Factors Associated with the Yield Variation Among Citron Watermelon (*Citrullus lanatus* var. *citroides* (L. H. Bailey) Mansf. ex Greb.) Genotype in South Africa

By

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PREFACE

The study presented in this thesis was completed by the candidate, while based at the Discipline of Crop Science, School of Agricultural, Earth and Environmental Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

I have supervised the final compilation of and carefully read through the thesis.

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(Isa Bertling, Ass. Prof. Horticultural Science)

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DECLARATION

I, Ntuthuko Hadebe, declare that:

(i) I am the sole author of the research disclosed in this dissertation, unless otherwise stated or recognized;

(ii) This dissertation has not been submitted in full or in part to another university for a degree or examination;

(iii) This dissertation does not include graphs, statistics, images, or any information that was not expressly cited as coming from another individual;

(iv) This dissertation does not include any writing by other people unless it is clearly cited as a source from another researcher. Where quotations from other written sources have been used, then:

a) their words have been changed, but the overall knowledge they are credited with has been cited;

b) where I have utilized their identical words, their writing has been quoted, and they have been cited;

(v) Where I used material for publications that followed, I specifically stated my contribution to the effort;

(vi) This dissertation is mainly a compilation of content I've written and has been published in journals or given as oral and poster presentations at conferences. In some instances, extra content has been added;

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ABSTRACT

Citron watermelon [*Citrullus lanatus* var. *citroides* (L. H. Bailey) Mansf. ex Greb.] is a plant species used for food and feed, belonging to the family Cucurbitaceae. Wide yield variation is exhibited among citron watermelon genotypes in South Africa. The objective of this study was to assess morphological and physiological factors associated with yield variation among citron watermelon genotypes in South Africa under drought stress and to recommend genotypes that are drought-tolerant and display desirable fruit traits for future crop production, breeding and conservation. The study also sought to understand, if the use of urine-based fertiliser sources, such as struvite, can improve phosphorus (P) availability in acidic soils, leading to increased root growth, nutrient uptake, and subsequently enhanced yield. The study was designed as a 6 x 2 x 3 factorial experiment with the following factors: citron watermelon genotype - 6 levels (WWM-1, WWM-16, WWM-30, WWM-31, WWM-35, and WWM-37), drought conditions - 2 levels (non-stress (NS) and (DS) drought-stress condition); and phosphorus source - 3 levels (struvite - a urine-derived fertilizer, single superphosphate (SSP), and no added fertilizer, Gromor® potting mix only). Data on quantitative leaf traits (leaf length, leaf width and leaf area), vine length (the longest vine length), root traits (root mass and root length), fruit mass, nutrient uptake (micro- and macronutrients) and leaf physiological data (gas exchange and chlorophyll fluorescence, indicating photosynthetic activity, stomatal conductance, electron transport rate, transpirational flow, intercellular carbon dioxide (CO_2) concentration, ratio of intercellular to atmospheric CO_2, and maximum fluorescence) were collected. Qualitative (phenotypic) traits, such as fruit colour, were also recorded and data subjected to analysis of variance using GenStat 20th edition.

A wide variety of quality parameters among citron watermelon genotypes that could be genetically improved, were observed. The results from this study showed no significant differences ($P>0.05$) between treatments with respect to leaf traits (leaf length, leaf width and leaf area) with all levels of P sources (struvite, SSP and no fertilizer), under both NS and DS. Struvite- and SSP-treated genotypes outperformed the control with respect to leaf traits (leaf length and leaf width) under NS, however, non-treated (control) genotypes outperformed both struvite- and SSP-treated genotypes, concerning plant leaf area. Application of struvite significantly improved the chlorophyll content index (CCI) of plants to levels higher than the SSP treatment and the control. The urine-based fertilizer was comparable to the SSP treatment concerning vine length of all six citron watermelon genotypes under both NS and DS. This

suggests that struvite improved vine growth of all selected genotypes. A significant difference ($p<0.05$) was observed between struvite and SSP treated genotypes in terms of fruit mass, and in terms of fruit nutrient (micro- and macronutrients) composition under both NS and DS.

The study revealed that both struvite and SSP effectively increased root development (resulting in greater root dry mass and longer roots) under DS. A significant difference ($p<0.05$) was observed in all levels of P sources (struvite, SSP, and no fertilizer) in terms of the root mass and root length. Therefore, the application of struvite improved root growth and development. The better uptake of micro- and macronutrients (higher mineral concentration than the control and the SSP treatment) by struvite-treated genotypes could be attributed to the high cation exchange capacity (CEC) of the Gromor. The increase of N uptake by citron watermelon fruit grown in SSP- and struvite-treated soils could be due to mineralization of organic matter in the soil, resulting in the release of initially unavailable N.

Results from this study revealed no significant differences ($P>0.05$) between treatments with respect to leaf gas exchange and chlorophyll fluorescence parameters. Struvite-treated genotypes performed well compared to both SSP-treated and control genotypes concerning the ratio of intercellular and atmospheric CO_2 (increased ratio) under NS, meaning struvite treatment improved the photosynthesis process of genotypes because of high intercellular CO_2 concentration in leaves. Struvite-treated genotypes performed well compared to SSP-treated genotypes concerning the stomatal conductance (reduced stomatal conductance), this indicates the efficient drought avoidance among the genotypes. Citron watermelon genotypes WWM-30, WWM-31, and WWM-37 recorded the highest maximum chlorophyll fluorescence in both NS and DS, meaning the effect of drought stress was reduced by enhancing P availability. The genotypes WWM-1, WWM-30, and WWM- 35 revealed high maximum quantum efficiency of photosystem II photochemistry and produced higher marketable fruit yields and a higher fruit number per plant than WM-16, WWM-31, and WWM-37. These traits are important as characteristics of parental genotypes in breeding programmes.

The present study recommends citron watermelon genotypes such as WWM-1, WWM-35, and WWM-37, for use as leafy vegetables, due to their higher leaf biomass, whereas genotypes including WWM-16, WWM-35, and WWM-37, are recommended for fruit production due to their large fruit size. In conclusion, the study shows that the use of urine-based fertiliser sources, such as struvite, seems to increase P availability in acidic soils and thereby leads to increased

root growth, nutrient uptake, and, ultimately, higher quality fruit.

Keywords: Citron watermelon, yield variation, physiological factors, morphological factors, soil phosphorus, nutrient uptake**.**

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GENERAL INTRODUCTION

Citron watermelon [*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.] is a plant species used for food and feed (Ngwepe *et al.*, 2021). The species is a vital source for breeding purposes, particularly of the dessert citron watermelon (*C. lanatus* var. *lanatus*) and other related species that are compatible (Ngwepe *et al*., 2021). There is a significant phenotypic diversity among South African citron watermelon genotype, which needs to be effectively researched for various purposes such as plant breeding and livestock feed, and cultivar designs (Ngwepe *et al*., 2019). The species is placed into the family Cucurbitaceae, which comprises different melons, gourds, and squashes. Its fruit is hard, has a white flesh, that cannot be eaten uncooked; however, it can be utilized to make jellies, jams and preserves. It is commonly pickled or utilized as cattle feed, but is especially valuable for fruit preserves, since it contains a high pectin concentration, which is used in food as a stabilizer, thickener and gelling agent (Méndez *et al.*, 2021).

Citron watermelon can grow an approximately 3 m long, herbaceous stem, and is, therefore, described as a vine creeper. Young stems and leaves are woody, whereas the older parts are hairless (Mandizvo *et al*., 2021). The crop is farmed in sub-Saharan Africa (SSA) for its tender leaves and fruit, eaten as boiled vegetables. The leaves contain bioactive substances with medicinal effects, including cucurbitacin (B and E and cucurbitacin E glycoside), which has anti-biotic and anti-tumor characteristics (Davidovich-Rikanati *et al*., 2015).

The crop has herbaceous leaves, usually 3-lobed and sometimes unlobed. Being monoecious, each plant possesses both female and male flowers. In the SSA region, citron watermelon fruit is frequently fed to livestock (pigs, goats, and cattle), and its fruits are a significant source of water (Nantoume *et al*., 2013). Citron watermelon is one of the unique species with the potential to reduce the micronutrient shortage frequent in SSA due to its nutritional content (micro- and macronutrients) (Chacha and Laswai, 2020). The fruit have numerous shapes (ellipsoid, globose and sub-globose) and the width can be approximately 200 mm. The ripe fruit's rind has diverse colours, often mottled with irregular longitudinal bands, and is smooth (Mashilo *et al*., 2017).

Protein fractions found in citron watermelon seeds include glutelins (14.0%), albumins (18.6%), and globulins (63.7%) (Singh and Matta, 2010). In South Africa, traditional healers cure hypertension with citron watermelon fruit and leaves (Semenya and Potgieter, 2015). Consuming roasted seeds can help with constipation and increase appetite (Semenya and Potgieter, 2014). A near relative of citron watermelon, the watermelon, *Citrullus lanatus* var. *lanatus*, contains mineral nutrients in substantial quantities in edible plant parts, including seeds (K: 0.17, K: 3.57 mg/g), fruit (N: 15.62, P: 7.81, k: 88.67 mg/g) and leaves (N: 56.36, P: 5.78, K: 78.59 mg/g).

In terms of fruit colour patterns, fruit size, seed coat colour and fruit form, citron watermelons exhibit a significant range in phenotypic appearance (Dane and Liu 2007; Mujaju *et al*., 2013). The specie's large yield diversity is crucial for choosing promising genotypes for a variety of applications (Singh *et al*., 2017). In describing citron watermelon genetic resources for breeding and genetic analysis, citron watermelon variation features are typically used (Lima *et al*., 2017). Small-holder farmers in South Africa frequently cultivate genetically different landrace genotypes of citron watermelons (based on fruit traits, leaf traits, vine length, and physiological traits) using low-input, low-fertilizer farming practices (Mashilo *et al*., 2017).

Climate change-related irregular rainfall patterns and prolonged dry periods put food security at risk by reducing crop yields. World population growth (estimated to reach 9.8 billion by 2050) (Dillard, 2019; Skaf *et al*., 2020) will make it more and more difficult to produce sufficient food in a changing environment and will continue to be a major hurdle for developing nations, especially those in SSA. Neglected, underutilized crop species (NUCS), including citron watermelon, have the potential to be cultivated under rather marginal conditions, improving crop diversification in the palette of smallholder farmers' fields and contribute to long-term food security, as these crop species are generally better adapted to poor, erratic rainfall and protracted dry spells.

The drought tolerance of cucurbit crops can be assessed by certain physiological parameters, including stomatal conductance, transpiration rate, photosynthetic efficiency as well as the ability of the leaves to use photochemical and non-photochemical quenching in order to reduce the stress load (Mashilo *et al*., 2017). The effectiveness of screening for and identifying drought-tolerant genotypes could be increased by using yield-based selection indices in conjunction with morphological and physiological traits to assess the drought tolerance of citron watermelon genotypes.

High-yielding cultivars, which are also drought-tolerant, can mitigate the impact of water stress and should be produced to reduce yield losses under drought conditions (Yavuz *et al*., 2020). In certain cucurbit species, drought tolerance is associated with lowering leaf transpiration rates and water loss, enhancing the uptake of water by the roots, and avoiding severe drought stress by earlier flowering (Kajikawa *et al*., 2010; Hakki *et al*., 2016). High drought resilience, high biomass production and fruit yield have all been connected to the plant's capacity to efficiently extract water from the soil and transport it to aboveground areas, enabling better/ more physiological activities (Guzzon *et al*., 2017).

Ensuring long-term access to phosphorus is one of the most pressing issues with natural resources that the world is currently confronting (Frissel, 1978). This element is a crucial component of fertilizers used worldwide in agricultural systems (Ashley *et al*., 2011). The fact that phosphate rock is a non-renewable resource contributes to the growing worry over phosphorus availability (Smil, 2000). One of the approaches, the diversion and recycling of human urine, is a practical and energy-efficient way to recover phosphorus from wastewater; an additional advantage of this method is that the recycling of human urine produces nutrients for the production of agricultural fertilizers that resemble conventional ones (Kalmykova *et al*., 2012).

Citron watermelon is grown in resource-restrained communities, where farming is characterized by drought and soils with low nutrient levels and high acidity. However, local farmers grow a range of citron watermelon genotype adapted to these conditions. It is hypothesized that variability exists among these genotypes concerning genetic potential (to survive under stress conditions), including physiological and morphological traits which can influence yield performance under drought and nutrient-deficient conditions. There is limited information on the yield variability under water stress conditions. Some research has been done by Mandizvo *et al*. (2022) and Ngwepe *et al*. (2019) on citron watermelon yield variation.

This study aims to gain knowledge on factors that may be associated with yield variation among citron watermelon genotype in South Africa, and whether this variability is associated with morphological and physiological traits, including root characteristics. Phosphorous (P) deficiency is a limiting factor under acidic conditions. Research is needed to determine if the use of urine-derived fertilisers could improve P availability and increase root growth and nutrient uptake in acidic soils. The study seeks to understand if the use of urine-based fertiliser sources, such as struvite, can improve P availability in acidic soils and lead to increased root growth, nutrient uptake, and yield performance.

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CHAPTER 1: Factors Affecting Yield Variation of Cucurbit Crops in South Africa

1.1 Background of cucurbit crops

The melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.), bitter gourd (*Momordica charantia*), pumpkin/squash (*Cucurbita* spp.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), and bottle gourd (*Lagenaria siceraria*), are members of the *Cucurbitaceae* family (cucurbit) (Paris, 2016). In terms of area planted, output, and monetary worth, these five cucurbits rank among the top vegetables (FAOstat, 2023). Although cucurbits perform better in warm climates, they are also produced under protected cultivation in cool and temperate climates. The cucurbit fruit family is particularly well-liked due to the sweetness and flavour of melons and watermelons, as well as the diverse culinary uses of cucumbers, pumpkins, and squash (Paris, 2016).

Cucurbit fruit provide minerals, vitamins, dietary fibre, and antioxidants, and are becoming widely acknowledged as important components of a balanced diet in many nations. Cucurbits are also more than fruit vegetables (Norrman and Haarberg, 1980). Scientific studies have verified that cucurbit leafy vegetables are beneficial in developing possible nutraceuticals to prevent diabetes mellitus. Dried leaves of watermelon are traditionally known as “kgwaile” in the indigenous South African Sepedi language. Kgwaile is pre-cooked, sun-dried and preserved leaves of citron watermelon which is used when desired as leafy vegetable.

1.1.1 Origin and botanical description of lesser-known cucurbits

Cucurbits provided ancient people with food and water. Since wild cucurbits typically contain small, bitter fruits, it must have been challenging for people to use them as water and/or food supplies. Very likely, cultivating the occasional cucurbit plant that produced non-bitter fruits was due to the absence of a single recessive gene, which causes bitterness; this was the first step into the usage of cucurbits by mankind (Pitrat and Navot *et al*., 1990; Paris and Brown 2005; and Call and Wehner, 2010;).

1.1.1.1 Squash and pumpkins

There are five varieties of cultivated *Cucurbita* species, encompassing squashes and pumpkins: *Cucurbita moschata, Cucurbita pepo, Cucurbita maxima, Cucurbita ficifolia* and *Cucurbita argyrosperma.* The most frequently cultivated and economically significant species is *Cucurbita pepo* L., followed by *Cucurbita moschata* Duch. and *Cucurbita maxima* Duch. (Ferriol and Pico, 2008). The other two related species are *Cucurbita argyrosperma* Huber and *Cucurbita ficifolia*

Bouché. According to Andrés and Robinson (2002), a sixth species, *Cucurbita ecuadorensis Cutler & Whitaker*, may have been farmed, but has since been forgotten. *Cucurbita* fruit and seeds were a significant part of the food of indigenous American people for thousands of years before the immigration of Europeans, according to archaeological evidence of farmed *Cucurbita* (Paris, 2018).

The crookneck, scallop, straight neck and acorn squashes (*C. pepo*) were products of this second domestication (Paris, 2000). According to Nee (1990) and Wessel-Beaver (2000), the origin of a further species, *Cucurbita moschata*, was probably in tropical, northern South America. In coastal Peru, seeds that are approximately 10000 years old have been discovered, providing proof of the early domestication of this species in this region (Dillehay *et al*., 2007). Although some cultivars thrive in more temperate areas and some in more arid ones, this species is ideally suited to the warm, humid tropics. In one location of Ecuador, phytoliths (microscopic plant silica bodies that record past plant communities and ecosystem) considered to be from *Cucurbita ecuadorensis* or possibly *Cucurbita moschata* have also been found. The fact that these 10000-year-old phytoliths are far larger than those of living wild *Cucurbita* crop indicates that they came from the fruits of domesticated plants, because they were selected (Piperno and Stothert, 2003).

It is recognized that the Cucurbitaceae family vegetable pumpkin has a number of bioactive compounds, such as sterols, proteins, peptides, polysaccharides, and para-aminobenzoic acid. These ingredients have been recognized as useful foods and are present in fruit meat, seeds, and leaves. One of the most significant vegetables in the world's traditional agricultural systems is the pumpkin fruit. Butternut squash, or *Cucurbita moschata,* has been utilized for many years in Asian countries as a traditional medicine and health food due to its purportedly unique medicinal qualities, which include anti-inflammatory, anti-tumor, cholesterol-lowering, hypertensive, anti-parasitic, and anti-diabetic effects. It's also a fantastic source of inexpensive, natural pectin (Zhu *et al*., 2015).

1.1.1.2 Watermelons

Watermelon is a member of the Cucurbitaceae family. It is known to botanists as a "pepo," a fruit with a meaty center and a thick rind. There are many varieties of watermelon including picnic, icebox, giant and seedless watermelon. *Citrullus lanatus* (Thunb.) has been found in 4000–5000-year-old archaeological sites in Egypt, Sudan and Libya, with most of the remains being seeds. Coptic, Egypt's pharaonic, post-pharaonic, and Roman sites all contained watermelon seeds (van der Veen, 2011; Asensi Amoros and De Vartavan, 1997). In Egypt, watermelon-related artefacts dating back 4000 years have been found (Feliks 2005; Keimer 1924). Even though watermelons were likely bland and not sweet at the time, this evidence shows that they were already produced in North-Eastern Africa (in Egypt) 4000 years ago (Paris, 2015).

Despite evidence from iconography (a picture formed by words), archaeobotany (a study of past human-plant interactions), and literature suggesting that *Citrullus lanatus* existed in North-Eastern Africa thousands of years ago, watermelons are believed to have originated from either *C. amarus Schrad*. (citron watermelon) of southern Africa, *C. colocynthis* (L.) Schrad. (colocynth) of northern Africa, or, more recently, *C. mucosospermus* species, after the cross breeding. Although the 'egusi' species is the dessert watermelon's closest relative (Fursa, 1983; Chomicki and Renner, 2015), crossing the two species has been a challenge (Gusmini *et al.*, 2004), which raises serious doubts about the 'egusi' being the dessert watermelon's ancestor. Although samples of modern wild (modern crossbred with wild species) and "semi-cultivated" (with enhanced growth) watermelons from Egypt and Sudan have not undergone genomic analysis, their simple presence suggests that the origin of *C. lanatus* is in the north-eastern African region (Paris, 2015).

The Mishna, a religious text written in Hebrew in the late 2nd century CE, mentions watermelons together with pomegranates, figs, and table grapes, indicating that by that time, watermelons were on par in sweetness, because of its sugar content, with these other fruits (Paris, 2015). By the end of the 5th century CE, sweet dessert watermelons had arrived in the Mediterranean regions of Europe. The Tacuinum Sanitatis, a late 14th-century Renaissance botanical herbal, and the document, both mention and illustrate the insipid, colourless, hard, citron watermelons as well as the red-fleshed, delicious dessert watermelons (Paris, 2015; Paris *et al*., 2013). The domestication of sweet dessert watermelons dates back nearly 2000 years; however, despite their diversity in shape, size, and interior and exterior fruit coloration, they exhibit little molecular genetic diversity (Nimmakayala *et al*., 2014; Levi *et al*., 2001), indicating that they may have gone through a "severe bottleneck", which shrunk the gene pool and resulted in genetic diversity, during their evolution.

1.1.1.3 Melons

The melon, *Cucumis melo* L., is indigenous to Asia (Sebastian *et al*., 2010). *Cucumis* findings are uncommon in comparison to the number of archaeobotanical discoveries of *Citrullus* and *Cucurbita cucumis* fruit do not possess lignified rinds, as wild *Cucurbita* fruit do, and their seed coats are not as hard and thick as those of *Citrullus* fruit. Cucumber (*Cucumis sativus* L.) and melon (*Cucurbita melo*) seeds have very similar morphologies (Bates and Robinson, 1995), making it nearly impossible to identify them according to appearance, hence, they were stored separately. Findings of *Cucumis* seeds from pre-dynastic Egyptian period (Newton, 2007), which date to a period more than 5,000 years ago, are undoubtedly *Cucurbita melo* because, in later periods, melons, rather than cucumbers, were more widely consumed throughout the Mediterranean (Janick *et al*., 2007).

The snake melons have continued to be a common crop in this area up to the present (Paris *et al.*, 2012). Pliny the elder, who lived in the first century, described it as a spherical fruit with a quince-like shape that turns yellow and breaks off from the plant when mature (Janick *et al*., 2007). The 'melopepo' was, therefore, a cultivar of melons, presumably from the Adana (a city in Turkey) group, whose fruit may be eaten when mature and had mealy, mildly sweet to bland flesh (Pitrat *et al*., 2000). Mosaics from the Roman and Byzantine eras feature rounded melons (Avital and Paris, 2014; Janick *et al*., 2007). These fruits originate from plants of several proposed subspecific classifications of the highly polymorphic *Cucumis sativus* (cucumbers) and *Cucumis melo*, but Pitrat *et al*. (2000) divided the two subspecies, *agrestis*, and *melo*, into 16 botanical varieties.

The most well-known botanical types with sweet and aromatic fruit flesh include muskmelons, cantaloupes and casabas. Writings from the 9^{th} and 10^{th} centuries provide the oldest proof of the presence of sweet melons, which have been traced to the ancient region of Khorasan in Central Asia (today Turkmenistan and portions of neighbouring countries) (Paris *et al*., 2012). Towards the end of the 11^{th} century, the casaba melons (inodorous group), the sweetest of all the melon varieties, had been introduced to Muslim Spain. The decorative, aromatic 'duda'im' melons (*Cucumis melo*) date back to modern-day Iran in the 10^{th} century (Paris *et al*., 2012).

1.1.1.4 Other melon types

Citrullus lanatus, the watermelon, gets its name from the fruit's high (around 93%) water content. The fruit's enormous, spherical form and its luscious, pulpy flesh are what gave it the name "melon." The scientific name for the watermelon is a hybrid of Latin and Greek roots, which is from the word 'karpouzi'. The genus name, which may refer to the globose fruit, is a diminutive of Citrus. The dense woolly hairs on young parts of the plant are referred to by the particular *epiphet lanatus*. The small hairs on the plant's leaves and stems are referred to as "woolly" in the Latin word *lanatus* (Baker *et al*., 2012). Watermelon grows frequently wild in southern Africa, where it also produces the greatest range of cultivars. *Citrullus lanatus* is a climbing or prostrate annual plant with numerous herbaceous, strong stems that can reach lengths of up to 3 meters (Robinson and Decker, 1997).

1.1.1.5 Cucumbers

The cucumber, *Cucumis sativus* L., is a well-known member of the Cucurbitaceae. Numerous locations on the Indian subcontinent have reported wild cucumbers (Bisht *et al*., 2004). A little more than 2,000 years ago, it appears that cucumber cultivation spread from India to China (Walters, 1989). Early in the Middle Ages, cucumbers spread west from India to Persia to Europe (Paris *et al*., 2012).

Chromosomes in the cucumber are divided into seven pairs, which sets it apart from the majority of other *Cucumis* species (Yang *et al*., 2019). Three of the chromosomes of the domesticated cucumber variety, *C.s*. var. *hardwickii* (Royle) Alef. were found to have been reshuffled in comparison to the wild variety (Yang *et al*., 2012). The cucumber's genome has been sequenced (Huang *et al*., 2009). There are hundreds of different varieties of cucumber, some better suited for pickling than others, while others are better used fresh in salads. Overall, however, cucumber phenotypic diversity is lower than that of melons, pumpkins, squash or watermelons. The diameter of mature cucumber fruit can vary. In the Far East, various botanical cucumber varieties are grown with melons. The most notable probably being *C.s*. var. *xishuangbannesis* Qi & Yuan; these plants produce huge fruits (5 kg) fluctuating in colour from yellow to orange in fruit flesh (Robinson and Decker-Walters, 1997).

1.1.2 Morphological description of cucurbit crops

Cucurbits are a group of herbaceous annual or perennial plants having primarily tendrils. The plants either spread out along the ground or use tendrils to climb, not developing into bushes (Pessarakli, 2016). From simple to palmately complex, leaf shape can vary form lobed, triangular, and pinnate with wavy leaf margins. Leaves which develop later on mature plants have deeper-lobed margins and frequently have extra-floral nectaries, except cucumbers, for the pollination. The plants are either male or female, and very seldom hermaphrodite since the flowers are unisexual (Pessarakli, 2016).

Large, big and bristly leaves (40-150 mm wide and 60-200 mm long) and individual yellow flowers are features of pumpkin plants. The fruit is linked to the plant by a woody stalk and is orange, huge (5 kg), spherical, and occasionally has greenish accents. Vertical grooves are present on the exterior, while the interior is composed of stringy flesh to which many flat, oval seeds are attached. Squashes and pumpkins are classified nearly exclusively based on fruit shape. The family's wider, rounder members are categorized as pumpkins, while other fruit forms are called squash. Some squash types have straight or wavy necks, as well as a range of hues, from vivid yellow to deep green, and frequently a combination of these colours. The stems have a rounder, softer shape than other parts of the plants, and a curve where they join the fruit. Cucumbers are immature fruit, green in colour, much longer than wide and cylindrical in shape (Pan *et al*., 2020).

Watermelon plants have forked tendrils, hairy leaves and stems, and occasionally three-lobed leaves with hairs, however, non-lobed leaves are infrequently produced. Oblong, ovate, or linear leaflet lobes with somewhat serrated edges make up the lobed leaf phenotype (Ngwepe *et al*., 2019). The crop is monoecious, meaning the same plant produces both male and female flowers. Within 40 to 60 days after planting, bright yellow blooms that are both male and female begin to appear. Usually, female flowers begin to bloom 5 to 15 days after male flowers first appear. Watermelon displays a wide range of physical and genetic variants as a result of cross-fertilization (Ngwepe *et al*., 2019; Mashilo *et al*., 2017).

While the older parts of watermelon plant are hairless, the younger parts have wool and are covered in brownish-to-yellowish hairs. Although they are normally severely 3-lobed, with the segments being further lobed or doubly lobed, the leaves are herbaceous, inflexible, and growing rough on both surfaces. They are 40-150 mm wide and 60-200 mm long (Laghetti and Hammer, 2007). The largest lobe is by far the middle one. The leaf stalks have some hair on them and can be up to 150 mm long.

Usually split in two, the upper part of the tendrils is fairly strong. Monoecious blooms have a hairy, up to 40 mm-long stalks. The fruit is indehiscent, subglobose, and up to 200 mm diameter in its natural state. The fruit stem can grow up to 50 mm in length (Van *et al*., 2004; Maynard, 2001; Fursa, 1981). The fruit weighs 0.1-3.0 kg and is typically globose to ellipsoid or oblong, occasionally oval. It is 5-70 cm long and weighs between 0.1 and 2.5 kilograms for egusi melons and 1.5 and 3.0 kg for watermelons. Sizes of the smooth, flattened, obovate to elliptical, 0.5-1.5 cm 0.5-1 cm, black, brown, or yellow seeds vary (Laghetti, and Hammer, 2007; Jackson, 1990).

Primordial fruits, ovaries, and sperm are glossy and smooth during development. From 1 kg to 25 kg, a fruit can weigh (Achigan-Dako *et al*., 2015). Fruits can be small to quite large and can have a round, elliptic, or broad-elliptic form. Light to dark green, without or with rind stripe patterns, is the colour spectrum of fruit that has reached complete growth and maturity. The rind's colour and striped pattern might vary depending on whether it is light, medium, or dark green. The fruit components most often consumed after cooking are the endocarp and mesocarp, which make up the meat. The flesh might be white-green, orange, or yellow and has a medium to hard, crisp texture (Ngwepe *et al*., 2019; Mashilo *et al*., 2017; Levi *et al*., 2013). Depending on the size of the fruit, there are 500 to 900 seeds within. The flat, firm, oval seeds are between 6.26 and 8.60 mm wide and 9.13 and 15.25 mm long.

1.1.3 Traditional uses of cucurbit crops

You can eat cucumbers either raw or pickled. Cucumbers are frequently preserved for longer periods by being pickled. In the past, it made them accessible well after the regular growing season. A brine, vinegar, and/or spice solution is used to soak cucumbers. The cucumber is preserved and given new flavours as a result. Certain cucumber characteristics have been linked to benefits in preventing cancer by the National Cancer Institute (Saboo *et al*., 2013).

One of the best food sources of carotenoids for humans is pumpkin. Carotenoids in plants produce vivid pigment to draw pollinators and protect the plant's exterior layer from sunlight exposure (Ngozi *et al*., 2014). In addition to assisting in the production of detoxifying enzymes, defending the body against free radicals, and regulating immunological response, carotenoids in humans also assist in protecting the skin and eyes from UV radiation. Alpha and beta carotene are two forms of carotenoids that in particular offer significant health advantages. Alpha-carotene can lower the effects of aging on the body, whereas beta-carotene is a strong antioxidant and anti-inflammatory. The pumpkin and squash seeds can be used as a taeniafuge (Saboo *et al*., 2013), as they are effective in exterminating intestinal parasites, such as roundworms and tapeworms. Cucurbitin, also known as (-)-3-amino-3-carboxypyrrolidine, is an amino acid that is present in various amounts in the seeds of many different types of *Cucurbita* species. The seeds can be blended or made into tea when used for this purpose of making a tea. Additionally, pumpkin seeds can aid males with enlarged prostate glands, because of the amino acid that is present in the seeds (Saboo *et al*., 2013).

There are numerous methods to prepare squash and pumpkin for human consumption (Saavedra *et al*., 2015). For use as a snack, the seeds are frequently baked. Even though pumpkin flesh is frequently stringy, it can be used to enhance the flavour of soups and pies. In the past, it was also combined with several different dishes, such as sauces, bread, beer, and pudding (Quinzio, 2013). Winter squash refers to squash that is harvested at the physiologically mature stage; therefore, the exocarp of summer squash can be cooked and consumed along with the flesh since they are softer (Saboo *et al*., 2013), while the winter squash skin and rind are inedible due to the hardened exocarp. Only the winter squash's flesh can be eaten when cooked.

Citrullus lanatus, often known as watermelon, belongs to the Cucurbitaceae family of cucurbits. Commercial crops are grown in regions with prolonged warm seasons without frost. Plants with long, trailing tendrils need to be spaced far apart (Wehner, 2008). The Bapedi and Venda tribes of South Africa refer to the watermelon plant as 'marotse', 'lerotse', 'motshatsha', or 'mutshatsha' (Mashilo *et al*., 2017). In the Sepedi language of South Africa, freshly collected leaves are referred to as 'motshatsha' or 'Morogo wa motshatsha,' even if dried leaves have traditionally been referred to as 'Kgwaile'. Fresh, luscious citron watermelon leaves are boiled with ripe tomatoes, salt, and other ingredients to make a meal known as motshatsha. It is typically consumed with sorghum or maize-based porridge. Citron watermelon leaves, known as 'kgwaile', are prepared (cooked as a green vegetable), sun-dried, and stored before usage as leafy vegetables (Mashilo *et al*., 2017).

Certain phytochemicals present in watermelon leaves give them a bitter taste (Kim *et al*., 2018); however, animals can be fed the dried vines (Hammer and Laghetti, 2007). The fully developed fruit is used to prepare many common dishes. The traditional cuisine of the Bapedi tribe in South Africa, for instance, is known and favoured and is referred to as 'kgodu', meaning 'melon'. The firm yellow or orange flesh is boiled until it is soft while preparing "kgodu". Then, a maize meal is added to make a porridge that can be enjoyed with soup, roasted watermelon seeds, or other foods. Moreover, 'mokgapu' is a soup produced by cooking meat with leaves and seasoning it with white or brown sugar. The fruit's seeds are extracted, roasted, salted, and eaten as a snack or with 'kgodu'. Red-brown, red, and white seeds are preferred for roasting because of their simple chewability, tasty seed with soft to medium-hard seed coats. White seeds have smooth seed coats, but maroon and black seeds have hard seed coats that make them difficult to chew and unsuitable for roasting (Ngwepe *et al*., 2019).

To be used as chicken feed, the raw and roasted seed is ground into a fine powder (Ngwepe *et al*., 2019). Both small and large livestock, such as donkeys, sheep, goats, pigs, and cattle, can be fed citron watermelon fruit as a feed addition. In particular during the dry winter season, when grazing and water are in short supply, this fruit supplies water and vital nutrients. Some of the citron watermelons' fresh roots are incredibly bitter. This might be because of natural phytochemicals such as phenolics, spingolipids, tetranortriterpenoid, and terpenoids, which could be useful in pharmacology and medicine. Little information is available regarding their pharmaceutical use (Ngwepe *et al*., 2019).

Roots of *C. lanatus* var. *lanatus* are ground into a powder by Bapedi traditional healers in the Limpopo Province of South Africa to treat tuberculosis (TB). Additionally, South African traditional healers mix cooked *C. lanatus* var. *lanatus* fruit with leaves of aloe species. The fact that watermelon is utilized in several unconventional ways in South Africa raises the possibility that the plant's edible parts contain important phytochemicals and minerals, thus the crop may be exploited to create nutraceutical and pharmaceutical products (Hammer and Laghetti, 2007).

1.2 Environmental factors affecting crop yield

The antioxidant concentration and organoleptic quality of the cucurbit fruit are influenced by environmental conditions. Plants produce antioxidant molecules to combat oxidative stress, which is caused by stressful circumstances, such as excessive light or heat (Martinez *et al*., 2021). Biotic (living organism) and abiotic (non-living organism) restrictions can be used to categorize environmental factors impacting cucurbit crop production. Actually, as a result of climate change brought on by global warming, these chemicals are present in higher concentrations. Abiotic stresses modify a plant's physiological, morphological, molecular, and biochemical processes and have a detrimental effect on growth and productivity. Soil parameters (physicochemical, soil pH, components, and biological), as well as climatic stresses (flood, drought, cold, heat stress, etc.) are examples of abiotic restrictions affecting the growth of cucurbit crops (Martínez *et al*., 2021). Biotic factors affecting the cucurbit crops on the other hand, include beneficial organisms (natural enemies of crops, decomposers of decaying organisms, and pollinators), pests (diseases, vertebrate pests, weeds, and arthropods), and humans.

1.2.1 Abiotic factors affecting yield variation among cucurbit crops

1.2.1.1 Effects of climatic conditions on cucurbit crops

Variations in average temperature, annual rainfall, the global increase of atmospheric CO_2, and variations in sea level are some of the major aspects of climate change that harm cucurbit crop yields (Raza *et al*., 2019). It is expected that changes in temperature and rainfall will have a significant negative impact on a variety of agricultural practices during the next few decades. Because of climate change, catastrophic weather events are making agriculture more difficult by drastically reducing food production. Due to their traditional selection for high yield rather than stress tolerance, plants are typically vulnerable to stress. Global warming is the cause of climate change (Raza *et al*., 2019).

It can indirectly, directly, and socioeconomically diminish crop production by up to 70% (Boyer, 1982) and has disastrous impacts on plant growth. Weather changes have both beneficial and bad effects on the environment, with negative consequences being particularly strongly expressed. Regression analysis of historical yield data for food crops over the past 30 years in Nepal revealed a mixed trend in precipitation and a rise in temperature of about 0.02-0.07°C each year in various seasons. The production of all crops was not significantly impacted by climate variables, and the regression analysis showed a negative correlation between summer precipitation and cucurbit.

1.2.1.2 Drought

Because the plants must deal with a very low water potential and resist salinity stress, oxidative stress, and drought stress are related (Seki *et al*., 2003). Cucurbitacin concentration was shown to be increased under drought stress, indicating these substances in drought resistance. A wide class of tetracyclic triterpenoids known as cucurbitacins are known for their diverse pharmacological activities, including free-radical scavenging capabilities and as antioxidants. The connection between various cucurbitacins and drought is not well understood (Mukherjee *et al*., 2013). Different cultivars of *Lagenaria siceraria* have been found to contain cucurbitacins E and I, and it is thought that they may have a role in how the plant responds to dryness. Plant cells experience oxidative stress when they lack water. Fan *et al*. (2017) discovered that drought stress increased the amount of malondialdehyde and hydrogen peroxide while decreasing the activity of antioxidant enzymes such CAT, SOD, and glutathione reductase. They treated cucumber seedlings with varied amounts of polyethylene glycol to subject them to various levels of water stress (Fan *et al*., 2017).

A drought happens when there is not enough water available from irrigation, rainfall, or both to meet the crop's evapotranspiration needs, according to Kumaraswamy and Shetty (2016). Changes in water availability (seasonal and volume distribution), as well as water demand from competing industries like agriculture, contribute to climate change. Abiotic forces such as temperature regimes and their impact on water availability change due to impending climate change, drought, an increase in the incidence of diseases and pests, and extreme weather events are known to result (Kumaraswamy and Shetty, 2016). Field agricultural productivity is reduced by 30–70% while under moisture or drought stress during the crop growing cycle. Under drought stress, an accumulation of ABA in guard cells can result in stomatal closure. Drought not only alters metabolism but also has an impact on plant growth and even has the potential to kill plants. Different stages of plant development are affected differently by drought, with the flowering stage being the most vulnerable (Li and Cui, 2014).

1.2.1.3 Floods

Plants are put through a variety of stressful scenarios based on the length and intensity of the inundation. Except for rice, which, like other wetland species, flourishes when plants are not completely submerged in water, soil waterlogging damages most crops. Flooding has increased in frequency in many lowlands and agricultural areas due to climate change, which harms people greatly and reduces crop and food production (Armstrong, 1979). Flooding frequently happens as a result of heavy rain, inadequate soil drainage, and ineffective irrigation techniques. The yield of crops is negatively impacted by soil waterlogging, particularly for dryland species, which include several crops. For crops that are not acclimated, the additional water causes complex physiological changes in the plants (Armstrong, 1979).

This leads to reduced gas exchange between the plant and its surroundings (low O_2 with high CO_2 ethylene in the root zone), hypoxia (where mitochondrial respiration is constrained by oxygen levels), and anoxia (where respiration is completely inhibited). These conditions are frequently accompanied by increased mobilization of "phytotoxins" in reduced soils. As a result, there is insufficient energy in plant cells, inadequate root metabolism (inability to absorb nutrients), and limited photosynthetic activity. The fundamental obstacle to plant growth in flood-prone areas is the abrupt lack of oxygen needed to maintain the aerobic respiration of submerged tissues (Voesenek *et al*., 2004).

1.2.1.4 Heat stress

Heat stress is the term used to describe temperature increases that remain long enough to permanently impair plant growth and development (Hasanuzzaman *et al*., 2013). The Intergovernmental Panel on Climate Change (IPCC) predicted that temperatures would increase by 3–4°C by 2050. Climate change-related high-temperature regimes have an impact on crop phenology, pollinator populations, reproductive biology, photosynthetic efficiency, flowering timing, and percentage of seed germination (Hasanuzzaman *et al*., 2013). Heat stress during the reproductive growth stage raises the temperature, which prevents pollen grains from growing and causes inadequate pollen to be released from the anther during dehiscence. Plant development is negatively impacted by heat stress, particularly the development and operation of reproductive organs. Furthermore, erratic temperature patterns could lead to unpredicted disease epidemics in many parts of the planet. Heat stress caused nearly 40% of the total yield loss in cucurbit crops, with maize suffering a daily yield loss of 1.0-1.7% for every degree over 30°C (Lobell *et al*., 2011).

1.2.1.5 Cold stress

Plants that experience cold or chilling stress between 0 and 15°C suffer significant agricultural losses. A wide range of tropical or subtropical crops can be harmed or killed by non-freezing low temperatures. Poor germination, chlorosis, growth retardation, or stunted seedlings, necrosis, wilting, and constrained leaf expansion are only a few of the signs these crops exhibit (Yadav, 2010). Plants often alter their pattern of gene expression and protein synthesis when exposed to cold temperatures. Plants from temperate temperature regions are often believed to be cold tolerant to varied degrees compared to tropical and subtropical crops. However, by cold acclimation, plants from these areas can improve their resistance to freezing (Yadav, 2010).

1.2.1.6 Soil property, soil salinity and acidity stress

The humus production, material mobility, and weathering of rocks are the main causes of the top layer of the earth's soils, or crust. Their physical characteristics, origins, personality quirks, and amount of production all vary. Soil fertility is the ability of the soil to supply the nutrients required for a specific crop's optimum growth. Soil fertility is one of the most important factors in crop productivity (Wang, 2014). It can sustain crop production depending on the full range of its biological, physical, and chemical characteristics. An essential component of soil productivity is soil fertility, which is a major source of the macronutrients (N, P, K, Mg, Ca, H, O, C, S) and micronutrients (Fe, Mn, B, Cl, Mo, Zn, Ni, Cu) required for plant growth (Wang, 2014).

The fertility of the soil can be assessed using a number of factors. According to research, the best indicator for improving sustainable land use management and achieving high agricultural productivity is the soil fertility index (Shang *et al*., 2014). In many regions of the world, croplands have suffered from soil degradation brought on by human activity, which has resulted in low yield production per area of harvested crops. Roughly 40% of agricultural regions are degraded due to human activity. Excessive use of fertilizers and chemicals, together with a disdain for agricultural sustainability, characterize intensive agricultural production, which leads to a loss of soil health, land degradation, and serious environmental problems. It is crucial to keep in mind that the loss of soil fertility often occurs over many years (Shang *et al*., 2014).

One of the most significant abiotic stresses and one of the most financially damaging to agriculture is salt stress. Because plants have a hard time capturing water and suffer from salt toxicity when they are under this kind of stress, their productivity is severely reduced. Reactive oxygen species are produced as a result of these two processes, and plants must adapt to this oxidative stress (Hasanuzzaman *et al*., 2018). Cucurbits have a wide range of tolerance to salt stress, with different species and even cultivars having varying levels of tolerance. In a study of three cucumber cultivars, responses to salt stress varied. Salt sensitivity typically results in reduced activity of the antioxidant enzymes glutathione peroxidase, sucrose ascorbate, and urate dehydrogenase in cucurbits as well as other plants (Erdinc, 2018).

In terms of crop productivity, salinity stress affects almost 30% of irrigated crops and 7% of dry-land agriculture globally (Schroeder *et al*., 2013). One of the main factors affecting agricultural production globally is salt, which affects 33% of irrigated lands and 20% of cultivated land. Salt causes ionic toxicity and osmotic stress in crop plants. Under typical conditions, the higher osmotic pressure in plant cells enables water and significant nutrients to be taken into the root cells from a soil solution (Schroeder *et al*., 2013). Nevertheless, under salt stress conditions, the high salt content of the soil solution inhibits water absorption and the uptake of necessary minerals but facilitates the entry of Na^+ and Cl^- ions into the cells, which will have toxic effects on cell membranes and metabolic processes in the cytosol.

The release of acidifying calcium, potassium, magnesium, and sodium ions, the nitrification of ammonia-based fertilizers, the breakdown of organic matter in the soil, the regeneration of organic acids, and poor land management techniques can result in a low soil pH. Low soil pH has a major negative impact on crop growth, which lowers output. For instance, soil acidity reduces production by up to 69% in maize (Tandzi *et al*., 2018).

1.2.2 Biotic factors affecting cucurbit crop yields

1.2.2.1 Weed management

Vegetable crops compete with weeds for soil nutrients, light, and space since weeds shade the crop, limiting its growth and eventually yield (Schonbeck and Tillage, 2011). Therefore, it's crucial to eliminate weeds while the crop is still in its early stages so that the crop canopy can grow and offer enough shade to hinder the growth of weeds. Research conducted outside of Australia indicates that weeds can significantly affect yield, particularly if infestations are severe during the crop's early growth stages. Weeds, according to one study responder, cause a crop yield drop of between 20 and 50 percent. Crop damage brought on by weed control operations and difficulty in collecting all the fruit in areas with heavy weed infestations are two specific effects of weeds on yield. Pests and diseases that affect the productivity and quality of vegetable crops can be carried by weeds. There is a lot of evidence that weeds, especially broadleaf weeds that resemble cucurbit plants in some ways, host a variety of viruses, diseases, and insect pests in cucurbit crops (Schonbeck and Tillage, 2011).

Overall, survey participants rated the effectiveness of their weed control plan as modest. The survey and field trip indicated that the most typical method for a specific crop includes the use of black plastic mulch, pre-plant herbicides, and early weed control in the inter-row before the crop vines have a chance to spread and be harmed by weed control activity (Sindel *et al*., 2012).

Depending on the grower's preferences, shielded spraying or cultivation may be used to control weeds between rows. To control larger weeds that may interfere with harvesting or those growing out of the crop holes in the plastic, chipping or manual weeding is used. Crop rotation is practiced largely for disease management benefits, with flexibility in weed control serving as a significant subsidiary benefit. To stop the spread of weed seeds, hygiene precautions are used. A successful weed control approach requires diligence. Growers may generally eliminate weeds before they establish seed before they are spread by cultivation or other activities, or from other properties, by taking a careful approach (Schonbeck and Tillage, 2011).

1.2.2.2 Diseases and pests

Plant diseases can be brought on by a variety of microbes, including viruses, fungi, and bacteria. Numerous soil-borne and above-ground insect pests affect crop productivity as well (Dresselhaus and Hückelhoven, 2017). The growth of illnesses is frequently encouraged by climate variation, which also harms soil fertility and plant productivity. Since there are fewer resources available to plants, they are unable to produce enough seeds, biomass, and thus, yield. Pathogens and pests can move due to migration brought on by the climate. Thus, the regionally adapted crop genotypes must cope with current biotic pressures. If plants interact with bacteria or chemicals connected to microbes, they may develop a resistance to secondary illnesses brought on by pathogens. This involves the synthesis and exchange of several low-molecular-weight plant metabolites, which are better understood in monocotyledonous plants, such as cereal crops, than in dicotyledonous plants (Dresselhaus and Hückelhoven, 2017).

According to predictions, climate change and variability will lead to the appearance of new diseases and pests as well as the possibility for current ones to become more virulent. The changing environment is bringing new forms of pests and diseases for which there are now no effective control measures (Shi *et al*., 2010). For instance, maize lethal necrosis (MLN) is one of the most serious diseases impacting maize in countries in Eastern and Central Africa. The maize chlorotic mottle virus (MCMV) and sugarcane mosaic virus (SCMV) combine synergistically to cause it. Depending on the timing of infestation, it reduces crop yields in farmers' fields by 30 to 100%. MLN is spread via rootworms, beetles, stem borers, thrips, several species of non-persistent aphids, contaminated soil, infected seeds, and any tools or materials used in the infected field (Durante *et al*., 2014).

It is pervasive in areas that cultivate cereals in Europe, the Middle East, Africa, Asia, North and South America, and most recently Australasia (Durante *et al*., 2014). Stunting wilting, necrosis, chlorosis, leaf striping with yellow, whitish, and, rolled leaves, purple longitudinal leaf lines, confined awns, and heads that do not develop are visual signs of infection in plants. These pests are extremely resilient to adverse weather conditions. RWA reduced wheat and barley yields by up to 80% and 100%, respectively. The main problem with the RWA is that an increasing number of biotopes are growing resistant to the pesticides that are now on the market. Certain biotopes also surpass the resistance of certain crop varieties. Furthermore, it has been discovered that the efficacy of different biotopes is impacted by rising atmospheric carbon dioxide (Durante *et al*., 2014).

1.3 Technological factors affecting cucurbit crop yield

Research programs have enabled a wide range of agricultural technological breakthroughs, including integrated nutrient and pest management, adaptive microbial technology, pesticides, farm machinery, fertilizer technology, and genetically modified varieties (Kumaraswamy *et al*., 2016). Eight kilograms of grain are said to be produced by 1 kg of nutrient fertilizer. Furthermore, as they can make up to 50% of the agricultural harvest product, fertilizers are typically considered essential to crop production (Shi *et al*., 2013). Nitrogen fertilizer increased 6.9 times and phosphorus fertilization increased 3.5 times in the 1990s, which helped to double world food production. Several factors negatively impact agriculture practices (Tilman *et al*., 2001).

Farmers in Bangladesh received chemical fertilizers and pesticides at discounted prices, which led them to use more fertilizer to maximize crop productivity. In the Philippines, excessive urea and lime used by farmers over many years led to the development of a lime layer in the subsoil, which led to a phosphorous deficit, while excessive potash usage by banana farms resulted in an unbalanced potassium and magnesium ratio. Jigawa State's average sesame yield production was reported to be 0.6 t/ha rather than 1.25 t/ha under well-managed farms (OLAM, 2007). Poor agronomic practices like improper plant spacing, late planting, improper planting technique, shallow sowing, ineffective disease and pest control, delayed weeding, inappropriate fertilizer use, late harvest, and the use of low-yielding varieties will, in general, result in a significant reduction in crop yields (OLAM, 2007).

1.3.1 Watermelon grafting and rootstock-mediated effects on fruit quality

To increase harvests, tolerate soil-borne diseases, and resist environmental challenges including salt and drought, horticultural grafting, which originated in Asia, has expanded around the world (Lee *et al*., 2010). The two most-commonly grafted cucurbit species are melons and cucumbers. Interspecific hybrids of *Cucurbita moschata* and *Cucurbita maxima* are the most often utilized rootstocks for watermelon and melon grafting; however, *Lagenaria siceraria* and wild *Citrullus* species are also used to graft watermelons. The most popular rootstocks for cucumbers are *Luffa cylindrica*, *Cucumis sp.* and interspecific hybrids of *Cucurbita* (Modarelli *et al*., 2020). When diverse species are grafted together to endure abiotic pressures such as salt and drought, the result is a higher activity of the antioxidant enzyme system (PX, CAT, SOD and other enzymes), as well as a lower level of ROS and other oxidative stressors like H_2O_2 or a lower concentration of MDA. Due to the upregulation of enzymes like guaiacol peroxidase, (GPOD), dehydroascorbate dehydrogenase (DHAR), SOD, and cucumber grafted on *Luffa* fared better under water stress (Modarelli *et al*., 2020).

Cucumber plants can resist the stress of high temperatures because of grafting. Concerning photosynthetic efficiency, grafted and self-grafted cucumber plants did not differ significantly from each other at 28°C, but at 42°C, grafted cucumber plants outperformed un-grafted and self-grafted plants. The authors hypothesized that the creation of antioxidant chemicals, as well as a larger production of polyamines and an increase in photosynthetic efficiency, were responsible for the cucumber seedlings' resistance to heat stress (Modarelli *et al*., 2020).

Grafting melon is also common. Cantaloupe melon plants were grafted onto *Cucurbita maxima* and grown under three different water stressors (20, 30, and 40 kPa) in an experiment to study the effects of water stress. The fruits of the grafted plants were firmer, contained more vitamin C, and had higher CAT and GPX activity at harvest, despite SOD activity being higher in the un-grafted plants. In essence, grafting enhanced the fruit's quality (Olguín *et al*., 2020). However, a recent study made the point that grafting itself does not increase fruit yield or fruit quality, therefore, nematode-resistant rootstocks for grafted melons are only required in nematode-infested soil. Therefore, it is anticipated that choosing the right rootstocks will be crucial to improving the fruit quality and antioxidant content of melon and other grafted crop species (Olguín *et al*., 2020).

Commercial watermelon production currently uses grafting onto rootstocks resistant to soilborne pathogens as an effective substitute for the prohibited use of chlorofluorocarbon soil fumigants, particularly in regions where intensive cultivation and a lack of available land prevent the implementation of broad rotation schemes (King *et al*., 2008). Additionally, grafting has been used to treat abiotic stress conditions such as organic pollutants, salinity, alkalinity, nutrient deficiency, water scarcity, acidity, thermal extremity, and heavy metal contamination. Interspecific hybrids of *Cucurbita moschata* (Duchesne ex Poir) and *Cucurbita maxima* (Duchesne) are the most frequently used rootstocks (King *et al*., 2008).

Watermelon grafting, which offers a productive method for trait stacking, has probably been motivated by resilience to biotic and abiotic stress conditions; by using suitable natural genetic resources, selecting independently for favourable rootstock features and desirable scion qualities while avoiding undesirable combinatorial or pleiotropic effects that impede breeding efforts (Brown and Caligari, 2011). Despite the convenience of breeding rootstocks and scions separately, rootstock-mediated impacts on scion fruit quality and widespread rootstock-scion interaction provide substantial hurdles for the watermelon industry's present widespread use of grafting. Although poor rootstock-scion compatibility may result in a loss in both yield and fruit weight, grafting watermelon onto robust *Cucurbita maxima, Cucurbita moschata*, and *L. siceraria* rootstocks can dramatically enhance yields by increasing the number of fruits per plant and unit fruit weight (Brown and Caligari, 2011).

Fruit weight increases are more common and frequently exceed 50% of non-grafted control fruit weight in genotypically large-fruited scions. Contrarily, it appears that grafting has little effect on fruit shape and that the genotype of the scion has a stronger genetic influence (Rouphael *et al*., 2008). The thickness of the fruit rind is a morphological characteristic that is impacted by grafting. When grafted onto strong interspecific and *L. siceraria* rootstocks, which may improve fruit storability, it typically has a small rind. Given that watermelon fruit skin thinning is linked to progressive ripening, rootstock-mediated rind thickening may, in part, result from delayed ripening seen on robust rootstocks (Soteriou *et al*., 2014). The pulp textural qualities have the most consistent impact on watermelon quality after grafting.

Grafting on interspecific *Cucurbita* across diploid and triploid scions increases pulp hardness. This is due to the development of more water-insoluble pectin portions in the cell walls of smaller, more numerous parenchymatic cells, as well as any potential osmotic cell pressure regulation by osmolytes like citrulline (Soteriou *et al*., 2014). Though such an effect typically enhances the postharvest performance and organoleptic quality of watermelon, it has the potential to produce an unfavourably hard texture in some circumstances, particularly when it comes to tiny (3 kg) triploid scions, such as the variety 'Extazy' (Soteriou and Kyriacou, 2015). Grafting on less popular rootstocks including *Cucurbita maxima*, *Cucurbita ficifolia*, *Cucurbita moschata* and *C. lanatus* var. *citroides* has also been linked to firmer pulp.

Only with *Cucurbita argyrosperma* as a rootstock and sporadically with *Lagenaria siceraria* rootstocks, rootstock-mediated reductions in pulp firmness have been observed (Zdemir *et al*., 2016; Bruton *et al*., 2009; Cushman and Huan, 2008; Davis and Perkins-Veazie, 2005). Morphological anomalies, such as a hollow heart or an overly rigid, yellow band (outside the fruit) and discoloured pith, which are infrequently noticed in watermelon fruit, are largely unrelated to commercially developed rootstocks. Rare reports on experimental grafting and some scion cultivars have been made (Kyriacou *et al*., 2017; Davis et al., 2008).

Contrarily, studies using the same rootstock types have not found any significant effects on glucose, sucrose, fructose, or total sugars, indicating that non-standardized harvest maturity and various cultural practices and climatic factors affecting fruit maturation and flowering events frequently lead to inconsistent results (Soteriou *et al*., 2014). Contrarily, because fruits from grafted plants had higher titratable acidity throughout the ripening process than fruits from non-grafted plants, the influence of harvest ripeness did not affect the effect of grafting on pulp acidity. Grafting onto interspecific rootstocks, principally *Citrullus lanatus* var. *citroides*, and *L. siceraria*, resulted in this increase in acidity, which was primarily seen as higher malate levels.

More research is required before a conclusive determination of the effects of grafting on this significant sensory aspect of watermelon quality can be reached, even though significant rootstock-mediated effects on the watermelon volatile profile have been identified. Although different effects were found among interspecific rootstocks concerning the levels of (Z)-6-nonenal (melon) and (E, Z)-2,6-nonadienal (cucumber), interspecific *Cucurbita* rootstocks increased several fruit aroma volatiles, including (E)-2-nonenal (fat, cucumber) and (Z, Z)-3,6-nonadien-1-ol (green, cucumber) (Fredes *et al*., 2016). The increase in (Z)-6-nonen-1-ol, the chemical in pumpkins that gives off a disagreeable stench, is a significant effect associated to interspecific rootstocks. Further verification of these findings under standardized maturity settings is required considering the greater quantities of lycopene breakdown products detected in the volatile profile of fruit from non-grafted plants, which hypothesizes that variations in harvest ripeness may be an underlying issue (Soteriou *et al*., 2014).

Watermelon fruit's lycopene content, which is often increased by interspecific rootstocks and less frequently by *L. siceraria* rootstocks, is the main way that grafting can increase the fruit's functional value (Soteriou *et al.,* 2014). The rise in lycopene, a lipophilic, membrane-bound carotenoid, may be related to the expansion of the cell wall brought on by grafting (Soteriou *et al*., 2017). The upregulation of catabolic genes and downregulation of scion carotenoid production genes in the case of wild watermelon and bottle gourd rootstocks suggests a rootstock-dependent effect on fruit lycopene accumulation. Lycopene, on the other hand, is extremely maturity-dependent and peaks in fruit from grafted plants approximately a week later, delaying the emergence of pulp colour (Soteriou *et al*., 2014). Only seldom have rootstock-scion combinations including *L. siceraria* and *Cucurbita argyrosperma* shown a decrease in lycopene content.

Citrulline, a bioactive non-essential amino acid, was more abundant in the watermelon fruit pulp derived from plants grafted onto interspecific rootstock during the ripening period together with lycopene (Soteriou *et al*., 2014). Citrulline is abundant in watermelon pulp and rind. Citrulline is an osmolyte with a potentially crucial role in pulp mechanical properties and osmotic cell expansion. It has been proposed that the build-up of citrulline in watermelon during drought conditions enhances the fruit's ability to reduce oxidative stress and scavenge free radicals (Soteriou *et al*., 2017).

1.3.2 Melon grafting and rootstock-mediated effects on fruit quality

Because grafting is effective against both biotic and abiotic stresses, it has become a widespread procedure for producing watermelon (*Citrullus lanatus*) in many parts of the world. Melon grafting is generally done to defend against Monosporascus and Fusarium wilts by using resistant *Cucumis melo* genotypes, *Cucurbita maxima* x *Cucurbita moschata* hybrids, and *Benincasa hispida* (white gourd) as rootstocks, as opposed to resistant *Cucumis metuliferus* and *Cucurbita melo ssp*. Due to the broad variety of melon genotypes, graft incompatibility and poor fruit quality are more prevalent concerns with melon than watermelon grafting (Soteriou *et al*., 2016). This diversity is related to the distinctive ripening physiology and aroma profiles of the many botanical melon varieties, which are essentially distinguishable as climacteric short-life aromatic varieties and non-climacteric long-life non-aromatic varieties (Soteriou *et al*., 2017).

Even though there is frequent rootstock-scion interaction, the feature of scion fruit weight is typically not altered by *Cucurbita* and *Cucumis melo* interspecific rootstocks. For example, grafting *Cucurbita* with muskmelon 'Proteo', while 'Energia' and 'Sting' rootstocks were not used, melo rootstocks 'Dinero' and 'Jador' caused a moderate increase in fruit weight (Zhao *et al*., 2011). The mass of the honeydew "Incas" fruit was unaffected by rootstock, neither melo rootstocks 'Griffin', 'Belimo', 'Energia', 'Sting' and 'ES Liscio′ nor by interspecific rootstocks 'AS10', 'P360', 'ES99-13' and 'Elsi'; nevertheless, it was moderately increased by interspecific hybrids 'RS841' and 'Polifemo'. The fruit weight of the climacteric Galia melons 'Elario' and 'Polynica' and the *ananas* melon 'Raymond' was unaffected by the interspecific hybrids 'TZ148', 'N101', 'Carnivor', and '30,900' (Soteriou *et al*., 2016). Strong *Tetsukabuto* and *Tosa*, interspecific hybrids of the *Shintosa* type, as well as some *Cucurbita melo* and *Shintoza* rootstocks that were grafted with muskmelon and honeydew, had no impact on the fruit weight of 'Athena' cantaloupe (Zhao *et al*., 2011).

Lesser-used rootstocks ‘Proteo’ and ‘Supermarket’ muskmelons’ fruit weights were unaffected by *Cucumis zeyheri*, *Cucumis metuliferus*, *Cucurbita maxima*, *Cucurbita moschata*, and *Cucurbita maxima* x *Cucurbita moschata* hybrids; nevertheless, 'Proteo' muskmelons' fruit weight dropped on *Benincasa hispida. Cucumis metuliferus* had no impact whatsoever on the fruit weight of the Galia '*Arava*' and Honeydew 'Honey Yellow' (Guan *et al*., 2014). According to research on a number of scions of the cantaloupe, honeydew, muskmelon, Galia, and ananas types, other fruit morphological features including pulp thickness, rind thickness, and shape, are typically unaffected by grafting and are strongly connected with the scion genotype. More frequently than watermelon, melon suffers from deterioration of fruit texture as a result of grafting (Soteriou *et al*., 2016).

Physiological incompatibility of varying degrees, sometimes latent and sometimes leading to plant mortality, was linked to the loss of pulp firmness seen in ananas and Galia type melons grafted on hybrid rootstocks (Soteriou *et al*., 2016). A widespread trend for decreased pulp firmness was seen in a variety of honeydew, muskmelon, and specialty melon cultivars that were grafted on interspecific hybrid rootstock. Additionally, muskmelon grafting on hybrids of the Shintosa type decreased pulp firmness in comparison to both self-grafted and non-grafted controls (Zhao *et al*., 2011). A loss in pulp hardness was seen in Galia-type melons grown organically in nematode-infested soil, but not in honeydew 'Honey Yellow' under the same circumstances (Guan *et al*., 2014). Contrary to the aforementioned findings, muskmelon grafted on *Cucurbita melo* and on hybrid *Cucurbita* rootstocks also showed an increase in pulp firmness after being grafted on interspecific 'P360' rootstocks for greenhouse cantaloupe.

Numerous studies showed that when the non-climacteric, inodorous melon 'Incas' was grafted on different interspecific hybrids and *Cucurbita melo* genotypes, grafting did not affect pulp firmness or dry matter content (Crin *et al*., 2007). Despite a decrease in total sensory rating, the same conclusion was reached for Galia '*Arava*' grafted on interspecific hybrids, *Cucurbita metuliferus*, and Piel de Sapo-type melons grafted on a genotype of *Cucurbita melo* subsp. *agrestis* and on interspecific hybrid RS841. Grafting appears to have a more nuanced impact on melon texture than it does on watermelon, where it tends to increase pulp hardness with time. Latent rootstock-scion incompatibility is a key indicator of this, and extensive genotypic variability concerning ripening behaviour further confuses matters (Kyriacou *et al*., 2017); however, melon grafting, regardless of rootstock type and cultural conditions, often has a negative or no influence on fruit firmness, as shown by the aforementioned research. Fruit sweetness is a crucial sensory attribute of melons, and like firmness, it is a frequent interaction between rootstock and scion that highlights the need to choose the optimum graft combinations (Liu *et al*., 2010).

Muskmelon underwent a minor (0.8°Brix) decrease in SSC while grafted on hybrid 'P360' cantaloupe saw a considerable (1.8°Brix) decline (Crino *et al*., 2007). Grafting the Piel de Sapo melon onto rootstocks that were interspecific hybrid and *Cucurbita melo* subsp. *agrestis* similarly reduced SSC to a commercially inconsequential degree. Muskmelons' reduced SSC was a result of grafting on *B. hispida* and *Cucurbita metuliferus*; however, Galia melon's reduced SSC and overall flavour resulted from grafting on the hybrids Strong 'Tosa' and 'Carnivor' rather than *Cucurbita metuliferus* (Guan *et al*., 2015, 2014). Increased fruit starch content and rootstock competition for soluble sugars have been linked to decreased fruit SSC in response to grafting, which is a frequent reaction (Liu *et al*., 2010); nevertheless, the extent of this decline is frequently immaterial to fruit quality and marketability as a whole (Kyriacou *et al*., 2017).

Furthermore, many studies have shown that grafting has no impact on melon SSC (Guan *et al*., 2014). According to Soteriou *et al*. (2016), only one of the four hybrid rootstocks onto which ananas melon was grafted, designated as "30,900", actually had an impact on the SSC, resulting in a 1.1°Brix decrease. Extensive field tests on hybrid rootstock 'Carnivor' with muskmelon, honeydew, and specialty melon kinds grafted on it revealed generally no difference in the SSC compared to self-grafted and non-grafted controls. As a result, graft compatibility has a significant impact on how grafting affects melon SSC, yet it frequently has little to no impact on overall quality and marketability. For honeydew and Galia, there have been a few isolated reports of SSC increases in response to grafting on *Cucurbita* hybrids (Soteriou *et al*., 2016).

Muskmelon and Galia grafting on hybrids or *Cucurbita melo* genotypes did not impact the titratable acidity of melon juice in previous investigations employing both odorous and inodorous scions, and an absolute rise of only 0.02-0.03% was seen on chosen hybrid rootstocks (Crin *et al*., 2007). Rootstock-mediated impacts on the scent profile of both odorous and inodorous melon species have not gotten as much attention as they did in the case of watermelon, even though they may be the most significant elements supporting assertions of a detrimental influence on overall sensory quality (Kyriacou *et al*., 2017). Lower levels of ester volatiles linked to *Cucumis melo*, *Cucurbita* hybrids, and rootstocks were produced in contrast to higher levels of non-critical alcohol and aldehyde volatiles like 1-octanol (fatty-green), 1-hexanol (flower-green), 2-methyl-1-butanol (fruity), (E)-2-butenal (green), ethanol (ethereal), and octanal (fresh lemon-green) (Kyriacou *et al*., 2017).

1.4 Agricultural input factors

To assure water availability for food production as well as competing human and environmental needs, water management and irrigation performance must be improved (Shah and Wu, 2019). Assessing how human activities affect soil fertility through the use of appropriate agricultural systems, such as the use of recommended rates and types of fertilizer, tillage, incorporation of farmyard manure or crop residues into the soil (which increases the supply of N, P, K, and other nutrients), and avoidance of sewage sludge irrigation are all necessary steps to increase crop productivity and sustainability. Through a long-term increase in the organic matter or physical characteristics of the soil, the use of these inputs encourages sustainable agriculture. According to Shang *et al*., (2014) little production variability and high crop yields can be reached in intensive cropping systems by increasing the integrated soil fertility quality index.

Cucurbit cultivation techniques have undergone extensive research due to their significance as prized traditional crops. The fertiliser dose and type used are the factors with the strongest influence on antioxidant content among all the techniques. In general, the antioxidant content declines as nitrogen fertilisation rates rise (Oloyede *et al*., 2012). As a result, the antioxidant content in the fruits was reduced by up to 30% when nitrogen concentrations in pumpkins exceeded 100 kg/ha. Other cucurbits, such as cucumber or melon, produced similar outcomes. The application of synthetic fertilizers was found to diminish the antioxidant content when nitrogen was readily available to plants, but plants acquire a larger concentration of flavonoids and other bioactive substances when nitrogen fertilisation is restricted (Kopczyńska *et al*., 2020).

As a result, compared to traditional farming methods, organic and low-input cucurbit crop farming systems, where fertilizers are of natural origin, were able to raise the level of antioxidants. As a result, Kopczyńska *et al*. (2020) discovered that under organic circumstances, the antioxidant content of zucchini fruits was increased, particularly in phenolic acids. Compared to conventional farming, organic melon production increased fruit antioxidant levels. When a watermelon is digested is used as a fertilizer in organic cucumber farming, it has been shown to increase the content of flavonoids and phenols as well as other anti-inflammatory, antioxidant, and antitumor compounds like neo-hesperidin and hesperidin as well as others like narirutin and naringin, which lower the risk of cardiovascular diseases (Kopczyńska *et al*., 2020).

1.6 Plant nutrition

The cucurbits are a family of relatively healthy foods. Cucumbers in particular have become important as "diet food". They contain only a few calories, a small amount of fibre, and 96% water (Saboo *et al*., 2013). Cucurbits offer a rich supply of vitamins A, C, and K in addition to having a lot of potassium. Numerous healthy nutrients and minerals are present in pumpkins. They have high concentrations of magnesium, phosphorus, thiamine, iron, and vitamin B6. They have even higher concentrations of copper, potassium, manganese, vitamin C and vitamin E. Additionally, pumpkin flesh is a good source of dietary fibre. One hundred grams of pumpkin flesh provides 148% of the daily value for vitamin A, which is a very high amount to find in pumpkins. The pumpkin's seeds are a further source of vital nutrients. Despite being relatively high in fat, they compensate for it by having higher levels of magnesium, zinc and protein (Saboo *et al*., 2013).

One of the most important preharvest determinants for crop productivity and nutritional quality is plant nutrition (Wang *et al*., 2008). Nitrogen (N) fertilizer has been the most successful method for raising crop productivity over the last forty years, particularly for vegetables. Excessive N application tends to reduce the amount of vitamin C, carbohydrates, and minerals (such as calcium and magnesium) in many fruits and vegetables (Lee and Kader, 2000). Since N-fertilization typically increases plant growth, a relative dilution effect may occur in various plant tissues, which has been largely blamed for the decline in nutritional quality (Lemaire *et al*., 2008). By increasing N fertilization, notably at 120 kg ha^{-1}, nutritional quality indicators of melon fruit 'Proteo', such as dry matter content and SSC, were severely impacted.

Other research has, however, shown that increasing the N fertilization rates from 80 to 240 mg/L, from 11 to 393 kg ha^{-1}, and from 0 to 250 kg ha^{-1} had no appreciable effects on the sugar content of Galia, Piel de Sapo, or watermelon 'Top Gun', respectively. The various melon and watermelon cultivars employed and the various settings in which the plants were produced may both provide explanations for this discrepancy (Wang *et al*., 2008). Squash and pumpkins have many of the same nutritional qualities because of how similar their chemical structures are. Depending on the type and age of the squash, the nutritional contents change. The mature winter squashes have substantially higher potassium and vitamin A levels than the physiologically immature summer squashes.

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Conclusion

Cucurbit crops provide minerals, vitamins, dietary fibre, and antioxidants, and are becoming widely acknowledged as important components of a balanced diet in many nations. Cucurbits are also more than fruit vegetables. Citron watermelon is utilized in several unconventional ways in South Africa which raises the possibility that the plant's edible parts contain important minerals and phytochemicals with beneficial health benefits and may be exploited to create nutraceutical and pharmaceutical products.

C. lanatus var. *lanatus* roots are ground into a powder by Bapedi traditional healers in the Limpopo Province of South Africa to treat tuberculosis (TB). Additionally, to treat TB, South African traditional healers mix cooked *C. lanatus* var. *lanatus* fruit with other aloe species.

Cucurbit crop production can be affected by abiotic factors. Actually, as a result of climate change brought on by global warming, these elements are more pronounced. Abiotic stresses modify a plant's physiological, morphological, molecular, and biochemical processes and have a detrimental effect on growth and productivity. The soil parameters (physicochemical, soil pH, components, and biological qualities), as well as climatic stresses (flood, drought, cold, heat stress, etc.), are examples of abiotic restrictions. Conversely, biotic factors include beneficial organisms (natural enemies, decomposers, and pollinators), pests (diseases, vertebrate pests, weeds, and arthropods), and anthropogenic evolution.

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Phosphorus is found in urine. They are mostly discharged into the environment, where they significantly disturb the biogeochemical cycles of the planet. These nutrients could be utilized in

Agriculture, if they were separated at the source. Phosphorus recovery and recycling from wastewater is becoming more popular due to engineered solutions that address the seriousness and urgency of the problems. Diversion and recycling of human urine is one of the solutions; it is a practical and energy-efficient way to recover phosphorus from wastewater. Recycling human urine as a fertilizer for crops improves the sustainability of food production.

This study aims to gain knowledge on factors that may be associated with yield variation among citron watermelon genotype in South Africa, and whether this variability is associated with morphological and physiological traits, including root characteristics. Phosphorous deficiency is a limiting factor under acidic conditions. Research is needed to determine, if the use of urine-derived fertilisers could improve P solubility and increase root growth and nutrient uptake in acidic soils. The study seeks to understand if the use of urine-based fertiliser sources, such as struvite, can improve P solubility in acidic soils and lead to increased root growth, nutrient uptake, and yield performance.

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CHAPTER 2: Morphological factors associated with yield variation among citron watermelon (*Citrullus lanatus* var. *citroides* (L. H. Bailey) Mansf. ex Greb.) genotype in South Africa

2.1 Abstract

In the Cucurbitaceae, the species C*itrullus lanatus* var. *citroides* (L. H. Bailey) Mansf. ex Greb. is used for food and feed in southern Africa. In South Africa, there is a significant variation in yield across the grown citron watermelon genotypes. This variation needs to be thoroughly investigated in the development of cultivars for various applications, such as plant breeding, crop production or livestock feed. The objective of this study was to assess morphological criteria associated with yield variation among citron watermelon genotypes in South Africa and to recommend desirable genotypes that are drought-tolerant and with desirable fruit traits to be used in future crop production and breeding. The study was designed as a 6 x 2 x 3 factorial experiment with the following factors: genotype - 6 levels (WWM-1, WWM-16, WWM-30, WWM-31, WWM-35, and WWM-37), drought condition - 2 levels (non-stress (NS) and drought-stress (DS)); and phosphorus source - 3 levels (struvite, single superphosphate (SSP), and no fertilizer). The six genotypes were planted in a completely randomized design (CRD). Data on key quantitative traits (leaf traits, vine length, root traits, and fruit mass) were collected, and subjected to ANOVA using GenStat 20th edition.

The genotypes showed wide variation and unique traits that could be selected to genetically improve the cultivars. No significant differences (P>0.05) were found in leaf traits (leaf length, leaf width and leaf area) with all levels of P source (struvite, SSP and control) under NS and DS. In terms of leaf characteristics (leaf length and leaf width), SSP treatment resulted in better fruit yield than struvite treatment, under NS. In contrast, struvite-treated genotypes had a reduced leaf area than both the SSP-treated and control genotypes. Urine-based fertilizer (struvite) application had similar vine length to the SSP treatment under both NS and DS. This shows that among all genotypes, struvite application enhanced vine growth under both NS and DS.

Under DS, both struvite and SSP effectively stimulated root growth (root dry mass and root length). Regarding the two root characteristics (root dry mass and root length), a significant difference (p<0.05) was found under both NS and DS. These larger and longer roots, brought about by the struvite treatment, allowed plants to take up more water, potentially resulting in increased yields.

Higher coefficients of variation were found for fruit attributes, such as fruit mass. The high-yielding citron watermelon genotypes WWM-1, WWM-35, and WWM-37 are advised for use in animal feed and fruit production for human use. High marketable fruit yields and high fruit numbers per plant were obtained by the genotypes WWM-1, WWM-30, and WWM-35, which are, hence, recommended as parental genotypes in breeding. The present study recommends the citron watermelon genotypes WWM-30, WWM-35, and WWM-37 for use as leafy vegetables due to their long vines and high branching ability, whereas the genotypes WWM-1, WWM-30, WWM-35, and WWM-37 are recommended for food consumption, as these produce larger fruit. The study also recommends the orange-fleshed genotype (WWM37) for cooking, fresh consumption, processing, or future breeding efforts, since the genotype responded positively to struvite treatment. In conclusion, the study highlights the potential to use urine-based fertiliser, such as struvite, to enhance above-ground growth, root growth and yield, which is possibly brought about by the better P solubility compared with producing citron watermelon in acidic soils.

Keywords: Citron watermelon, yield variation, morphological factors, P soils, root characteristics

2.2 Morphological factors associated with yield variation among citron watermelon genotypes

Citron watermelon, a diploid (2n = 2x = 22) monoecious vining crop, is a member of the Cucurbitaceae. The market value of citron watermelon is determined by requirements (production and transportation inputs) along the value chain, as well as by consumer preferences (Houessou *et al*., 2022). Seedlessness, sweet flesh, large fruit size, a specific fruit shape (round, elongated, and oblong), light to dark green exterior fruit colour, and striped or non-striped fruit patterns are some of the characteristics of citron watermelon fruit that certain markets prefer. Additionally, the watermelon breeding program, North Carolina (NC), incorporates the following quantitative and qualitative traits: high number of fruits per plant, high leaf biomass, early flowering, certain fruit mass and size, high lycopene and sugar content of the fruit flesh, high rind and flesh firmness, diverse seed coat colour, as well as a red fruit flesh colour are some of the characteristics targeted in the breeding process of watermelon. Thus, sufficient genetic variation is necessary to satisfy the demand for the aforementioned qualities to produce diverse citron watermelon types that are desired by consumers or needed in the value chains (Ngwepe *et al*., 2023).

Certain features, including leaf shape, fruit colour and shape, rind stripe patterns, seed shape, seed coat colour and flesh colour make up the phenotypic diversity of the crop (Guzzon *et al*., 2017; Mashilo *et al*., 2016; Achigan-Dako *et al*., 2015). There are two citron watermelon leaf types: lobed and non-lobed. According to Laghetti and Hammer (2007), there are different leaf lobation degrees, ranging from tri- to penta-lobate with broad, smooth, circular lobes or small, serrated lobes. A distinct physical characteristic of standard citron watermelon is its non-lobed leaf shape, although the majority of other melon types (such as citron watermelon and sweet desert melon) have lobed leaves (Wei *et al*., 2017). This lobed leaf structure of desert watermelon is controlled by a single dominant gene, CILL1 (Wei *et al*., 2017).

Citron watermelon fruit appear in three different shapes: elongated elliptic, elliptic, and broad elliptic; fruit are dark green or bright green in colour. This crop also has a range of rind stripe patterns, from none to spotty, narrow, medium (medium sized stripe), or reticulate stripes that can either be green or dark green in colour (Mashilo *et al*., 2016). The colour of the flesh varies from green-white for non-cooking varieties, known as "kiti" in the indigenous Sepedi language of South Africa (Achigan-Dako *et al*., 2015; Guo *et al*., 2015), to orange and yellow in cooking varieties (Mashilo *et al*., 2016; Achigan-Dako *et al*., 2015).

The ability of a crop to tolerate heat and drought is attributed to its ability to produce sufficient above-ground biomass to support the development of fruit and seed yield under conditions of water deficit (Guzzon *et al*., 2017). These strategies also allow the crop to avoid heat stress and survive untimely and/or insufficient precipitation. Accumulation of certain metabolites, such as glutamate, citrulline, and arginine, as well as modifications in the expression of oxidative stress-related genes (such as ascorbate peroxidase, glutathione peroxidase, and glucose-6-phosphate-dehydrogenase) are among the mechanisms of citron watermelon's resistance to drought (Akashi *et al*., 2002). Due to its extreme drought tolerance, the crop has become known as a viable rootstock for a sweet citron watermelon (*Citrullus lanatus* var. *lanatus*), particularly when grown in water-limited areas, with the goal of increasing fruit yield and quality under such conditions (Seymen *et al*., 2021; Yavuz *et al*., 2020).

One of the main elements needed for high crop yield is phosphorus. Application of phosphorus (P) fertilizer, can, however be problematic, due to P immobilization (crop residues are incorporated in the soil), because of soil alkalinity. The applied fertilizers' phosphorus may change into a form that is not available to plants to absorb (El-Dahtory *et al*., 1989). Furthermore, from the economical point of view, high prices of such fertilizers may increase the production costs of the crop. A practical and energy-efficient method of recovering particularly phosphorus, but also nitrogen and potassium from wastewater is to divert and recycle human urine. This method allows obtaining nutrients needed in the manufacture of agricultural fertilizers (Kalmykova *et al*., 2012). Phosphorous deficiency is a limiting factor under acidic conditions. This research was carried out to determine, if the use of urine-derived fertilisers (struvite) can improve P availability and increase root growth and yield performance of citron watermelon.

Understanding the morphological characteristics that cause variation among citron watermelon genotype under water stress is, at best, poorly understood. This study, therefore, aimed to gain knowledge on morphological factors, including root characteristics, that may be associated with yield variation among citron watermelon genotype in South Africa under drought-stress conditions in acidic soils.

2.3 Materials and methods

2.3.1 Plant material

Six citron watermelon genotypes (WWM-01, WWM-16, WWM-30, WWM-31, WWM-35, and WWM-37) were used in the study (Figure 1). These genotypes were obtained from the Limpopo Department of Agriculture and Rural Development in Modjadjiskloof, South Africa. These chosen genotypes, used by smallholder farmers in South Africa, are frequently under stress due to extended heat and drought and are, hence, morphologically and genetically different from each other, because they have been grown by various smallholder farmers in rain-fed environments (Ngwepe *et al*., 2021; Mashilo *et al*., 2017). These selected genotype exhibit variation in several traits, including seed coat colour, flesh colour, fruit shape, exterior fruit colour, and rind stripe patterns. Figure 1 depicts seeds of the selected citron watermelon genotype.

Figure 1: Seeds of six citron watermelon genotypes used in the study (obtained from the Limpopo Department of Agriculture and Rural Development, Modjadjiskloof, South Africa)

2.3.2 Struvite processing

Struvite, a phosphorus-rich urine-derived solid precipitate, was made using source-separated urine in a straightforward reactor in a field setting. Magnesium sulphate ($MgSO_4$) was added to the urine obtained from Pietermaritzburg community (using containers) to create struvite (magnesium ammonium phosphate hexahydrate). Since the liquid obtained from the pit latrines is mainly urine (urine diversion toilets (Larsen and Gujer, 1996), it contains a lot of waste (human excretion). Thus urine-containing liquid can, hence, be used as a source for the manufacturing of fertilizers (Larsen and Gujer, 1996).

According to Cordell *et al*. (2009), phosphorus (P), which is an essential macro-nutrient for crop growth and development, is directly tied to food security. However, if over-applied, P can pollute groundwater and surface waters, resulting in eutrophication.

2.3.2.1 Urine source and storage

Urine was collected from different people from Pietermaritzburg community, using containers, for three weeks to obtain 20 L. In a typical pit-latrine setup, soak-away pits would be used to dispose of the urine. At a field location, collected urine was mixed from different containers, into a 20 L container and stored. Due to the urine's naturally high magnesium and calcium content, phosphorus salts spontaneously precipitate when urine is stored (Udert *et al*., 2003). The urine was, however, contaminated with certain debris (e. g., cigarette butts (Karak and Bhattacharyya, 2011).

2.3.2.2 Magnesium source

Industrial grade magnesium sulphate heptahydrate ($MgSO_4 \cdot 7H_2O$) was selected as the magnesium source, as it is available locally in South Africa (Strathmore Mine, Malelane, Mpumalanga, SA).

2.3.2.3 Simple struvite reactor design

The construction of a simple, portable, manually controlled 40 L struvite reactor was based on Etter *et al.* (2011). The reactor had the following measurements: a diameter of 300 mm, a height of 840 mm, and a freeboard of 160 mm. Four vertical baffles and two mixing impellers were installed in the reactor to improve mixing (Figure 2). The mixing crank was free-moving and simple to handle due to the bearing ring arrangement on the mixing shaft. The reactor's content was drained between reaction cycles using a manual valve. A concrete platform with a slope and a drain was used to support the reactor. The sanitary sewer received the effluent's drainage. After each use, the reactor was rinsed with tap water.

Figure 2: Manual, simple, controlled struvite reactor, containing dual impellers; vertical baffles in interior, mixing tank, mixing handle and isolation valve in exterior (Hadebe, 2022)

2.3.2.4 Magnesium dosing strategy

Using the historical average measurement of 280 mg/L P as the stoichiometric quantity of P, dissolved magnesium was injected at a 10% excess (Kumar and Pal, 2015). It would be necessary to measure the incoming phosphate concentration to create the dosing solution to provide a magnesium (Mg) dose tailored to each batch of stored urine. This strategy would have been in opposition to the goal of creating straightforward techniques.

The reactor operators assisted in the establishment of the dosing process. Twenty litres of tap water were mixed with four measuring cups (1 000 ml or 980 g) of magnesium sulphate in a mixing tank with a valve. The preparation of the dosage solution occurred before processing of the struvite. Four 20-litre batches of urine were mixed with magnesium solution (0.5 L).

2.3.2.5 Process sequence

A submersible pump was used to pump urine into the reactor until the solution reached the 20 L mark, thereafter, 0.5 L of the magnesium dosing solution was filled into the vessel's top. After mixing, the valve was opened, allowing the mixture to drain into a filter bag (which separate dry matter from the liquid). To assess phosphorus recovery and the amount of struvite that the filter bag was unable to catch, wastewater (the unwanted liquid) was typically discharged. The liquid had drained out of the filter bag in about 30 minutes. To speed up the draining process, pressure was applied to the outside of the filter using a mixing handle (Etter *et al*., 2014).

2.3.2.6 Calculations

Mg: P dosage ratio

The amount of magnesium dissolved during the experiment was divided by the initial mass of phosphate in the urine to determine the molar Mg: P dose ratio.

$$\text{Mg: P dosage ratio} = \frac{\text{mMg; dissolved (t)}}{\text{mP (t0)}} \text{ x } \frac{\text{mp}}{\text{mMg}}$$

The available phosphorus content was calculated using the formula according to (Dijk, 2007):

Where mMg = Magnesium mass, and mP= Phosphate mass

2.3.3 Experimental design and crop establishment

The experiment was conducted at the Controlled Environment Facility (CEF) of the University of KwaZulu-Natal, Pietermaritzburg, South Africa (29◦37′37.5″ S and 30◦24′ 10.4″E). The study was designed as a 6 x 2 x 3 factorial experiment with the following factors: genotype - 6 levels (WWM-01, WWM-16-WWM-30, WWM-31, WWM-35, and WWM-37), drought condition - 2 levels (no-stress (NS) and drought-stress (DS)) and phosphorus source - 3 levels (struvite, single superphosphate (SSP) and control (No P)), with four replications per combination, giving a total of 144 experimental (30 cm thermoform) units laid out in a completely randomized design (CRD) (Figure 16).

Each genotype's seeds were placed in a separate pot containing Gromor® Potting Mix (Gromor®, Cato Ridge, South Africa). Each genotype's plant pot (10 L pot) was randomly placed (6 pots per row) onto raised benches inside the plastic tunnel (tunnel L2). One seed for each genotype was placed into each pot (144 pots) with Gromor® (8 L Gromor® per pot). Based on the P requirements of citron watermelon (30 mg P pot^{-1}), the potting mix that contained 20% of P was amended withstruvite and/or SSP (single superphosphate) fertilizer. Each genotype's plants were subjected to well-watered NS or DS plants until at least one or two male flower buds appeared. This timing corresponded to the development of six to ten fully expanded leaves. To maintain the soil water potential, NS plants were continually irrigated to 100% FC, whereas DS plants were irrigated to 50% FC according to Thameur *et al*. (2012). Under NS, 6 L of water was applied in every 24 hours, and under DS, 3L of water was applied in every 24 hours until maturity stage.

2.3.4 Data collection

After planting, all 144 pots, both DS and NS, were irrigated for 30 days from planting. After 30 days, drought stress was imposed. Using a tape measure to record leaf traits (leaf length and leaf width) and vine length, and the Easy Leaf Area Free (ELAF) software (https://github.com/heaslon/Easy-Leaf-Area, University of California, California, CA, USA), quantitative measurements of leaf length, leaf breadth, seed length, and root length, were recorded. While whole plant and fruit attributes were assessed only after harvest, leaf characteristics were recorded throughout the vegetative growth stage, from selected leaf per plant. Following a descriptor list for citron watermelon (IPGRI 2003; UPOV 2004), qualitative data (phenotypic traits) were gathered during the seedling, flowering and mature fruit stages (i.e., until about 120 days after planting). Fruit mass was measured using a precision scale (UW4200H Shimadzu, Kyoto, Japan) for all treatments at harvest. The root system was carefully cleansed of the medium before being oven-dried at 70ºC to a constant mass and dry mass was recorded.

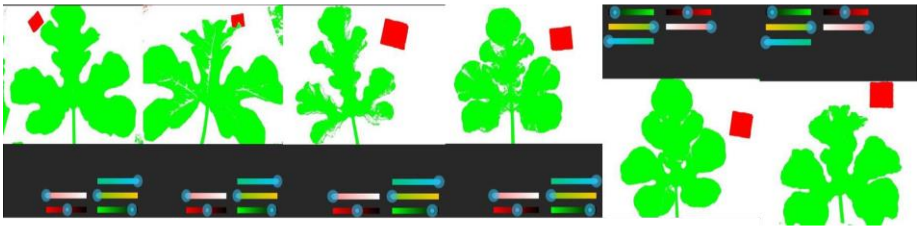

Figure 3: Leaf area of citron watermelon leaves measured with the erase background option selected after selecting the greenest and reddest pixels in the simple leaf area free (ELAF) program (red square equivalent to 2.5 cm^2), with the bars underneath showing delete background options

2.3.5 Statistical analysis

GenStat 20th edition (VSN International, Hemel Hempstead, UK) was used to carry out the analysis of variance (ANOVA) on averaged data from citron watermelon genotype (leaf traits, root traits, vine length, and fruit traits). When treatments indicated significant variations in the measured parameters at 5% level of significance, the means were separated using Fisher's protected least significant difference (LSD) test using XLSTAT software (Data Analysis and Statistical Solution for Microsoft Excel, Addinsoft, Paris, France, 2017). Agglomerative Hierarchical Clustering (AHC) was performed using drought tolerance indices following Ward's technique using the squared Euclidean distance to quantify similarities. Using XLSTAT, tolerant and susceptible genotypes were identified by principal component analysis (PCA) and biplot diagrams. With the use of GraphPad Prism Version 9.2.0 (GraphPad Software, San Diego, CA, USA), Pearson correlation heatmaps were created based on mean values.

2.4 Results

The evaluated quantitative traits showed no significant differences (P > 0.05) across the genotypes under NS, during vegetative until maturity stages, with leaf length ranging from 3.47 to 21.18 cm, leaf width ranging from 3.20 to 27.90 cm and leaf area ranging from 5.25 to 16.32 cm (Table 1). No significant differences (P> 0.05) were detected across the genotypes under DS, with leaf length ranging from 3.57 to 17.17 cm, leaf width ranging from 3.20 to 23.0 cm and leaf area ranging from 13.60 to 22.90 cm (Table 2).

Table 1: Means of leaf traits of citron watermelon genotypes irrigated to field capacity (NS)

Genotype	Control			Struvite			SSP		
	LL	LW	LA	LL	LW	LA	LL	LW	LA
WWM-1	3.63[a]	4.10[a]	13.52[a]	18.35[a]	21.52[a]	7.35[a]	20.88[a]	27.90[a]	5.65[a]
WWM-16	3.47[a]	3.20[a]	16.32[a]	17.23[a]	24.35[a]	5.25[a]	15.70[a]	22.55[a]	5.60[a]
WWM-30	3.67[a]	4.83[a]	14.25[a]	16.40[a]	21.05[a]	7.95[a]	16.80[a]	22.85[a]	7.10[a]
WWM-31	4.00[a]	6.35[a]	13.30[a]	17.30[a]	21.40[a]	7.55[a]	17.78[a]	22.58[a]	6.60[a]
WWM-35	4.30[a]	4.53[a]	15.50[a]	18.83[a]	24.77[a]	6.45[a]	18.33[a]	16.60[a]	6.02[a]
WWM-37	4.46[a]	5.0[a]	14.12[a]	19.55[a]	24.95[a]	7.40[a]	21.18[a]	26.27[a]	4.65[a]
LSD	0.874	1.500	1.598	3.745	5.330	7.950	3.563	3.540	5.109
P-value	0.092	0.886	0.644	0.352	0.378	0.566	0.386	0.013	0.461

LL: Leaf length (cm), **LW**: Leaf width (cm), **LA**: Leaf area (cm^2)

No significant differences (P> 0.05) were detected across the genotypes under DS, with leaf length ranging from 3.57 to 17.17 cm, leaf width ranging from 3.20 to 23.0 cm and leaf area ranging from 13.60 to 22.90 cm (Table 2). Struvite tended to increase leaf length and leaf with of citron watermelon genotype compared to SSP and control.

Table 2: Means of leaf traits of citron watermelon genotypes grown under DS (drought-stress)

Genotype	Control			Struvite			SSP		
	LL	LW	LA	LL	LW	LA	LL	LW	LA
WWM-1	3.83[a]	4.10[a]	22.67[a]	15.60[a]	20.57[a]	15.30[a]	16.13[a]	18.80[a]	15.37[a]
WWM-16	3.60[a]	3.20[a]	22.60[a]	14.93[a]	19.47[a]	15.77[a]	17.17[a]	22.70[a]	15.03[a]
WWM-30	3.57[a]	3.70[a]	22.60a	17.17[a]	21.03[a]	14.57[a]	16.23[a]	19.70[a]	14.70[a]
WWM-31	3.97[a]	3.50[a]	22.47[a]	16.83[a]	19.40[a]	14.57[a]	16.80[a]	20.20[a]	15.23[a]
WWM-35	3.70 [a]	3.33[a]	22.60[a]	15.60[a]	18.67[a]	15.17[a]	14.80[a]	16.60[a]	15.70[a]
WWM-37	3.73[a]	4.30[a]	22.90[a]	16.77[a]	23.00[a]	13.97[a]	9.67[a]	13.60[a]	17.40[a]
LSD	0.686	1.181	1.187	3.403	2.647	1.747	5.967	9.16	3.698
P-value	0.792	0.322	0.977	0.649	0.046	0.320	0.138	0.390	0.660

LL: Leaf length (cm), **LW**: Leaf width (cm)**, LA**: Leaf area (cm^2)

2.4.1 Leaf length under different P treatments

No significant differences (P>0.05) between treatments were observed with respect to leaf length of the citron watermelon genotype under NS. Leaves of the genotypes WWM-35 and WWM-37 in the control, subjected to NS conditions (Table 1) averaged 4.30 to 4.46 cm, respectively. Genotype WWM-1 and WWM-16 had leaves ranging from 3.63 and 3.47 cm, respectively in the control, under NS. The longest leaves in the struvite treatment were ranging from genotype WWM-37 (19.55 cm), followed by WWM-35 (18.83 cm). The shortest leaves were from WWM-30 (16.40 cm) plants with struvite treatment. In the SSP treatment, leaves were from the WWM- 37 (21.18 cm) genotype, followed by WWM-1 (20.88 cm), with the shortest leaves on WWM-16 (15.70 cm). Overall, struvite treatment tended to increase leaf length.

There was no significant difference between treatments (P>0.05) with respect to the leaf length of citron watermelon genotypes under DS. Leaves were averaged on the genotype WWM-31 (3.97 cm) under control (no fertilizer), subjected to a DS (Table 2), followed by WWM-1 (3.83 cm), under the same condition (control, subjected to DS). Genotype WWM-30 had the average leaf length of 3.57 cm under control. The average of leaves in the struvite treatment on WWM-30 were17.17 cm, followed by WWM-31 (16.83 cm) ones. The shortest leaves grew on WWM-16 (14.93 cm) plants with struvite application. When SSP was used, leaves ranged from the WWM-

16 genotype (17.17 cm), followed by WWM-31 (16.80 cm), with the leaves ranged from WWM-37 (9.67 cm). Generally, DS tended to reduce leaf length.

2.4.2 Leaf width under different P treatments

There were no significant differences ($P>0.05$) between treatments concerning final leaf width of all citron watermelon genotypes. Leaves of the genotype WWM-31 were averaged (6.35 cm) under control (without fertilizer), and when grown at field capacity (Table 1), followed by genotype WWM-37 (5.0 cm), while genotype WWM-16 had the leaves averaged on (3.20 cm) under NS. Amongst the genotypes, WWM-37 bore the widest (24.95 cm) leaves in the struvite treatment, followed by WWM-35 (24.77 cm), also in the struvite treatment. The leaves ranged from WWM-30 (21.05 cm) in the struvite treatment. In the SSP treatment, the leaves were ranging from the WWM-1 genotype (27.90 cm), followed by WWM-37 (26.27 cm), with WWM-16 having the (22.55 cm). Struvite treatment tended to increase leaf width in the control.

There were no significant differences between treatments ($P>0.05$) concerning the leaf width of citron watermelon genotypes under DS. Genotype WWM-30 had the widest leaves (4.30 cm) under the control (Table 2), followed by WWM-1 (4.10 cm). The narrowest leaves were in WWM-16 (3.20 cm). The widest leaf in the struvite treatment was in the WWM-37 (23.00 cm), followed by WWM-30 (21.03 cm). The widest leaves were measured in the WWM-35 genotype (18.67 cm) from the struvite treatment. In the SSP treatment, the widest leaves were from the WWM-16 genotype (22.70 cm), followed by WWM-31 (20.20 cm), while WWM-35 produced the narrowest leaves (13.60 cm). DS tended to reduce leaf width, but the effect was not statistically significant.

2.4.3 Leaf area under different P treatments

There were no significant differences ($P>0.05$) between treatments with respect to leaf area among the citron watermelon genotypes. The largest leaf area was observed in the WWM-16 genotype (16.32 cm^2) subjected to no stress (Table 1), followed by genotype WMM-35 (15.50 cm^2) of the control. Genotype WWM-31 had the lowest leaf area (13.30 cm^2), while the highest leaf area was on plants of the WWM-30 genotype in the struvite treatment (7.95 cm^2), followed by WWM-31 (7.55 cm^2) with the same P source. The lowest leaf area was recorded for the WWM-30 genotype in the struvite treatment (5.25 cm^2). For the SSP treatment, the largest leaf area was determined in genotype WWM-30 (7.10 cm^2), followed by WWM-31 (6.60 cm^2), with the lowest leaf area in the WWM-37 genotype (4.65 cm^2). Struvite and SSP treatments tended to show a decreased leaf area under NS. Mandizvo *et al*. (2022) noted that citron watermelon genotypes' decreased leaf area is an indication of their adaptable phenotype and effective drought-avoidance technique.

There were no significant differences between P treatments ($P>0.05$) concerning citron watermelon leaf area among the genotypes under DS. Genotype WWM-37 had the highest average leaf area under the control (Table 2), followed by WWM-1. The lowest leaf area was in WWM-31. The highest average leaf area in the struvite treatment was in WWM-16, followed by WWM-1. The lowest average leaf area was in WWM-37 under struvite treatment. In the SSP treatment, the highest area was in WWM- 37, followed by WWM-35, and the lowest average was in WWM-30. The DS tended to increase in leaf area in all treatments.

2.4.4 Vine length

Highly significant differences ($P<0.05$) were observed between the treatments concerning vine length among citron watermelon genotypes. Vine length varied from 42.1 cm to 141.2 cm. When no stress was applied, genotype WWM-1 produced the longest vines (59.8 cm), followed by WWM-16 (52.1 cm) (Figure 4), whereas the shortest vine length (42.1) was recorded for genotype WWM-31. The longest vines were recorded in the struvite treatment for genotype WWM-35 (140.2 cm), followed by WWM-30 (139 cm), while the shortest was in genotype WMM-16 (116 cm). Under DS, the longest vines were produced by WWM-16 (43.4 cm) in the control (no fertilizer), whereas WWM-35 (32.6 cm) recorded the shortest vines in the no stress control (Figure 5). When struvite was applied, WWM-37 and WWM-16 recorded the longest vines (138.5 and 137.8 cm, respectively), whereas the shortest vine length of control plants (111.2 cm) was found in WWM-35. WWM-30 measured the longest vine length, followed by WWM-16 in a struvite treatment under DS, whereas WWM-35 recorded the shortest vine length. In the SSP treatment, WWM-35 recorded the longest vine length, followed by WWM-30 under NS condition, whereas the shortest vine length was recorded for WWM-16. Under DS condition, the longest vine length was recorded for WWM-37, followed by WWM-31. Struvite treatment tended to increase vine length in both NS and DS.

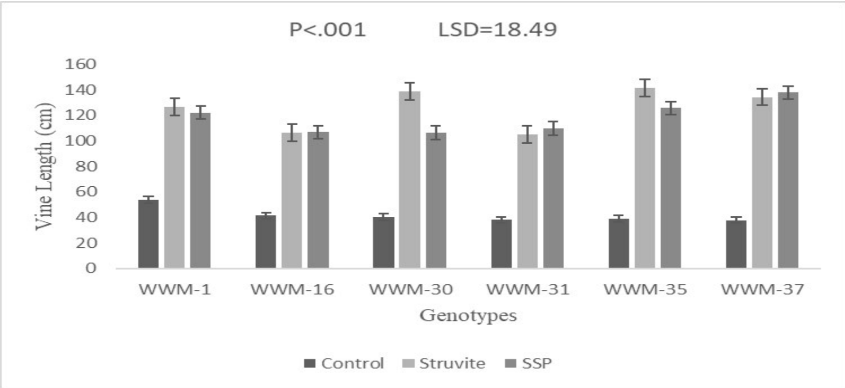

Figure 4: Effect of P amendment (struvite, SSP) on citron watermelon vine length on plants of six genotypes grown under NS

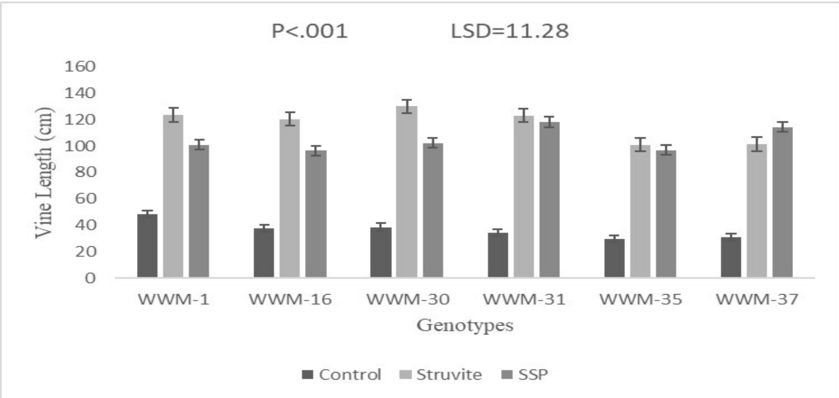

Figure 5: Effect of P amendment (struvite, SSP) on vine length of six citron watermelon genotypes grown under DS

2.4.5 Fruit size and mass

Fruit mass varied significantly among the six genotypes. Highly significant differences ($P<0.05$) between treatments were observed with respect to fruit mass, except the control (non-treated) genotypes under DS ($P>0.05$). Fruit mass ranged from 0.54 to 1.89 kg per plant among all citron watermelon genotypes (Table 3). Genotypes WWM-16, WWM-30, and WWM-37 were high-yielding (1.45, 1.33, and 1.27 kg) under NS, whereas WWM-1 and WWM-31 and WWM-35 were low-yielding (1.22, 1.18 and 1.14 kg) under the same condition (NS). Under DS, WWM-1 and

WWM-37 produced the highest yield (0.64 and 0.62 kg, respectively), followed by WWM-30 with a fruit mass of 0.61 kg. In the struvite treatment, WWM-35 and WWM-16 obtained the highest fruit mass (1.85 and 1.72 kg under NS), whereas WWM-31 acquired the lowest weight (1.48 kg under NS). WWM-35 and WWM-1 were high-yielding (0.88 and 0.84 kg) under DS. In the SSP treatment, WWM-35 and WWM-37 obtained the highest fruit mass of 1.89 and 1.76 kg under the NS, whereas WWM-31 had the lowest yield (1.52 kg) under NS. Under DS, WWM-1 and WWM-35 were high-yielding (0.86 and 0.80 kg) in the SSP treatment. The DS tended to decrease fruit mass of all genotypes, while the struvite treatment tended to increase fruit mass of genotypes under NS and DS. Figures 6 and 7 show six citron watermelon genotypes variation with different fruit colour from yellow to dark green, under different P treatments.

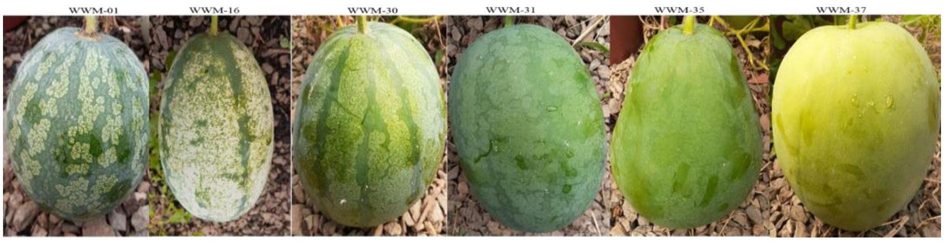

Figure 6: Six citron watermelon genotypes showing different shapes and rind stripe patterns, as well as colours under NS

Figure 7: Six citron watermelon genotypes showing different shapes, rind stripe patterns and colours under DS

Table 3: Mean values for fruit yield (kg) of citron watermelon genotypes evaluated under NS and DS

Genotype	Control (kg)		Struvite (kg)		SSP (kg)	
	NS	DS	NS	DS	NS	DS
WWM-1	1.22[c]	0.64[a]	1.58[d]	0.84[a]	1.65[d]	0.86[a]
WWM-16	1.45[a]	0.56[a]	1.72[b]	0.65[b]	1.72[c]	0.73[b]
WWM-30	1.33[b]	0.61[a]	1.66[c]	0.79[a]	1.67[d]	0.65[c]
WWM-31	1.18[c]	0.54[a]	1.48[e]	0.76[a]	1.52 [e]	0.76[b]
WWM-35	1.14[cd]	0.56[a]	1.85[a]	0.88[a]	1.89[a]	0.80[b]
WWM-37	1.27[bc]	0.62[a]	1.56[d]	0.78[a]	1.76[b]	0.77[b]
LSD	0.054	0.066	0.026	0.065	0.026	0.045
P-value	<.001	0.027	<.001	<.001	<.001	<.001

NS: Non-stress, **DS**: Drought stress

2.4.6 Dry root mass and length under different P treatments

2.4.6.1 Dry root mass under different P treatments

Highly significant differences ($P < 0.05$) were observed between the treatments concerning root dry root mass (Table 4). Under NS, WWM-37, WWM-30, and WWM-35 measured the greatest root dry mass (50.6, 49.3, and 48.1 g) in the struvite treatment, while in the SSP treatment, WWM-1, WWM-37, and WWM-16 (51.8, 48.9 and 45.9 g) measured the greatest root dry mass (Figure 8). WWM-31 and WWM-1 measured the least root dry mass (32.3 and 32.9 g) in the control under NS. Under the DS, the greatest dry root mass was recorded for WWM1, WWM-37, and WWM-35 (53.7, 51.5, and 50.9 g) in the struvite treatment, while WWM-1 and WWM-16 recorded the greatest root dry mass (47.8 and 44.4 g) in the control. Struvite treatment tended to increase root mass under both NS and DS.

Table 4: Mean root mass of citron watermelon genotypes measured under NS and DS

Genotype	Control		Struvite		SSP	
	NS	DS	NS	DS	NS	DS
WWM-1	32.9^{d}	47.8^{a}	35.6^{d}	53.7^{a}	51.8^{a}	51.8^{a}
WWM-16	34.6^{c}	44.4^{b}	36.2^{d}	37.5^{c}	45.9^{c}	48.6^{a}
WWM-30	39.1^{b}	40.9^{c}	49.3^{a}	48.1^{b}	40.7^{d}	47.7^{a}
WWM-31	32.3^{d}	40.7^{c}	38.7^{c}	49.4^{b}	39.7^{d}	50.2^{a}
WWM-35	40.2^{ab}	40.4^{c}	48.1^{b}	51.5^{b}	37.3^{d}	48.6^{a}
WWM-37	41.2^{a}	41.7^{c}	50.6^{a}	50.8^{b}	48.9^{b}	50.0^{a}
LSD	1.148	1.526	1.287	1.476	1.281	1.879
P-value	<.001	<.001	<.001	<.001	<.001	0.003

NS: Non-stress, **DS**: Drought-stress

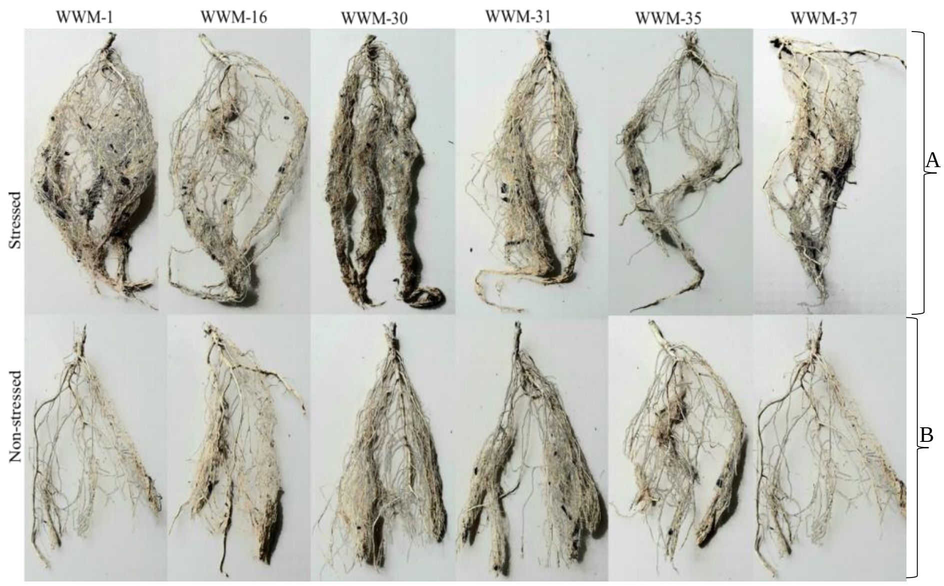

Figure 8: Root shape of six citron watermelon genotypes under non-stress and drought-stress seen using Root-Snap analysis®. Bar A shows the average of 60 cm root length, while bar B shows the average of 57 cm root length.

2.4.6.2 Root length under different P treatments

Significant differences ($P < 0.05$) were observed between the treatments with respect to root length (Table 5). Under NS, WWM-16 and WWM-35 produced the longest roots (63.2 and 60.9 cm, respectively) in struvite treatment, while under SSP treatment, WWM-37 and WWM-35 measured the longest roots (62.3 and 59.9 cm, respectively). Genotypes WWM-31 and WWM-30 produced the shortest roots (46.5 and 47.6 cm) in the control, under NS. Under DS, the longest roots were recorded for WWM-1, WWM-37 and WWM-35 (66.4, 66.3, and 64.6 cm, respectively) in the struvite treatment, while WWM-37 and WWM-16 recorded the longest roots (63.5 and 61.2 cm) in the control. Struvite treatment tended to increase root length under both NS and DS.

Table 5: Mean values for root length of citron watermelon genotype evaluated under NS and DS

Genotype	Control		Struvite		SSP	
	NS	DS	NS	DS	NS	DS
WWM-1	49.5^{c}	48.5^{a}	59.6^{c}	66.4^{a}	57.2^{c}	59.8^{c}
WWM-16	50.9^{c}	51.8^{a}	63.2^{a}	60.7^{d}	51.4^{de}	61.2^{b}
WWM-30	47.6^{d}	44.7^{b}	59.3^{d}	59.2^{de}	54.1^{d}	58.8^{d}
WWM-31	46.5^{d}	44.9^{b}	60.9^{b}	62.5^{c}	56.9^{c}	57.4^{e}
WWM-35	55.1^{b}	49.7^{a}	61.5^{b}	64.6^{b}	59.1^{b}	59.0^{e}
WWM-37	57.3^{a}	43.2^{b}	58.3^{d}	66.3a	62.3^{a}	63.5^{a}
LSD	0.996	2.813	0.823	1.125	0.921	0.727
P-value	<.001	<.001	<.001	<.001	<.001	<.001

NS: Non-stress, **DS**: Drought stress

2.5 Discussion

Struvite treatment enhanced citron watermelon leaf traits (leaf length and leaf width) under NS and DS (Tables 1 and 2). This could be due to the release of mineral elements from the growing medium and the resultant enhanced phosphorus uptake, which promoted plant growth due to improved cell division and enlargement. The current study discovered that the citron watermelon genotypes produced a reduced leaf area (Table 1) under DS, a sign of phenotypic adaptation and an effective drought-avoidance tactic. A reduction in leaf area in the struvite and SSP treatments, as well as the controls among all citron watermelon genotypes was observed under DS (Table 2).

This corroborates findings of Queenborough and Porras (2013), who observed specific leaf area (SLA) alterations (a ratio indicating how much leaf area a plant builds with a given amount of leaf biomass) as a useful indicator of a plant's ability to tolerate drought.

In the present study, an increase in both root length and root dry mass was observed under DS compared with NS. Citron watermelon genotypes obtained a greater dry root mass in the struvite treatment than in the control (Table 4). This might be explained by the crop's capacity to partition carbohydrates to the water-acquiring regions, such as roots, to be able to maintain water uptake, resulting in a higher dry mass than in those leaf areas under DS than under NS. The results of this study are consistent with the optimal partitioning theory, which contends that plants direct resources to organs that will result in the promotion of growth of the entire plant (Comas *et al*., 2013; Weiner, 2004). A high root dry mass among the NS citron watermelons could also be attributed to the high root N content, which would have been partitioned to these areas, since N positively impacts root growth (Yavuz *et al*., 2020).

The most important characteristics that indicate variability among citron watermelon genotypes are fruit qualitative traits, including marbling, stripes, and the main colour of the fruit. Fruit stripes were present in genotypes WWM-16, WWM-30 and WWM-37 in all treatments under NS and DS. Genotypes WWM-16, WWM-30 and WWM-37 were high-yielding under NS conditions. Marbling was observed only in WWM-1, WWM-16, and WWM-35 under NS and DS. A yellow flesh colour was observed for WWM-16 and WWM-35, while a green flesh colour was observed for WWM-1, WWM-30 and WWM-31 (Figure 10). This could be attributed to the lack of the carotenoid lycopene, which gives watermelon its pink to red flesh colour (Ilahy *et al*., 2019).

The study revealed a positive relationship between chlorophyll content index and leaf growth traits (leaf length, leaf width and leaf area) (Table 1 and 2), indicating the important role of phosphate, nitrogen and magnesium in leaf growth and chlorophyll production of the assessed citron watermelon genotypes. The enhanced vine length of citron watermelon genotypes in the SSP and struvite treatments under NS conditions could be attributed to the readily available nutrients in these fertilizers (Hertzberger *et al*., 2020). A positive relationship between chlorophyll content index and vine length was observed in all treatments, indicating the importance of the minerals provided in the applied fertilizers for plant growth.

2.6 Conclusion

The present study aimed to gain knowledge on morphological factors, including root characteristics, that may be associated with yield variation among citron watermelon genotype in South Africa. This study sought to contribute to understanding if the use of urine-based fertiliser sources, such as struvite, can improve P availability in acidic soils. It indeed led to increased performance of citron watermelon genotypes due to the enhanced nutrients uptake.

Varied responses were revealed in the study for measured morphological (leaf length, leaf width, leaf area, dry root length, vine length, and fruit weight) traits among the assessed citron watermelon genotypes. Results obtained from this study revealed that the rate of growth, in terms of leaf traits, was significantly higher in the SSP treatment under NS, as compared to the struvite treatment and the control. The fast growth in terms of leaf length, leaf width and leaf area in the SSP treatment could be attributed to the efficiency of this fertilizer to provide nutrients necessary for leaf growth and development of citron watermelon. The high growth rate due to SSP fertilizer application to treated genotypes could be due to the easy availability of nutrients in this fertilizer. The low growth rate in control plants could be due to the loss of N through denitrification (Appendix Figure 18) (Qasim *et al*., 2022). Volatilization of ammonia from the urine-based fertilizer could have resulted in a reduced P availability, and thereby a slow growth rate of the leaves in the struvite treatment compared to the SSP treatment.

The current study assessed the morphological diversity among six citron watermelon genotypes (WWM-1, WWM-16, WWM-30, WWM-31, WWM-35, and WWM-37) that were planted under three P soil treatments (SSP, struvite, and control), planted under NS or DS. In the present study, wide variations were observed in the assessed watermelon genotypes for fruit mass (Table 3). Fruit mass was greater in the struvite treatment under NS compared to the control in all genotypes. This could be attributed to the high phosphorus (P) content of struvite, which is distributed throughout the citron watermelon plant (Table 3); moreover, P application increased citron watermelon fruit mass and promoted higher yields (Figure 6). Fruit mass of the SSP-treated plants was, however, greater compared to both the struvite and the control, indicating that SSP is superior in enhancing leaf performance, plant growth as well as fruit mass, compared with the other treatments. In conclusion, struvite improved morphological characteristics (leaf traits, root traits, vine length and fruit mass), and also improved the stripe sizes of the assessed citron watermelon genotypes.

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CHAPTER 3: Nutritional composition and physiological factors associated with the yield variation among citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.) genotype in South Africa

3.1 Abstract

Citron watermelon [*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.] is a plant species used for food and feed, belonging to the family Cucurbitaceae. Wide yield variation is exhibited among citron watermelon genotypes in South Africa. The objective of this study was to assess morphological and physiological factors associated with yield variation among citron watermelon genotype in South Africa and to recommend desirable genotypes that are drought-tolerant and have desirable fruit traits positively impacting future citron watermelon production, breeding, and conservation. The study was designed as a 6 x 2 x 3 factorial experiment with the following factors: genotype - 6 levels (WWM-1, WWM-16, WWM-30, WWM-31, WWM-35, and WWM-37), drought conditions - 2 levels (non-stress (NS) and drought stress (DS) conditions); and phosphorus source - 3 levels (struvite, single superphosphate (SSP), and control (no fertilizer)) with four replications giving a total of 144 experimental units. The experiment was laid out using six genotypes in a completely randomized design (CRD). Physiological data (leaf gas exchange and chlorophyll fluorescence parameters), including photosynthesis activity, stomatal conductance, electron transport rate, transpiration flow, intercellular carbon dioxide (CO_2) concentration, ratio of intercellular and atmospheric CO_2, and maximum fluorescence data were collected using a LI-6400XT. The chlorophyll content index (CCI) was measured using a SPAD meter, and averaged data were subjected to analysis of variance using GenStat.

A wide variety and distinct qualities for genetic improvement were seen in the evaluated genotypes. The results from this study showed no significant differences ($P>0.05$) between treatments concerning leaf gas exchange and chlorophyll fluorescence parameters. Struvite treatment outperformed both SSP and the control concerning the ratio of intercellular and atmospheric CO_2, under NS. Genotypes under struvite treatment also outperformed SSP treatment concerning stomatal conductance. A significant difference ($p<0.05$) was in stomatal conductance among struvite-treated genotypes under NS. The urine-derived fertilizer improved the chlorophyll content index (CCI) of the plants, compared to SSP and the control. The urine-based fertilizer was comparable to SSP treatment concerning maximum quantum efficiency of photosystem among citron watermelon genotypes under non-stress, whereas struvite outperformed both SSP and control under DS. This suggests that the struvite improved the leaf gas exchange and chlorophyll fluorescence parameters among genotypes. The study revealed that there was a high uptake of nutrients among genotypes that were treated with struvite and SSP; this could be attributed to the high CEC of the soil. The increase of N uptake in the SSP and struvite- treated soil could be due to the mineralization of the organic matter in the soil, thus releasing N that was initially unavailable. Citron watermelon genotypes WWM-30, WWM-31, and WWM- 37 recorded high maximum florescence in both NS and DS and are recommended to farmers for livestock feed and fruit production for food.

Keywords: Citron watermelon, yield variation, physiological factors, morphological factors, soil P, and nutrient uptake

3.2 Nutritional composition and physiological factors affecting yield in citron watermelon

Africa is the native home of citron watermelon, which belongs to the tribe Benincaseae, subtribe Benincasinae, and subfamily Cucurbitoideae in the Cucurbitaceae family (Maynard 2001). There are three distinct kinds of the annual *C. lanatus*: feral, cultivated, and wild, classified as the subspecies *vulgaris* (Schrad. ex Eckl. et Zeyh.) Fursa, as well as *lanatus*, and subspecies mucosospermus Fursa are the three subspecies that Fursa (1972) identified within the species. The subspecies *vulgaris* is thought to include the most commonly grown varieties with red, delicious fruits (Fursa 1972). The subspecies *lanatus*, also known as tsamma (var. *caffer* (Schrad.) Mansf., is a vital source of food and water in the Kalahari Desert. Originating from the tsamma melon, the citroides group (*C. lanatus* var. *citroides* [L. H. Bailey] Mansf. ex Greb.) is a collection of old cultigens from southern Africa. Because not only the fruit flesh can be consumed, but the fruit rind can be used in conserves, jellies, and preserves, it is nicknamed "preserving melon" (Dar *et al*., 2022).

According to reports by Nkoana *et al*. (2022), citron watermelon has special phytochemical and nutritional characteristics that give the fruit certain medicinal and pharmacological features. The seeds are rich in protein and oil, containing unsaturated fatty acids, such as palmitic, linoleic, stearic and oleic acids (Singh and Matta 2010; Jarret and Levy, 2012). The non-sweet fruit has a high concentration of organic acids and carotenoids, particularly β-carotene, and low levels of sugars (fructose, sucrose, and glucose) (Yoo *et al*., 2012). Cucurbitacins and their glycoside derivatives, which have therapeutic potential, are among the phytochemical compounds found in the fruit and leaves (Davidovich-Rikanati *et al*., 2015). Traditional healers in South Africa treat hypertension with the leaves and fruit of the citron watermelon (Semenya and Potgieter, 2015).

Citron watermelon has been shown to be drought tolerant (Malambane *et al*., 2018; Zhang *et al*., 2011). Various physiological features are responsible for this: an effective photosynthetic and photoprotective system, as well as the special compounds, including the amino acids citrulline (Zhang *et al*., 2011). The drought tolerance is achieved by certain physiological characteristics, including its low transpiration rate, high photosynthetic efficiency, control of stomatal conductance, and certain protective mechanisms (e.g., photochemical and non-photochemical quenching ability) (Mashilo *et al*., 2017). The effectiveness of screening and identifying drought-tolerant germplasm could be improved by using yield-based selection indices in conjunction with morphological and physiological features for drought tolerance assessment. Assessing the amount of genotypic diversity for drought tolerance in this crop is necessary for the efficient use of citron watermelon genetic resources in the breeding for abiotic stress tolerance of citron watermelon.

These will make it possible to identify and ultimately choose useful sources of genes linked to drought adaptation for breeding purposes of the entire watermelon group (Mashilo *et al*., 2017).

Human urine has been reported as a good source of phosphorus (Krishnamoorthy *et al*., 2021). This mineral is, hence, commonly discharged into the environment, where it can significantly disturb the biogeochemical cycles of the planet, leading to eutrophication. These nutrients derived from human urine could be utilized in agriculture, if they were separated at the source. Hence, means to undertake phosphorus recovery and recycling from wastewater is becoming popular. Diversion of human urine from the faeces is one of the solutions; it is a practical and energy-efficient way to recover phosphorus from wastewater (Kalmykova *et al*., 2012). Recycling human urine and its subsequent use as a fertilizer leads to sustainability of food production (Wu *et al*., 2016).

Information regarding the variability of citron watermelon production under water stress is scarce, with only few studies (Mandizvo *et al*., 2021; Ngwepe *et al*., 2021) reporting on its potential. The purpose of this study was, therefore, to investigate physiological variables or characteristics that might be linked to yield variability in South African genotype of citron watermelon under DS. In acidic soil environments, phosphorus deficiency is a growth-limiting factor. It was, therefore, researched whether using the urine-derived fertilizer struvite enhances P availability to citron watermelon, as well improve the physiological efficiency as well as the nutrient uptake of citron watermelon grown in acidic soils.

3.3 Materials and methods

3.3.1 Plant material

The research employed six citron watermelon genotypes: WWM-01, WWM-16, WWM-30, WWM-31, WWM-35, and WWM-37 (Figure 1). These genotypes were obtained from the South African Department of Agriculture and Rural Development in the Limpopo Province. These selected genotypes are morphologically and genetically distinct, having been farmed by small-holder farmers under rain-fed conditions in Limpopo, where plants are often stressed by prolonged heat and drought (Ngwepe *et al*., 2021; Mashilo *et al*., 2017). The chosen genotype differed in several characteristics, such as patterns of fruit rind stripes, fruit shape, flesh colour, and seed coat colour (Mashilo *et al*., 2017).

3.3.2 Soil analysis of medium used

Seeds of the above-mentioned genotype were planted into 10 L plastic pots filled with Gromor® potting mix (Gromor®, Cato Ridge, South Africa). Medium analysis was carried out before planting at the KwaZulu-Natal Department of Agriculture and Rural Development's Analytical Services Unit (Cedara College of Agriculture, Cedara, South Africa), to determine the initial nutrient availability. This analysis was undertaken using ICP-AES (inductively coupled plasma atomic emission spectrometry).

3.3.3 Experimental design and crop establishment

The experiment was conducted at the Controlled Research Facility (CEF) of the University of KwaZulu-Natal, Pietermaritzburg, South Africa (29◦37′37.5′′ S and 30◦24′ 10.4′′E) in plastic tunnel L2. The study was designed as a 6 x 2 x 3 factorial experiment with the following factors: genotype - 6 levels (WWM-01, WWM-16, WWM-30, WWM-31, WWM-35, and WWM-37); drought conditions - 2 levels (no-stress (NS) and drought-stress (DS)); phosphorus source - 3 levels (struvite, single superphosphate (SSP) and control (no added P fertilizer)) with four replications per combination, giving a total of 144 experimental units, which were laid out in a completely randomized design (CRD) (Figure 10). One seed for each genotype was planted per pot (10 L pot) and these pots were arranged randomly on three elevated benches within the tunnel. The seeds were planted 6 cm deep into individual 10 L pots, with 8 L medium (Gromor® potting mix). The P fertilizer, either struvite (30 mg P pot^{-1}) and SSP (40 mg P pot^{-1}) was applied into the medium in accordance with medium (Gromor® potting mix; 30 dm^3) nutrients composition and was mixed with a concrete mixer. For all replications, 6 L of water was applied once daily to each pot under NS, and 3 L of water was applied once per day to each genotype in the DS. To maintain soil water potential, pots under NS were continuously irrigated to 100% FC (6 L of water), whereas DS was simulated by irrigating pots to 50% FC (3 L of water).

3.3.4 Anatomical and physiological data collection

3.3.4.1 Determination of nutrients in plant tissues

Before tissue analysis, plant tissue sample groups were collected after harvest. Coffee grinders were used to grind the samples. Citron watermelon leaf samples were pulverized in a wide-form porcelain crucible with a capacity of roughly 20 mL and weighed at approximately 0,5 g to the nearest milligram. The crucible was clearly labelled. It was placed in a desiccator to cool after 2 hours of ignition in a cold muffle furnace with a temperature setting of 500°C. It was wetted with water before 10 mL of a 4 M HCl/HNO_3 acid combination was gradually added. Then digested for 20 minutes in a sand bath. To minimize losses, it was placed onto filter paper with distilled water and then into a 250 mL volumetric flask. Both the precipitate on the filter paper and the crucible were thoroughly cleaned with distilled water. Made to the desired volume with distilled water. To avoid exceeding 500°C, the furnace temperature was increased gradually (Habibullah *et al*., 2020).

3.3.4.1.1 Determination of calcium (Ca) and magnesium (Mg)

A 5 mL aliquot of extract was pipetted into a test tube. One mL strontium buffer solution (2500 mg/L strontium was added in a solution of 7,61 g/L $SrCl_2$ 6 H_2O). The solutions were mixed thoroughly. The test tubes were retained for Ca and Mg readings by AAS. Results were reported as % Ca and % Mg in plant material (Ibrahim, 2012).

3.3.4.1.2 Determination of potassium (K)

A 1 mL aliquot of the extract was pipetted into a test tube. Then 5 mL caesium buffer solution (1200 mg Cs/L) - (1, 52 g CsCl/L) was added. The buffer and the sample were mixed by gently shaking. The test tubes were stoppered and retained for K analysis by AAS. The results were reported as % K in plant material. The vanadomolybdate yellow method is preferred to the more sensitive molybdate blue method because it is less sensitive to interference from other elements and small variations in acidity and experimental conditions and the colour formed is more stable. Because it is less sensitive, it is suitable for plant material where the initial concentration will be greater than 100 mg P/kg but is not suitable for soil extracts (Okalebo *et al*., 2002).

3.3.4.1.3 Determination of nitrogen (N)

Leco TruMac equipment was used to perform dry digestion to measure plant nitrogen. About 3, 8212 g NH_4Cl/L of distilled water was created as a 0,1 M nitrogen standard. A range of standards up to 1 x 10-5 M were included in the 0,1 M standard. They were created frequently and kept in plastic bottles with labels. A 100 mL Kjeldahl flask was filled with a sample weighing 0.5 g. The addition of 0.15 g (0.14 spatula) of K_2SO_4 was made. This was done to increase the acid's boiling point. To catalyse the acid, 0.5mL of $HgSO_4$ from a burette was added. Ten mL of concentrated H_2SO_4 was pipetted in, using an automated dispenser. In the fume cupboard, the flasks were set up on digesting racks with the heater set to 6 and the fan turned on. To ensure that they all digest equally, it was occasionally shaken and their position on the rack was altered. The heater was turned off and left to cool for two to three hours after the solution had gone from brown, red, and yellow to clear (Mupambwa *et al*., 2023).

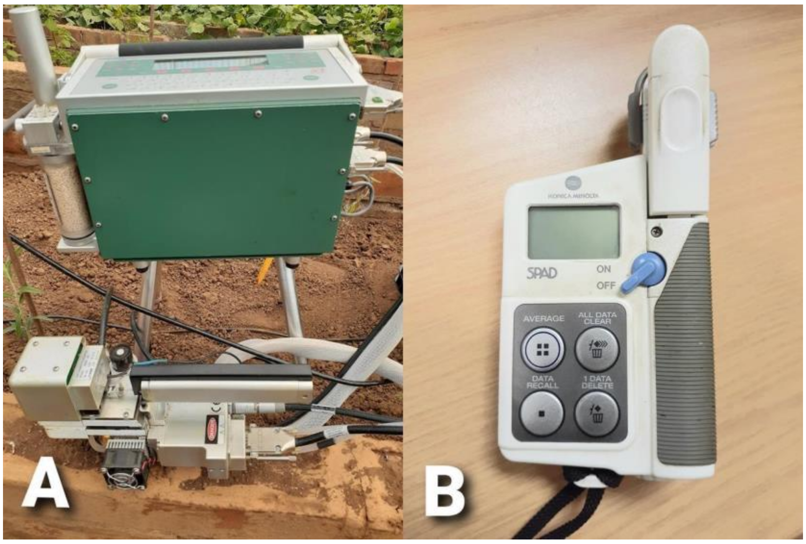
Each flask received a spritz of distilled water (about 10 mL) and was allowed to cool again while remaining within the fume cupboard. It was then transferred to a 250 mL volumetric flask after cooling, rinsed with distilled water, and filled to the specified volume. The flask was stopped and shaken. A 100 mL clean, labelled beaker was filled with 50 mL of each sample extract (using a measuring cylinder). After rinsing, the ammonia electrode was dried with a tissue: The auto dispenser was used to add 7 mL of 10 M NaOH, which was then placed under the electrode and the stirrer turned on. The mV meter was measured after 45 seconds, and data from the standard curve were acquired (Mupambwa *et al*., 2023).

3.3.4.1.4 Measurement of mineral nutrients in citron watermelon fruit

The fruit rind and flesh powders were dehydrated in a 5:1 volume ratio solution of H_2SO_4 and H_2O_2. Three replicates were included in each treatment. The regular Kjeldahl method (Bremner, 1965) was used to determine the concentration of N, and the phosphomolybdate blue method (Murphy and Riley, 1962) was used to measure the concentration of P. Atomic absorption spectrometry was used to determine the concentrations of K, Ca, Mg, Fe, Mn, and Cu (Varian spectra AA 220, Varian, Palo Alto, CA, USA).

3.3.4.2 Physiological data collection

Physiological factors (leaf gas exchange parameters and chlorophyll fluorescence parameters) as photosynthesis activities, stomatal conductance, electron transport rate, transpiration flow, intercellular carbon dioxide (CO_2) concentration, ratio of intercellular and atmospheric CO_2, and maximum fluorescence data were collected using LI-6400XT (LI-COR Inc., Lincoln, state, USA) (Figure 9A). The chlorophyll content index (CCI) was measured using a SPAD meter (Minolta Camera Co., Osaka, Japan) (Figure 9B), and the measurements were taken on the third leaf from the apical meristem in each pot.



size: 15.56 X 21.44 cm

Figure 9A: LI-6400XT used to measure plant physiological factors. **Figure 9B:** SPAD-502 meter used to measure chlorophyll content index (CCI)

3.3.5 Statistical analysis

Genstat 20th edition (VSN International, Hemel-Hempstead, United Kingdom) was used to do an analysis of variance on data from the citron watermelon genotype. When treatments indicated significant variations in the measured parameters at a 5% level of significance, the means were separated using Fisher's protected least significant difference (LSD) test using XLSTAT software (Data Analysis and Statistical Solution for Microsoft Excel, Addinsoft, Paris, France,); Agglomerative hierarchical clustering (AHC) was performed using drought tolerance indices following Ward's technique using squared Euclidean distance to quantify similarity (Mandizvo *et al*., 2022).

3.4 Results

3.4.1 Nutrient uptake

A significant difference ($p<0.05$) on soil nutrient content was revealed by medium fertility analysis when comparing treatments before planting and after harvest (Table 6). Struvite and SSP application tended to increase micro- and macro-nutrients availability in the medium. High significant differences ($p<0.05$) were observed among the genotypes with respect to nutrient composition in leaves and vines. High N concentration (44.12 mg kg^{-1}) in citron watermelon leaves was in struvite-treated genotypes, followed by SSP-treated genotypes (33.96 mg kg^{-1}) reflected in the intensity of the green leaf colour as compared to plants in the SSP and control (Tables 7 and 8) in leaves. High concentration of P (9.41 and 4.64 mg kg^{-1}) was recorded for struvite treated genotypes in both leaves and vines, followed by SSP-treated genotypes (7.69 and 3.87 mg kg^{-1}). Genotypes under SSP treatment recorded the highest concentration of calcium (62.65 and 18.26 mg kg^{-1}) in both leaves and vines, under NS and DS. The application of struvite and SSP treatments tended to increase the nutrient concentration in both leaves and vines of the genotypes, while DS tended to decrease nutrient concentration in both leaves and vines.

Table 6: Nutrient composition of the medium before planting and after planting

Element (mg/kg)	Before Planting			After Harvest		
	Medium 1 (control)	Medium 2 (struvite)	Medium 3 (SSP)	Medium 1 (control)	Medium 2 (struvite)	Medium 3 (SSP)
Nitrogen (N)	8.03^{a}	10.50^{c}	2.60^{d}	7.50^{a}	8.73^{c}	2.20^{d}
Phosphorus (P)	5.66^{b}	12.30^{a}	13.45^{a}	4.54^{b}	11.33^{a}	12.87^{a}
Potassium (K)	4.60^{c}	5.20^{d}	5.70^{b}	4.53^{b}	4.87^{d}	5.53^{b}
Calcium (Ca)	0.17^{e}	1.63^{e}	1.53^{e}	0.17^{c}	1.43^{e}	1.33^{e}
Magnesium (Mg)	1.25^{d}	11.40^{b}	2.97^{c}	1.25^{c}	10.29^{b}	2.95^{c}
Copper (Cu)	0.55^{e}	1.02^{f}	1.03^{f}	0.53^{c}	0.98^{f}	1.07^{f}
Manganese (Mn)	0.66^{e}	0.97^{f}	0.88^{g}	0.65^{c}	0.95^{f}	0.86^{g}
Iron (Fe)	0.63^{e}	0.21^{f}	0.13^{h}	0.63^{c}	0.18^{g}	0.11^{h}
LSD	0.316	0.207	0.129	0.815	0.340	0.150
P-value	<.001	<.001	<.001	<.001	<.001	<.001

SSP (Single superphosphate), **Struvite** (Urine derived fertilizer), **Control** (Without treatment)

Table 7: Leaf and vine nutrient composition of citron watermelon genotypes under NS after fruit harvest

Element (mg/kg)	Leaf			Vine		
	Control	Struvite	SSP	Control	Struvite	SSP
Nitrogen (N)	36.24^{b}	47.53^{c}	19.25^{c}	31.71^{b}	38.47^{b}	21.76^{b}
Phosphorus (P)	3.64^{d}	7.84^{d}	6.71^{d}	2.64^{d}	3.24^{e}	3.65^{e}
Potassium (K)	53.15^{a}	66.75^{a}	64.09^{a}	67.50^{a}	75.50^{a}	77.77^{a}
Calcium (Ca)	47.24^{c}	57.23^{b}	61.34^{b}	13.79^{c}	17.77^{c}	15.51^{c}
Magnesium (Mg)	4.33^{d}	7.43^{d}	4.04^{e}	3.55^{d}	5.04^{d}	4.18^{d}
Copper (Cu)	0.01^{e}	0.04^{e}	0.04^{f}	0.01^{e}	0.03^{f}	0.03^{f}
Manganese (Mn)	0.02^{e}	0.05^{e}	0.04^{f}	0.01^{e}	0.17^{f}	0.01^{f}
Iron (Fe)	0.13^{e}	0.18^{e}	0.16^{f}	0.02^{e}	0.07^{f}	0.06^{f}
LSD	0.945	1.093	0.670	1.110	0.663	0.270
P-value	<.001	<.001	<.001	<.001	<.001	<.001

SSP (Single superphosphate), **Struvite** (Urine derived fertilizer), **Control** (Without treatment)

T**able 8**: Nutrient composition in the leaves and vine of citron watermelon genotypes under DS after fruit harvest

Element (mg/kg)	Leaf			Vine		
	Control	Struvite	SSP	Control	Struvite	SSP
Nitrogen (N)	33.96^{c}	44.12^{c}	16.07^{b}	31.85^{b}	38.28^{b}	15.07^{b}
Phosphorus (P)	4.57^{d}	9.41^{d}	7.69^{c}	2.73^{d}	4.64^{d}	3.87^{c}
Potassium (K)	53.43^{a}	66.01^{a}	63.50^{a}	56.07^{a}	65.89^{a}	67.31^{a}
Calcium (Ca)	48.22^{b}	58.21^{b}	62.65^{a}	14.28^{c}	17.93^{c}	18.26^{b}
Magnesium (Mg)	4.53^{d}	7.67^{e}	5.59^{d}	3.65^{d}	5.18^{d}	4.75^{c}
Copper (Cu)	0.01^{e}	0.04^{f}	0.01e	0.01^{d}	0.12^{d}	0.03^{c}
Manganese (Mn)	0.02^{e}	0.05^{f}	0.05^{e}	0.02^{d}	0.02^{d}	0.02^{c}
Iron (Fe)	0.16^{e}	0.21^{f}	0.18^{e}	0.03^{d}	0.08^{d}	0.06^{c}
LSD	1.620	1.215	1.862	6.696	5.355	6.258
P-value	<.001	<.001	<.001	<.001	<.001	<.001

SSP (Single superphosphate), **Struvite** (Urine derived fertilizer), **Control** (Without treatment)

High significant differences ($p<0.05$) with respect to micro- and macro-nutrients composition, except calcium concentration in both rind and flesh of citron watermelon fruit were observed under NS and DS. The concentration of all elements increased in the fruit rind of struvite- and SSP-treated genotypes in comparison with the control. The total mineral concentration in the rind of fruit of genotypes under struvite treatment was increased by 10.98% and 11.02%, respectively, compared with that of the control, under NS (Tables 9.1 and 9.2). Highly significant effect on the concentration of K, Mg, Mn, and Cu was observed in the flesh of fruits with SSP and struvite treated genotypes in comparison with the control values under DS (Tables 10.1 and 10.2). The concentration of P was increased in the flesh of fruits with struvite treatment, whereas the concentration of Mn was substantially decreased under DS. The application of struvite and SSP treatments tended to increase nutrient concentration more in fruit rind than in fruit flesh under both NS and DS.

Table 9.1: Nutrient composition in the rind of citron watermelon fruit under NS after harvest

Element (mg/kg)			Fruit Rind		
	Control	Struvite	SSP	LSD	P-value
Nitrogen (N)	12.37^{b}	16.31^{a}	10.43^{b}	2.137	0.004
Phosphorus (P)	5.53^{b}	7.67^{a}	7.47^{a}	0.907	0.005
Potassium (K)	81.61^{c}	88.23^{a}	84.98^{b}	2.730	0.007
Calcium (Ca)	3.60^{a}	4.37^{a}	4.37^{a}	0.684	0.056
Magnesium (Mg	2.18^{c}	2.85^{a}	2.61^{b}	0.115	<.001
Copper (Cu)	0.06^{b}	0.08^{a}	0.05^{b}	0.008	0.002
Manganese (Mn)	0.98^{a}	0.90^{b}	0.09^{c}	0.019	<.001
Iron (Fe)	0.48^{b}	0.64^{a}	0.68^{a}	0.068	0.003

SSP (Single superphosphate), **Struvite** (Urine derived fertilizer), **Control** (Without treatment)

Table 9.2: Nutrient composition in the flesh of citron watermelon fruit under NS after harvest

Element (mg/kg)			Fruit Flesh		
	Control	Struvite	SSP	LSD	P-value
Nitrogen (N)	9.03^{b}	13.43^{a}	8.62^{b}	1.677	0.002
Phosphorus (P)	4.22^{c}	5.51^{b}	6.65^{a}	0.159	<.001
Potassium (K)	15.62^{c}	18.81^{a}	16.76^{b}	0.881	0.001
Calcium (Ca)	2.49^{b}	2.95^{a}	1.76^{c}	0.250	<.001
Magnesium (Mg	1.54^{c}	2.78a	1.96^{b}	0.179	<.001
Copper (Cu)	0.23^{a}	0.23^{a}	0.24^{a}	0.043	<.001
Manganese (Mn)	0.50^{a}	0.45^{b}	0.53^{a}	0.022	0.001
Iron (Fe)	0.87^{a}	0.91^{a}	0.90^{a}	0.026	<.001

SSP (Single superphosphate), **Struvite** (Urine derived fertilizer), **Control** (Without treatment)

Table 10.1: Nutrient composition in the rind of citron watermelon fruit under DS after harvest

Element (mg/kg)	Fruit Rind				
	Control	Struvite	SSP	LSD	P-value
Nitrogen (N)	9.39^{b}	11.84^{a}	9.22^{b}	0.438	<.001
Phosphorus (P)	6.29^{b}	7.22^{a}	7.19^{a}	0.358	0.003
Potassium (K)	57.65^{c}	68.28^{a}	63.44^{b}	1.204	<.001
Calcium (Ca)	3.40^{b}	3.70^{a}	3.60^{a}	0.034	<.001
Magnesium (Mg	1.95^{c}	2.64^{a}	2.12^{b}	0.037	<.001
Copper (Cu)	0.05^{a}	0.08^{a}	0.03^{a}	0.035	<.001
Manganese (Mn)	0.93^{a}	0.85^{b}	0.85^{b}	0.022	<.001
Iron (Fe)	0.38^{b}	0.57^{a}	0.55^{a}	0.033	<.001

SSP (Single superphosphate), **Struvite** (Urine derived fertilizer), **Control** (Without treatment)

Table 10.2: Nutrient composition in the flesh of citron watermelon fruit under DS after harvest

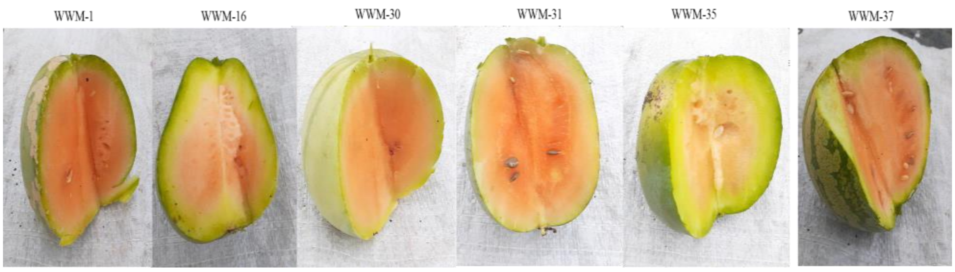
Element (mg/kg)	Fruit Flesh				
	Control	Struvite	SSP	LSD	P-value
Nitrogen (N)	6.14^{c}	10.55^{a}	7.65^{b}	0.969	<.001
Phosphorus (P)	5.06^{b}	5.43^{a}	4.99^{b}	0.274	0.023
Potassium (K)	13.14^{b}	15.70^{a}	14.89^{a}	0.810	0.002
Calcium (Ca)	2.31^{a}	2.26^{a}	1.72^{b}	0.520	0.061
Magnesium (Mg	1.58^{c}	2.67^{a}	1.99^{b}	0.081	<.001
Copper (Cu)	0.17^{b}	0.19^{b}	0.23^{a}	0.030	0.008
Manganese (Mn)	0.49^{b}	0.41^{c}	0.52^{a}	0.008	<.001
Iron (Fe)	0.83^{b}	0.88^{a}	0.54^{c}	0.008	<.001

SSP (Single superphosphate), **Struvite** (Urine derived fertilizer), **Control** (Without treatment)

Citron watermelon flesh ranged from orange to pink with rind from thick to thin (Figure 10,11,12,13,14 and 15), under different P treatments.

Size: 7.2 X 39.64 cm

Figure 10: Flesh and rind colour, and rind thickness of six citron watermelon genotypes with struvite treatment under NS



Size: 8.79 X 41.34 cm

Figure 11: Flesh and rind colour, and rind thickness of six citron watermelon genotypes with SSP treatment under NS.

Size: 8.1 X 39.51 cm

Figure 12: Flesh and rind colour, and rind thickness of six citron watermelon genotypes with control under NS

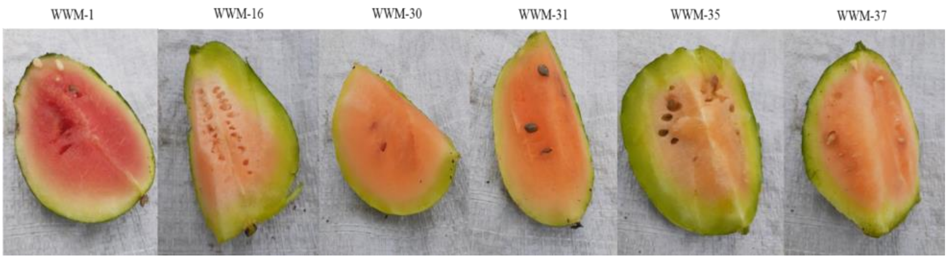
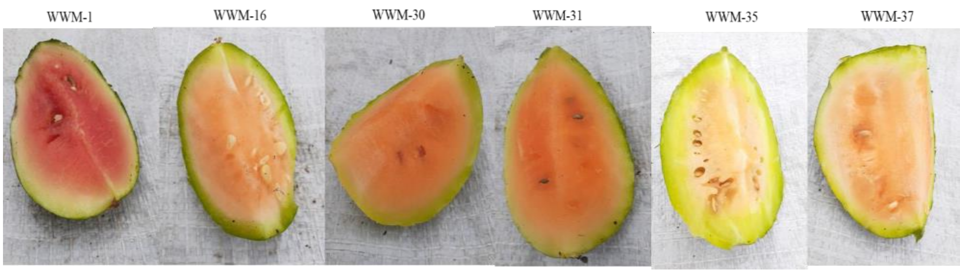
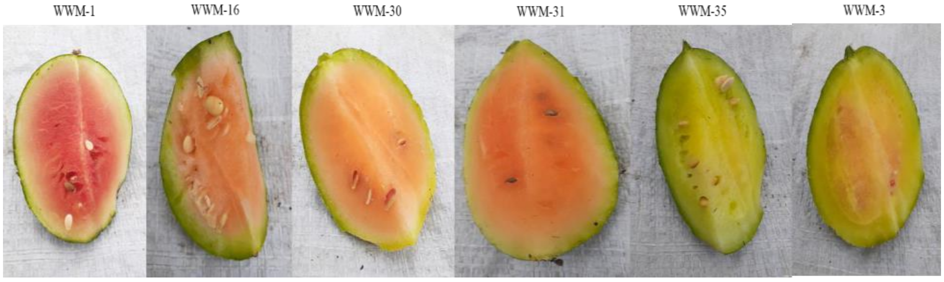


Figure 13: Flesh and rind colour, and rind thickness of six citron watermelon genotypes with struvite treatment under DS



Size: 8.48 X 35.75 cm

Figure 14: Flesh and rind colour, and rind thickness of six citron watermelon genotypes with SSP treatment under DS



Size: 8.37 X 36.34 cm

Figure 15: Flesh and rind colour, and rind thickness of six citron watermelon genotypes with control under DS

3.4.2 Photosynthesis

Drought has a significant impact on photosynthesis, which is a vital process that plants use to produce oxygen and energy in the form of sugar from sunlight, water, and carbon dioxide. No significant differences ($P>0.05$) were observed among genotypes with respect to leaf gas exchange and chlorophyll fluorescence parameters under NS with SSP (Table 12) and control (Table 11), while there were significant differences ($p<0.05$) observed among struvite-treated genotypes with respect to leaf gas exchange under NS (Table 13). The citron watermelon genotypes (WWM-1, WWM-16, WWM-30, WWM-31, WWM-35, and WWM-37) in this study responded to P soils (SSP, struvite, and control) in both NS and DS with similar trends but variable degrees of alterations in photosynthetic rates. Light-saturated photosynthesis (Asat) values were determined at 1000 mol m^{-2} s^{-1} light intensity and atmospheric CO_2 concentration using measurements of photosynthesis (stomatal activity, photosynthetic activity, internal CO_2, and transpiration rate).

Under NS, genotype WWM-31 recorded highest photosynthetic rates (50.80 μmol CO_2 m^{-2} s^{-1}), followed by WWM-30 and WWM-31 (50.20 and 5.20 μmol CO_2 m^{-2} s^{-1}) under struvite treatment (Table 13), respectively. Genotype WWM-16 and WWM-37 recorded lowest photosynthetic rates (46.80 and 47.80 μmol CO_2 m^{-2} s^{-1}). Genotype WWM-30 recorded highest photosynthetic rates (45.19 μmol CO_2 m^{-2} s^{-1}), followed by WWM-37 (44.47 μmol CO_2 m^{-2} s^{-1}) under SSP treatment (Table 12). Genotype WWM-35 recorded highest photosynthetic rates (43.38 μmol CO_2 m^{-2} s^{-1}), followed by WWM-31 (41.96 μmol CO_2 m^{-2} s^{-1}) under control (Table 9). ANOVA indicated that drought stress significantly decreased the stomatal conductance (gs), CO_2 assimilation rate, and transpiration rate (T) among the evaluated citron watermelon genotypes. Genotype WWM-1, WWM-30, WWM-31, WWM-35, and WWM-37 recorded gs values of ≥0.75 mmol m^{-2} s^{-1} under NS, with control (Table 9), whereas genotype WWM-16 recorded the highest gs value of 0.504 mmol m^{-2} s^{-1}. Genotypes recorded gs values of ≥0.061 mmol m^{-2} s^{-1} under NS condition, with struvite treatment (Table 13), whereas genotype WWM-37 recorded the highest gs value of 0.532 mmol m^{-2} s^{-1}, followed by genotype WWM-16 (0.396 mmol m^{-2} s^{-1}). Genotypes recorded gs values of ≥0.344 mmol m^{-2} s^{-1} under NS, with SSP treatment (Table 12), whereas genotype WWM-1 recorded the highest gs value of 0.666 mmol m^{-2} s^{-1}, followed by genotype WWM-16 (0.623 mmol m^{-2} s^{-1}).

Genotypes WWM-31, WWM-35, and WWM-37 recorded highest T values (≥10.6 mmol H_2O m^{-2} s^{-1}) under NS, with control (Table 9), whereas genotype WWM-16 and WWM-31 recorded highest T values (≥ 20.8 mmol H_2O m^{-2} s^{-1}) under NS, with struvite treatment (Table 13). Genotypes WWM-1 and WWM-16 recorded highest T values (≥25.4 mmol H_2O m^{-2} s^{-1}) under NS, with SSP treatment (Table 10). C*i* values ≥ 735 µmol m^{-1} were recorded for WWM-30, WWM-31, and WWM-35, whereas C*i* values ≤618 µmol m^{-1} were recorded for genotype WWM-1, WWM-16 and WWM-37 under NS, with struvite treatment (Table 13). Genotypes WWM-30 and WWM-37 recorded C*i* values ≥173 µmol m^{-1} under NS, with SSP treatment (Table 12). Under NS, with SSP treatment, significant genotypic variation for C*i*/C*a* was observed. Genotype WWM-30 and WWM-35 recorded the highest C*i*/C*a* (≥88.1 mol m^{-2} s^{-1}), whereas WWM-1, WWM-16, WWM-31 and WWM-37 recorded the lowest C*i*/C*a* (≤64.5 mol m^{-2} s^{-1}) under NS, with struvite treatment, while genotype WWM-16 and WWM- 30 recorded the highest C*i*/C*a* (≥121.7 mol m^{-2} s^{-1}) under DS.

Under DS, with control, genotypes WWM-16, WWM-35, and WWM-37 recorded the highest ETR values (≥3470 µmol e^{-1} m^{-2} s^{-1}), whereas WWM-1, WWM-30 and WWM-31 recorded the lowest ETR values (≤609 µmol e^{-1} m^{-2} s^{-1}) (Table 12). Based on the results, DS tended to reduce the ETR of all genotypes. The average of the maximum fluorescence (Fm*'*) was significantly increased under drought stress, with control. Highest Fm*'* values (≥2002) were recorded for genotypes WWM-16, WWM-30, and WWM-35 under DS (Table 12), with the control, whereas Fm*'* values ≤2120 were recorded for genotypes WWM-1, WWM- 31 and WWM-37 under the same treatment. Moreover, with struvite treatment, DS significantly increased the average values of Fm*'* among genotypes compared to the NS condition with ±13.8. Fm*'* values ranged from 2041 to 2364 under the NS, whereas under DS values ranged from 2010 to 2270 with struvite treatment.

Table 11: Means of leaf gas exchange and chlorophyll fluorescence parameters of citron watermelon genotypes under NS (no stress) without P treatment

Genotype	Leaf gas exchange parameters				Chlorophyll fluorescence parameters			
	gs	T	C*i*	Ci/Ca	Fv/Fm	ETR	Fm*'*	Photo
WWM-1	0.175[a]	7.4[a]	364[a]	40.7[a]	0.0643[c]	3861[a]	2443[a]	39.34[a]
WWM-16	0.166[a]	7.6[a]	532[a]	58.8[a]	0.0741[c]	2851[a]	2360[a]	40.93[a]
WWM-30	0.206[a]	9.0[a]	333[a]	34.9[a]	0.0745[c]	332[a]	2384[a]	41.96[a]
WWM-31	0.375[a]	11.1[a]	287[a]	32.2[a]	0.1090[b]	3518[a]	2208[a]	41.80[a]
WWM-35	0.266[a]	10.6[a]	569[a]	59.4[a]	0.0968[b]	681[a]	2300[a]	43.38[a]
WWM-37	0.504[a]	18.7[a]	253[a]	27.3[a]	0.1450[a]	7930[a]	2060[a]	41.75[a]
LSD	0.387	14.11	531.6	62.48	0.0592	8458.8	186.5	5.755
P-value	0.399	0.547	0.719	0.797	0.096	0.406	0.009	0.764

Stomatal conductance (**gs**), mmol m^{-2} s^{-1}; transpiration rate (**T**), mol cm^{-2} s^{-1} intercellular CO_2 concentration (**Ci**), μmol CO_2; ratio of intercellular and atmospheric CO_2 (**Ci/Ca**); maximum quantum efficiency of photosystem II photochemistry (**Fv/Fm**); electron transport rate (**ETR**), μmol m^{-2} s^{-1}); maximum fluorescence (**Fm**'); photosynthetic rate (photo), mol m^{-2} s^{-1}.

Table 12: Means of leaf gas exchange and chlorophyll fluorescence parameters of citron watermelon genotypes under NS with SSP treatment

Genotype	Leaf gas exchange parameters				Chlorophyll fluorescence parameters			
	gs	T	C*i*	C*i*/C*a*	Fv/Fm	ETR	Fm*'*	Photo
WWM-1	0.666[a]	25.5[a]	114[a]	13.03[a]	0.150[a]	7622[a]	2074[a]	40.01[a]
WWM-16	0.623[a]	25.4[a]	117[a]	12.95[a]	0.158[a]	9192[a]	2056[a]	41.35[a]
WWM-30	0.483[a]	23.0[a]	173[a]	17.51[a]	0.120[a]	3226[a]	2159[a]	45.19[a]
WWM-31	0.576[a]	25.3[a]	126[a]	13.64[a]	0.261[a]	4750[a]	2032[a]	43.12[a]
WWM-35	0.492[a]	22.6[a]	162[a]	16.65[a]	0.132[a]	1632[a]	2088[a]	43.74[a]
WWM-37	0.486[a]	23.4[a]	150[a]	16.07[a]	0.146[a]	2713[a]	2051[a]	44.47[a]
LSD	0.344	14.95	92.7	6.943	0.182	8823	123.4	3.550
P-value	0.771	0.994	0.647	0.575	0.609	0.421	0.347	0.061

Stomatal conductance (**gs**), mmol m^{-2} s^{-1}; transpiration rate (**T**), mol cm^{-2} s^{-1}; intercellular CO_2 concentration (**Ci**), μmol CO_2; ratio of intercellular and atmospheric CO_2 (**Ci/Ca**); maximum quantum efficiency of photosystem II photochemistry (**Fv/Fm**); electron transport rate (**ETR**), μmol m^{-2} s^{-1}); maximum fluorescence (**Fm**'); photosynthetic rate (photo), mol m^{-2} s^{-1}.

Table 13: Means of leaf gas exchange and chlorophyll fluorescence parameters of citron watermelon genotypes under NS with struvite treatment

Genotype	Leaf gas exchange parameters				Chlorophyll fluorescence parameters			
	gs	T	C*i*	C*i*/C*a*	Fv/Fm	ETR	Fm*'*	Photo
WWM-1	0.210[b]	6.8[b]	618[a]	54.8[b]	0.040[a]	1493[a]	2359[a]	50.20[a]

WWM-16	0.396[a]	20.8[a]	192[a]	20.2[b]	0.121[a]	3562[a]	2051[a]	46.80[a]
WWM-30	0.079[b]	4.9[b]	996[a]	88.1[b]	0.040[a]	3267[a]	2364[a]	50.80[a]
WWM-31	0.133[b]	6.5[b]	735[a]	64.5[b]	0.117[a]	422[a]	2191[a]	50.20[a]
WWM-35	0.061[b]	3.7[b]	1331[a]	116.4[a]	0.059[a]	4146[a]	2233[a]	50.80[a]
WWM-37	0.532[a]	27.6[a]	141[a]	14.1[b]	0.141[a]	1716[a]	2041[a]	47.80[a]
LSD	0.175	10.69	486.2	46.46	0.149	10681	304.8	7.83
P-value	<.001	0.001	0.001	0.003	0.530	0.595	0.143	0.812

Stomatal conductance (**gs**), mmol m^{-2} s^{-1}; transpiration rate (**T**), mol cm^{-2} s^{-1}; intercellular CO_2 concentration (**Ci**), μmol CO_2; ratio of intercellular and atmospheric CO_2 (**Ci/Ca**); maximum quantum efficiency of photosystem II photochemistry (**Fv/Fm**); electron transport rate (**ETR**), μmol m^{-2} s^{-1}); maximum fluorescence (**Fm'**); photosynthetic rate (photo), mol m^{-2} s^{-1}.

Table 14: Means of leaf gas exchange and chlorophyll fluorescence parameters of citron watermelon genotypes under DS with control treatment

Genotype	Leaf gas exchange parameters				Chlorophyll fluorescence parameters			
	gs	T	Ci	C*i*/C*a*	Fv/Fm	ETR	Fm*'*	Photo
WWM-1	0.419[a]	17.4[a]	453[a]	36.1[a]	0.077[a]	2433[a]	2120[a]	49.0[a]
WWM-16	0.228[a]	13.8[a]	694[a]	51.6[a]	0.099[a]	5993[a]	2138[a]	50.1[a]
WWM-30	0.209[a]	12.9[a]	817[a]	62.0[a]	0.099[a]	609[a]	2142[a]	50.4[a]
WWM-31	0.232[a]	17.2[a]	369[a]	28.8[a]	0.093[a]	998[a]	2037[a]	48.7[a]
WWM-35	0.208[a]	12.3[a]	639[a]	45.9[a]	0.110[a]	3470[a]	2237[a]	49.3[a]
WWM-37	0.225[a]	14.8[a]	484[a]	37.9[a]	0.050[a]	3617[a]	2067[a]	49.0[a]
LSD	0.364	29.36	692.5	58.33	0.147	10493	314.2	16.71
P-value	0.786	0.989	0.726	0.840	0.956	0.513	0.795	1.000

Stomatal conductance (**gs**), mmol m^{-2} s^{-1}; transpiration rate (**T**), mol cm^{-2} s^{-1}; intercellular CO_2 concentration (**Ci**), μmol CO_2; ratio of intercellular and atmospheric CO_2 (**Ci/Ca**); maximum quantum efficiency of photosystem II photochemistry (**Fv/Fm**); electron transport rate (**ETR**), μmol m^{-2} s^{-1}); maximum fluorescence (**Fm'**); photosynthetic rate (photo), mol m^{-2} s^{-1}.

Table 15: Means of leaf gas exchange and chlorophyll fluorescence parameters of citron watermelon genotypes under DS with SSP treatment

Genotype	Leaf gas exchange parameters				Chlorophyll fluorescence parameters			
	gs	T	C*i*	C*i*/C*a*	Fv/Fm	ETR	Fm'	Photo
WWM-1	0.224[a]	21.3[a]	251[a]	20.2[a]	0.087[a]	3885[a]	2058[a]	52.53[a]
WWM-16	0.366[a]	19.9[a]	287[a]	22.1[a]	0.092[a]	4923[a]	2029[a]	53.48[a]
WWM-30	0.293[a]	22.5[a]	248[a]	19.6[a]	0.178[a]	4663[a]	1876[a]	53.16[a]
WWM-31	0.218[a]	11.4[a]	560[a]	38.6[a]	0.125[a]	5733[a]	2162[a]	56.36[a]
WWM-35	0.348[a]	24.6[a]	214[a]	17.0[a]	0.100[a]	1299[a]	1985[a]	52.30[a]
WWM-37	0.257[a]	15.8[a]	719[a]	50.0[a]	0.068[a]	7184[a]	2153[a]	52.22[a]
LSD	0.248	14.18a	661.1	38.98	0.139	13280	160.7	6.908
P-value	0.698	0.403	0.487	0408	0.605	0.242	0.019	0.663

Stomatal conductance (**gs**), mmol m^{-2} s^{-1}; transpiration rate (**T**), mol cm^{-2} s^{-1}; intercellular CO_2 concentration (**Ci**), μmol CO_2; ratio of intercellular and atmospheric CO_2 (**Ci/Ca**); maximum quantum efficiency of photosystem II photochemistry (**Fv/Fm**); electron transport rate (**ETR**), μmol m^{-2} s^{-1}); maximum fluorescence (**Fm**'); photosynthetic rate (photo), mol m^{-2} s^{-1}.

Table 16: Means of leaf gas exchange and chlorophyll fluorescence parameters of citron watermelon genotypes under DS with struvite treatment

Genotype	Leaf gas exchange parameters				Chlorophyll fluorescence parameters			
	gs	T	Ci	Ci/Ca	Fv/Fm	ETR	Fm'	Photo
WWM-1	0.279[a]	21.01[a]	256[a]	20.1[b]	0.0622[a]	1193[a]	2010[a]	54.6[a]
WWM-16	0.182[a]	4.41[b]	1830[a]	121.7[a]	0.0158[a]	910[a]	2201[a]	58.1[a]
WWM-30	0.043[a]	3.15[b]	2068[a]	138.6[a]	0.0280[a]	2567[a]	2261[a]	59.2[a]
WWM-31	0.121[a]	10.47[b]	627[a]	45.7[b]	0.0466[a]	2045[a]	2222[a]	57.6[a]
WWM-35	0.127[a]	9.79[b]	643[a]	45.2[b]	0.0718[a]	1361[a]	2270[a]	58.0[a]
WWM-37	0.140[a]	9.43[b]	776[a]	57.1[b]	0.0181[a]	1559[a]	2165[a]	57.4[a]
LSD	0.1698	6.654	1081.7	58.70	0.0712	5400.3	247.3	10.01
P-value	0.147	0.001	0.017	0.005	0.442	0.417	0.288	0.945

Stomatal conductance (**gs**), mmol m^{-2} s^{-1}; transpiration rate (**T**), mol cm^{-2} s^{-1}; intercellular CO_2 concentration (**Ci**), μmol CO_2; ratio of intercellular and atmospheric CO_2 (**Ci/Ca**); maximum quantum efficiency of photosystem II photochemistry (**Fv/Fm**); electron transport rate (**ETR**), μmol m^{-2} s^{-1}); maximum fluorescence (**Fm**'); photosynthetic rate (photo), mol m^{-2} s^{-1}.

3.4.3 Chlorophyll content index (CCI)

Highly significant differences ($p<0.05$) among the treatments with respect to chlorophyll content index (CCI) were observed (Table 17). The highest CCI was in the WWM-1 and WWM-37 under the control, followed by WWM-31 under both NS and DS, whereas genotype WWM-35 recorded the lowest CCI under the same conditions (NS and DS). The highest CCI in struvite treatment was observed in WWM-37 followed by WWM-16, and the lowest CCI was in WWM-31 under both NS and DS. In the SSP treatment, the highest CCI was observed in WWM-35 followed by WWM-16, whereas genotype WWM-1 recorded the lowest CCI. Struvite and SSP treatments tended to increase CCI, while DS tended to decrease the CCI of citron watermelon genotypes.

Table 17: Means for plant chlorophyll content index in citron watermelon genotypes during the growth stage

Chlorophyll Content Index (CCI)						
Genotype	Control		Struvite		SSP	
	NS	DS	NS	DS	NS	DS
WWM-1	28.50[a]	26.40[a]	41.33[d]	38.17[d]	42.33[f]	38.99[f]
WWM-16	13.67[b]	11.33[b]	45.43[a]	41.56[a]	47.60[b]	44.25[b]
WWM-30	25.00[a]	22.11[a]	42.57[c]	39.77[c]	43.23[d]	41.41[d]
WWM-31	27.27[a]	24.70[a]	41.47[d]	38.11[d]	42.70[e]	39.77[e]
WWM-35	6.77[c]	5.32[c]	44.50[b]	42.89[b]	49.40[a]	46.98[a]
WWM-37	30.77[a]	27.87[a]	45.60[a]	40.21[a]	44.73[c]	41.32[c]
LSD	2.387	2.117	0.642	0.611	0.288	0.243
P-value	<.001	<.001	<.001	<.001	<.001	<.001

SSP (Single superphosphate), **Struvite** (Urine derived fertilizer), **Control** (Without treatment)

3.5 Discussion

The increase in plant tissue (leaf and vine) nutrients in the struvite-treated soil (Tables 7 and 8) could suggest an improved nutrient uptake of these elements compared with what the SSP fertilizer or chemical commercial fertilizer supplies. The high amount of sesquioxides in the urine-derived fertilizer (struvite) could have resulted in P fixation (Biswas *et al*., 2022), manifesting as a high P content in this treatment. Under non-stress conditions, the increase in N uptake by plants in the struvite treatment could be attributed to the mineralization of the organic matter present in the struvite, thus releasing N to plants. This additional N is likely to have contributed to the green leaf colour (Figure 17**)** of the plants grown in struvite-treated soil as compared to the chlorotic citron leaves of the watermelon genotypes grown in the control soils which showed N deficiency symptoms (yellowish colour) (Figure 18).

A higher uptake of Na, Mg, K, Zn, Cu, Mn, and Fe by fruit in struvite treatment compared with the control under both non-stress and stressed conditions could be credited to the availability of these nutrients in urine-derived fertilisers, especially in struvite. Gell *et al.* (2011) revealed that struvite contains an array of nutrients (sulphur, potassium, calcium and magnesium) at levels near 1%, indicating that there could be an added value to the use of struvite that extends beyond N and P presence compared with conventional fertilizers. Generally, the high uptake of nutrients from SSP could be attributed to the high CEC of the soil due to its high clay content. Hazelton and Murphy (2007) noted that soils with clay particles can attract (bind) cations, since they have a negative charge, and release them to the soil water for plant uptake.

According to Hansch and Mendel (2009), macronutrients are essential for plant growth and are involved in nearly all cellular and metabolic processes, including hormone perception, energy metabolism, primary and secondary metabolisms, cell protection, gene regulation, signal transduction, and reproduction. Struvite-treatment of citron watermelon fruit resulted in an increase in flesh P concentration, while under drought stress conditions it led to a significant decrease in Mn concentration. In contrast to the control, struvite treatment significantly raised N (23.1%), P (6.2%), and K (7.5%) concentrations under non-stress (NS) but had no effect on the Cu concentration of the flesh (Table 9.2). According to this research, applying urine-derived fertilizers greatly improves the nutrient concentrations present in fruit rind and flesh of citron watermelon genotypes (Shinbori *et al*., 1981).

Plant growth and nutrition have also been shown to be negatively impacted by micronutrient deficiencies (Bonilla *et al*., 2004; Uchida, 2000). For example, deficiencies in zinc (Zn) primarily impact growth, hormone production, and flowering (Hafeez *et al*., 2013); deficiencies in copper and manganese, on the other hand, primarily affect protein synthesis, respiration, and chlorophyll production, resulting in delayed maturity (Uchida, 2000). The present citron watermelon study revealed a higher iron concentration in the fruit flesh compared to the fruit rind. This could be attributed to an increase in light energy transformation efficiency, CO_2 conductivity, dark reaction activity, and photosynthetic rate (Davis *et al*., 2008). Genotypes WWM-1 and WWM-31 exhibiting red and orange, this could be attributed to high concentration of lycopene (Figure 10). Yellow-flesh colour that was observed in genotype WWM-35 (Figure 11) could be attributed to a higher concentration of carotenoids such as violaxanthin, neoxanthin and lutein, whereas orange-fleshed colour was observed for genotype WWM-31, which could be attributed to the concentration of *β*-carotene, pro-lycopene, and phytoene, coupled with a low lycopene content. Pink-fleshed colour was observed in Genotype WWM-30, which could be attributed to its high concentration of lycopene (Liu *et al*., 2012; Bang *et al*., 2010).

The evaluated citron watermelon genotypes showed a range of responses in physiological traits measured in the study. In the citron watermelon genotypes of this study, water deficit led to the impairment of physiological processes, including stomatal conductance, transpiration, and net CO_2 assimilation (Table 11, Table 12, and Table 13) under NS. Water conservation is linked to decreased stomatal conductance and reduced transpiration rates, assisting plants in preventing dehydration and the loss of physiological functions (Ncama *et al*., 2022). Among the examined citron watermelon genotypes, reduced stomatal conductance, transpiration rates and photosynthetic activity are indicative of effective drought avoidance (Mandizvo *et al*., 2022). This enables the crop to endure severe drought conditions, such as those found in semi-arid and arid regions, like in the Limpopo Province, where the genotypes were gathered from.

Citron watermelon genotypes that were evaluated exhibited high maximum chlorophyll fluorescence following struvite and SSP treatments in both NS and DS. This indicates that in the assessed genotypes, higher amounts of photons were emitted from the chloroplasts, with the extent of emission depending on the concentration of the excited molecules. This excitation was created by the excessive absorption of energy (light) by these fluorescent molecules. This study revealed that highly drought-tolerant genotypes exhibited a higher maximum ETR compared to other genotypes under NS. Citron watermelon genotypes WWM-30, WWM-31, and WWM-37 recorded a higher maximum fluorescence under both NS and DS than genotypes WWM-1, WWM-16 and WWM-35.

The chlorophyll content index (CCI) is indicative of the total amount of chlorophyll present in a plant, as it measures the nitrogen (N) content of the plant (Liu *et al*., 2019). A high chlorophyll content was recorded for the SSP and struvite treatments under both NS and DS; this could mean that (nitrogen) N was efficiently taken up by plants from the soil and transported to other parts of the plant (Table 17). The control exhibited the lowest chlorophyll content index under both NS and DS; this could be due to the very low inherent N content in citron watermelon genotypes, compared with N in leaves of SSP and struvite-treated plants. DS tended to decrease the chlorophyll content in all treatments (struvite, SSP and control). This could be due to water deficiency which resulted in plants not able to transport nutrients, particularly N, to other parts of the plant.

3.4.3 Conclusion

The present study aimed to gain knowledge on the nutritional composition and on key factors, including root characteristics, that may be associated with yield variation among citron watermelon genotype in South Africa. Citron watermelon is grown by resource-strained farmers, whose land is characterized by frequent drought occurrence and poor soils with low nutrient levels and high acidity. His study provided evidence that local farmers already grow a range of genotype adapted to these conditions.

Through this study, it has been established that the urine-derived fertilizers (struvite) improved the plant performance more so than the control. Treatment with SSP and struvite outperformed the control concerning nutrient composition and physiological factors under NS and DS. Urine-derived fertilizer improved the chlorophyll content index (CCI) of plants, compared to SSP treatment and the control.

In conclusion, application of urine-derived fertilizer significantly increases nutrient uptake and plant physiological performance of citron watermelon, especially when genotypes were planted under NS. Nutrient concentration of citron watermelon genotype was higher in struvite and SSP treatments than that under the control.

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CHAPTER 4: SUMMARY AND CONCLUSIONS

4.1 General discussion

Citron watermelon is a monoecious plant, which necessitates cross-pollination, leading to both genetic and phenotypic diversity. Assessing the factors that lead to yield variation present among citron watermelon genotypes is crucial for the development of new cultivars and the improvement of existing ones. These genotypes are frequently cultivated by small-holder farmers, who produce this crop under water-scarce conditions. Selection of superior genotypes for breeding purposes and trait transfer to genetically improve watermelon requires the evaluation of genotypes under water-restricted conditions. Studies by Ngwepe *et al.* (2021) and Mandizvo *et al*. (2021) on citron watermelon yield variation do not pinpoint the cause(s) of yield variation among citron watermelon genotypes. The present study, therefore, assessed the factors associated with yield variation among citron watermelon genotypes and, additionally, investigated the potential of urine-based P fertilizer on growth and development of this crop.

Varied responses were revealed by the study for the measured morphological (leaf length, leaf width, leaf area, root length, vine length, and fruit mass) and physiological (leaf gas exchange and chlorophyll fluorescence) parameters among the assessed citron watermelon genotypes.

Results obtained in this study reveal that the rate of leaf growth under NS and control conditions was significantly lower than that of the SSP treatment and the struvite treatment. The fast-vegetative growth of plants in the SSP treatment (greater leaf length, leaf width and leaf area) could be attributed to the inherent low soil fertility (improved by P application). This higher growth rate could be due to the nutrients readily available in the SSP fertilizers. The slow growth rate of control plants could also be due to the loss of N through denitrification. The volatilization of ammonia from the struvite fertilizer during processing could have been the reason for the slow leaf growth rate compared to the SSP treatment.

A high growth rate was, however, revealed in the citron watermelon genotypes from the struvite treatment under drought-stress compared with the control. This could be due to the release of mineral elements and their subsequent uptake, particularly of P, which promoted plant growth through positively impacting cell division and cell enlargement. The current study discovered that the citron watermelon genotypes had a reduced leaf area under NS compared to DS, indicating the plant’s phenotypic flexibility and the ability to use an effective drought-avoidance method. This reduction in leaf area was observed in the struvite, the SSP, and in the control treatments among all citron watermelon genotypes under drought stress. This verifies findings by Queenborough and Porras (2013), who found specific leaf area (SLA) to be a useful indicator of a plant's ability to tolerate drought; as the SLA declines, less water is lost from the leaf, thereby increasing the plant's water use efficiency.

The chlorophyll content index (CCI) can be used as a measure of a leaf's nitrogen (N) concentration. Citron watermelon genotypes under NS exhibited high CCI compared to genotypes under DS, and this could be due to water deficiency that resulted in failure of plants to transport nutrients, particularly N, to other parts of the plant. High CCI was recorded in the struvite treatment than in the SSP and control treatments in both NS and DS. This could mean that the N supplied in these treatments was efficiently taken up by plants. The control exhibited the lowest CCI under both NS and DS, and this could be due to the very low inherent N in the soil (medium). This corroborates findings of Wang *et al*. (2014) who observed a relationship between N concentration and CCI. The study revealed a positive relationship between CCI and leaf growth traits (leaf length, leaf width, and leaf area), possibly indicating the major role that N plays in the leaf growth of the assessed citron watermelon genotypes. The longer vine length of citron watermelon genotypes in the struvite treatment compared to SSP and control treatments under NS could be attributed to the readily available nutrients, particularly P and N, in this fertilizer. A positive relationship between CCI and vine length was found for all P treatments, further emphasizing that P is required for plant growth.

The high concentration of Na, Mg, K, Zn, Cu, Mn and Fe in the leaves of the struvite-treated genotypes under both no-stress and stressed conditions, is likely due to the availability of these nutrients in the struvite. Gell *et al.* (2011) revealed that struvite contains the nutrients S, K, Ca and Fe at levels near 1%, which indicates that there could be an added value to the use of struvite that extends beyond N and P presence.

Generally, the high uptake of nutrients by the citron watermelon plants in the SSP and struvite treatments, particularly P, could be due to the availability of these nutrients in the soil (medium). The increase in the concentration of plant nutrients in the struvite-treated soil suggests an improved nutrient retention when struvite is applied compared to the application of SSP fertilizer. The higher amounts of sesqui-oxides in the urine-derived struvite fertilizer could have resulted in a slight P fixation (Biswas *et al*., 2022), allowing plants to make use this P and transporting it to the leaves. Under no-stress conditions, the increase in N uptake by the struvite treatment could be attributed to organic matter mineralization, thus, releasing N from the soil that was initially unavailable. This, seemingly, contributed to the greener leaf colour (Figure 11**)** of plants grown in struvite-treated soil, as compared to the chlorosis of citron watermelon genotypes grown in the control soils (Figure 12). The enhancement of K, N, Ca, and Mg in struvite-treated soil is contrary to Postma's (2003) explanation that these nutrients are unavailable for uptake in acidic soils.

The evaluated citron watermelon genotypes exhibited a higher maximum fluorescence following struvite and SSP application, whether not stressed (NS) or drought-stressed (DS). This indicates that the assessed genotypes under struvite and SSP treatments had absorbed a high amount of light (photons), since fluorescence is created by the absorption of energy (light) by fluorescent molecules, called fluorophores (Buschmann *et al*., 2000). This study revealed that highly drought-tolerant genotypes exhibited a higher electron transport rate (ETR) under DS compared to other genotypes under NS. According to Abraha *et al.* (2021), drought-tolerant genotypes use water more efficiently, produce stable yields, reduce water loss, and maintain the tissue water status, when water is a restricting resource. Such genotypes can produce higher fruit yield with very limited rainfall (Sofo *et al*., 2012).

Certain physiological processes were reduced under DS, such as transpiration rate, stomatal conductance and net CO_2 assimilation. In the control, however, the activity of these physiological processes was higher. This could be due to a higher transpiration rate and a higher stomatal conductance, resulting in a higher water loss from such plants. Furthermore, irrigated citron watermelon genotypes with struvite treatment exhibited a higher photosynthetic efficiency than those that received SSP treatment and the control whether under NS or DS. Under NS, an increment in intercellular CO_2 concentration (C*i*) was observed for all citron watermelon genotypes in the struvite treatment compared with no fertilizer (control) and SSP application; this could be attributed to the flux of CO_2 into the leaf. According to Mashilo *et al*. (2017), elevated Fv/Fm under drought stress conditions is an indicator of drought stress tolerance. An effective defence against excessive energy being emitted in the PSII reaction centres is the increase in genotype C*i*/C*a* levels (Zivcak *et al*., 2013; Dambrosio *et al*., 2006).

In the present study, the increase in both root length and dry mass, was observed under DS compared to NS. Citron watermelon genotypes had a greater root dry mass in the struvite treatment than in the control. This could be due to the fact that root growth is encouraged under DS (water-scarce), so the plant will partition more CHO and other resources to that region to be able to just maintain the reduced above-ground growth. The results of this study are consistent with the optimal partitioning theory, which states that "plants preferentially allocate biomass to acquire the resource that most limits growth" (Kobe et al., 2010; Weiner, 2004). Greater root dry mass among citron watermelons genotypes can also be attributed to the high content of nitrogen (N) in roots, since N affects root growth (Mandizvo *et al*., 2022).

Under DS, genotypes had longer roots in all levels of treatments, particularly in the struvite treatment; this offers resistance to water stress and allows drought tolerance, as the plant will be able to obtain water from deep in the soil profile to support plant growth. The results of the current study are consistent with the functional balancing theory developed by Ritchie (1998), which states that plants respond to limited water availability by a relative increase in the flow of assimilates to the root, which results in an increase root fresh and dry mass.

In the present study, wide variations were observed in the assessed citron watermelon genotypes in terms of fruit mass. Fruit mass was greater following struvite treatment under NS compared to the control in all genotypes. This could be attributed to the high phosphorus (P) content in the struvite, which resulted in greater citron watermelon fruit mass leading to high yields. Fruit mass of all citron watermelon genotypes was lower under DS, and this could be due to water deficiency. However, struvite treated genotypes exhibited greater fruit mass compared to SSP and control under DS.

The fruit qualitative traits also confirm the variability present among citron watermelon genotypes, with fruit differing in marbling, stripes and flesh colour. Genotypes WWM-16, WWM-30, and WWM-37 were high-yielding under NS. Fruit stripes were present in genotypes WWM-1, WWM-16, and WWM-30, independent of the fertilizer and stress application. The stripes of these genotypes (WWM-1, WWM-16 and WWM-30) were thicker and dark under NS, while the stripes under DS were thinner and slightly light. Marbling was observed only in genotype WWM-1, WWM-16, and WWM-35, whether under NS or under DS. A yellow flesh colour was observed in WWM-16 and WWM-35, while a green flesh colour was observed in WWM-1, WWM-30, and WWM-31. This could be attributed to the high composition of lycopene. An orange flesh colour was only observed in WWM-37 (Bang *et al*., 2010).

4.2 Conclusions and recommendations

The present study shows that struvite can be successfully used as a fertilizer for citron watermelon. Urine-derived fertiliser has been considered by the current study as a useful fertilizer that can improve citron watermelon morphologically (Table 1), physiologically (Table 11) and it can also improve nutrient uptake and plant performance. Many studies have demonstrated the fertilizer value of urine, nonetheless, these studies used synthetic urine. More studies are needed to investigate the effects of urine-derived fertilizers (struvite) on different soils and crops to confirm their comparability to chemical fertilizers.

The current study assessed six citron watermelon genotypes (WWM-1, WWM-16, WWM-30, WWM-31, WWM-35, and WWM-37), planted with two different P fertilizers (SSP and struvite) and without additional P. These citron watermelon genotypes were planted under NS or DS. A wide range of variations were observed for quantitative and qualitative traits among genotypes in different levels of fertilizers, under both NS and DS. This variation could assist South African breeders in developing new cultivars to improve the production of citron watermelon.

Through this study, it has been established that the urine-derived fertilizer, struvite, improves plant performance above the 'no fertilizer' treatment. Application of standard SSP fertilizer and struvite treatment improved citron watermelon growth concerning the leaf characteristics (leaf length and leaf width) under NS, while struvite application outperformed both the control and the SSP treatment, under DS. The study recommends the identified genotypes for citron watermelon production.

Urine-derived fertilizer improved the chlorophyll concentration of citron watermelon plants under both NS and DS (Table 17). The high concentration of chlorophyll improved the plants' physiology, including photosynthetic rate, resulting in high yielding genotypes. This indicates that struvite improved plant growth and development. Struvite and SSP effectively increased plant root growth (root dry mass and root length) under drought-stress condition. However, struvite application outperformed SSP application, producing greater root mass under both NS and DS conditions. The study recommends the application of struvite in citron watermelon production under NS and DS.

The high uptake of nutrients by citron watermelon genotypes with struvite treatment, as discovered in this experiment, could be attributed to the high CEC of the soil used (Tables 9.1 and 9.2). The increase in N uptake by citron watermelon genotypes with struvite treatment could be due to the mineralization of the organic matter, thus, releasing N that was initially unavailable. This contributed to the greener leaf colour of plants grown in struvite-treated soil (Figure 11) compared to the chlorosis of plants grown in the control (Figure 12).

Citron watermelon genotypes WWM-1, WWM-35, and WWM-37 were high yielding; therefore, the present study recommends them for livestock feed and even food production. The present study recommends the citron watermelon genotypes WWM-30, WWM-35, and WWM-37 for use as leafy vegetables, due to their longer vines and profuse branching ability, whereas the genotypes WWM-1, WWM-30, WWM-35, and WWM-37 are recommended for fruit production due to their inherent larger fruit size. The study also recommends the orange-fleshed genotype WWM-37 for cooking, fresh consumption and processing. Results of the present study corroborate the findings of Solmaz and Sari (2009) and Szamosi *et al*. (2009) who reported a wide range of variation in several fruit and seed traits in citron watermelon.

Presently, there are no genetically improved citron watermelon cultivars in South Africa that are used for processing, commercial production or marketing. The identified genotypes are recommended for various uses, including production for direct consumption, further citron watermelon commercialization, and potential product development and processing. Urine-derived fertilizer (struvite) could provide a viable option for the production of this crop; however, management practices, application type, and timing of fertilizer must be optimized. In conclusion, the study shows that the use of urine-based fertiliser, such as struvite, can improve P solubility in acidic soils and lead to increased root growth, nutrient uptake, and yield of citron watermelon. Further research on how to use struvite fertilizer, the amount applied, mixing with other P source fertilizers and breeding efforts on citron watermelon grown for different purposes should be carried out.

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Appendices

Size: 11.75 X 16.3 cm

Appendix Figure 1: The randomized layout of citron watermelon genotypes (144 experimental units)

size: 10.88 X 12.38 cm

Appendix figure 2: The green colour on the citron watermelon plant leaf indicates the higher content of nitrogen (N)

Size: 11.65 X 9.2 cm

Appendix figure 3: The yellow colour on the citron watermelon plant leaf indicates nitrogen (N) deficiency

size: 11.56 X 13.97 cm

Appendix figure 4: Citron watermelon roots were oven dried at 70ºC to a constant mass