level), South Africa, during the 2020 growing season. Three seeds from each parental accession were planted per hole at an inter- and intra-row spacing of 8 × 8 m. Citron watermelon is profusely branching crop with primary and secondary branches reaching lengths of up to 10m. The higher spacing within and between plants allow for ease of data collection (Ngwepe et al. 2021a, b). Two rows of each parental accession were planted. Three weeks after emergence, two plants were thinned out to retain a single plant per hole. Each accession had five plants per row (i.e., 10 plants in total). Plants were grown under rain-fed until male and female flowers were developed, approximately 60 days after planting. The five parents were crossed in a 5 × 5 half diallel mating design to generate 10 F_1 hybrids. Briefly, near-open male and female flowers were closed a day before with small brown or white envelopes and secured with staples at the bottom for making crosses. The following day, the male and female flowers were uncovered and hand pollinated. The male flowers were gently dusted onto the female flower to induce cross-pollination. The pollinated female flowers were then re-covered with envelopes to prevent cross-contamination from any cross-pollinating agent. The cross-pollinated flowers were tagged and labelled accordingly. At least 10 to 25 crosses were made per combinations. When the fruit of the pollinated female flower started developing and the petals had fallen off, the envelopes were removed, and the fruit was allowed to grow until maturity. At maturity (i.e., when the blossom-end vine attached to the fruit had dried out), the fruit from successful crosses were harvested, seeds were removed and sun-dried, labelled and stored for later use. The crossing scheme employed to develop the F_1 hybrids is shown in Figure 5.1.

5.2.3. Study site

Parents and F_1 hybrids were evaluated in 2021/22 and 2022/23 growing seasons under dry-land conditions at TADC, Bela-Bela, South Africa. The soil types are of the Hutton soil form (Botha et al., 2014). During the two growing seasons soil samples were collect before planting to determine the fertility status. The soil samples were analysed at Research and Technology Development, Analytical Services, KwaZulu-Natal Department of Agriculture and Rural Development. Soil physical and chemical properties during the 2021/22 and 2022/23 season are presented in Table 5.2.

During the experimental period, a total rainfall of 735.8 and 561 mm was received during the 2021/22 and 2022/23 seasons, respectively. Temperature averaged a minimum of 13.22^{o}C to a maximum of 28.81^{o}C during the 2021/22 season and from 13.96^{o}C to 29.28^{o}C during the 2022/2023 season. The weather data was collected from the weather station located at TADC and the data provided by South African Weather Services (SAWS) during the two growing seasons (www.weathersa.co.za).

Table 5.2 Soil physical and chemical properties at Towoomba Agricultural Development Centre during the 2021/2022 and 2022/2023 growing seasons.

Season	Organic C (%)	N (%)	P (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	Total cations (cmol/L)	Acid saturation (%)	pH (KCl)	Clay (%)	Zn (mg/L)	Mn (mg/L)	Cu (mg/L)
2021/22	< 0.5	< 0.5	5	217	687	262	6.19	6	4.58	< 30	3.2	21	6.8
2022/23	< 0.5	< 0.5	3	221	447	173	4.48	6	4.03	< 29	1.2	25	6.0

C = carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Ca = Calcium; Mg = Magnesium; KCl = Potassium chloride, Zn = Zinc; Mn = Manganese, Cu = copper.

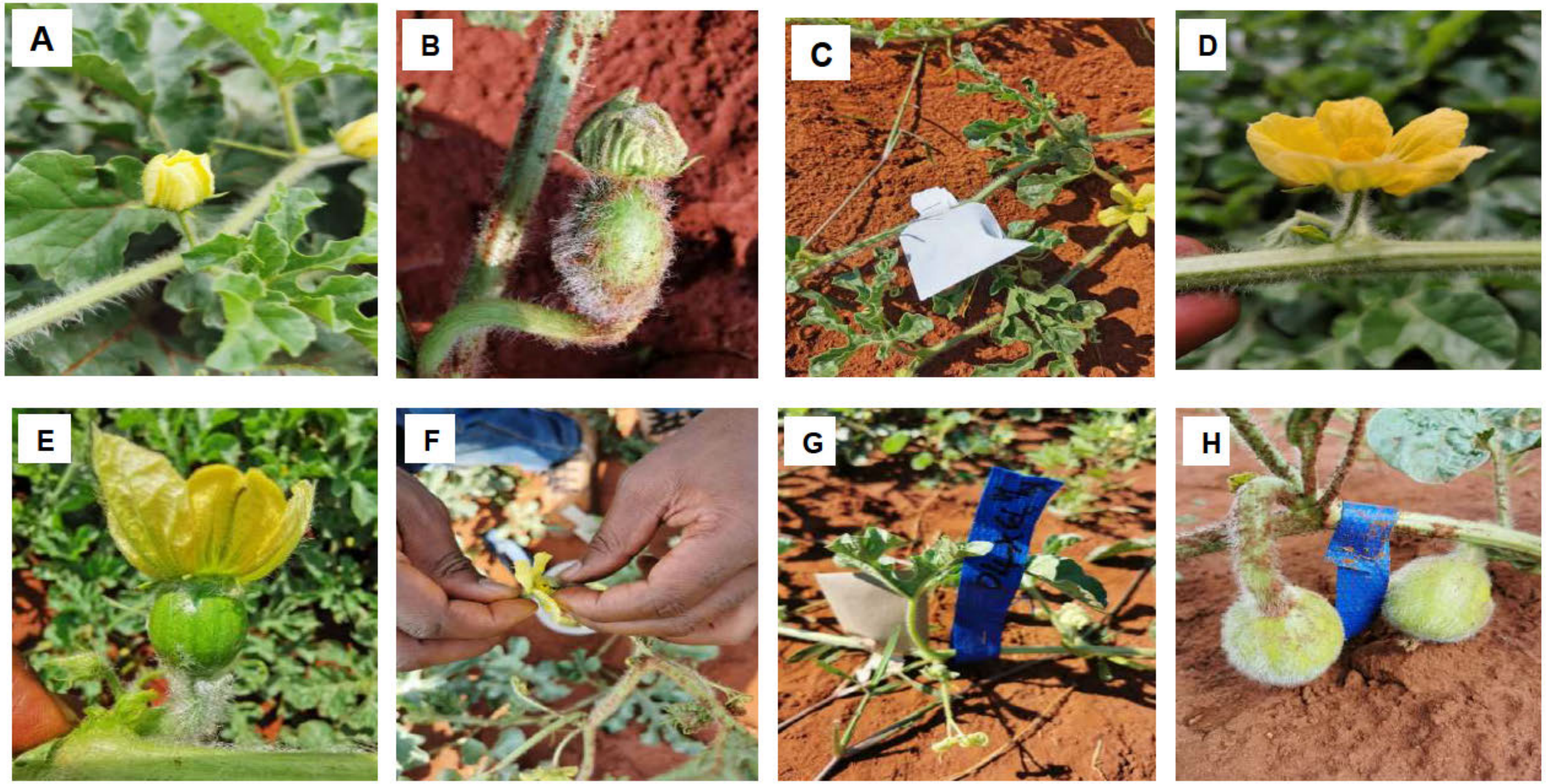

Figure 5.1: Crossing procedure to develop F_1 citron watermelon hybrids. Note: A = Near-open male flower, B = Near-open female flower, C = Tagged and covered near-opened male/female flower with an envelope, D = Fully opened male flower ready for pollen collection , E = Fully opened female flower ready for pollination, F = Cross-pollination of female flower with male flower by gently rubbing the pollen on the receptive stigma, G = re-bagged female flower with a tag designated with the pollen donor, and H = An expanding fruit of successfully pollinated female flower.

5.2.4. Evaluation of the parents and crosses

The five parents and the 10 F_1 hybrids were evaluated across two growing seasons 2021/22 and 2022/23 at TADC. The two seasons provided two contrasting testing environments. The experiment was conducted under field conditions using a randomized complete block design (RCBD) with three replicates. Three seeds were directly sown per planting hole in the field at inter-row and intra-row spacing of 8 × 8 m. The seedlings were thinned three weeks after emergence (at 3–4 true leaf stages) to retain one plant per hill. Weeding was done manually, and no fertilizer and pesticides were applied to mimic the citron watermelon production predominantly practiced by small holder farmers in the study areas.

5.2.5. Data collection

Data was collected on the following agronomic traits, total number of male flowers per plant (NMFL) were regularly counted and added as flower peduncles attached to the plant without the ovary, total number of female flowers per plant (TNFfl) were regularly counted and added based on flower peduncles showing well-developed ovary, and productive and fully developed fruits, plant height (PH) measured from the base to the tip of the main vine/stem at harvest and expressed in meters (m), number of primary branches per plant (NPBr) counted as branches emerged from the main stem, number of secondary branches per plant (NSBr) counted as branches developed from the primary branches, total number of fruits per plant (TNFr) counted at harvest, number of marketable fruits per plant (NMFr) counted as total fully grown fruits without defects, marketable fruit yield per plant (MyId) weight expressed in kilograms (kg) of total fully grown fruits per plant, total fruit yield (TFYId, kg) as the weight of total number of fruits per plant, fruit weight (FrW, kg) of a single fully grown fruit per plant, fruit length (FrL, cm) and fruit diameter (FrD, cm) were measured with ruler, rind diameter (RD, mm) measured with a Vernier calliper (Metr-ISO), and total soluble solids (TSS) measured using a portable refractor-meter ATAGO Brix (0~53), number of seed per fruit (NSPFr) were counted, seed yield per fruit (SYPFr, g) as the total weight of total number of seeds per fruit, seed diameter (SD, mm) and seed length (SL, mm) were measured with a digital Vernier calliper (Metr-ISO), and weight of hundred seed (HSW, g) expressed as the weight of 100 seeds without defects.

5.2.6. Data analysis

A combined analysis of variance (ANOVA) was performed using the metan package (Olivoto et al., 2020) in R 4.2.0 (R Core Team, 2020). Entries (i.e., parents and F_1 hybrids) were considered fixed effects, whereas environment and replications were considered as random effects. The following linear model was used for combined analysis across environments as proposed by Barreto and Barnett (1999):

$$Y_{ijkl} = \mu + G_i + E_j + GE_{ij} + E(R)_k + \varepsilon_{ijkl}$$

where $\boldsymbol{Y_{ijkl}}$ is the observed performance of the *i*th genotype in the *l*th replication of the *j*th environment, $\boldsymbol{\mu}$ is the grand mean, G_i is the effect of the *i*th genotype, E_j the effect of the *j*th environment, $\boldsymbol{GE_{ij}}$ is the effect of the interactions of the *i*th genotype with the *j*th environment; $\boldsymbol{E(R)_j}$ is the effect of the *k*th replication in the *j*th environment and $\boldsymbol{\varepsilon_{ijkl}}$ is the residual effect associated with each $\boldsymbol{Y_{ijkl}}$.

5.2.6.1 Diallel analysis

Data on agronomic traits was subjected to a half-diallel mating design using Griffing's (Griffing, 1956) Method IV (i.e., F_1s only) and Model I (i.e., fixed effect) using AGD-R (Rodríguez et al., 2020) using the following linear model:

$$X_{ij} = \mu + g_i + g_j + s_{ij} + E_k + E_k g_i + E_k g_j + ES_{ij} + e_{ijk}$$

where, X_{ij} is the mean of i × jth genotype (g) over kth Environments (Env), μ is the population mean, g_i and g_j are the GCA effects, s_{ij} are the SCA effects such that $s_{ij} = s_{ji}$ (thus, assuming the absence of reciprocal effects) and e_{ijk} is the random error term. $E_k g_i$, $E_k g_j$, and ES_{ij} are Environment × GCA and Environment × SCA interaction effects, respectively. Test of significance for the effects were determined using a t-test at 5% level of significance.

5.2.6.2 Estimates of genetic components and gene action

Variance components such as GCA and SCA, and GCA/SCA ratio were computed in R package using the Diallel analysis (Muhammad and Kent, 2023).

The predominance of additive versus non-additive gene action was compared from the ratio of components of GCA variance to SCA variance as follows (Baker, 1978):

GCA/SCA ratio that is greater than unity indicates the predominance of additive gene action, whereas ratios of less than unity indicate the predominance of non-additive gene action for the trait (Baker, 1978).

5.2.6.3 Heritability estimates for agronomic traits

Broad-sense heritability (H^2b) was was calculated using META-R version 6.0 (Alvarado et al., 2020) using the following formula:

$$H^2b = \frac{\sigma^2 g}{\sigma^2 g + \sigma^2 ge/nLoc + \sigma\varepsilon^2/(nLoc\ x\ nRep)}$$

Where, σ^2g = genotypic variance, $\sigma^2\varepsilon$ = error variance, *n*Rep = number of replicates, σ^2ge = G × E interaction variance, *n*Loc = number of test environments.

5.3. Results

5.3.1. Genotype, environment and their interaction effects on agronomic traits

Combined analysis of variance showing mean square values and significant tests are presented in Table 5.3. The effects of environment, genotypes and genotype × environment are summarised on agronomic traits of citron watermelon parental accessions and their derived F_1 hybrids across two testing environments. The environment was significant *($p < 0.05$ and 0.001)* for most traits except TNFfl, RD, and TSS. The genotypic effect was significant *($p < 0.05$ and 0.001)* for NMF, PH, NPBr, TNFr, NMFr, Myld, FRL and FD, RD, NSPFr, SYPFr, SD and HSW. A significant *($p < 0.05$ and 0.001)* genotype × environment effect was recorded for most traits except for FW, TNFfl, NPBr, TFYld, SL and TSS.

Table 5.3 Analysis of variance showing mean squares for agronomic traits assessed among five citron watermelon parental genotypes and 10 F$_1$ hybrids evaluated under field conditions across two environments.

Traits	Sources of variation					
	Env	Rep (Env)	Gen	Gen × Env	Residuals	
df	1	4	14	14	56	CV (%)
NMFL	32308864.18**	32947.02	292000.33*	317959.11**	125249.09	2.81
TNFfl	179.78^{ns}	58.18	675.94^{ns}	404.94**	138.04	3.24
PH	120.94**	0.17	2.01**	0.88*	0.46	11.20
NPBr	10.68*	1.24	6.61**	3.20^{ns}	2.02	21.13
NSBr	23008.01**	220.31	442.38^{ns}	346.65^{ns}	349.41	4.50
TNFr	1672.71**	2.28	127.23**	59.76**	9.88	2.78
NMFr	113.34**	3.61	85.03**	37.27**	8.81	3.66
MyId	72618.80**	530.03	1794.70**	1471.26**	421.35	4.98
TFYld	61538.80**	1565.55	1559.15^{ns}	968.05^{ns}	1048.83	6.82
FrW	373.89*	66.29	97.88^{ns}	91.58^{ns}	66.68	9.51
FrL	713.74**	5.98	74.02**	82.47**	6.80	10.45
FrD	342.26**	3.92	67.16**	36.85**	5.61	9.89
RD	27.64^{ns}	20.81	79.67**	50.25*	23.60	19.82
TSS	1.32^{ns}	5.53	6.32^{ns}	6.59^{ns}	5.91	7.64
NSPF	2720488.26**	20960.66	99838.91**	56404.95*	29470.98	2.94
SYPFr	74189.51**	333.42	2398.62**	1252.99*	563.33	2.97
SD	8.53**	0.07	0.99**	0.52**	0.11	4.66
SL	1.45*	0.55	1.20^{ns}	0.52*	0.23	3.86
HSW	217.78**	0.38	16.26**	3.28**	0.72	6.39

Env = Environment, REP = Replications; GEN = Genotype; GEN × Env = Genotype by Environment; NMFl = Total number of male flowers ; TNFfl = Total female flowers; PH = Plant height; NPBr = Number of primary branches; NSBr = Number of secondary branches; TNFr = Total number of fruits; NMFr = Number of marketable fruits; Myld = Marketable fruit yield; TFYld = Total fruit yield; FrW = Fruit weight; FrL = Fruit length; FrD = Fruit diameter; RD = Rind diameter; TSS = Total soluble solids; NSPFr = Number of seed per fruit; SYPFr = Seed yield per fruit; SD = Seed diameter; SL = Seed length; and HSW = Hundred seed weight. * and ** = Significant at $p < 0.05$ *and* $p < 0.001$, respectively, ns = non-significant, and CV = coefficient of variance.

5.3.2. Performance of citron watermelon parents and F_1 hybrids for agronomic traits

Best linear unbiased predictors (BLUPs) for agronomic traits of the citron watermelon parents and F_1 hybrids evaluated across two test environments in South Africa are presented in Table 5.4. Total number of female flowers (TNFfl) for the parents ranged from 31 to 45, with WWM16 exhibiting the highest TNFfl. For the hybrids, TNFfl varied from 32 for WWM66 × WWM50 to 45 for WWM04 × WWM03, with a mean of 36. Among the parents, the PH varied from 5.79 m for WWM66 to 6.19 m for WWM16. The hybrids exhibited PH ranging from 5.63 to 6.72 m (mean = 6.09 m) with WWM04 × WWM16 and WWM16 × WWM50 possessing longer plants. The NPBr and NSBr for parents ranged from 6.35 to 7.3 (mean = 6.68), and 43.97 to 39.64 (mean = 41.42), respectively. The NPBr and NSBr for the hybrids varied from 5.75 to 7.4 (mean = 6.75), and 39.07 to 44.73 (mean = 41.57), respectively. Parent WWM03 and hybrid WWM04 × WWM03 recorded the highest NPBr, whereas parent WWM66 and hybrid WWM16 × WWM66 recorded the lowest NSBr.

Three parents, WWM16, WWM50 and WWM04, recorded the highest TNFr (> 10). Hybrids WWM66 × WWM50 and WWM03 × WWM66 recorded the lowest TNFr (< 10), whilst the remainder of the crosses recorded TNFr (> 10). WWM16 × WWM66 recorded the highest TNFr (> 15) with a mean of 11.41 across hybrids. The highest NMFr was recorded for parent WWM16 (13.33) compared to the total parental mean of 7.49, whereas parent WWM03 recorded the lowest NMFr of 5.3. Hybrids WWM04 × WWM50 recorded the highest NMFr of 13.58, whereas WWM66 × WWM50 recorded the lowest NMFr of 6.37 (mean = 8.44). Marketable yield (Myld) varied from 43.64 to 50.03 kg for parents, with a mean of 46.02 kg. The mean Myld was 48.62 kg for hybrids, with WWM16 × WWM66 recording the highest Myld of 54.17 kg, followed by WWM03 × WWM16 (50.49 kg) and WWM03 × WWM50 (52.08 kg) (Table 5.4).

Parents WWM16 and WWM50 recorded the highest TFYld of 56.24 and 50.17 kg respectively (mean = 48.09 kg). Three hybrids: WWM04 × WWM16, WWM03 × WWM50 and WWM04 × WWM03 recorded the highest TFYld (> 50 kg) with a total mean of 47.19. FRW of 8.71 and 8.52 were recorded for parents and hybrids, respectively. The highest

Fruit weight (FrW) was recorded for parent WWM16 at 9.29 kg and hybrid WWM04 × WWM16 at 8.98 kg. Fruit Diameter (FrD) varied from 22.57 to 24.96 cm for parents and 21.36 to 27.40 cm for hybrids. Fruit Diameter (FrD) was the highest for parent WWM50 (24.96 cm) and hybrid WWM04 × WWM16 (27.40 cm). Rind diameter (RD) varied from 22.70 to 26.97 mm (mean = 24.55) for the parents and 23.05 and 26.39 mm (mean = 24.49) for hybrids. Parents WWM16 and WWM03 had thicker RD (> 25 mm), whereas hybrids WWM03 × WWM16, WWM16 × WWM50, WWM03 × WWM66 and WWM04 × WWM66 had the thickest RD (> 25 mm) (Table 5.4).

The parents' NSPFr varied from 548 to 655 (mean = 582). Parent WWM50 recorded the highest NSPFr (> 600). Hybrids WWM04 × WWM66, WWM16 × WWM50, WWM03 × WWM16, WWM03 × WWM66 and WWM04 × WWM16 recorded the highest NSPFr (> 600) with a mean of 585.88. Parent WWM03 and hybrid WWM66 × WWM50 recorded the lowest NSPFr of 584 and 467.54, respectively. Seed yield per fruit (SYPFr) ranged from 67.42 to 90.59 g for the parents, with a mean of 79.87 g. For the hybrids, SYPFr varied from 62.49 g for WWM66 × WWM50 to 98.23 g for WWM03 × WWM16, with a mean of 80.06 g.

Seed length (SL) varied from 12.04 to 12.79 mm for the parents and 12.10 to 12.89 mm (mean = 12.46 mm) for hybrids. The highest SL 12.79 and 12.89 mm was recorded for parent WWM16 and hybrid WWM16 × WWM50, respectively. Seed diameter (SD) varied from 6.59 to 7.36 mm with a mean of 7.04 for the parents. Hybrid WWM04 × WWM03 recorded the highest SD of 7.31 mm, whilst cross WWM04 × WWM50 recorded the lowest SD of 6.87 mm, with a mean of 7.07 mm recorded across hybrids. For HSW, parents recorded values ranging from 10.14 to 14.66 g (mean = 13.06), whereas hybrids recorded values ranging from 12.26 and 15.19 g (mean = 13.44). Overall, the performance of the hybrids was better for most agronomic traits compared to that of parents. Also, the newly developed hybrids displayed variations in qualitative traits such as fruit and seed parameters (Figure 5.2).

Table 5.4 Mean values of agronomic traits for five citron watermelon parents and 10 F_1 crosses evaluated under field conditions across two environments.

		Traits								
		NMFL	TNFfl	PH	NPBr	NSBr	TNFr	NMFr	Myld	TFYld
Parents	WWM03	1137	30.89	5.80	7.38	39.64	8.58	5.53	43.64	40.85
	WWM04	1196	35.11	5.88	6.61	39.97	10.88	7.49	44.83	46.42
	WWM16	1370	44.93	6.19	6.52	42.49	14.50	11.33	50.03	56.27
	WWM50	1210	39.59	6.10	6.52	43.97	12.56	6.56	45.22	50.17
	WWM66	1497	34.51	5.79	6.35	41.05	9.02	6.56	46.40	46.73
	Parent mean	1282	37.00	5.95	6.68	41.42	11.11	7.49	46.02	48.09
Hybrids	WWM04 × WWM03	1619	34.11	5.63	7.04	41.66	10.62	7.49	50.49	55.55
	WWM04 × WWM16	962	33.57	5.94	7.64	39.28	9.20	6.65	45.94	45.14
	WWM04 × WWM66	1271	38.92	6.60	6.52	40.69	11.23	7.68	46.20	47.94
	WWM04 × WWM50	1041	37.11	6.38	5.75	42.92	16.89	13.58	54.17	44.82
	WWM03 × WWM16	1748	34.78	5.92	6.95	42.42	11.06	8.52	49.38	46.30
	WWM03 × WWM66	1070	37.98	6.00	6.95	44.19	13.36	9.55	52.08	45.66
	WWM03 × WWM50	1312	33.1	5.68	6.35	40.98	10.44	7.59	43.43	41.75
	WWM16 × WWM66	1101	32.9	6.72	7.21	39.79	10.35	7.49	48.09	50.21
	WWM16 × WWM50	1117	44.73	6.24	7.21	44.73	13.27	9.46	49.24	56.24
	WWM66 × WWM50	1207	32.24	5.82	5.83	39.07	7.70	6.37	47.21	38.26
	Crosses mean	1244	35.94	6.09	6.75	41.57	11.41	8.44	48.62	47.19
	Grand mean	1257.27	36.30	6.04	6.72	41.52	11.31	8.12	47.76	47.49
	p-value	*	**	*ns*	*ns*	*ns*	**	**	**	*ns*
	LSD	0.00	15.78	0.87	1.59	10.72	6.97	5.67	20.17	23.22
	CV (%)	2.75	3.17	10.97	20.86	4.45	2.70	3.58	4.98	6.77

Table 5.4 (Continued).

		Traits									
		FrW	FrL	FrD	RD	TSS	NSPFr	SYPFr	SD	SL	HSW
Parents	WWM03	8.47	22.37	22.57	25.89	2.92	548.24	67.42	6.59	12.04	10.14
	WWM04	8.40	28.19	23.43	23.91	2.97	569.77	79.60	7.36	12.58	13.33
	WWM16	9.29	19.43	24.55	26.97	2.77	555.85	80.96	7.15	12.79	14.66
	WWM50	8.69	27.82	24.96	23.27	2.85	655.33	90.59	7.13	12.24	13.59
	WWM66	8.70	30.08	24.64	22.70	2.87	579.27	80.80	6.97	12.56	13.59
	Parent mean	8.71	25.58	24.03	24.55	2.88	581.69	79.87	7.04	12.45	13.06
Hybrids	WWM04 × WWM03	8.48	21.28	22.48	23.70	2.83	631.33	80.72	6.96	12.24	12.53
	WWM04 × WWM16	8.61	26.62	23.61	25.74	3.25	653.88	84.46	6.92	12.70	12.26
	WWM04 × WWM66	8.64	22.03	25.44	25.44	3.07	606.32	83.66	7.14	12.89	14.93
	WWM04 × WWM50	8.39	26.68	23.62	24.36	3.03	535.98	74.19	7.01	12.39	13.86
	WWM03 × WWM16	8.51	22.45	24.60	23.69	2.87	605.95	84.46	7.05	12.11	13.46
	WWM03 × WWM66	8.57	30.35	25.02	25.25	2.82	622.01	98.23	7.17	12.55	14.66
	WWM03 × WWM50	8.36	28.03	22.73	23.05	2.90	544.68	71.80	6.87	12.44	12.53
	WWM16 × WWM66	8.98	23.28	27.40	26.39	3.12	664.39	90.59	7.27	12.70	15.19
	WWM16 × WWM50	8.39	21.08	22.66	24.18	2.83	526.70	70.05	7.31	12.47	12.66
	WWM66 × WWM50	8.30	24.55	21.36	23.05	2.63	467.54	62.49	6.98	12.24	12.26
	Crosses mean	8.52	24.64	23.89	24.49	2.94	585.89	80.06	7.07	12.47	13.44
	Grand mean	8.59	24.95	23.94	24.51	3.18	584.48	80.00	7.06	12.46	13.31
	p-value	*ns*	**	**	*ns*	*ns*	*ns*	*	**	*	**
	LSD	3.01	5.05	0.00	5.33	0.00	193.98	30.29	0.68	0.62	2.00
	CV	9.51	9.79	10.41	19.74	7.63	2.91	2.93	3.86	4.61	6.29

NMFl = Total number of male flowers ; TNFfl = Total female flowers; PH = Plant height; NPBr = Number of primary branches; NSBr = Number of secondary branches; TNFr = Total number of fruits; NMFr = Number of marketable fruits; MyId = Marketable fruit yield; TFYld = Total fruit yield; FrW = Fruit weight; FrL = Fruit length; FrD = Fruit diameter; RD = Rind diameter; TSS = Total soluble solids; NSPFr = Number of seed per fruit; SYPFr = Seed yield per fruit; SD = Seed diameter; SL = Seed length; and HSW = Hundred seed weight. * and ** = Significant at $p < 0.05$ and $p < 0.001$, respectively, ns = non-significant, LSD = least square difference; and CV = coefficient of variance.

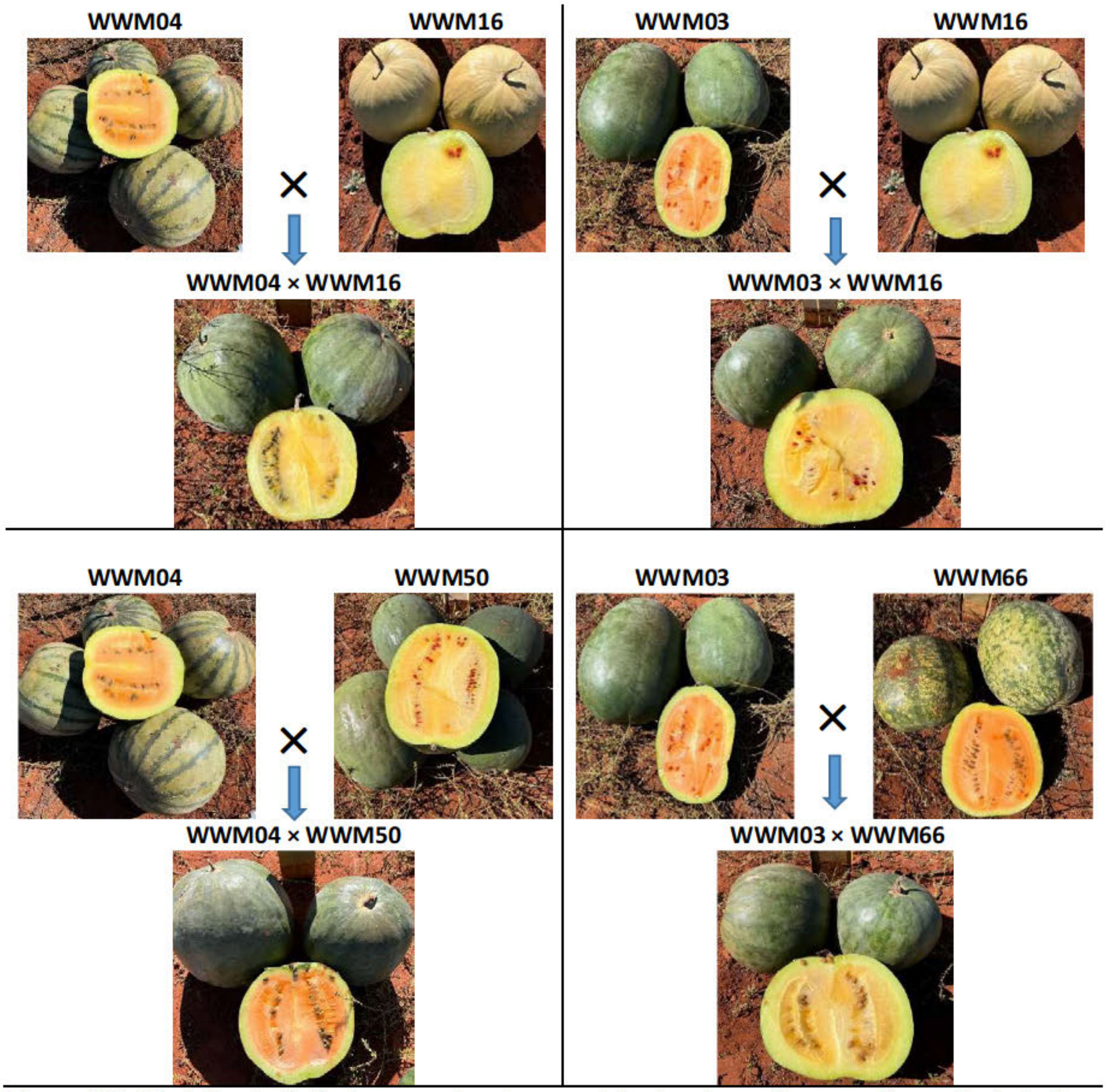

Figure 5.2: Variation in fruit shape and colour, and flesh colour of among five selected citron watermelon parents and derived F_1 hybrids.

5.3.3. Combining ability effects

Combining ability analysis of variance showing mean squares and test of significance for assessed agronomic traits among citron watermelon parents and F_1 hybrids evaluated across two environments is presented in Table 5.5. Significant *($p < 0.001$)* GCA effects were observed for TNFfl, TNFr, FL, RD, SYPFr, SD and HSW, whereas significant ($p <$ 0.05*; $p < 0.001$)* SCA effects were observed for TNFfl, TNFr, NMFr, FD, FL, RD, NSPFr, SD, SYPFr. Significant *($p < 0.05$ and $p < 0.001$)* effects were observed for environment × GCA (Env × GCA) effects on TNFr, FrD and FrL, whereas environment × SCA (Env × SCA) was significant for NMFL, PH, NSBr, TNFr, Myld, FrD, FrL, NSPFr, SYPFr, SL and HSW.

5.3.4 Estimates of GCA effects among citron watermelon parental accessions for agronomic traits

Estimates of GCA effects among citron watermelon parental accessions for the assessed agronomic traits are presented in Table 5.6. For NMFL, parent WWM04 recorded positive and significant *($p < 0.05$)* GCA effects of 153.83, whereas WWM03 recorded negative GCA effects of -114.96. The parent WWM04 exhibited a significant *($p < 0.001$)* and positive GCA effect of 5.82 for TNFfl. For PH, GCA effects of 0.20 and -0.16 were recorded for parents WWM16 and WWM04, respectively. WWM16 recorded significant and positive GCA effects of 0.66 for NPBr.

Positive GCA effects of 3.64 and 3.87 were recorded for NSBr for WWM04 and WWM50. Parent WWM16 recorded a significant and positive GCA effect of 1.67 for NMFr. For Myld, WWM16 recorded the highest and positive GCA value of 12.98. Also, WWM16 recorded a significant *($p < 0.001$)* positive GCA effect of 14.61 for TFYld. Parent WWM16 recorded a positive and significant GCA value of 3.48 for FW. Positive GCA effects for FRD were 0.81 and 0.63 for parents WWM66 and WWM16. Negative GCA effect was recorded for WWM03 for FrL, whereas GCA effects were positive for WWM66 and WWM50. Parent WWM16 exhibited high significant *($p < 0.001$)* and positive GCA effects of 3.67 for RD, whereas WWM50 recorded an undesirable GCA estimate (Table 5.6).

Table 5.5 Combined analysis of variance showing mean square values due to Environment (Env), replications (Rep), Genotypes (Gen), GCA and SCA effects, General combining ability (GCA) by Environment (Env × GCA), specific combining ability (SCA) by Environment (Env × SCA) for yield and yield-related traits citron watermelon.

	Sources of variation								
Traits	Env	Rep (Env)	Gen	Gen × Env	GCA	SCA	Env × GCA	Env × SCA	Residual
NMFL	32308864.18**	32947.02	292000.33^{ns}	317959.11**	178472.22^{ns}	149630.17^{ns}	106296.18^{ns}	726456.53**	125249.09
TNFfl	179.78^{ns}	58.18	675.94^{ns}	404.94**	595.11**	701.54**	302.55^{ns}	321.82^{ns}	138.04
PH	120.94**	0.17	2.01^{ns}	0.88**	0.34^{ns}	4.48^{ns}	0.83^{ns}	1.48**	0.46
NPBr	10.68*	1.24	6.61^{ns}	3.20^{ns}	4.87^{ns}	4.69^{ns}	2.74^{ns}	1.29^{ns}	2.02
NSBr	23008.01**	220.31	442.38^{ns}	346.65^{ns}	213.74^{ns}	328.24^{ns}	26.25^{ns}	847.75*	349.41
TNFr	1672.71**	2.28	127.23^{ns}	59.76**	38.71**	88.71**	34.11**	28.84**	9.88
NMFr	113.34**	3.61	85.03^{ns}	37.27**	16.25^{ns}	71.22**	15.46^{ns}	7.53^{ns}	8.81
Myld	72618.80**	530.03	1794.70^{ns}	1471.26**	1048.34^{ns}	1282.68*	327.53^{ns}	1497.35**	421.35
TFYld	61538.80**	1565.55	1559.15^{ns}	968.05^{ns}	1954.73^{ns}	938.30^{ns}	855.63^{ns}	694.87^{ns}	1048.83
FrW	373.89*	66.29	97.88^{ns}	91.58^{ns}	134.66^{ns}	87.38^{ns}	145.47^{ns}	123.67^{ns}	66.68
FrD	342.26**	3.92	67.16^{ns}	36.85**	11.44^{ns}	113.54**	16.02*	69.60**	5.61
FrL	713.74**	5.98	74.02**	82.47**	2.00**	135.54**	28.29**	174.85**	6.80
RD	27.64^{ns}	20.81	79.67^{ns}	50.25*	105.60**	107.97**	32.19^{ns}	23.25^{ns}	23.60
TSS	1.32^{ns}	5.53	6.32^{ns}	6.59^{ns}	8.37^{ns}	9.74^{ns}	11.57^{ns}	8.70^{ns}	5.91
NSPFr	2720488.26**	20960.66	99838.91^{ns}	56404.95**	47744.65^{ns}	82787.88*	29801.71^{ns}	71922.51*	29470.98
SYPFr	74189.51**	333.42	2398.62^{ns}	1252.99**	758.79^{ns}	1922.98**	309.76^{ns}	2907.51**	563.33
SL	1.45*	0.55	1.20^{ns}	0.52**	1.35**	1.99^{ns}	0.58^{ns}	0.75**	0.23
SD	8.53**	0.07	0.99^{ns}	0.52**	0.39**	1.40**	0.00^{ns}	0.17^{ns}	0.11
HSW	217.78**	0.38	16.26**	3.28**	6.54**	33.44**	1.29^{ns}	6.06**	0.72

NMFl = Total number of male flowers ; TNFfl = Total female flowers; PH = Plant height; NPBr = Number of primary branches; NSBr = Number of secondary branches; TNFr = Total number of fruits; NMFr = Number of marketable fruits; Myld = Marketable fruit yield; TFYld = Total fruit yield; FrW = Fruit weight; FrL = Fruit length; FrD = Fruit diameter; RD = Rind diameter; TSS = Total soluble solids; NSPFr = Number of seed per fruit; SYPFr = Seed yield per fruit; SD = Seed diameter; SL = Seed length; and HSW = Hundred seed weight. * and ** = Significant at $p < 0.05$ *and* $p < 0.00,1$ respectively and ns = non-significant.

Table 5.6 Estimate of the general combining ability (GCA) effects for agronomic traits of citron watermelon parental accessions evaluated across two environments.

Traits	Parents and GCA				
	WWM04	WWM03	WWM16	WWM66	WWM50
NMFL	153.83*	-114.06^{ns}	-32.61^{ns}	27.22^{ns}	-34.39^{ns}
TNFfl	5.82*	-9.35**	2.16^{ns}	-0.95^{ns}	2.33^{ns}
PH	-0.16^{ns}	-0.02^{ns}	0.20^{ns}	0.06^{ns}	-0.08^{ns}
NPBr	-0.40^{ns}	0.32^{ns}	0.66	0.04^{ns}	-0.62*
NSBr	3.64^{ns}	-3.02^{ns}	-2.41^{ns}	-2.08^{ns}	3.87^{ns}
TNFr	1.03^{ns}	-1.63*	1.03^{ns}	-1.58**	1.14^{ns}
NMFr	-0.11^{ns}	-0.61^{ns}	1.67*	-0.44^{ns}	-0.50^{ns}
Myld	-4.15^{ns}	-5.52^{ns}	12.98^{ns}	0.73^{ns}	-4.03^{ns}
TFYld	1.63^{ns}	-13.08^{ns}	14.61**	-6.17^{ns}	3.01^{ns}
FrW	2.32^{ns}	-1.87^{ns}	3.48*	-1.20^{ns}	-2.73*
FrL	0.17^{ns}	-0.49^{ns}	-0.19^{ns}	0.27^{ns}	0.25^{ns}
FrD	-0.35^{ns}	0.07^{ns}	0.63^{ns}	0.81^{ns}	-1.17*
RD	0.09^{ns}	0.26^{ns}	3.67**	-1.06^{ns}	-2.96*
TSS	0.67^{ns}	-0.38^{ns}	-0.57^{ns}	0.82^{ns}	-0.53^{ns}
NSPFr	-60.84^{ns}	-42.01^{ns}	66.93^{ns}	20.93^{ns}	14.99^{ns}
SYPFr	-4.48^{ns}	-8.31^{ns}	7.36^{ns}	5.02^{ns}	0.41^{ns}
SD	-0.12*	-0.17**	0.19^{ns}	0.04^{ns}	0.06^{ns}
SL	-0.14^{ns}	-0.35**	0.32*	0.22*	-0.05^{ns}
HSW	-0.49*	-0.77**	0.62**	0.46*	0.18^{ns}

NMFl = Total number of male flowers ; TNFfl = Total female flowers; PH = Plant height; NPBr = Number of primary branches; NSBr = Number of secondary branches; TNFr = Total number of fruits; NMFr = Number of marketable fruits; Myld = Marketable fruit yield; TFYld = Total fruit yield; FrW = Fruit weight; FrL = Fruit length; FrD = Fruit diameter; RD = Rind diameter; TSS = Total soluble solids; NSPFr = Number of seed per fruit; SYPFr = Seed yield per fruitz SD = Seed diameter; SL = Seed length; and HSW = Hundred seed weight. * and ** = Significant at $p < 0.05$ *and* $p < 0.001$, respectively and ns = non-significant.

For TSS, GCA effects were positive for WWM04 and WWM66, and negative for WWM03, WWM16 and WWM50. General combining ability (GCA) effect was high and positive for WWM16 and negative for WWM04 and WWM03 for NSPFr. For, SYPFr, the parents WWM04 and WWM03 recorded negative GCA effects contrary to positive GCA effects recorded for WWM16. For SD and SL, parent WWM03 recorded highly significant *($p < 0.05$)* and negative GCA effects of -0.17 and -0.35. WWM16 and WWM66 recorded positive and significant GCA effects for SL. Parent WWM16 exhibited significant *($p < 0.001$)* and positive GCA effects of 0.62 for HSW followed by WWM66, whereas WWM03 recorded significant *($p < 0.001$)* and negative GCA effect of -0.77 for the same trait (Table 5.6).

5.3.5. Estimates of SCA effects among citron watermelon crosses

Estimates of SCA effects among citron watermelon F_1 hybrids for the studied agronomic traits are presented in Table 5.7. Specific combining ability (SCA) effects for NMFRL were negative for six hybrids and positive for four hybrids. For TNFfl, only cross WWM04 × WWM16 revealed significant *($p < 0.001$)* and positive SCA effects of 14.98. Specific combining ability (SCA) effects for PH were positive and significant *($p < 0.05$)* for WWM03 × WWM16 and WWM66 × WWM50, whereas negative and significant SCA effects were recorded for WWM03 × WWM50 and WWM16 × WWM50. For NPBr, WWM04 × WWM03 and WWM16 × WWM66 had positive SCA effects, whereas WWM04 × WWM16, WWM04 × WWM66 and WWM03 × WWM50 recorded negative effects. The hybrids WWM04 × WWM16 and WWM03 × WWM66 recorded positive SCA effects for TNFr. For NMFr, a positive and significant *($p < 0.001$)* SCA effect of 5.19 was recorded for WWM04 × WWM16. Contrastingly, WWM04 × WWM03 and WWM03 × WWM66 recorded undesirable SCA effects for NMFr. WWM04 × WWM16, and WWM03 × WWM66 recorded high positive SCA effects for TFYld (Table 5.7).

For FrW, WWM03 × WWM16 and WWM66 × WWM50 recorded positive SCA effects. Hybrids WWM04 × WWM03, WWM04 × WWM50 and WWM16 × WWM50 had significant *($p < 0.001$)* and positive SCA effects of 2.26, 3.41 and 4.04 for FrL, respectively. A significant *($p < 0.001$)* and positive SCA effects were recorded for crosses WWM03 × WWM16, WWM04 × WWM50 and WWM66 × WWM50 for FrD, in that order. WWM04 ×

WWM16 recorded SCA effect in a negative direction. WWM66 × WWM50 recorded a positive and significant *($p < 0.001$)* SCA effect for RD. Hybrids WWM04 × WWM66 exhibited positive and significant *($p < 0.05$)* SCA effects of 2.01 for TSS. The hybrid WWM04 × WWM50 exhibited positive and significant SCA effects of 162.64 for NSPFr. For SYPFr WWM04 × WWM50 and WWM03 × WWM16 recorded positive and significant *($p < 0.05$)* SCA effects of 23.00 and 19.89, respectively. For SD, WWM04 × WWM50 and WWM03 × WWM16 recorded significant *($p < 0.05$)* and positive SCA effects. Positive and significant *($p < 0.05$)* SCA effects of 0.57 and 0.44 were recorded for WWM66 × WWM50 and WWM03 × WWM16 for SL. The hybrids WWM03 × WWM16 and WWM66 × WWM50 recorded significant *($p < 0.001$)* and positive SCA effects of 2.53 and 1.42 for HSW (Table 5.7).

Table 5.7 Estimate of the specific combining ability (SCA) for agronomic traits among citron watermelon F$_1$ hybrids evaluated across two environments.

	Hybrids and SCA									
Traits	WWM04 × WWM03	WWM04 × WWM16	WWM04 × WWM66	WWM04 × WWM50	WWM03 × WWM16	WWM03 × WWM66	WWM03 × WWM50	WWM16 × WWM66	WWM16 × WWM50	WWM66 × WWM50
NMFL	-46.53^{ns}	45.86^{ns}	-174.14^{ns}	174.81^{ns}	-94.42^{ns}	154.75^{ns}	-13.81^{ns}	114.47^{ns}	-65.92^{ns}	-95.08^{ns}
TNFfl	-8.54^{ns}	14.98**	-7.91^{ns}	1.48^{ns}	0.14^{ns}	7.92^{ns}	0.48^{ns}	-6.59^{ns}	-8.53^{ns}	6.58^{ns}
PH	-0.24^{ns}	0.24^{ns}	-0.34^{ns}	0.35^{ns}	1.04**	-0.25^{ns}	-0.54*	-0.43^{ns}	-0.84**	1.03**
NPBr	1.11*	-0.89^{ns}	-0.61^{ns}	0.39^{ns}	-0.28^{ns}	-0.17^{ns}	-0.67^{ns}	0.83^{ns}	0.33^{ns}	-0.06^{ns}
NSBr	-7.81^{ns}	4.75^{ns}	-2.25^{ns}	5.31^{ns}	-1.08^{ns}	10.75^{ns}	-1.86^{ns}	-4.36^{ns}	0.69^{ns}	-4.14^{ns}
TNFr	-3.50*	5.00*	-2.72*	1.22^{ns}	-0.17^{ns}	3.78**	-0.11^{ns}	-2.39*	-2.44*	1.33^{ns}
NMFr	-2.86*	5.19**	-1.19^{ns}	-1.14^{ns}	-1.14^{ns}	2.81*	1.19^{ns}	-2.81*	-1.25^{ns}	1.19^{ns}
MYld	-8.33^{ns}	8.62^{ns}	0.73^{ns}	-1.02^{ns}	-0.75*	18.68^{ns}	-9.60^{ns}	-18.95*	11.08^{ns}	-0.46^{ns}
TFYld	-10.00^{ns}	7.97^{ns}	0.51^{ns}	1.52^{ns}	4.75^{ns}	13.96^{ns}	-8.71^{ns}	-17.19^{ns}	4.47^{ns}	2.71^{ns}
FrW	-3.55^{ns}	3.70^{ns}	-0.74^{ns}	0.59^{ns}	3.19^{ns}	0.59^{ns}	-0.24^{ns}	-3.19^{ns}	-3.70^{ns}	3.34^{ns}
FrL	2.26*	-6.80**	1.12^{ns}	3.41**	1.05^{ns}	0.66^{ns}	-3.97**	1.70^{ns}	4.04**	-3.48**
FrD	-3.56**	0.28^{ns}	0.30^{ns}	2.98**	6.16**	-0.22^{ns}	-2.38*	-2.96**	-3.48**	2.88**
RD	2.93^{ns}	2.44^{ns}	-4.40*	-0.96^{ns}	0.69^{ns}	-1.89^{ns}	-1.73^{ns}	0.24^{ns}	-3.37^{ns}	6.06**
TSS	-0.68^{ns}	-0.69^{ns}	2.01*	-0.63^{ns}	0.84^{ns}	-0.77^{ns}	0.61^{ns}	-0.71^{ns}	0.56^{ns}	-0.53^{ns}
NSPFr	-26.53^{ns}	-117.97^{ns}	-18.14^{ns}	162.64*	112.69^{ns}	24.36^{ns}	-110.53^{ns}	25.58^{ns}	-20.31^{ns}	-31.81^{ns}
SYPFr	-16.78^{ns}	-4.11^{ns}	-2.11^{ns}	23.00*	19.89*	9.39^{ns}	-12.50^{ns}	-6.28^{ns}	-9.50^{ns}	-1.00^{ns}
SD	-0.57**	0.24^{ns}	0.01^{ns}	0.33*	0.52*	0.22^{ns}	-0.17^{ns}	-0.4**	-0.35*	0.19^{ns}
SL	-0.26^{ns}	0.39*	0.09^{ns}	-0.22^{ns}	0.44**	-0.51*	0.34^{ns}	-0.14^{ns}	-0.69**	0.57**
HSW	-2.69**	1.58**	0.42^{ns}	0.69*	2.53**	0.53^{ns}	-0.36^{ns}	-2.36**	-1.75**	1.42**

NMFl = Total number of male flowers ; TNFfl = Total female flowers; PH = Plant height; NPBr = Number of primary branches; NSBr = Number of secondary branches; TNFr = Total number of fruits; NMFr = Number of marketable fruits; MYld = Marketable fruit yield; TFYld = Total fruit yield; FrW = Fruit weight; FrL = Fruit length; FrD = Fruit diameter; RD = Rind diameter; TSS = Total soluble solids; NSPFr = Number of seed per fruit; SYPFr = Seed yield per fruit; SD = Seed diameter; SL = Seed length; and HSW = Hundred seed weight. * and ** = Significant at $p < 0.05$ *and* $p < 0.001$, respectively and ns = non-significant.

5.3.6. GCA and SCA variances, Baker's ratio and traits heritability

Variance components due to GCA and SCA variances, GCA/SCA ratio and heritability estimates for assessed agronomic traits are presented in Table 5.8. Baker's ratio was less than unity for most traits except for PH with a value of 4.57. Low H^2b (< 0.4) were recorded for NMFL, NSBr, Myld, TFYld, FrW, FrL, RD and TSS, whereas moderate H^2b (>0.41 to 0.56) were recorded for TNFfl, PH, NPB, TNFr, NSPFr, SYPFr and SD. The highest H^2b was recorded for HSW (0.80).

Table 5.8 Estimates of variance components for GCA, SCA, GCA/SCA and broad-sense heritability (H^2b) for agronomic traits.

	Variance components			
Traits	GCA	SCA	GCA/SCA	H^2b
NMFL	57.78	178.17	0.32	0.00
TNFfl	16.76	68.02	0.25	0.40
PH	0.25	0.05	4.57	0.56
NPBr	0.01	0.00	0.00	0.52
NSBr	6.67	18.11	0.37	0.22
TNFr	-1.59	3.55	-0.45	0.53
NMFr	-0.32	8.2	-0.04	0.56
Myld	84.03	213.04	0.39	0.18
TFYld	7.57	39.65	0.19	0.34
FrW	4.86	22.45	0.22	0.06
FrL	-4.61	8.42	-0.55	0.00
FrD	-1.50	12.51	-0.12	0.45
RD	2.73	8.57	0.32	0.37
TSS	0.53	1.35	0.39	0.00
NSPFr	52.85	100.17	0.53	0.44
SYPFr	17.10	31.91	0.54	0.48
SD	0.00	0.18	0.00	0.48
SL	0.04	0.22	0.18	0.56
HSW	-0.09	4.21	-0.02	0.80

NMFl = Total number of male flowers ; TNFfl = Total female flowers; PH = Plant height; NPBr = Number of primary branches; NSBr = Number of secondary branches; TNFr = Total number of fruits; NMFr = Number of marketable fruits; Myld = Marketable fruit yield; TFYld = Total fruit yield; FrW = Fruit weight; FrL = Fruit length; FrD = Fruit diameter; RD = Rind diameter; TSS = Total soluble solids; NSPFr = Number of seed per fruit; SYPFr = Seed yield per fruit; SD = Seed diameter; SL = Seed length; and HSW = Hundred seed weight. GCA variance component for General combining ability, SCA variance component for specific combining ability. (H^2b) =Broad-sense heritability.

5.4. Discussion

Assessment of combining ability and hybrid performance for desired traits is useful for population development, cultivar development and release. In this study, the environment had an impact on most agronomic traits indicating that the expression of genes that regulate these traits are subject to environmental effects (Table 5.4). Environmental impact on agronomic traits was also reported in sweet watermelon (Bahari et al., 2012; Dia et al., 2016; Kumar and Wehner, 2011; Singh et al., 2022). Quantitative trait loci (QTL) conditioning various agronomic traits in a population of sweet × citron watermelons were highly variable under diverse environments, indicating environmental influence on gene expression governing quantitative traits (Sandlin et al., 2012).

The parental genotypes and newly developed F_1 hybrids performed differently for the studied agronomic traits, including fruit and seed yields (Table 5.4). Achigan-Dako et al. (2015) and Ngwepe et al. (2021b) reported varied performances of citron watermelon for agronomic traits. The varied responses could be linked to the extensive genetic variation reported in the crop (Mujaju et al., 2013; Nantoumé et al., 2013; Levi et al., 2013; Mashilo et al., 2017). The phenotypic and genetic variation in citron watermelon allows the selection of parents with suitable traits for breeding. Genotype × environment interaction effect caused variable responses of agronomic traits, including fruit and seed yields (Table 5.5). These suggested best-performing genotypes of citron watermelon could be selected based on targeted production environments. There are limited studies available that explored genotype × environment effects on yield of citron watermelon.

Combining ability analysis allows for selecting parental genotypes for breeding and best-performing hybrids for direct release or genetic advancement. Therefore, the parental genotype possessing high GCA effects for traits of interest could serve as vital sources of beneficial alleles for hybrid breeding. Flowering capacity (i.e., number of male and female flowers per plant) in citron watermelon is a key trait that enhances fruit set and yield capacity. Parental accession WWM04 with high positive GCA effects for the number of female flowers per plant (Table 5.6) is recommended for breeding, given its profuse flowering capacity. The hybrids WWM04 × WWM50 and WWM04 × WWM66 with a common parent WWM04 had high positive SCA and recorded a relatively higher number of female flowers per plant. Ngwepe et al. (2021a, b) reported that the number of female flowers per plant is positively correlated with the number of fruits per plant, thus improving fruit yield. Therefore, parental accessions with more female flower production per plant are worthy of breeding and fruit production.

Fruit yield is an economic trait for breeding in citron watermelon. The ripened yellow-and-orange fleshed fruits of the crop are used to derive various dishes in Africa (Mashilo et al., 2017; Ngwepe et al., 2019, 2021a). In the current study, parent WWM16 with positive GCA effects for number of marketable fruits per plant, marketable fruit yield per plant, total fruit yield per plant and single fruit weight was identified as a good combiner for these traits. The F_1 hybrids WWM04 × WWM16, WWM03 × WWM16, WWM16 × WWM66 and WWM16 × WWM50 derived from parental accession WWM16 were identified as best performers for these traits, performing better than either of one of their parents. The F_1 hybrids involving the parental accession WWM16 also showed variation in fruit colour, rind stripe patterns and flesh colour (Figure 5.2). For example, the hybrid WWM04 × WWM16 had green fruit colour and green seeds, suggesting green fruit colour is dominant over light-green fruit colour, and green seed coat colour is dominant over red seed coat colour. Further, crosses involving round and elliptic shapes developed a hybrid of round fruit shapes. These suggest that the round fruit shape is dominant over elliptic fruit shape. Crosses involving yellow and orange flesh produced yellow flesh in the F_1 generation (i.e., WWM04 × WWM16, WWM03 × WWM16 and WWM04 × WWM50), indicating the dominance of yellow flesh colour over orange flesh colour. Studies on the genetic regulation of fruit and seed traits are lacking in citron watermelon. In agreement with the present findings, Maragal et al. (2019) reported that green fruit colour is dominant over light green colour.

Seed yield is a valuable end-product of citron watermelon whereby the seeds are consumed as a food snack, and preparation of animal feeds in Africa (Mashilo et al., 2016; Ngwepe et al., 2019, 2021a; Mandizvo et al., 2022). The seeds possess high nutritive values, including protein (≥ 23%), minerals, essential vitamins and amino acids (Singh et al., 2010; Achu et al., 2013; Yoo et al., 2013). Breeding for high seed yield potential can enhance the crop's economic potential. The following F_1 hybrids WWM04 × WWM50 and WWM03 × WWM16 with positive SCA effects for seed yield per fruit had enhanced seed yield (Table 5.7). Also, the hybrid WWM03 × WWM16 had heavier seeds. The parents WWM04, WWM03 and WWM16 showed the potential to increase the number of seeds per fruit. The F_1 hybrids derived from these parents recorded high number of seeds per fruit compared to their parents, suggesting increased genetic gains for seed traits. Seed-related traits (i.e., number of seeds per fruit and seed weight) have positive effects on seed yield for enhanced selection (Ngwepe et al., 2021a).

Understanding the mode of gene action controlling agronomic traits in citron watermelon can enhance breeding and selection gains. Non-additive gene action governed most of the studied agronomic traits in the present study (Table 5.8). Conversely, the inheritance of plant height was conditioned by additive genetic effects (Table 5.8). Higher heritability is linked with a higher response to selection. In the present study, most traits had low to moderate heritability values vital for subsequent selection (Table 5.8). The high performance of the present experimental hybrids suggests that hybrid breeding is a suitable breeding strategy. Furthermore, the recurrent selection method could be pursued to select citron watermelon populations for breeding or direct production.

5.5. Conclusions

The present study estimated the combining ability and hybrid performance in citron watermelon for agronomic traits for new variety development. The parental genotypes WWM16, with positive GCA effects for fruit yield and seed yields, and WWM66, with positive GCA effects for the number of seeds per fruit and seed yield, were identified for hybrid breeding. The F_1 hybrids WWM04 × WWM16, WWM03 × WWM66 and WWM16 × WWM50 with positive SCA effects on total fruit yield per plant and marketable fruit yield per plant, and WWM04 × WWM50, WWM03 × WWM16 and WWM03 × WWM66 were best performers for number of seeds per fruit and total seed yield per fruit. The high performance of the present experimental hybrids suggests that hybrid breeding is a suitable breeding strategy. Furthermore, the recurrent selection method could be pursued to select citron watermelon populations for breeding or direct production.

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Overview and implications of the study

Introduction and objectives of the study

Citron watermelon [*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.] is a multi-purpose food crop in sub-Saharan Africa (SSA). The fresh leaves are consumed as leafy vegetables, the ripened fruit is used for cooking, and the seeds are prepared as a roasted snack. In SSA, smallholder farmers mainly cultivate the crop using unimproved landrace varieties. There is no dedicated breeding programme in the region, including South Africa, to develop farmer and consumer-preferred varieties with desirable product profiles. Genetic and phenotypic analyses are prerequisites for pre-breeding and cultivar development programs. Therefore, this study was undertaken to initiate a pre-breeding program for citron watermelon through identification and selection of unique and complementary genotypes for production, value-adding and breeding.

The specific objectives of the study were:

i. To determine the extent of genetic diversity among South African citron watermelon landrace accessions using selected simple sequence repeat (SSR) markers to identify genetically divergent accessions for trait integration and variety development.

ii. To assess the phenotypic diversity of citron watermelon landrace accessions of South Africa to select desirable genotypes with suitable agronomic and horticultural traits for direct production, breeding and conservation.

iii. To estimate variance components, heritability and genetic advance of phenotypic traits in citron watermelon to guide the selection of superior genotypes for direct production and breeding.

iv. To determine the combining ability and hybrid performance of citron watermelon genotypes for agronomic traits for breeding.

Research findings in brief

Characterization of genetic diversity of citron watermelon (Citrullus lanatus var. *citroides [L.H. Bailey] Mansf. ex Greb.*) *landrace accessions of South Africa through simple sequence repeat markers*

Forty-eight citron watermelon landrace collections widely grown in the Limpopo Province of South Africa were genotyped using 11 selected SSR markers. Ten seeds per accession were grown in black polyethylene plastic bags in a net-house. After four weeks, young and healthy fresh leaves were sampled from 10 plants per accession for bulked DNA sampling and genetic analysis.

The core findings of the study were:

- The SSR markers amplified 24 alleles, with a mean expected heterozygosity value of 0.38, indicating moderate genetic diversity among the studied accessions.
- Analysis of molecular variance attributed 8%, 75%, and 17% of the genetic variation between populations, among accessions and within accessions, respectively.
- Three distinctive genetic groups were identified based on cluster analysis.
- The following distantly related genotypes are recommended as breeding parents namely: WWM03, WWM04, WWM15, WWM16, WWM18, WWM22, WWM23, WWM24, WWM25, WWM26, WWM28, WWM33, WWM34, WWM35, WWM38, WWM39, WWM41, WWM66, WWM76, WWM78, WWM81, WWM84, WWM86 and WWM89 (selections from Cluster I), WWM14, WWM37, WWM42, WWM44, WWM46, WWM50, WWM65, WWM79, WWM85 and WWM87 (Cluster II), and WWM38, WWM47 and WWM48 (Cluster III).
- These are useful parental lines for pre-breeding to develop and release new varieties with multiple uses.

Variation in South African citron watermelon (Citrullus lanatus var. *citroides [L.H. Bailey] Mansf. ex Greb.) landraces assessed through qualitative and quantitative phenotypic traits.*

Thirty-six selected citron watermelon landrace accessions were evaluated for qualitative and quantitative phenotypic traits using a 6 × 6 lattice design in three replicates. The evaluation was carried out under field conditions across two environments.

The major findings of the study were:

- The accessions showed wide phenotypic variation and unique traits for genetic improvement.
- Positive and significant correlations *($p < 0.001$)* were recorded between total fruit yield per plant with plant height (r = 0.64), number of harvestable fruits (r = 0.70), number of marketable fruits (r = 0.73) and marketable fruit yield (r = 0.96). Seed yield per plant positively and significantly *($p < 0.001$)* correlated with number of male flowers (r = 0.68), plant height (r = 0.61) and total fruit yield (r = 0.79).
- The principal component analysis identified nine components, accounting for 86.38% of total variation amongst accessions for assessed phenotypic traits.
- The study recommended citron watermelon accessions such as WWM14, WWM16, WWM39, WWM41, WWM67 and WWM79 for use as leafy vegetables owing to their profuse branching ability and longer vine production. Whereas accessions including WWM03, WWM17, WWM35, WWM40, WWM50, WWM67, WWM79 and WWM85 are selected with larger fruit size.
- Accessions WWM05 and WWM09 are sour-flesh types which are suitable genetic stocks for breeding sweet-and-sour and sweet dessert watermelons.
- Orange-fleshed accessions such as WWM03, WWM04, WWM46, WWM64, WWM66 and WWM67 are recommended for fresh consumption, cooking, processing or variety design.
- Accessions WWM02, WWM03, WWM08, WWM14, WWM16, WWM23, WWM38, WWM40, WWM41 and WWM67 have red and white seed coat colour which are superior selections to prepare roasted citron watermelon seed snack.

Estimates of the variance components, heritability and genetic gains of phenotypic traits in citron watermelon (*Citrullus lanatus* var. *citroides [L.H. Bailey] Mansf. ex Greb.*).

Thirty-six citron watermelon landrace accessions were evaluated to estimate variance components, heritability and genetic advance of phenotypic traits for breeding. The accessions were evaluated across two seasons using a 6 × 6 lattice design with three replications.

The key summaries from this study were:

- High broad-sense heritability and genetic advance as percent of the mean were recorded for fruit length at 83.86 and 4730.45%, seed length (77.73 and 1731.27%), hundred seed weight (73.73 and 4027.36%), fruit diameter (70.44 and 2949.64%) and fruit weight (70.39 and 8490.05%), respectively.
- Step-wise regression analysis revealed marketable fruit yield and the total number of fruits per plant, explaining 89% ($R^2 = 0.89$) of the total variation for total fruit yield per plant, whereas the number of seeds per fruit and hundred seed weight explained 92% ($R^2 = 0.92$) of the total variation for seed yield per fruit.
- Citron watermelon landrace accessions WWM03, WWM14, WWM16, WWM39, WWM65, WWM67 and WWM79 with high total fruit yield and seed yield per fruit were selected for production or breeding programme.

Combining ability and hybrid performance in citron watermelon (*Citrullus lanatus* var. *citroides [L.H. Bailey] Mansf. ex Greb.*) *for agronomic traits.*

To determine the combining ability effects and select best-performing hybrids, five contrasting and relatively high-yielding citron watermelon genotypes were crossed in a 5 × 5 half-diallel mating design to develop 10 F_1 hybrids. The five parents and 10 F_1 hybrids (15 genotypes) were evaluated across two environments under field conditions.

The core findings of the study were:

- General combining ability (GCA) and specific combining ability (SCA) effects were significant *($p < 0.001$)* for most assessed traits.
- Environment × GCA interaction effects were non-significant, whereas Environment × SCA effects were significant *($p < 0.001$)* for most traits.
- The ratios of GCA/SCA variances were less than unity for most traits, indicating non-additive gene action of the traits.
- Broad-sense heritability varied from low to moderate, implying variable selection response of the assessed traits among the F1 hybrids.
- The parental genotypes WWM16 with positive GCA effects for fruit and seed yields, and WWM66, with positive GCA effects for the number of seeds per fruit and seed yield, were identified for hybrid breeding.

- The following F_1 hybrids, namely: WWM04 × WWM16, WWM03 × WWM66 and WWM16 × WWM50 with positive SCA effects on total fruit yield per plant and marketable fruit yield per plant, and WWM04 × WWM50, WWM03 × WWM16 and WWM03 × WWM66 with positive SCA effects for number of seeds per fruit and total seed yield were identified.
- The study identified novel and best-performing F1 hybrids of citron watermelon for economic traits and are recommended for multi-environmental evaluations, variety registration and commercialization.

The implications of the study findings

- The study revealed genetic and phenotypic variation in South African citron watermelon collections. The genetic variability enables selecting and recommending suitable genotypes for production and breeding new generation varieties based on market needs and consumer preferences.
- The study recommends accessions such as WWM14, WWM16, WWM39, WWM64, WWM67, WWM76 and WWM79 with high fruit yield, and WWM03, WWM04, WWM14, WWM15, WWM16, WWM24, WWM28, WWM37, WWM46, WWM50, WWM66 and WWM68 for direct production and for breeding for high fruit and seed yields.
- Crosses involving the selected complementary parents will enhance citron watermelon breeding programs and product development.
- The parents WWM04, WWM03 and WWM16 were identified as good combiners for fruit or seed yield and related-component traits for future breeding.
- The following F_1 experimental hybrids derived from the above parents: WWM04 × WWM16, WWM03 × WWM66, WWM16 × WWM50, and WWM04 × WWM50, WWM03 × WWM16 and WWM03 × WWM66 were best performing for fruit and -related traits and for new breeding population development.
- There is a need for multi-environmental evaluations of the candidate selections and F_1 hybrids for cultivar registration and commercialization in South African conditions or similar agro-ecological zones.