Identifying suitable citron melon (*Citrullus lanatus* var *citroides*) genetic resources for improved cultivation under low-nitrogen growing conditions

By

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Thesis abstract

Low-nitrogen production environments, an example of abiotic stress in agricultural crop production, affect productivity and breeding in citron melon (*Citrullus lanatus* var. *citroides*). Citron melon is an underutilised and nutritious crop possessing natural adaptation to stress-prone environmental growing conditions. Despite being naturally adapted to environmental stress, the crop still requires optimisation for performance under poor growing conditions. Variable performance of citron melon under differential growing conditions affects breeding progress for important traits in breeding programs. Furthermore, citron melon is a significant genetic resource for aiding breeding and enhancing environmental stress tolerance in related cucurbits, such as sweet watermelon. Soil nitrogen deficiency is among the key limiting factors affecting crop productivity, influencing plant growth, photosynthetic capacity, yield, and compositional attributes. Additionally, there is limited information and research output reporting nitrogen requirements for optimum citron melon productivity. These make the nitrogen use efficiency important objective for inclusion in citron melon breeding, to enhance the crop's productivity under limited growing conditions and to improve response to selection in breeding activities. This study aimed to improve citron melon for enhanced growth and accumulation of compositional attributes under low-nitrogen production/ growing conditions. The specific study objectives were (1) to identify suitable citron melon genotypes possessing enhanced adaptive attributes for cultivation under low-nitrogen availability for production and breeding, and (2) to determine accumulated metabolites in citron melon genotypes cultivated under low and optimal nitrogen applications to aid selection of suitable genotypes for cultivation.

The first study assessed morpho-physiological traits of four selected citron melon genotypes (WWM-Ranoto, WWM-16, WWM-58, and WWM-65) under low nitrogen (LN) and optimum nitrogen (ON) conditions in greenhouse (GH) and shadehouse (SH) production sites. The following morpho-physiological traits were evaluated: leaf Area (*LA*), vine length (*VL*), nitrogen use efficiency (*NUE*), fruit weight (*FW*), fruit length (*FL*), fruit diameter (*FD*), rind thickness (*RT*), seed per fruit (*SPF*), root length (*RL*) and root weight (*RW*), photosynthetic rate (*A*), stomatal conductance (*gs*), transpiration rate (*E*), intercellular CO_2 concentration (*Ci*), maximum quantum yield of photosystem II (*PSII*) in photosynthesis (*Fv'/Fm'*), the effective quantum efficiency of PSII photochemistry (*ΦPSII*), partial pressure of CO_2 (*PhiCO₂*), photochemical quenching (*qP*), electron transport rate (*ETR*), amount of light absorbed by the leaf (*leafAbs*). Analysis of variance revealed a significant genotype effect ($p \leq 0.01$) for morpho-physiological attributes *LA*, *VL*, *FL*, *RT*, *SPF*, *gs, E*, and *Ci* across all study environments. The nitrogen level effect was significant ($p \leq 0.05$) for morpho-physiological traits *VL*, *SPF*, *An*, *E*, *Ci* and $PhiCO_2$, suggesting differential genotype performance under varied levels of nitrogen availability. Morpho-physiological traits *LA*, *VL*, *NUE*, *FW*, *FL*, *FD*, *RT*, *RL*, *A*, *gs*, *E*, *Ci*, *Fv'/Fm'*, ΦCO_2, *qP*, and *LeafAbs* deferred significantly ($p \leq 0.05$) based on production site. Pearson's correlation analysis revealed strong positive associations between morpho-physiological traits *LA*, *VL*, *NUE*, *FW*, *FD*, RT, *A*, *E* and *qP* under LN production conditions across all sites. Principal components analysis (PCA) revealed a strong positive interrelationship between genotype WWM-58 with morpho-physiological traits *NUE, FW*, *FL*, *RT*, *RW*, *RL*, $PhiCO_2$ and *qP* under LN conditions in GH. Principal components analysis (PCA) revealed a strong positive interrelationship between genotype WWM-16 with morpho-physiological traits *RW*, *FD*, *RT*, *LA*, *VL*, *ETR*, $\Phi PSII$, *Fv'/Fm'* and $PhiCO_2$ under LN conditions in SH. Also under SH, genotype WWM- 58 associated positively with morpho-physiological traits *VL*, *LA*, *RW*, *FL* and *NUE*. This suggests genotype WWM-58 is a selected candidate for further breeding.

The second study assessed the biochemical responses of the citron melon genotypes under LN and ON conditions in GH and SH production sites. The following biochemical traits were sampled: Total soluble solids (*TSS*), titratable acid (*TA*), sugar-acid ratio (*TSS/TA*), total carotenoids (*TC*), sucrose (*S*), glucose (*G*), fructose (*F*), ascorbic acid/ vitamin C (*AA*), and water content (*WC*). The analysis of variance revealed a significant ($p \leq 0.01$) genotype effect for *TSS, TA*, *TSS/TA*, *TC*, G, *AA*, and *WC*. The contents of all evaluated metabolites differed significantly based on nitrogen application. The test site significantly affected ($p \leq 0.01$) most of the studied traits, except TSS/TA, TC, and *%WC*. Pearson's correlation revealed a positive correlation between *AA* and *TC* in LN conditions. Only negative bi-variate correlations were observed for *TSS/TA* with *TA*, and for *AA* with *G*. Principal components analysis (PCA) associated with citron melon genotypes revealed that these citron melon genotypes, WWM-65 and WWM-58, exhibited high *TA*, *TSS*, *TSS/TA*, *TC*, and *AA*, highlighting their potential for commercialisation and selection for fruit quality improvement.

Keywords: Abiotic stress, biochemical attributes, citron melon, low-nitrogen, morpho-physiological traits, nitrogen use efficiency

Conference presentation

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Declaration

I, **Nkanyezi Halala Khaba**, 224026919, hereby declare that the research presented in this thesis is my original research. I confirm that this work does not report any information or data from others without proper acknowledgement or citation. This thesis has not yet been submitted for examination or to obtain a degree at any university or research institute.

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Date 04/ 05/ 2025

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Signed Dr Zamalotshwa Goodness Thungo (*Supervisor*)

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Preface

This thesis consists of individual manuscripts, each chapter functioning as an independent entity, resulting in unavoidable repetition between chapters.

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CHAPTER 1

1.1 Introduction to thesis

Citron melon [*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. Ex Greb.], commonly known as wild watermelon, is an underutilised fruit, vegetable, and forage crop of the *Cucurbitaceae* family and Sub-Saharan Africa origin (Dane & Liu, 2007; Paris, 2015). The citron melon (*Citrullus lanatus var. citroides*) is botanically a fruit (a type of melon or gourd), but is functionally used as a vegetable in culinary applications due to its edible leaves, stems, seeds, and fruits. The curcubit is widely cultivated for its edible vegetative parts (i.e., seeds, succulent leaves and stems, and fruits) (Mandizvo et al., 2022c; Mashilo et al., 2017, 2022; Ngwepe et al., 2021). Citron melon is rich in key compositions including mineral elements (e.g., calcium, iron, manganese, zinc, copper, etc.), macronutrients (e.g., nitrogen, phosphorus, potassium, magnesium, etc.) and antioxidants (e.g., such as β-carotene, phytoene and lutein) (Mandizvo et al., 2022a; Mashilo et al., 2022; Nkoana et al., 2022). As a result, citron melon presents an opportunity to alleviate food and nutrition security through increased consumption of its nutrient-dense vegetative parts.

Citron melon is also known for its persistence under production conditions confounded by various environmental stresses resulting from biotic and abiotic factors (Guzzon et al., 2017; Katuuramu et al., 2024; Mandizvo et al., 2022a; Mo et al., 2016). Consequently, citron melon has been adopted for implementation as rootstock to improve other cucurbits (i.e., watermelon, cantaloupe and cucumber), representing a genetic resource for environmental stress tolerance improvement (Keinath et al., 2019; Kurtar et al., 2024; Robledo-Torres et al., 2024; Semenya & Maroyi, 2019). These signify citron melon as an important food and nutrient resource, as well as a source of resistance genes in cucurbit crop breeding. Despite the presence of natural adaptation against various environmental stress factors, citron melon shares limited productivity under low-input agricultural systems (Katuuramu et al., 2024; Tembo & Maphosa, 2025).

Important abiotic-based environmental stresses in citron melon and other cucurbits include heat and drought, low-temperature, and poor soil fertility (Cavalcante et al., 2019; Katuuramu et al., 2024; Parvathi et al., 2022; Sinha & Tandon, 2020). For example, reduced fruit yield was associated with heat and drought, and low-temperature stresses in citron melon (Mandizvo et al., 2022a; Nanasato et al., 2010; Parvathi et al., 2022). There is limited research investigating the adaptability of citron melon to varied regimes of these abiotic stresses, especially poor soil fertility as a result of low soil nitrogen. Nitrogen is a key plant nutrient required for optimum growth and development, influencing crop yield and quality (Ye et al., 2022; Zhao et al., 2005). This plant macro-nutrient is mostly limited in low-input farming systems, especially in arid and semi-arid regions characterised by combined drought and poor soil quality (Assogba et al., 2023), suggesting the requirement for combined nitrogen and drought stress tolerances in crop production.

Poor fruit yield and quality, early flowering, stunted vine length and leaf deterioration were partly attributed to limited soil nitrogen in cucurbits (Nowaki et al., 2017; Özmen et al., 2015).. Therefore, the genetic potential of citron melon for enhanced performance under low-nitrogen environments should be increased in order to maintain high productivity levels. There is a need to identify adaptive plant growth attributes to low-nitrogen stress in citron melon, for recommendations for improvement to increase genetic advance. The significant advancements in drought tolerance breeding for citron melon (Guzzon et al., 2017; Ngwepe et al., 2023; Yoshimura et al., 2007) signify that additional genetic resistance for other abiotic stress factors would result in the development of more resilient cultivars for cultivation and breeding, and for the genetic improvement of related cucurbits.

There is limited breeding research targeting the improvement of low-nitrogen stress tolerance in citron melon, which hinders the identification of suitable germplasms for cultivation and breeding. In other cucurbits, limited breeding progress has been reported for nitrogen use efficiency (Nawaz et al., 2018). The metabolic response of citron melon under cultivation in low-nitrogen environments is under-researched. An understanding of the changes in the phenotype and metabolic profiles of citron melon in response to cultivation under low-nitrogen conditions can serve as a platform for identifying biomarkers for breeding low-nitrogen stress. Phenotyping using morpho-physiological and metabolomic plant traits can aid the identification of adaptive attributes to low-nitrogen stress and the isolation of superior germplasms for cultivation and breeding. The bi-variate and multi-variate correlation analyses procedures are important to reveal associations present among plant growth attributes in citron melon (Mandizvo et al., 2022b; Tembo & Maphosa et al., 2025), important to improve breeding objectives, methods and strategies in citron melon.

1.2 Aim of the study

The aim of this study was to identify citron melon genotypes possessing adaptive plant growth attributes to cultivation under low-nitrogen production conditions for cultivation and abiotic stress breeding.

1.3 Specific objectives

i. To identify suitable citron melon genotypes possessing enhanced adaptive attributes for cultivation under low-nitrogen availability for production and breeding.
ii. To determine accumulated metabolites in citron melon genotypes cultivated under low and optimal nitrogen applications to aid selection of suitable genotypes for cultivation.

1.4 Structure of thesis

This thesis comprises five chapters, partly in accordance with the specified objectives (**Table 1.1**). Chapter 1 serves as an introduction to the thesis, whereas Chapter 2 presents the meta-narrative review. Chapters 3 to 4 represent separate and unequal experimental reports. Lastly, Chapter 5 presents a general discussion, followed by a conclusion and future perspective sections of the study. The introduction to the thesis, literature review and experimental chapter reports were compiled into interconnected reports following a recommendation by the University of KwaZulu-Natal.

Table 1.1 Thesis structure

Chapter	Title
1	Introduction to thesis
2	Literature Review
3	The morpho-physiological responses of selected citron melon genotypes were evaluated under a low-nitrogen environment.
4	The Metabolomic responses of selected citron melon genotypes under low nitrogen environment.
5	General discussion, recommendations and conclusion

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CHAPTER 2

Variation in the phenotype of abiotic stress-adaptive attributes affects citron melon (*Citrullus lanatus var citroides*) cultivation: Implications for productivity and plant breeding selection: A review

Abstract

There is a need to enhance the genetic potential of citron melon (*Citrullus lanatus* var. *citroides*) for adaptive traits to cultivation under various abiotic stress types, thereby improving productivity and response to selection in plant breeding programs. Citron melon is a climate-smart crop, cultivated for its edible vegetative plant structures, including leaves and fruit. Significant quantities of nutrients and phytochemicals are present in citron melon, making the crop suitable for food and nutrition security, as well as for health. As a result, increased productivity in citron melon is crucial for enhanced global access to adequate, healthy food crops. Citron melon possesses natural adaptability to various abiotic stress factors, suggesting an opportunity to develop superior cultivars possessing enhanced productivity. Subsequently, citron melon presents as a significant genetic resource for abiotic stress adaptation genes, which are important for enhancing fruit production and quality in other cucurbits, including sweet watermelon. Nevertheless, variation in the phenotype of adaptive attributes affects productivity and reduces the efficiency of plant breeding selection involving citron melon. The objective of this review is to discuss the influence of varied abiotic stress types on the variation of phenotype for adaptive attributes in citron melon, using morphological, physiological and metabolomic attributes, to inform cultivation for production and plant breeding selection. The variation of phenotype for adaptive morphological attributes in response to regimes of different abiotic stress factors is discussed. The performance of adaptive physiological attributes to varied regimes of different abiotic stresses is also discussed. The review also incorporates a discussion of molecular markers (i.e., candidate genes, metabolites, DNA markers, and quantitative trait loci) that underpin variation in the phenotype of abiotic-stress-adaptive morphological and physiological attributes in citron melon cultivation and breeding.

Keywords: Abiotic stresses, adaptive attributes, citron watermelon, cucurbits, metabolites, morpho-physiological traits, phenotypic variation

2.1 Introduction

Citron melon *(Citrullus lanatus* var *citroides* (L.H. Bailey), a monoecious member of the Cucurbitaceae family, is cultivated in various regions of the world for its edible succulent leaves, seed and fruit, which are important for human consumption and animal feed formulation (Dane & Liu, 2006; El-Sayed et al., 2021). The citron melon originated from Sub-Saharan Africa, making it well-suited for production in arid and semi-arid regions (Dane & Liu, 2006; Levi et al., 2014). The following nutrients are present in citron melon leaves, seed and fruit: carbohydrates (e.g., fructose, glucose, cellulose, malic acid, etc.) vitamins (e.g., vitamin A, vitamin C etc.), minerals (e.g., potassium, calcium, iron, etc.), protein (e.g., globulins, albumins, glutelins, etc.) and lipids (e.g., linoleic acid, oleic acid, stearic acid, etc.) (El-Sayed et al., 2021; Masoko et al., 2022; Nkoana et al., 2022). Carotenoids such as β-carotene, prolycopene, phytoene and neoxanthin are also present in citron melon, especially β-carotene in orange-fleshed cultivars.

The roots of citron melon contain bioactive compounds (e.g., cucurbitacin, flavonoids, etc.), which are useful to treat tuberculosis (El-Sayed et al., 2021; Maoto et al., 2019; Masoko et al., 2022; Semenya & Maroyi, 2019). Furthermore, the crop exhibits antibacterial properties against *Staphylococcus aureus, Bacillus. subtilis, Escherichia. coli, Proteus. vulgaris and Pseudomonas. aeruginosa* (Hassan et al., 2011), signifying effectiveness as a standard antimicrobial drug. Antifungal properties against *Candida. albicans* were also reported in citron melon (Hassan et al., 2011; Masoko et al., 2022). An overripe fruit flesh helps alleviate fever and acts as a natural diuretic to relieve kidney problems (Braide et al., 2012; Hassan et al., 2011; McGregor, 2012), while the seeds and rind help against high blood pressure and alcohol poisoning, respectively (Erhirhie & Ekene, 2013). These signify the importance of citron melon as a food and nutrition crop. Therefore, the cultivation of citron melon could be promoted to support the goal of the Group of Twenty (G20) international forum to increase the supply of food products (Srivastava & Kashyap, 2025).

The cultivation of citron melon is influenced by varied abiotic environmental stress factors, including heat and drought, low temperature, and poor soil quality (Coşkun, 2025; Guzzon et al., 2017; Kurtar et al., 2024; Ramirez et al., 2014). Abiotic environmental stress factors limit productivity and alter the performance of breeding materials in crop improvement programs, reducing the efficiency of selection. The development of improved citron melon germplasms is crucial for enhanced productivity and breeding progress. The cultivation of citron melon varieties possessing superior yield and compositional attributes is crucial for food and nutrition security. Additionally, the rootstocks of citron watermelon are utilised as sources of drought tolerance genes in sweet watermelon breeding for improved fruit yield (Levi et al., 2014; Wimer et al., 2015; Yavuz et al., 2020). The inherent genetic potential against multiple abiotic stresses signifies the potential of citron melon as a foundational genetic resource for breeding programs in other cucurbit crops. Furthermore, these findings highlight the significance of abiotic stress in affecting the performance of citron melon across diverse production/ growing regions. Rapid progress in the genetic improvement of various abiotic stress-adaptive attributes in citron melon is crucial for enhancing global food and nutrition security, as well as for responding to selection in citron and related cucurbits breeding. Therefore, there is a need to explore and utilise the untapped genetic potential of citron melon in plant breeding.

Genetic potential exists for several abiotic stress-adaptive attributes in citron melon, as identified through the implementation of classical and molecular-based plant breeding strategies. As a result, citron melon is a recognised genetic resource for environmental stress tolerance breeding in related cucurbits, including sweet watermelon (Edelstein et al., 2014; García-Mendívil et al., 2019; Keinath et al., 2019; Mahapatra et al., 2023). For example, high root biomass activity was associated with drought tolerance in citron melon (Yoshimura et al., 2008). Antioxidant enzymes (e.g., superoxide dismutase, glutathione reductase, catalase and ascorbate), the catalysts behind reactive oxygen species scavenging in plants, are elevated under environmental stress (Mo et al., 2016). Enhanced contents of total soluble sugars and antioxidants were associated with drought tolerance in citron melon (Kirnak et al., 2009). The improved citron melon genotypes exhibited varied performances in terms of yield across South Africa, Botswana, and India’s production regions (Maragal et al., 2019; Mashilo et al., 2017a; Ngwepe et al., 2023b).

Citron melon breeding programs exist globally, including South Africa, China and the United States of America (Levi et al., 2002; Li et al., 2022; Mashilo et al., 2017; Ngwepe et al., 2023). Initiatives such as the Vision for Adapted Crops and Soils (VACS), involving the United States Department of State, the African Union, and the Food and Agriculture Organisation (FAO), support plant breeding programs that target the development of climate-smart germplasms, particularly for underutilised crops such as cucurbits. An assembly of available scientific information in citron melon breeding under diverse production regions could be key to enhancing selection efficiency in current and future breeding programs. This includes information on the available breeding programs, gene banks, improved cultivars, breeding germplasms, and genomic data. There is a need to promote the communication of breeding advances, opportunities and challenges for citron melon and related cucurbits to improve selection efficiency in improvement programs.

A strategic review of the literature to provide an analysis of progress in genetic improvement of tolerance through selection, using various morphological, physiological, and metabolomic attributes in response to abiotic stresses, could be key to accelerating cultivar design and development for enhanced productivity in citron melon. Therefore, this review aims to discuss the genetic variability of citron watermelon in response to varied abiotic stress factors, using adaptive morphological, physiological and biochemical attributes to inform cultivation and plant breeding selection. The review is comprised of two sections: the first section outlines the influence of different abiotic stress factors on the performance and selection of adaptive morphological attributes, and the second section unpacks the role of abiotic stress factors in influencing performance and selection in adaptive physiological attributes in citron melon. Additionally, the review incorporates discussion of molecular markers (i.e., candidate genes, metabolites, DNA markers and quantitative trait loci) that regulate variation in the phenotype of adaptive morphological and physiological attributes in citron melon cultivation and breeding.

2.2 The variation of abiotic-stress-adaptive morphological attributes in citron melon

Important abiotic stress factors affecting cultivation and response to plant breeding selection in citron melon and related cucurbits include heat and drought, low temperatures, poor soil fertility, salinity, and flooding or waterlogging (Colla et al., 2006; Guzzon et al., 2017; Madumane et al., 2024). Published literature on citron melon responses to abiotic stress availability is limited to heat, drought, and low-temperature stress. There is no empirical data for other abiotic stressors such as salinity, flooding, poor soil fertility, heavy metals, or UV stress. The key abiotic-stress-adaptive morphological attributes in citron melon are related to seedling root, stem and leaf, and fruit traits (Fredes et al., 2017; Madumane et al., 2024; Mo et al., 2015; Ngwepe et al., 2023b). Data showing variation in the phenotype of certain morphological attributes in response to different abiotic stressors, recorded under both stress and non-stress production conditions globally, are presented in **Table 2.1**.

2.2.1 The influence of varied regimes of heat and drought stress regimes on adaptive morphological attributes

2.2.1.1 Seedling root traits

Key adaptive root and seedling traits for heat and drought tolerance include increased root biomass, length, depth, width, and the ratio of root to shoot biomass in citron melon and its relatives (Guzzon et al., 2017; Mo et al., 2015; Mandizvo et al., 2022b; Yoshimura et al., 2008). Vigorous root growth is crucial for ensuring adequate moisture and plant nutrient absorption in crop plants, including citron melons. There is significant genetic variation for adaptive root traits in citron melon plant materials cultivated under varied regimes of heat and drought stress (Yoshimura et al., 2008; Guzzon et al., 2017; Madumane et al., 2024; Mandizvo et al., 2022a; Mo et al., 2015). An increased accumulation of genetic advance for root traits adaptive to heat and drought stress could aid productivity and selection in citron melon and related species.

Root biomass, estimated as the total weight of below-ground plant structures, is a key morphological marker for heat and drought tolerance in citron melon. High root biomass is identified as an adaptive morphological attribute for enhanced soil moisture extraction and productivity under drought-prone growing conditions in citron melon. Root biomass increases between 36 and 54 mg per plant was reported in citron melon evaluated under drought conditions, representing a ~50% change compared to the control treatment (Yoshimura et al., 2008). Up to 19% and 26% increases were reported for root system width and depth, respectively, in citron melon tested under drought-prone growing conditions compared to the well-watered treatment (Mandizvo et al., 2022b). Root length increased by 13 and 15% in citron melon, stimulated by exposure to limited soil moisture growing conditions (Mandizvo et al., 2022b; Mo et al., 2016). A significantly higher root-shoot ratio was reported under dry test conditions than in wet treatment for citron melon landraces evaluated at the seedling growth stage (Guzzon et al., 2017; Mo et al., 2016). Contrary to the findings showing increased root expansion under drought, a reduced root-shoot ratio was reported under drought-prone conditions compared to the wet treatment in citron melon (Guzzon et al., 2017). This highlights the significance of the genotype-by-environment interaction effect in influencing the response to selection in citron melon breeding for heat and drought stress tolerance. Proteins actin and a-tubulin were reported in the roots of drought stress-tolerant citron melon (Guzzon et al., 2017). The HSP90 family of heat shock proteins regulates the genetic control of adaptive root attributes in citron melon (Akashi et al., 2011).

Overall, these findings indicate the remodelling of the root system in citron melon subject to water deficit. The enhanced root system attributes (e.g., root biomass, root expansion, etc.) represent the activation of heat and drought stress avoidance mechanisms and tolerance for accessing soil water and nutrients. Also, there is a positive association between root biomass and root length, root width, root expansion, root depth, and root shoot ratio in citron melon and related cucurbits (Guzzon et al., 2017; Mandizvo et al., 2022b; Yoshimura et al., 2008). This suggests that genetic advances for these adaptive root traits can be accumulated simultaneously in citron melon breeding programs. The available heritable phenotypic variation for these adaptive root traits (i.e., increased root biomass, length, depth, width, and the ratio of root to shoot biomass) can be incorporated in current citron melon crop improvement research to increase genetic advance in heat and drought tolerance breeding. The plant breeding programs aimed at identifying heat- and drought-tolerant citron melon genotypes can be conducted under simulated soil moisture stress growing conditions to separate citron melon germplasms possessing genetic potential for an enhanced root system and architecture under heat and drought stress.

2.2.1.2 Stem and leaf traits

Adaptive stem and leaf traits include reduced vine length, leaf area, and number, as well as shoot biomass, to improve water conservation under environmental stress. Heat and drought stress increase leaf temperature in citron melon (Akashi et al., 2011), which can be partly attributed to excessive evapotranspiration from tissues and stomatal closure. Improved citron melon germplasms should exhibit suitable performance and productivity under heat and drought conditions, despite adverse changes in leaf temperature. A decrease in leaf area (17-25%) and vine length (15-24%) was reported in citron melon tested under moisture-deficient conditions, compared to the well-watered treatment (Mandizvo et al., 2022a). This identifies reduced leaf growth and stem elongation as adaptive traits to supporting citron melon growth under deficient moisture. Under simulated drought stress involving citron melon and sweet watermelon, the former was found to have a higher above-ground biomass, ranging from 3.1 to 3.4 g per plant, compared to sweet watermelon (Guzzon et al., 2017). Low leaf epidermal tissue and increased trichome density were reported in citron melon grown under moisture stress compared to the control (Mo et al., 2016). These (i.e., low leaf epidermal tissue and increased trichome density) are significant morphology-based genetic markers for heat and drought resistance in citron melon. Reduced transpiration was associated with resistance to moisture stress in the citron melon genotype [*Citrullus lanatus* (Thumb.) sp. No. 101117-1]. For example, transpiration rate reduced from 622 mmol H_2O^{-2} s^{-1} to 12 mmol H_2O^{-2} s^{-1} within 5 days of moisture stress (Akashi et al., 2011).

Using gel-electrophoresis, dynamics in the leaf proteome were revealed in citron melon *Citrullus lanatus* (Thumb.) sp. No. 101117-1, compared between moisture-stress and non-stress growing conditions. This identified an upregulation of HSP90 family heat shock proteins, which are also linked to the regulation of adaptive root attributes (Akashi et al., 2011). This suggests that genetic advances for adaptive leaf and root traits can be achieved simultaneously in citron melon breeding. The xerophyte citron melon genotype *Citrullus lanatus* (Thumb.) sp. No. 101117-1 can be utilised as a genetic resource to aid in breeding heat and drought tolerance in citron melon. The increased secretion of proteins that are related to antioxidative defence (i.e., lactoylglutathione lyase, glutathione S-transferase, and phospholipid hydroperoxide glutathione peroxides) and carbohydrate metabolism (cytosolic phosphoglumatase and glucose-6-phosphate) is associated with tolerance to moisture stress in citron melon and other terrestrial plant crops (Lytovchenko et al., 2002; Akashi et al., 2011). Additionally, the content of thioredoxin-like proteins, which enhance photosynthesis, increased in the leaf tissues of citron melon plants growing under moisture stress (Akashi et al., 2011). Current crop improvement programs in citron melon and other cucurbits can utilise the thioredoxin-like protein as a biotechnological tool to aid in genetic variation in citron melon, thereby identifying superior parents for breeding that possess enhanced adaptive attributes. Drought stress-responsive genes such as SOD (superoxide dismutase), CAT (catalase), PPH (pheophytin pheophorbide hydrolase), and PAO (pheidea oxygenase) were significantly upregulated in the leaf tissues of wild watermelon (*C. lanatus* var. *citroide*) M20 and Chinese domesticated watermelon (C. *lanatus* var. *lanatus*) *Y34* tested under drought stress conditions (Mo et al., 2016). Leaf tissue influences gaseous exchange and chlorophyll content parameters; therefore, plant breeders should target utilising these genetic resources (i.e., genotypes, genes and proteins) to improve the phenotype of adaptive leaf and stem traits, and response to selection under moisture stress in citron melon and related cucurbit species. These findings highlight the significance of heat and drought stress in shaping the phenotypes of adaptive leaf and stem traits in citron melon cultivation and breeding.

2.2.1.3 Fruit traits

Increased fruit yield, fruit mass and fruit number per plant are criteria for identifying and selecting superior citron melon germplasms in the face of various abiotic stress factors, including heat and drought stress. These traits (i.e., fruit yield, fruit mass, and fruit number per plant) are positively correlated in citron melon, providing an opportunity for simultaneous enhancement in breeding programs (Mandizvo et al., 2022a; Tembo & Maphosa, 2025). Heat and drought stress were linked to fruit yield loss, resulting in a range of 0.63-2.05 kg/plant fruit yield loss across drought-stress and non-stress conditions. This resulted in the identification of the superior citron melon genotype WWM-24, which exhibits high fruit yield (0.99 kg/plant) under drought stress. This genotype (WWM-24) is a potential source of genetic information for enhancing simultaneous heat and drought tolerance, as well as fruit yield, in citron melon. The average number of fruits per plant increased from two to five due to the introduction of 10 citron melon accessions (Tembo & Maphosa, 2025), indicating the presence of genetic variation. Intermating between these citron melon accessions could aid cultivar design and development for enhanced yield per unit area in citron melon. Limited genetic variation was reported for vitamin C content (6.33 mg/100 g to 7.37 mg/100 g), total soluble solids (6.3% to 6.95%) and pH (5.20 to 5.27) in mature fruit, suggesting limited opportunity for selection to enhance fruit quality in the citron melon. The genotypes identified by Tembo et al. (2025) as possessing adaptive attributes to drought stress, including accessions 185 (172 kg/ha), 2766 (114 kg/ha), 179 (160 kg/ha), and 378 (190 kg/ha), could be subjected to further improvement to enhance compositional attributes such as vitamin C, soluble solids, and pH. Qualitative traits, including fruit shape, skin colour, and seed colour, were identified with significant phenotypic variation in citron melon (Tembo & Maphosa, 2025).

Overall, the reported insight highlights the significance of heat and drought stress in influencing fruit yield and related traits, thereby impacting productivity and selection efficiency. There is a lack of research reporting variation in fruit yield and related traits in response to heat and drought regimes in citron melon. The reported citron melon genotypes (e.g., WWM-24), identified with suitable performance under moisture deficiency, could be targeted for incorporation in subsequent breeding endeavours to increase genetic advance for fruit yield.

2.2.2 The influence of varied regimes of low-temperature stress on adaptive morphological attributes

Low-temperature stress affects the choice of planting date, slows growth and maturation, and decreases yield in citron melon (Korkmaz & Dufault, 2001; Katuuramu et al., 2024). Plant materials susceptible to low-temperature stress, among others, have low chlorophyll content, photosynthetic capacity, and biomass production (Kidokoro et al., 2017). Low-temperature stress was linked to poor leaf health and reduced resistance to diseases in watermelon seedlings (Korkmaz & Dufault, 2001). Tolerant citron melon genotypes should possess suitable productivity under low-temperature cultivation conditions. Progress in identifying improved varieties, candidate genes, and genomic regions that harbour low-temperature stress tolerance is crucial for enhancing citron melon cultivation and breeding.

There is limited reported research output targeted to revealing the genetic control of low-temperature stress tolerance in watermelon, including citron melon. Nevertheless, significant variation in shoot biomass was reported due to the effects of genotype, environment, and their interaction (Katuuramu et al., 2024). For instance, shoot biomass ranged between 3 and 65 g/plant under low-temperature stress among 122 citron melon accessions. This was a wider range compared to the 22-72 g/plant recorded under non-stress conditions. A wider variation for shoot biomass under low-temperature stress identifies chilling as a significant source of phenotypic variation in citron melon cultivation and selection. The highest shoot biomass was recorded under non-stress conditions, highlighting the need to achieve genetic gains for combined low-temperature stress tolerance and high above-ground biomass production in citron melon crop improvement programs.

Low-temperature stress reduced vine length by 4-6%, with reported ranges of 114-516 mm and 236-660 mm for stress and non-stress conditions, respectively (Katuuramu et al., 2024).

The adaptive morphological attributes discussed herein should be subjected to breeding to increase genetic advance for chilling stress tolerance in citron melon and its relatives.

Citron melon genotypes USVL_114 and PI_532664 exhibited high shoot biomass of 65.1 and 44.2 g per plant, respectively, under cold stress (Katuuramu et al., 2024). Candidate genes CaU08G17330 and CaU08G17440) and SNPs S8_269 were associated with 04287 high shoot biomass under cold stress citron melon (Katuuramu et al., 2024). (i.e., COR15a, RD29a, EXPA1, EXPA8, EXPA10) (Katuuramu et al., 2024). SNP markers Ca02, Cao5, and Ca06 are associated with the genetic regulation of increased vine length under cold stress conditions in citron melon, explaining 4-16% variance in the test population (Katuuramu et al., 2024). The low deviation in biomass production highlights the crop's potential and resilience under cold temperatures, indicating the presence of genetic potential for cold stress tolerance. Six quantitative trait loci (i.e., CMCT505_Chr.1, DE1329_Chr.2, SSR015829_Chr.3, SSR015 399_Chr.3, SSR018207_Chr.4, and CMAGN75_Chr.7) were linked to the genetic control of low-temperature stress tolerance in melon (Cucumis melo) (Hou eta al. 2018). Genetic markers, including genes such as RD29A, EXPA1 and EXPA-A, were associated with cell expansion, inducing flexibility and loosening of the root cell wall during growth (Katuuramu et al., 2024). The genetic resources for regulating low-temperature stress tolerance reported in this review can be useful for enhancing breeding in citron melon and related cucurbit crops.

2.2.3 The influence of poor soil fertility stress on morphological attributes in citron melon

There is limited research investigating the variation in morphological attributes of citron melon resulting from changes in soil fertility regimes. Infertile soils were associated with an increased development of male flowers and a reduced number of fruits per plant in sweet watermelon (*Citrullus lanatus L.*), a close relative of citron melon (Eifediyi et al., 2017). Supporting research findings reported a low mean fruit yield in watermelon growing under a control treatment (38 t/ha), compared to fertilisation with inorganic nitrogen-phosphorus-potassium

(NPK) fertiliser (334 t/ha) (da Silva et al., 2020). These imply that limited soil fertility cultivation conditions hinder watermelon cultivation, reducing productivity and limiting response selection in breeding programs. For sustainable agriculture, the quantities of inorganic plant nutrient sources used in watermelon production should decrease. The genetic potential of watermelon for increased fruit development under poor soil fertility should be improved.

Soil amended with NPK resulted in a significantly higher mean value for vine length (161mm) in sweet watermelon, compared to the control treatment of no fertiliser application (114 cm) (LSD = 4.59 cm) (Eifediyi et al., 2017). High soil fertility is crucial for longer vines, which correlates to an increased number of flowers and fruits formed. Watermelon varieties that are tolerant to growing under poor soil fertility production conditions should maintain a suitable fruit yield despite the limited availability of plant nutrients. The number of fruits per plant varied significantly in response to variations in soil fertility, with different values reported under fertilisation with inorganic fertiliser (334), cow dung (242), poultry manure (306), and compost (170) (Eifediyi et al., 2017). These were higher than the average number of fruits recorded under no fertiliser application (i.e., 38 fruits per plant). Increased vine length is linked to a larger number of fruits per plant, as demonstrated by combined high records for average vine length and fruit yield under fertilisation with inorganic fertilisers. Therefore, screening for enhanced fruit yield under low-fertility production conditions may fail to select superior genotypes due to limited vine development and fruit production. This will result in a subsequent loss of selection efficiency in improvement programs. Furthermore, this implies that producers and breeders should mitigate stress caused by poor soil fertility in watermelon cultivation.

Table 2.1: Morphological traits of citron melon (*Citrullus lanatus var citroides*) genotypes under abiotic stress (heat, drought, cold stress) conditions

Stress factor	Trait (unit)	Non-stressed	Stressed	Cultivar name	Year of study	Country	Reference
Heat and Drought	Root: shoot ratio	0,73	0,47	RSA (Republic of South Africa)	2017	Italy	Guzzon et al., 2017
		0,69	0,49	UZB (Zimbabwe)	2017	Italy	Guzzon et al., 2017
		0,52	0,49	USA (United States of America)	2017	Italy	Guzzon et al., 2017
		0,54	0,53	RUS (Russia)	2017	Italy	Guzzon et al., 2017
		0,51	0,52	UKR (Ukraine)	2017	Italy	Guzzon et al., 2017
		0,46	0,45	CAN (Canada)	2017	Italy	Guzzon et al., 2017
		0,7	0,4	ITA6 (Italy)	2017	Italy	Guzzon et al., 2017
	Root biomass (mg)	36	54	101117-1	2008	Japan	Yoshimura et al., 2008
	Root length (cm)	16	18	M20	2016	China	Mo et al., 2016
	Root length (day^{-1})	2,192	2,33	WWM-09	2022	South Africa	Mandizvo et al., 2022b
		2,22	2,36	WWM-41 (A)	2022	South Africa	Mandizvo et al., 2022b
		2,49	2,2	WWM-37 (2)	2022	South Africa	Mandizvo et al., 2022b
		2,13	2,46	WWM- 76	2022	South Africa	Mandizvo et al., 2022b
		1,55	1,72	WWM-46	2022	South Africa	Mandizvo et al., 2022b
		1,78	1,74	WWM-64	2022	South Africa	Mandizvo et al., 2022b
		1,67	1,77	WWM-15	2022	South Africa	Mandizvo et al., 2022b
	Shoot length (cm)	9,43	8,21	RSA (Republic of South Africa)	2017	Italy	Guzzon et al., 2017
		10,09	9,31	UZB (Zimbabwe)	2017	Italy	Guzzon et al., 2017
		9,27	8,7	USA (United States of America)	2017	Italy	Guzzon et al., 2017
		8,89	7,94	RUS (Russia)	2017	Italy	Guzzon et al., 2017
		9,18	8,38	UKR (Ukraine)	2017	Italy	Guzzon et al., 2017
		9,67	8,91	CAN (Canada)	2017	Italy	Guzzon et al., 2017
		10,5	9,09	ITA6 (Italy)	2017	Italy	Guzzon et al., 2017
	Shoot biomass (g)	57	57	101117-1	2008	Japan	Yoshimura et al., 2008
	Leaf Trichome density (mm^{-1})	12	13	M20	2016	China	Mo et al., 2016
	Fruit yield (kg)	2,9	2,14	WWM-46	2022	South Africa	Mandizvo et al., 2022a
		2,54	1,94	WWM-68	2022	South Africa	Mandizvo et al., 2022a
		2,89	2,21	WWM-41(A)	2022	South Africa	Mandizvo et al., 2022a
		2,68	2,01	WWM-15	2022	South Africa	Mandizvo et al., 2022a
		2,8	2,1	WWM-64	2022	South Africa	Mandizvo et al., 2022a
		2,66	2	WWM-57	2022	South Africa	Mandizvo et al., 2022a
		2,73	2,13	WWM-47	2022	South Africa	Mandizvo et al., 2022a
		2,75	2,05	WWM-37(2)	2022	South Africa	Mandizvo et al., 2022a
		2,62	1,98	WWM-79	2022	South Africa	Mandizvo et al., 2022a
		2,61	1,97	WWM-05	2022	South Africa	Mandizvo et al., 2022a
		2,78	2,02	WWM-50	2022	South Africa	Mandizvo et al., 2022a
Cold stress	Shoot biomass (g)	13	7,8	PI 244018	2024	South Africa	Katuuramu et al., 2024
		23,5	14,1	PI 270563	2024	South Africa	Katuuramu et al., 2024
		9,8	5,9	PI 482265	2024	Zimbabwe	Katuuramu et al., 2024
		9,7	5,8	PI 482257	2024	Zimbabwe	Katuuramu et al., 2024
		4,8	2,9	PI 271769	2024	South Africa	Katuuramu et al., 2024
		108,3	65,1	USVL_114	2024	Botswana	Katuuramu et al., 2024

Values represent measurements under non-stressed vs stressed conditions.

Table 2.1: continued

Stress factor	Trait (unit)	Non-stressed	Stressed	Cultivar name	Year of study	Region	Reference
Cold stress	Shoot biomass (g)	68,2	41	PI_532644	2024	Eswatini	Katuuramu et al., 2024
		47,1	28,3	PI_596669	2024	South Africa	Katuuramu et al., 2024
		66,1	39,7	PI_542114	2024	Botswana	Katuuramu et al., 2024
		76,9	46,2	PI_532666	2024	Eswatini	Katuuramu et al., 2024
		147,3	192,8	PI 244018	2024	South Africa	Katuuramu et al., 2024
		132,5	173,4	PI 270563	2024	South Africa	Katuuramu et al., 2024
	Vine length (mm)	136,2	178,3	PI 482265	2024	Zimbabwe	Katuuramu et al., 2024
		158	206,8	PI 482257	2024	Zimbabwe	Katuuramu et al., 2024
		108,4	141,9	PI 271769	2024	South Africa	Katuuramu et al., 2024
		655,8	501	USVL_114	2024	Botswana	Katuuramu et al., 2024
		651,8	498	PI_532644	2024	Eswatini	Katuuramu et al., 2024
		619,5	473,3	PI_596669	2024	South Africa	Katuuramu et al., 2024
		642,8	491,1	PI_542114	2024	Botswana	Katuuramu et al., 2024
		594,9	454,5	PI_532666	2024	Eswatini	Katuuramu et al., 2024

Values represent measurements under non-stressed vs stressed conditions.

2.3 The variation of abiotic-stress-adaptive physiological attributes in citron melon

Important physiological parameters for assessment of abiotic stress effect in citron melon breeding include the leaf-gaseous exchange parameters stomatal conductance (g_s), photosynthetic rate (*An*), transpiration rate (*E*), water use efficiency intrinsic (*WUEi*), water use efficiency instantaneous (*WUEinst*), intercellular CO_2 concentration (*Ci*), and chlorophyll fluorescence parameters such as minimum fluorescence, maximum fluorescence, chlorophyll content (*SPAD*), maximum quantum yield of PSII, effective quantum yield of PSII, photochemical quenching coefficient (*qP*), non-photochemical quenching (*qN*) and electron transport rate (*ETR*) (Fredes et al., 2017; Madumane et al., 2024; Mo et al., 2015; Ngwepe et al., 2023). Changes in the phenotype of certain physiological attributes in response to various abiotic stressors, as recorded under both stress and non-stress production conditions, are presented in **Table 2.2**. Due to limited published data on the physiological responses of citron melon, this section focuses exclusively on attributes reported under heat, drought, and cold stress conditions.

2.3.1 The influence of varied regimes of heat and drought stress regimes on adaptive physiological attributes

Heat and drought stress reduce stomatal conductance, intracellular carbon dioxide concentration, and the rate of transpiration and carbon dioxide assimilation in citron melon (Mo et al., 2016; Mandizvo et al., 2022a). The decreased responses in these physiological parameters are identified as drought-adaptive traits for water conservation in citron melon. However, a reduction in the responses of these physiological parameters is linked to low photosynthesis capacity and assimilation of primary metabolites in plants. This signifies the importance of heat and drought stress as a source of variation in the phenotype of leaf and gaseous exchange parameters in citron melon. The development of citron melon varieties with enhanced genetic potential for leaf gas exchange parameters in response to heat and drought stress is crucial for improving productivity and response to selection in breeding programs. The extent of variation in adaptive physiological attributes under heat and drought stress should be analysed to inform cultivation and breeding strategies.

The Citron melon genotype M20 was reported to have a reduced transpiration rate and a rapid decrease in stomatal conductance under heat and drought conditions, compared to its relative, i.e., sweet watermelon. The citron melon genotype later showed a recovery of 45.36% in transpiration rate compared to sweet watermelon (Y34), which showed 12.71% recovery (Mo et al., 2016). This demonstrated adaptability in conserving moisture in the face of drought stress. Citron melon breeding programs should target developing varieties that exhibit high productivity despite reduced transpiration rates under heat and drought stress. The effective quantum yield of PSII was reduced by 52 and 62% in citron melon M20 and sweet watermelon Y34, respectively, under drought stress (Mo et al., 2016). This reveals citron melon as a more adaptable cucurbit to heat and stress, indicating that heat and drought are sources of variation influencing the phenotype of adaptive physiological attributes in citron melon. Similar findings reporting reduced effective quantum efficiency were made elsewhere, showing ranges of 0.13-0.33 and 0.05-0.26 under non-stress and stress conditions, respectively. This resulted in the identification of citron melon accession WWM-38 as a potential genetic resource for photosynthetic capacity under drought-prone production conditions (Mandizvo et al., 2022a). A high maximum quantum yield of photosystem II, at 0.12, was reported among citron melon plants growing under drought conditions, compared to the control (0.04). This represents an increased potential for citron melon to convert absorbed light into chemical energy as an adaptive response to maintain suitable photosynthetic capacity under conditions of limited moisture.

Chlorophyll content varied significantly among 40 genetically diverse citron melon genotypes tested under drought and non-drought growing conditions (Mandizvo et al., 2022a). This highlights the importance of the genotype-by-environment interaction effect in shaping the response to selection in citron melon breeding for heat and drought tolerance. The overall reduction in chlorophyll content under heat and drought suggests that tolerant varieties should possess high productivity despite loss of leaf pigmentation. The genetic potential of citron melon to stay green under heat and drought stress should be enhanced to aid cultivation and selection. High water use efficiency is an indication of tolerance to heat and drought in citron melon. As a result, water use efficiency increased under drought stress in citron melon. Accession WWM-35 was reported with the highest (1225.6 μmol (CO_2) m^{-2}) intrinsic water use efficiency under drought stress, a potential genetic resource for high instantaneous water-use efficiency under drought stress (Mandizvo et al., 2022a). This genotype can be subjected to marker-assisted selection to identify genetic regulators that enhance water use efficiency under heat and drought stress.

The MYB967-like genetic regulator mediates the action of abscisic acid, which is reported to be upregulated in citron melon growing under drought stress (Mo et al., 2016). Additionally, the WRKY70-like gene is associated with the negative regulation of stomatal closure and is downregulated in the citron melon genotype M20 under drought-stressed conditions. This is evidence that heat and drought stress impacts the phenotype of adaptive physiological attributes in citron melon, indicating the need to develop more stable varieties that are less susceptible to changes in soil moisture regimes during cultivation. Germplasms exhibiting a high genomic representation of the MYB967-like gene are crucial for enhancing heat and drought tolerance breeding in citron melon and its relatives. Similarly, a reduced occurrence of the WRKY70-like gene in the citron melon genome could aid in increasing the accumulation of genetic advance for enhanced adaptive physiological attributes in watermelon.

2.3.2 The influence of varied regimes of low-temperature stress regimes on adaptive physiological attributes

Citron melon cultivation under low-temperature conditions has physiological effects on chlorophyll content. The chlorophyll content index decreased by 11% in response to low-temperature growing conditions in citron melon cultivation, compared to the control treatment (Katuuramu et al., 2024). The chlorophyll content parameter is crucial for photosynthesis, suggesting that selection to identify superior citron melon genotypes with enhanced productivity may be hindered by cultivation under low-temperature stress conditions. Citron melon screening trials aimed at identifying high-yielding germplasms should not be conducted under low-temperature production regions to increase the response to selection. Heritability estimates of 0.35 and 0.42 were reported for the chlorophyll content index and maximum quantum yield of photosystem II, respectively (Katuuramu et al., 2024), indicating the presence of a considerable amount of heritable variation for these traits. These traits (i.e., chlorophyll content index and maximum quantum yield of photosystem II) are positively associated with enhanced photosynthetic capacity: the present heritable variation can be exploited to aid cultivar design and development for improved low-stress tolerance in citron melon.

Citron melon germplasm resources, such as PI_296339, PI_482319, and PI_596669, were identified as having a high chlorophyll content index of above 40 under low-temperature stress (Katuuramu et al., 2024). These germplasm resources could aid in cultivar design and development, targeting the development of citron melon germplasm with enhanced productivity under cold stress. High photosynthetic capacity under cold stress was associated with chromosomes Ca03, Ca04, Ca05, and Ca07 in citron melon, responsible for 2-17% of the total phenotypic variation present among the tested citron melon germplasm resources. Candidate genes (i.e., CaU04G07240 and CaU04G07290) and peak SNP S4_21563874 associated with enhanced maximum photosynthetic capacity under cold stress in citron melon were revealed. Genes CaU05G14340 and SNP S5_12205786 were identified with the genetic up-regulation of zinc finger protein synthesis for cold stress tolerance. The regulation of ethylene, a ripening hormone in citron melon, was linked to the CaU01G00790 gene, which is closely linked to SNP marker SNP S1_451332 (Katuuramu et al., 2024). The reported chromosomal regions, genes and markers associated with suitable plant physiology for enhanced photosynthetic capacity under low-temperature stress should be considered by present stakeholders, including researchers and farmers, involved in citron melon improvement.

Table 2.2: Physiological traits of citron melon (*Citrullus lanatus var. citroides*) genotypes under abiotic stress (heat, drought and cold stress) conditions

Stress factor	Trait (unit)	Non-stressed	Stressed	Cultivar name	Year of study	Country	Reference
Heat and Drought	Photosynthetic rate (A_n)	42,2	15,89	WWM-46	2022	South Africa	Mandizvo et al., 2022a
		36,21	14,31	WWM-68	2022	South Africa	Mandizvo et al., 2022a
		41,46	15,04	WWM-41(A)	2022	South Africa	Mandizvo et al., 2022a
		35,18	14,69	WWM-15	2022	South Africa	Mandizvo et al., 2022a
		39,92	15,13	WWM-64	2022	South Africa	Mandizvo et al., 2022a
		40,89	13,74	WWM-57	2022	South Africa	Mandizvo et al., 2022a
		40,89	15,36	WWM-47	2022	South Africa	Mandizvo et al., 2022a
		38,16	18,35	WWM-37(2)	2022	South Africa	Mandizvo et al., 2022a
		36,16	17,38	WWM-79	2022	South Africa	Mandizvo et al., 2022a
		30,97	16,78	WWM-05	2022	South Africa	Mandizvo et al., 2022a
		41,02	14,84	WWM-50	2022	South Africa	Mandizvo et al., 2022a
		24,8	10,2	M20	2016	China	Mo et al., 2016
	Stomatal conductance (gs)	0,117	0,038	WWM-46	2022	South Africa	Mandizvo et al., 2022a
		0,125	0,02	WWM-68	2022	South Africa	Mandizvo et al., 2022a
		0,122	0,031	WWM-41(A)	2022	South Africa	Mandizvo et al., 2022a
		0,111	0,034	WWM-15	2022	South Africa	Mandizvo et al., 2022a
		0,113	0,052	WWM-64	2022	South Africa	Mandizvo et al., 2022a
		0,129	0,055	WWM-57	2022	South Africa	Mandizvo et al., 2022a
		0,123	0,046	WWM-47	2022	South Africa	Mandizvo et al., 2022a
		0,124	0,028	WWM-37(2)	2022	South Africa	Mandizvo et al., 2022a
		0,116	0,026	WWM-79	2022	South Africa	Mandizvo et al., 2022a
		0,109	0,029	WWM-05	2022	South Africa	Mandizvo et al., 2022a
		0,119	0,032	WWM-50	2022	South Africa	Mandizvo et al., 2022a
		0,31	0,08	M20	2016	China	Mo et al., 2016
	Transpiration rate (E)	7,981	3,543	WWM-46	2022	South Africa	Mandizvo et al., 2022a
		7,281	2,581	WWM-68	2022	South Africa	Mandizvo et al., 2022a
		8,956	2,509	WWM-41(A)	2022	South Africa	Mandizvo et al., 2022a
		6,008	1,531	WWM-15	2022	South Africa	Mandizvo et al., 2022a
		7,961	2,613	WWM-64	2022	South Africa	Mandizvo et al., 2022a
		7,193	2,803	WWM-57	2022	South Africa	Mandizvo et al., 2022a
		9,216	3,352	WWM-47	2022	South Africa	Mandizvo et al., 2022a
		6,812	3,421	WWM-37(2)	2022	South Africa	Mandizvo et al., 2022a
		8,096	2,735	WWM-79	2022	South Africa	Mandizvo et al., 2022a
		6,987	2,763	WWM-05	2022	South Africa	Mandizvo et al., 2022a
		8,649	2,984	WWM-50	2022	South Africa	Mandizvo et al., 2022a
		6,52	2,13	M20	2016	China	Mo et al., 2016
	Intercellular CO_2 (Ci)	306,8	445,9	WWM-46	2022	South Africa	Mandizvo et al., 2022a
		323,4	426,7	WWM-68	2022	South Africa	Mandizvo et al., 2022a
		325,3	467,3	WWM-41(A)	2022	South Africa	Mandizvo et al., 2022a
		288,1	405,2	WWM-15	2022	South Africa	Mandizvo et al., 2022a
		414,7	451,4	WWM-64	2022	South Africa	Mandizvo et al., 2022a
		415,6	446,3	WWM-57	2022	South Africa	Mandizvo et al., 2022a
		379,6	471,6	WWM-47	2022	South Africa	Mandizvo et al., 2022a

Values represent measurements under non-stressed vs stressed conditions.

Table 2.2: continued.

Stress factor	Trait (unit)	Non-stressed	Stressed	Cultivar name	Year of study	Country	Reference
Heat and Drought	Intercellular CO_2 (Ci)	323,1	409	WWM-37(2)	2022	South Africa	Mandizvo et al., 2022a
		288,9	409,8	WWM-79	2022	South Africa	Mandizvo et al., 2022a
		279,6	393,6	WWM-05	2022	South Africa	Mandizvo et al., 2022a
		348,4	464,3	WWM-50	2022	South Africa	Mandizvo et al., 2022a
	water use efficient intrinsic (WUEi)	360,7	543,5	WWM-46	2022	South Africa	Mandizvo et al., 2022a
		288,8	754,2	WWM-68	2022	South Africa	Mandizvo et al., 2022a
		343,5	794,3	WWM-41(A)	2022	South Africa	Mandizvo et al., 2022a
		317,2	497,9	WWM-15	2022	South Africa	Mandizvo et al., 2022a
		352,2	394,1	WWM-64	2022	South Africa	Mandizvo et al., 2022a
		315,9	306,8	WWM-57	2022	South Africa	Mandizvo et al., 2022a
		331,8	372,4	WWM-47	2022	South Africa	Mandizvo et al., 2022a
		310	704,9	WWM-37(2)	2022	South Africa	Mandizvo et al., 2022a
		306,8	680,4	WWM-79	2022	South Africa	Mandizvo et al., 2022a
		284,7	674,5	WWM-05	2022	South Africa	Mandizvo et al., 2022a
		343,1	471,6	WWM-50	2022	South Africa	Mandizvo et al., 2022a
	water use efficient instantaneous (WUEinst)	5,364	4,516	WWM-46	2022	South Africa	Mandizvo et al., 2022a
		5,034	5,927	WWM-68	2022	South Africa	Mandizvo et al., 2022a
		4,644	6,111	WWM-41(A)	2022	South Africa	Mandizvo et al., 2022a
		5,877	9,54	WWM-15	2022	South Africa	Mandizvo et al., 2022a
		5,05	5,797	WWM-64	2022	South Africa	Mandizvo et al., 2022a
		5,659	4,993	WWM-57	2022	South Africa	Mandizvo et al., 2022a
		4,596	4,972	WWM-47	2022	South Africa	Mandizvo et al., 2022a
		5,583	5,57	WWM-37(2)	2022	South Africa	Mandizvo et al., 2022a
		4,616	6,382	WWM-79	2022	South Africa	Mandizvo et al., 2022a
		4,469	6,874	WWM-05	2022	South Africa	Mandizvo et al., 2022a
		5,058	5,399	WWM-50	2022	South Africa	Mandizvo et al., 2022a
	Fv/Fm	0,4	0,729	WWM-46	2022	South Africa	Mandizvo et al., 2022a
		0,591	0,671	WWM-68	2022	South Africa	Mandizvo et al., 2022a
		0,392	0,593	WWM-41(A)	2022	South Africa	Mandizvo et al., 2022a
		0,426	0,664	WWM-15	2022	South Africa	Mandizvo et al., 2022a
		0,485	0,719	WWM-64	2022	South Africa	Mandizvo et al., 2022a
		0,499	0,606	WWM-57	2022	South Africa	Mandizvo et al., 2022a
		0,376	0,717	WWM-47	2022	South Africa	Mandizvo et al., 2022a
		0,399	0,67	WWM-37(2)	2022	South Africa	Mandizvo et al., 2022a
		0,337	0,638	WWM-79	2022	South Africa	Mandizvo et al., 2022a
		0,411	0,637	WWM-05	2022	South Africa	Mandizvo et al., 2022a
		0,343	0,609	WWM-50	2022	South Africa	Mandizvo et al., 2022a
		0,812	0,776	M20	2016	China	Mo et al., 2016
	PhiPSII	0,214	0,177	WWM-46	2022	South Africa	Mandizvo et al., 2022a
		0,254	0,129	WWM-68	2022	South Africa	Mandizvo et al., 2022a

Values represent measurements under non-stressed vs stressed conditions.

Table 2.2: continued.

Stress factor	Trait (unit)	Non-stressed	Stressed	Cultivar name	Year of study	Country	Reference
Heat and Drought	PhiPSII	0,273	0,214	WWM-41(A)	2022	South Africa	Mandivzo et al., 2022a
		0,332	0,065	WWM-15	2022	South Africa	Mandivzo et al., 2022a
		0,237	0,18	WWM-64	2022	South Africa	Mandivzo et al., 2022a
		0,238	0,234	WWM-57	2022	South Africa	Mandivzo et al., 2022a
		0,177	0,158	WWM-47	2022	South Africa	Mandivzo et al., 2022a
		0,223	0,113	WWM-37(2)	2022	South Africa	Mandivzo et al., 2022a
		0,187	0,102	WWM-79	2022	South Africa	Mandivzo et al., 2022a
		0,193	0,14	WWM-05	2022	South Africa	Mandivzo et al., 2022a
		0,187	0,052	WWM-50	2022	South Africa	Mandivzo et al., 2022a
		0,742	0,601	M20	2016	China	Mo et al., 2016
	qP	0,441	0,294	WWM-46	2022	South Africa	Mandivzo et al., 2022a
		0,514	0,189	WWM-68	2022	South Africa	Mandivzo et al., 2022a
		0,488	0,288	WWM-41(A)	2022	South Africa	Mandivzo et al., 2022a
		0,49	0,176	WWM-15	2022	South Africa	Mandivzo et al., 2022a
		0,385	0,315	WWM-64	2022	South Africa	Mandivzo et al., 2022a
		0,501	0,272	WWM-57	2022	South Africa	Mandivzo et al., 2022a
		0,355	0,247	WWM-47	2022	South Africa	Mandivzo et al., 2022a
		0,334	0,211	WWM-37(2)	2022	South Africa	Mandivzo et al., 2022a
		0,38	0,245	WWM-79	2022	South Africa	Mandivzo et al., 2022a
		0,4	0,259	WWM-05	2022	South Africa	Mandivzo et al., 2022a
		0,307	0,165	WWM-50	2022	South Africa	Mandivzo et al., 2022a
	ETR	118436	94277	WWM-46	2022	South Africa	Mandivzo et al., 2022a
		140379	74589	WWM-68	2022	South Africa	Mandivzo et al., 2022a
		150892	87847	WWM-41(A)	2022	South Africa	Mandivzo et al., 2022a
		180027	65194	WWM-15	2022	South Africa	Mandivzo et al., 2022a
		128502	71795	WWM-64	2022	South Africa	Mandivzo et al., 2022a
		131488	124501	WWM-57	2022	South Africa	Mandivzo et al., 2022a
		117078	57159	WWM-47	2022	South Africa	Mandivzo et al., 2022a
		123308	58693	WWM-37(2)	2022	South Africa	Mandivzo et al., 2022a
		103675	44724	WWM-79	2022	South Africa	Mandivzo et al., 2022a
		106490	75044	WWM-05	2022	South Africa	Mandivzo et al., 2022a
		103210	57370	WWM-50	2022	South Africa	Mandivzo et al., 2022a
cold	Chlorophyll content (SPAD)	42	39,1	USVL_114	2024	Botswana	Katuuramu et al., 2024
		43,1	40	PI_482312	2024	Zimbabwe	Katuuramu et al., 2024
		42,5	38,9	PI_532666	2024	Eswatini	Katuuramu et al., 2024
		43	39,7	PI_606135	2024	Russia	Katuuramu et al., 2024
		42,8	40,7	PI_532664	2024	Eswatini	Katuuramu et al., 2024
		41,9	37,1	PI_542114	2024	Botswana	Katuuramu et al., 2024
		47	46,4	PI_482246	2024	Zimbabwe	Katuuramu et al., 2024

Values represent measurements under non-stressed vs stressed conditions.

Table 2.2: continued.

Stress factor	Trait (unit)	Non-stressed	Stressed	Cultivar name	Year of study	Region	Reference
cold	Chlorophyll content (SPAD)	47,2	46,8	PI_500335	2024	Zambia	Katuuramu et al., 2024
		46	44,9	PI_596669	2024	South Africa	Katuuramu et al., 2024
		49,1	48	PI_296339	2024	South Africa	Katuuramu et al., 2024
	Fv/Fm	0,83	0,81	USVL_114	2024	Botswana	Katuuramu et al., 2024
		0,82	0,8	PI_482312	2024	Zimbabwe	Katuuramu et al., 2024
		0,83	0,81	PI_532666	2024	Eswatini	Katuuramu et al., 2024
		0,82	0,81	PI_606135	2024	Russia	Katuuramu et al., 2024
		0,83	0,81	PI_532664	2024	Eswatini	Katuuramu et al., 2024
		0,82	0,8	PI_542114	2024	Botswana	Katuuramu et al., 2024
		0,83	0,81	PI_482246	2024	Zimbabwe	Katuuramu et al., 2024
		0,82	0,81	PI_500335	2024	Zambia	Katuuramu et al., 2024
		0,82	0,79	PI_596669	2024	South Africa	Katuuramu et al., 2024
		0,83	0,81	PI_296339	2024	South Africa	Katuuramu et al., 2024

Values represent measurements under non-stressed vs stressed conditions.

2.4 Discussion

Variation in the phenotype of different morphological and physiological citron melon attributes was revealed under varied regimes of the reported abiotic stressors, namely heat, drought, low temperature, and poor soil fertility. These abiotic stresses tend to lead to adaptive responses that involve both morphological and physiological attributes, as indicated in the literature. The reported morpho-physiological plant attributes include root (root biomass, length, depth, width, and the ratio of root to shoot biomass), stem and leaf (vine length, leaf area and number, shoot biomass, gaseous exchange and chlorophyll content parameters), and fruit (fruit yield, fruit weight and number of fruits per plant) traits. Variation in biochemical attributes (i.e., nutritional and phytochemical traits) in response to varied regimes of different abiotic stressors was revealed. Variation in adaptive phenotypic responses is crucial for enhancing cultivation in the face of abiotic stress, leading to unstable productivity levels and a reduced response to selection in breeding programs due to the genotype-by-environment interaction effect.

There is limited research investigating variation in citron melon performance in response to major sources of abiotic stress during cultivation. As a result, research findings on related cucurbits species, such as sweet watermelon and *Cucumis melo*, were useful sources of insight. Heat and drought, as well as low-temperature stresses, are better researched than other available sources of abiotic stress in agricultural production. Taken together, the cited studies indicate broad agreement that morpho-physiological plasticity underpins citron melon responses to abiotic stress; however, the strength and direction of these responses vary with genotype, growth stage, stress intensity/duration, and experimental setting, which limits direct comparability across studies. Accordingly, insights drawn from related cucurbits are useful for context but should be interpreted as indicative rather than definitive for citron melon, given species-level anatomical and phenological differences.

The reviewed studies revealed adaptive parameters for drought and heat stress tolerance in citron melon. Increased root development, which is supported by high root length, width, expansion and shoot-root ratio, is an adaptive morphological response in citron melon. Heat and drought-tolerant citron melon germplasms have been identified in previous studies, providing valuable resources for improving stress tolerance in breeding programmes (Mandizvo et al., 2022; Mo et al., 2016; Yoshimuri et al., 2008). Adaptive stem and leaf traits to cultivation under heat and drought stress include reduced vine length, leaf area, and number, as well as shoot biomass. The significant declines in vine length and shoot biomass are structural adaptations to reduce the utilisation of metabolic reserves for growth in citron melon imposed by heat and stress (Akashi et al., 2011; Katuuramu et al., 2024). The morphological alterations indicate that some genotypes of citron melon may lengthen their vines to maximise light capture, while others prioritise survival overgrowth by reducing shoot biomass to conserve energy. Notably, the relative contribution of these traits to tolerance appears to vary by genotype and study context: some investigations report strong associations between root allocation and performance, whereas others highlight canopy or architectural adjustments (e.g., vine elongation) under ostensibly similar stress, indicating strategy diversification rather than a single canonical response. Such variation is plausibly explained by differences in stress intensity and duration, growth stage assessed, and measurement windows (instantaneous vs. integrated metrics), suggesting that trait effects should be interpreted comparatively across designs rather than assumed to be uniform.

The ability to maintain suitable levels of fruit yield under moisture-deficient growing conditions represents tolerance to heat and drought stress in citron melon. Fruit yield under heat and drought stress is influenced by other morphological traits, including the number of fruits per plant and individual fruit weight (Mandizvo et al., 2022a; Tembo & Maphosa, 2025). The fruit-based biochemical attributes, vitamin C, soluble solids and pH, also vary in response to heat and drought stress. Physiological processes, such as reduced stomatal conductance and density, as well as increased water-use efficiency, support photosynthesis under stress (Guzzon et al., 2017; Mandizvo et al., 2022; Mo et al., 2016; Yoshimura et al., 2008). The significant decreases in chlorophyll content index, photosynthetic performance, and the rates of carbon dioxide assimilation and transpiration are adaptive physiological attributes to heat and drought stress tolerance in citron melon (Akashi et al., 2011; Mo et al., 2016; Katuuramu et al., 2024).

A synchronised adaptation approach is seen in the interaction between morphological and physiological responses. The physiological changes, such as stomatal closure and enhanced WUE, enhance root parameters under stress, enhancing water uptake. These reactions are likely caused by genes that control stomatal behaviour, photosynthetic efficiency, and root architecture (Erez et al., 2020; Guo et al., 2013). Low-temperature stress is reported to cause variations in the phenotypes of root, stem, leaf, and fruit traits in citron melon. However, the relative contribution of these traits to yield stability appears to differ among studies, with some reports emphasising root-driven water capture and others highlighting canopy-level efficiencies, suggesting that tolerance arises from different trait combinations rather than a single mechanism. These differences are likely influenced by variation in stress severity, plant developmental stage and experimental conditions, indicating that findings should be interpreted comparatively rather than assumed to be uniform across studies.

Adaptive attributes under low-temperature cultivation conditions include reduced chlorophyll content, decreased photosynthetic capacity, and decreased biomass production (Kidokoro et al., 2017). Lack of tolerance to low-temperature stress results in poor leaf health, reduced resistance to diseases, decreased vine length, and low shoot biomass production (Korkmaz & Dufault, 2001). Soil fertility regimes reported with significant variation in the morphological attributes of watermelon, a relative of citron melon. The findings obtained using sweet watermelon to reveal the role of varied soil fertility levels on fruit yield and related traits can be useful in informing cultivation and breeding in citron melon. Because nitrogen is necessary for the synthesis of chlorophyll and the operation of photosynthetic enzymes, traits such as root length and stomatal conductance influence nitrogen intake and assimilation in nitrogen dynamics, and these should be optimised in citron melon and other cucurbits cultivation. Identifying genotypes with efficient nutrient utilisation under poor soil fertility stress or reduced fertiliser application is desirable, as stress-induced reductions in photosynthesis may limit productivity and selection responses in breeding programs.

Despite these findings, significant gaps remain in understanding the variation in citron melon plant growth and development attributes in response to all available sources of abiotic stress. Limited research has been conducted on other factors, such as heavy metal toxicity, UV exposure, and salinity. The different adaptive antioxidant activity and osmolyte accumulation parameters are less researched. Citron melon has no set nutrient requirements, which makes it difficult to optimise fertilisation techniques. Gene-level mechanisms remain largely unknown due to the scarcity of molecular research. Developing resilient cultivars will require filling these gaps using integrative methods that combine morpho-physiology, biochemistry, and genetics.

2.5 Conclusion

Abiotic stress alters the phenotype of adaptive morphological, physiological, and biochemical attributes in citron melon, both in production and in screening trials for plant breeding selection. Variation in the phenotype of adaptive attributes could be attributed to differences in the genetic potential of citron and differences in environmental production factors, including heat, drought, low-temperatures, and poor soil fertility. A significant interaction between genotype and production environmental factors, i.e., genotype-by-environment interaction, influences productivity and response to selection in breeding programs. Nevertheless, the available variation in adaptive traits can be leveraged to enhance the abundance of genetic regulators for abiotic stress tolerance in the citron melon genome. Simultaneous genetic advancement for adaptive morpho-physiological and biochemical attributes, as well as for increased fruit yield, is crucial. This can be achieved by incorporating molecular-based research tools to identify candidate genes and allelic variants associated with superiority for adaptive traits in response to abiotic stress. This review highlights citron melon as a vital genetic resource for cultivation to enhance food and nutrition security and for incorporation in cucurbits' abiotic stress tolerance breeding.

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CHAPTER 3

The morpho-physiological responses of selected citron melon (*Citrullus lanatus var citroides*) genotypes evaluated under low nitrogen conditions

Abstract

Citron melon (*Citrullus lanatus var. citroides*), an underutilised crop that is rich in essential nutrients and important phytochemical compositions, possesses natural adaptations to various biotic and abiotic stress factors. There is limited research investigating variation in the adaptive attributes of citron melon for increased productivity under poor soil fertility, particularly low nitrogen availability. This study aims to identify suitable citron melon genotypes possessing enhanced adaptive attributes for cultivation under low-nitrogen availability for production and breeding. Four genetically diverse citron melon genotypes (WWM-Ranoto, WWM-16, WWM-58, and WWM-65) were evaluated under greenhouse (GH) and shade house (SH) environments using a randomised complete block design with three replications. Two nitrogen application regimes, i.e., low nitrogen (LN) and optimum nitrogen (ON), were investigated using polyethene pots. The morphological traits [(nitrogen use efficiency (*NUE*), fruit length (*FL*), fruit diameter (*FD*), root weight (*RW*), seeds per fruit (*SPF*) and fruit weight (*FW*), leaf area (*LA*), vine length (*VL*)) and physiological traits (i.e., photosynthetic rate (*A*), stomatal conductance (g_s), intercellular carbon dioxide concentration (*Ci*), transpiration rate (*E*), partial pressure of CO_2 ($PhiCO_2$))] were recorded. The analysis of variance revealed a significant genotype effect ($p \leq 0.01$) for *LA*, *FL*, *FW*, *RT*, *SPF*, and *NUE*. Significant site effect was recorded for all study traits except *RW* and *SPF*. Pearson's correlation analysis revealed strong positive relationships in the greenhouse LN, $PhiCO_2$ with *RL* (r = 0.97), *VL* with *Ci* (r = 0.97), *FW* with *NUE* (r = 0.99) under GH and LN conditions. Under SH and LN, strong positive associations were observed in the following traits *VL* with $PhiCO_2$ (r = 0.96), *FW* with *Ci* (r = 0.99) and *FD* with *qP* (r = 0.98). Additionally, PCA revealed a genotype-trait association, consistent across genotypes WWM-58 and WWM-16, indicating that fruit yield, nitrogen use efficiency, and physiological traits were maintained under low nitrogen conditions across environments. Overall, for breeding selection, genotype WWM-58 exhibited superior performance across environments, with high fruit yields, indicating enhanced nitrogen efficiency in low-input systems.

Keywords: Citron melon, low-nitrogen, morpho-physiology, nitrogen use efficiency, photochemistry.

3.1 Introduction

Citron melon (*Citrullus lanatus var. citroides*) is a cucurbit and food crop cultivated globally in various agro-ecologies. The crop is important for its edible leaves, fruit, and seeds (Dane & Liu, 2006; El-Sayed et al., 2021; Ngwepe et al., 2023), which are rich in minerals and macronutrients, as well as secondary compounds with antioxidant properties (El-Sayed et al., 2021; Masoko et al., 2022; Nkoana et al., 2022; Semenya & Maroyi, 2019). The crop is renowned for its remarkable tolerance to various abiotic stresses, including heat and drought (Mandizvo et al., 2022a,b; Mo et al., 2017). Unlike other cultivated cucurbits such as watermelon, pumpkin and squash, citron melon has evolved under harsh environmental conditions, enabling it to thrive in marginal soil conditions (Grumet et al., 2021). The cultivation of citron melon could play a key role in meeting the global Sustainable Development Goals (SDGs) (SDG 2; SDG 12, and SDG 13) of increasing access to food crops with limited dependency on production inputs (Atukunda et al., 2021; Campbell et al., 2018; Viana et al., 2022).

Furthermore, citron melon is recognised as a source of key genetic regulators for breeding abiotic stress tolerance in related cucurbit crops, such as sweet watermelon (*Citrullus lanatus*) (Fredes et al., 2017; Kombo & Sari, 2019; Yavuz et al., 2020). The contribution of citron melon to improving food and nutrition security, as well as to breeding for abiotic stress tolerance in related cucurbits, should be increased (Colla et al., 2006; Nawaz et al., 2018; Ngwepe et al., 2023). Despite the natural adaptability of citron melon to various environmental stresses, these stresses cause variations in its performance during cultivation, affecting productivity and response to selection in breeding programs (Katuuramu et al., 2024; Mandizvo et al., 2022a,b). Therefore, there is a requirement for stable citron melon germplasms that possess enhanced tolerance to abiotic stress, thereby improving productivity and facilitating genetic advances in breeding programs.

With increasing concerns over poor soil fertility (Aleminew & Alemayehu, 2020), particularly nitrogen availability, especially in low-input agricultural systems, an improved genetic potential of resilient species like citron melon for enhanced productivity under low-nitrogen conditions could increase global access to adequate and nutritious food crops (Cavalcante et al., 2019; Hirel et al., 2007). There is limited research reporting the variability of performance and productivity in response to diversified regimes of the different abiotic stress types, particularly low soil nitrogen growing conditions. Nitrogen is a key macronutrient essential for plant growth, and its deficiency often limits productivity, affecting fruit yield (Sinha & Tandon, 2020). However, not all plant species respond equally to nitrogen stress (Cavalcante et al., 2019; Ye et al., 2022; Jangam & Raghuram, 2015). There is a knowledge gap in understanding how different citron melon genotypes perform when exposed to nitrogen-limited conditions, and whether certain landraces can be improved for use in low-input or degraded agroecosystems. Leaching, particularly in high-rainfall areas, prompts soil fertility loss, resulting in poor productivity (Agostini et al., 2010; Aleminew & Alemayehu, 2020). Without crop improvement, cultivation remains dependent on synthetic fertilisers, which is a hindrance to sustainable agriculture and to enhancing selection in plant breeding screening trials.

Adaptive morphological and physiological attributes to abiotic stress tolerance have been reported in citron melon and its relatives using stem and leaf, root, and fruit plant traits. Regarding stem- and leaf-based morphological traits; vine length, leaf area, leaf expansion, shoot biomass, and leaf-gaseous-exchange parameters (i.e., stomatal conductance, transpiration rate, chlorophyll content, intracellular carbon dioxide concentration, etc.) are important indicators of response to abiotic stress regimes (Guzzon et al., 2017; Katuuramu et al., 2024). These, among others, are the important root-based traits: root length, width, expansion, depth, root-shoot ratio (Guzzon et al., 2017; Mandizvo et al., 2022b; Yoshimura et al., 2008). Significant variation has been reported in these morphological and physiological traits in response to varied regimes of abiotic stresses (Katuuramu et al., 2024; Kurtar et al., 2025; Mandizvo et al., 2022a), linked to variable productivity levels and reduced efficiency of breeding programs.

For example, low heritability was reported for important selection criteria/ traits in citron melon, e.g., chlorophyll content (35%) and Fv/Fm (42%) (Katuuramu et al., 2024). There is a lack of research investigating the variation in these morphological and physiological plant traits in response to cultivation under low-nitrogen conditions. There is a need to identify citron melon germplasms possessing heritable genetic potential for superior performance under low-nitrogen conditions, together with estimates of nitrogen use efficiency (NUE), an index relating plant yield to nitrogen application (Grumet et al., 2021).This study aims to identify citron melon genotypes with adaptive traits suitable for low-nitrogen cultivation and breeding. This research will contribute to the existing genetic information on enhancing abiotic stress tolerance in citron melon and related cucurbits.

3.2 Materials and methods

3.2.1 Genetic materials

Four citron melon genotypes sourced from different districts of Limpopo province in South Africa, were selected based on the capability to resist abiotic stresses (Mashilo et al., 2017 b,c; Mandizvo et al., 2021; Ngwepe et al., 2023). The genotype panel was limited to four lines due to germplasm availability; while adequate for contrasting responses, this may constrain broad generalisability.

3.2.2 Study site and trial layout

The study was conducted in the controlled environment facility (CEF) at the University of KwaZulu-Natal (UKZN) (29°37′37.5″S; 30°24′10.4″E) under greenhouse (GH), which maintained a temperature range of 25 to 32 ºC, Air temperature was actively regulated using a wet-wall evaporative cooling system with extraction fans during hot periods and shadehouse (SH) (**Figure 3.1 A & B**). Conditions reflected ambient outdoor weather moderated by the SH, therefor, no active climate control. Four selected citron melon genotypes, namely WWM-Ranoto, WWM-16, WWM-58, and WWM-65, were evaluated for their tolerance to low nitrogen application. The experiment followed a randomised complete block design (RCBD) with three replications. Each environment had two nitrogen application levels, i.e., low nitrogen (LN) and optimum nitrogen (ON). Each genotype was grown in a 10-L polyethene pot filled with Duzi-turf compost-based growth medium, which had an N: P: K ratio of 1.18:0.60:0.29, in that order. The experiment consisted of one plant per pot, and three replications were used based on the available germplasm at the time of trial establishment. The study was conducted as a single-season experiment.

Figure 3.1: Experimental sites **A**: greenhouse and **B**: shadehouse.

Before planting, the seeds were grown into seedlings for two weeks. The seedling trays were filled with Duzi-turf compost. Each seed genotype was inserted into seedling trays and then placed in a tunnel equipped with a high-heat absorbent and an automatic irrigation misting system, set to release mist water in the morning, during the day, and in the evening, to keep the growing media moist. The seedlings were then allowed to grow in seedling trays until a true leaf had developed, a process that took approximately two weeks. After two weeks, they were transplanted into pots. The plants were irrigated using a watering can at least once a day on rainy days in the greenhouse and twice on hot/ sunny days. In the shadehouse, they were rain-fed and irrigated only once a day, if necessary. The fertiliser requirement was calculated based on the recommendations made by Mandivzo et al. (2022a).

To balance NPK in compost, 4g/pot (124 kg/ha) of Urea (Optimum), 28 g/pot (855 kg/ha) of SSP, and 3 g/pot (95 kg/ha) of KCl were applied. To achieve the desired nitrogen Levels, LN treatments were reduced to 40% (50 kg/ha) of the optimal level, while ON remained at its complete nitrogen requirement. The first application of Low nitrogen (Urea) was at planting with 0.7 g/pot (25 kg/ha), and the second application of 0.7 g/pot (25 kg/ha) applied as top dressing after 30 days of transplant. Plants were allowed to grow for six weeks until they reached the flowering stage. The male flowers appeared first, between 39 and 60 days after growth, and then the female flowers developed 4 to 10 days later. All flowers were covered with a 12 x 9 cm pollinating bag and secured to prevent any cross-pollination among genotypes, as demonstrated by Ngwepe et al. (2023), as presented in **Figure 3.2**. Once the female flower was ready to receive pollen, the male and female flowers were uncovered, and the male flower was picked off the plant and rubbed onto the female flower to stimulate cross-pollination. The pollinated female was covered with a pollinating bag to prevent disruption from crosspollinating agents, and then the pollinated flowers were labelled accordingly. Upon visible signs of fruit development, the hand-pollinated flower was uncovered to facilitate optimal growth and development.

Figure 3.2: Controlled pollination to prevent cross-pollination among genotypes. **A**: partially open male flower, **B**: partially female flower, **C**: enclosed partially opened male/female flower with an aerated bag, **D**: ready male flower for pollen collection, **E**: receptive female flower ready to be pollinated, **F**: hand pollination of the female flower by rubbing the pollen on the stigma, **G**: covering back the female flower after pollination, and **H**: a successfully pollinated female flower.

3.3 Data collection

The first data were collected after six weeks of growth at the flowering stage, and then bi-weekly thereafter for six weeks. The following attributes were collected: morphological and physiological traits

3.3.1 Morpho-physiological attributes

A measuring tape was used to measure in cm the vine length (VL) at harvest. After the sixth week of transplanting, leaf length (*LL*) and leaf width (*LW*) were measured weekly using a cm ruler, averaged from three selected healthy leaves in each genotype. Fully expanded leaves were selected to provide a representative average of leaf size while minimising within-plant variability, as standard practice in non-destructive leaf-area estimation. Leaf area (*LA*) (cm^2) was estimated using equation 1 by Rouphael et al. (2010) as follows:

$$\boldsymbol{LA = 2.99 + 0.496LW} \qquad \text{Equation 1}$$

Fruit parameters were recorded at harvest. The fruit for each genotype was harvested, weighed using an Ohaus 2-kg scale (OHAUS Europe Corporation, Parsippany, NJ, USA; model SKX2202) to determine fruit weight (*FW*) and each fruit was measured for its length (*FL*), diameter (*FD*) and rind thickness (*RT*) using a cm-ruler. Seeds were counted in each fruit to determine the seed number per fruit (*SPF*). Hundred seed weight (*100-SW*) was estimated by weighing a random sample of 100 seeds and reporting the result in g/100 seeds.

Root parameters were taken after harvest. Irrigation was terminated to allow the soil to dry. Plants were carefully removed from the pot and gently shaken to dislodge compost aggregates and particles. It was then laid out in a dry area on plastic to allow it to air dry. Once the roots have dried, they are cut at the stock area to separate the root system from the vine. The roots were measured for root length (*RL*) using a cm ruler and root weight (*RW*), which was determined by placing the root system on a weighing scale. Because roots were air-dried prior to weighing, the measurement reflects apparent air-dry root mass rather than true fresh or oven-dry mass.

3.3.2 Physiological parameters

A portable LI-6400XT photosynthesis system (LI-COR Biosciences, Lincoln, Nebraska, USA) was utilised to record the physiological parameters. The data were collected mid-morning between 9:00 and 11:00 a.m., starting from the 6th week after transplant, and then collected biweekly for six weeks. Measurements were taken on the upper surface of citron melon leaves; one new leaf was selected from each experimental unit at each sampling event to ensure a consistent developmental stage and to avoid variability associated with measuring the same leaf as it aged. The system light intensity was set to 1000 μmol/m^2/s, leaf temperature to 25 °C and 400 μmol/mol of carbon dioxide (CO_2). The measured physiological traits included photosynthetic rate (*A*), stomatal conductance (*gs*), transpiration rate (*E*), intercellular CO_2 concentration (*Ci*), maximum quantum yield of PSII in photosynthesis (*Fv'/Fm'*), effective quantum efficiency of PSII photochemistry (*ΦPSII*), partial pressure of CO_2 ($PhiCO_2$), photochemical quenching (*qP*), electron transport rate (*ETR*) and amount of light absorbed by the leaf (*LeafAbs*).

To obtain nitrogen use efficiency (*NUE*), all fruits were marked/ labelled at harvest according to their respective treatments and genotypes. The individual fruits were collected at maturity and weighed using an Ohaus 2-kg scale (OHAUS Europe Corporation, Parsippany, NJ, USA;

model SKX2202). Agronomic NUE (g/g), the following equation 2 was used with modifications (Hong et al., 2021):

$$(NUE) = \frac{Y}{N} \qquad \text{Equation 2}$$

where: Y and N denote total fruit yield per plant and the amount of nitrogen applied per pot, measured in grams.

3.4 Statistical analysis

GenStat, 23rd Edition (VSN International, Hempstead, UK) was used to perform an analysis of variance (ANOVA). Normality and homogeneity of variance were assessed using residual diagnostics in GenStat, and the data met the assumptions required for ANOVA. Fisher's protected least significant difference (LSD) test was used to evaluate genotype mean differences for the measured variables at a 5% significance level. In OriginPro 2025b (OriginLab Corporation), Pearson correlation coefficients and Principal Component Analysis (PCA) were used to assess the direction and intensity of linear correlations between morpho-physiological traits linked to selected genotypes based on mean values. OriginPro 2025b was also used to form multivariate plots to identify the parameters affected by nitrogen levels and environmental factors.

3.5 Results and discussion

3.5.1 Genotype, environment, and interaction effects on studied morpho-physiological attributes

Analysis of variance for morphological and physiological parameters evaluated among the studied citron melon genotypes, nitrogen application, and test environments is presented in **Tables 3.1** and **3.2**. Genotype effect was significant ($p \leq 0.01$) for most studied morphological and physiological traits, except *NUE*, *FW*, *FD*, *RL*, *RW*, *Fv'/Fm'*, *ΦPSII*, *ETR* and *LeafAbs*. The test site significantly ($p \leq 0.05$) affected the response of most studied traits except *SPF*, *RW*, $PhiCO_2$ and *ETR*. The nitrogen level effect was significant ($p \leq 0.05$) for the following evaluated attributes: *VL*, *SPF*, *100-SW*, *A*, *E*, and $PhiCO_2$. These suggest differential performance of citron melon genotypes due to environmental production conditions. Significant genotype and environmental effects on the morphological and physiological attributes of citron melon have been reported elsewhere (Kutar et al., 2024; Mandizvo et al., 2022a, b). The G × S interaction effect was highly significant ($p \leq 0.01$) for the following studied morphological and physiological traits, *LA*, *RT*, *100-SW*, *A*, g_s and *Ci*, this suggests that the relative performance of genotypes for these traits depended on the production site (greenhouse vs shadehouse); meaning, genotype rankings changed between sites, so genotypes should be compared within each site rather than across sites. The G × N interaction effect was significant ($p \leq 0.01$) for the following studied morphological and physiological traits: *100-SW* and *A*. This suggests that genotype responses varied with nitrogen level; the effect of nitrogen on *100-SW* and photosynthesis was not uniform across genotypes, so genotype comparisons should be made within each nitrogen level. Moreover, the G × S × N interaction effect was highly significant ($p \leq 0.05$) for the following studied morphological and physiological traits, *A*, *ETR* and $PhiCO_2$, this suggests that these photosynthetic traits were jointly contingent on genotype, site and nitrogen, indicating context-dependent responses; therefore, simple-effect means should be interpreted by site within each nitrogen level (and vice versa) for each genotype.

Table 3.1: Analysis of variance matrix showing the effect of genotype, site and nitrogen application on morphological traits of the studied citron melon.

Source	d.f.	LA (cm^2)	VL (m)	NUE	FW (g)	FL (cm)	FD (cm)	RT (cm)	SPF	RL (cm)	RW (g)	100-SW
Genotype (G)	3	33178**	4.48**	69194^{ns}	234807^{ns}	42.94**	7.72^{ns}	1.93**	9028**	304.0^{ns}	0.35^{ns}	32.68**
Site (S)	1	60692**	1.79*	666633*	3140006**	118.76**	93.02**	2.32**	91^{ns}	2990.9*	0.28^{ns}	65.80**
Nitrogen (N)	1	5838^{ns}	1.36*	228048^{ns}	20247^{ns}	0.02^{ns}	0.00^{ns}	0.05^{ns}	5376*	397.3^{ns}	0.33^{ns}	5.09*
G × S	3	74813**	0.24^{ns}	103714^{ns}	336553*	7.48^{ns}	13.68*	1.36**	2615^{ns}	208.3^{ns}	0.40^{ns}	8.09**
G × N	3	4725^{ns}	0.16^{ns}	30013^{ns}	55857^{ns}	5.98^{ns}	3.87^{ns}	0.05^{ns}	1543^{ns}	80.0^{ns}	1.99^{ns}	7.93**
S × N	1	2874^{ns}	0.73*	24330^{ns}	7618^{ns}	4.50^{ns}	1771^{ns}	0.04^{ns}	2241^{ns}	442.3^{ns}	0.21^{ns}	27.63**
G × S × N	3	3597^{ns}	0.23^{ns}	65060^{ns}	30128^{ns}	2.32^{ns}	3.74^{ns}	0.03^{ns}	555^{ns}	64.7^{ns}	0.89^{ns}	5.17*
Residual	30	2146	0.1502	111359	98134	3.923	3.117	0.1732	1247	271.2	1.114	0.756

d.f; degrees of freedom, ***LA***; leaf area, ***VL***; vine length, ***NUE***; nitrogen use efficiency, ***FW***; fruit weight, ***FL***; fruit length, ***FD***; fruit diameter, ***RT***; rind thickness, ***SPF***; seed number per fruit, ***RL***; root length, ***RW***; root weight, ***100-SW***; 100 seed weight, ×; interaction between factors, **; $p \leq 0.01$ Highly significant, *; $p \leq 0.05$ significant and ***ns***; not significant.

Table 3.2: Analysis of variance matrix showing the effect of genotype, site and nitrogen application on physiological traits of studied citron melon genotypes.

Gaseous exchange						Chlorophyll fluorescence					
Source	d.f.	A (µmol CO$_2$ m^{-2} s^{-1})	g_s (mmol m^{-2} s^{-1})	E (H$_2$O m^{-2} s^{-1})	C$_i$ (µmol mol^{-1})	Fv'/Fm'	ΦPSII	PhiCO$_2$ (mol mol^{-1})	qP	ETR (m^{-2} s^{-1})	LeafAbs
Genotype (G)	3	3.23*	1.4E-3**	0.07**	5981445**	0.15^{ns}	6.96E-3^{ns}	1.66E-7*	0.03*	2.51E+8^{ns}	20.5^{ns}
Site (S)	1	124.51**	0.04**	2.84**	133051435**	0.57*	7.54E-3^{ns}	2.70E-7*	0.26*	1.44E+7^{ns}	19693.7**
Nitrogen (N)	1	48.42**	6E-4^{ns}	0.07*	2145^{ns}	0.23^{ns}	0.03^{ns}	2.35E-7*	6E-3^{ns}	1.11E+9^{ns}	2732.4^{ns}
G × S	3	3.99**	1.3E-3**	0.06**	3957086**	0.16^{ns}	0.01^{ns}	1.65E-7*	0.04^{ns}	3.65E+8^{ns}	43.1^{ns}
G × N	3	5.60**	2E-4^{ns}	0.03*	1020252^{ns}	0.03^{ns}	2E-3^{ns}	1.75E-7*	0.02^{ns}	8.52E+7^{ns}	185.8^{ns}
S × N	1	54.67**	2.4E-3**	2.49E-4^{ns}	1961399^{ns}	3E-4^{ns}	0.01^{ns}	1.33E-7^{ns}	6.8E-4^{ns}	1.22E+8^{ns}	166.0^{ns}
G × S × N	3	2.60*	2.3E-4^{ns}	0.02^{ns}	2219808^{ns}	0.22^{ns}	0.02^{ns}	1.77E-7*	6.57E-3^{ns}	8.89E+8*	433.0^{ns}
Residual	30	0.75	1.8E-4	0.01	524065	0.1056	0.00631	4.508E-08	0.02	2.82E+8	659.7

d.f; degrees of freedom, ***A***; photosynthetic rate, ***gs***; stomatal conductance, ***E***; transpiration rate, ***Ci****;* intercellular CO$_2$ concentration*,* ***Fv'/Fm'***; maximum quantum yield of photosystem II (PSII) in photosynthesis, Φ***PSII***; the effective quantum efficiency of PSII photochemistry, ***PhiCO$_2$***; partial pressure of CO$_2$, ***qP***; photochemical quenching, ***ETR***; electron transport rate, ***LeafAbs***; amount of light absorbed by the leaf, ×; interaction between factors, **; $p \leq 0.01$ Highly significant, *; $p \leq 0.05$ significant and ***ns***; not significant.

3.5.2 Mean performances of morpho-physiological traits among the studied citron melon genotypes

Mean values for morphological and physiological parameters of the tested citron melon genotypes evaluated in low-nitrogen (LN) and optimal-nitrogen (ON) conditions across GH and SH production sites are presented in **Tables 3.3** and **3.4**. The studied citron melon genotypes differed significantly based on morphological traits *LA*, *VL*, *NUE*, *FL*, *FD*, *FW*, *RL*, *RW*, *SPF* and *100-SW* recorded across all environments (**Table 3.3**). The following ranges were recorded for *LA* (89-133 cm^2), *VL* (1-3 m), *NUE* (385-659), *FL* (9-14 cm), *FD* (10-11 cm), *FW* (681-1124 g), *RL* (23-32 cm), *RW* (1-2 g) *SPF* (56-118 seeds per fruit) and *100-SW* (11-13 g) under low-nitrogen conditions. Variation in the phenotype of these morphological traits suggests differential adaptation strategies to low-nitrogen stress among the studied citron melon. The following ranges were observed for the same morphological traits under optimum-nitrogen conditions, i.e., *LA* (92-124 cm^2), *VL* (1-2 m), *NUE* (285-413), *FL* (9-14 cm), *FD* (9-12 cm), *FW* (737-998 g), *SPF* (78-150 seeds per fruit) and *100-SW* (10-15 g) (**Table 3.3**). Vegetative expansion under nitrogen stress is crucial for light interception and photosynthetic productivity (Yan et al., 2022). This suggests that low-nitrogen stress tolerant citron melon germplasms maintain high above-ground biomass under low-nitrogen environments. Based on Sun et al. (2020), crop plants re-allocate resources to support shoot biomass production and metabolism under limited nitrogen supply. Citron melon genotype WWM-Ranoto was identified with the highest *LA* (133 cm^2), making it suitable for selection to impact cultivation under low-nitrogen stress. This genotype can be subjected to further selection to improve fruit yield even under low-nitrogen cultivation conditions.

Low-nitrogen stress conditions can influence the growth and development of fruit and its related traits (Wahocho et al., 2017). The rind of citron melon fruit is a significant food and nutrient resource for livestock (Shaayau & Bashir, 2025). This signifies rind thickness as a key selection criterion in citron melon crop improvement programs. In the present study, the highest *RT* was recorded in citron melon genotype WWM-16 (1.7 cm), followed by WWM-58 and WWM-Ranoto (1.5 cm), and WWM-65 (0.8 cm) (**Table 3.3**). Citron melon seeds are rich in oil, which is predominantly linoleic acid, along with a high protein content (Mandizvo et al., 2022c; Shaayau & Bashir, 2025), making them an important food source for human consumption and nutrition. Genotypes with high *SPF* and *100-SW* are suitable for selection in low-nitrogen cultivation environments for production and breeding. In the present study, the highest values for *SPF* (118 seeds per fruit) and *100-SW* (13.3 g) were identified in citron melon

WWM-Ranoto under low-nitrogen stress environments (**Table 3.3**), indicating the suitability of WWM-Ranoto for selection to improve seed yield in environments with limited soil nitrogen production. Furthermore, *SPF* was lower under LN (118 seeds per fruit) conditions compared to the control treatment (150 seeds per fruit), suggesting that low-nitrogen stress reduces seed productivity. Although many species elongate roots under nitrogen limitation to increase soil exploration, *RL* in this study was consistently higher under ON (32.14- 26.38 cm). This pattern likely reflects the physical constraints of the pot system, where restricted volume and dense root mats at the base limited further elongation under LN. In contrast, *RW* showed genotype-specific adjustments (1.39-1.23 g), suggesting that citron melon foraging responses under LN were expressed more through changes in root mass allocation than through elongation. These results are consistent with reports that container systems can suppress typical LN-foraging traits, even when nitrogen is limiting (Cassiano et al., 2025; Poorter et al., 2012)

The studied citron melon genotypes differed significantly based on physiological traits *A*, *gs*, *E, Ci, ΦPSII*, *PhiCO₂* and ETR recorded across all environments (**Table 3.4**). The following ranges were recorded for *A* (64-66 µmol CO_2 m^{-2} s^{-1}), *gs* (0.06-0.07 mmol m^{-2} s^{-1}), *E* (1.33-1.42 H_2O m^{-2} s^{-1}), *Ci* (2231-3122 µmol mol^{-1}), *ΦPSII* (0.072-0.154), $PhiCO_2$ (1.4E-4 - 1.5E-4 mol mol^{-1}) and ETR (15180-31545 m^{-2} s^{-1}) under LN conditions (**Table 3.4**). Low nitrogen conditions result in a slight reduction in *A*, indicating that nitrogen limitation decreases carboxylation efficiency but does not entirely prevent CO_2 assimilation (Chen et al., 2025; Mishra et al., 2024). This finding aligns with previous research that nitrogen deficiency decreases Rubisco activity and chlorophyll content, thereby limiting photosynthesis (Nawaz et al., 2018; Hong et al., 2021). The *gs* (0.08-0.07 mmol m^{-2} s^{-1}), *Ci* (3420-3122 µmol mol^{-1} and) *E* (1.44-1.37 mmol H_2O m^{-2} s^{-1}) were relatively low, suggesting biochemical limitations in stomatal regulation to conserve water under stress and due to low CO_2 assimilation demand (Mishra et al., 2024; Tantray et al., 2020).

Together, the reductions in *E* and the constrained maxima of *g*s and *Ci* imply that photosynthesis under LN is primarily limited by diffusive factors (such as water-use), where stomata restrict gas-exchange to conserve water, thereby reducing the maximum potential CO_2 availability for assimilation. This can also reflect that greenhouse temperature was actively stabilised (wet-wall), whereas the shadehouse tracked ambient conditions, which can further modulate *g*s, *Ci*, and *E* independently of N. The *ΦPSII* and *ETR* were low, reflecting impaired photochemical efficiency due to reduced nitrogen availability, which is significant for chlorophyll synthesis and photosystem II function (Mu & Chen, 2021; Xu et al., 2025).

Similarly, *PhiCO₂* indicates limited efficiency in converting absorbed light into CO_2 fixation, a response observed in other crops, such as apples (Cheng et al., 2001; Mu & Chen, 2021; Zhu et al., 2008).

The following ranges were observed for the same physiological traits under optimum-nitrogen conditions, i.e., *A* (66.5-67 µmol CO_2 m^{-2} s^{-1}), *gs* (0.05-0.08 mmol m^{-2} s^{-1}), *E* (1.36-1.62 H_2O m^{-2} s^{-1}), *Ci* (1379-3420 µmol mol^{-1}), *ΦPSII* (0.04-0.07), *PhiCO₂* (1.6E-4 - 6.4E-4 mol mol^{-1}) and *ETR* (8637.09-15458.04 m^{-2} s^{-1}) (**Tables 3.4**). The *A* was slightly higher under optimum, indicating improved carboxylation efficiency due to sufficient nitrogen for Rubisco and chlorophyll synthesis (Cheng et al., 2001; Hong et al., 2021). The *gs* values exhibited a wider range, suggesting flexible stomatal regulation under favourable N supply (Silva et al., 2024). The *E* was slightly higher under ON, which is consistent with improved water relations when nitrogen is not limiting (Matimati et al., 2013). However, *Ci* was lower on average under ON compared to LN, indicating reduced biochemical limitations and better CO_2 fixation efficiency.

Similarly, the ΦPSII was lower under ON compared to low N, which is possibly due to saturation effects at high *ETR* values rather than stress (Mu and Chen et al., 2021; Xu et al., 2025). Moreover, the declines in ON could have occurred when the absorbed photon flux is high relative to sink demand, because a larger fraction of energy is safely dissipated rather than used photochemically. This can occur even when biochemical capacity is good. While *PhiCO₂* is significantly high, reflecting better conversion of absorbed light into CO_2 fixation (Cheng et al., 2001). Citron melon genotypes WWM-16 and WWM-65, identified with the highest values for *A*, *gs*, *E*, *Fv'/Fm'*, *ΦPSII* and *LeafAbs* under low-nitrogen conditions, indicate genotype physiological resistance. The lowest values for the same traits (i.e., *A*, *gs*, *E*, *Fv'/Fm'*, *ΦPSII* and *LeafAbs*) were observed in citron melon genotypes WWM-58 and WWM-Ranoto, under the same low-nitrogen conditions (**Tables 3.4**).

Table 3.3: Mean values of evaluated genotypes for morphological traits under optimum nitrogen and low nitrogen across environments.

	LA (cm)		VL (m)		NUE		FL (cm)		FD (cm)		FW (g)	
Genotype	ON	LN	ON	LN	ON	LN	ON	LN	ON	LN	ON	LN
WWM-16	94.04	126.04	1.90	2.55	413.36	456.58	10.25	9.40	11.62	10.67	930.72	796.47
WWM-58	106.61	119.40	1.85	2.21	381.54	659.29	13.53	13.68	10.92	11.33	964.66	1121.13
WWM-65	91.64	89.34	1.04	1.14	285.33	385.37	10.60	9.47	11.22	10.32	736.99	680.51
WWM-Ranoto	123.89	133.07	2.38	2.61	378.74	509.16	8.66	10.65	8.74	10.15	997.60	867.57
Grand mean	104.05	116.96	1.79	2.13	364.74	502.59	10.76	10.80	10.62	10.62	907.49	866.42
Min. mean	91.64	89.34	1.04	1.14	285.33	385.37	8.66	9.40	8.74	10.15	736.99	680.51
Max. mean	123.89	133.07	2.4	2.61	413.36	659.29	13.53	13.68	11.62	11.33	997.60	1121.13
LSD (5%)	27.31		0.23		196.7		1.17		1.04		184.7	
$p \leq 0.05$	0.11		0.01		0.16		0.95		0.99		0.65	

LA; leaf area, ***VL***; vine length, ***NUE***; nitrogen use efficiency, ***FW***; fruit weight, ***FL***; fruit length, ***FD***; fruit diameter, **LSD;** least significant differences of means (5% level), ***p* ≤ 0.05;** significance, **ON**; optimum nitrogen, **LN**; low nitrogen.

Table 3.3: Continued.

	100-SW (g)		RT (cm)		RL (cm)		RW (g)		SPF	
Genotype	ON	LN	ON	LN	ON	LN	ON	LN	ON	LN
WWM-16	11.54	11.61	1.72	1.67	26.02	24.52	1.25	1.79	81.33	76.00
WWM-58	9.77	11.08	1.35	1.58	27.28	26.07	0.91	1.29	103.83	56.17
WWM-65	13.34	11.39	0.80	0.77	41.03	32.03	1.48	1.17	77.83	78.33
WWM-Ranoto	15.36	13.33	1.48	1.58	34.22	22.92	1.93	0.67	149.83	117.67
Grand mean	12.50	11.85	1.33	1.40	32.14	26.38	1.39	1.23	103.21	82.04
Min. mean	9.77	11.08	0.80	0.77	26.02	22.92	0.91	0.67	77.83	56.17
Max. mean	15.36	13.33	1.72	1.67	41.03	32.03	1.93	1.79	149.83	117.67
LSD (5%)	2.55		0.25		9.71		0.62		20.82	
$p \leq 0.05$	0.01		0.60		0.24		0.59		0.05	

RT; rind thickness, ***SPF***; seed number per fruit, ***RL***; root length, ***RW***; root weight, ***100-SW***; 100 seed weight.

Table 3.4: Mean values of evaluated genotypes for physiological traits under optimum nitrogen and low nitrogen across environments.

	A (μmol CO_2 m^{-2} s^{-1})		gs (mmol m^{-2} s^{-1})		E (H_2O m^{-2} s^{-1})		Ci (μmol mol^{-1})		Fv'/Fm'	
Genotype	ON	LN	ON	LN	ON	LN	ON	LN	ON	LN
WWM-16	67.31	66.19	0.05	0.06	1.38	1.38	3375.19	3004.89	0.38	0.45
WWM-58	67.45	64.11	0.05	0.06	1.39	1.33	3307.04	3122.39	0.33	0.35
WWM-65	66.51	65.94	0.08	0.07	1.62	1.42	1379.06	2231.46	0.51	0.69
WWM-Ranoto	67.14	64.14	0.05	0.06	1.36	1.34	3420.43	3069.49	0.29	0.55
Grand mean	67.10	65.09	0.05	0.06	1.44	1.37	2870.43	2857.06	0.37	0.51
Min. mean	66.51	64.10	0.05	0.06	1.36	1.33	1379.06	2231.46	0.29	0.35
Max. mean	67.45	66.19	0.08	0.07	1.62	1.42	3420.43	3122.39	0.51	0.69
LSD (5%)	0.51		0.01		0.05		426.8		0.19	
$p \leq 0.05$	< 0.01		0.07		0.02		0.95		0.16	

A; photosynthetic rate, ***gs***; stomatal conductance, ***E***; transpiration rate, ***Ci****;* intercellular CO_2 concentration*,* ***Fv'/Fm'***; maximum quantum yield of photosystem II (PSII) in photosynthesis **,LSD;** least significant differences of means (5% level), ***p* ≤ 0.05;** significance, **ON**; optimum nitrogen, **LN**; low nitrogen.

Table 3.4: Continued

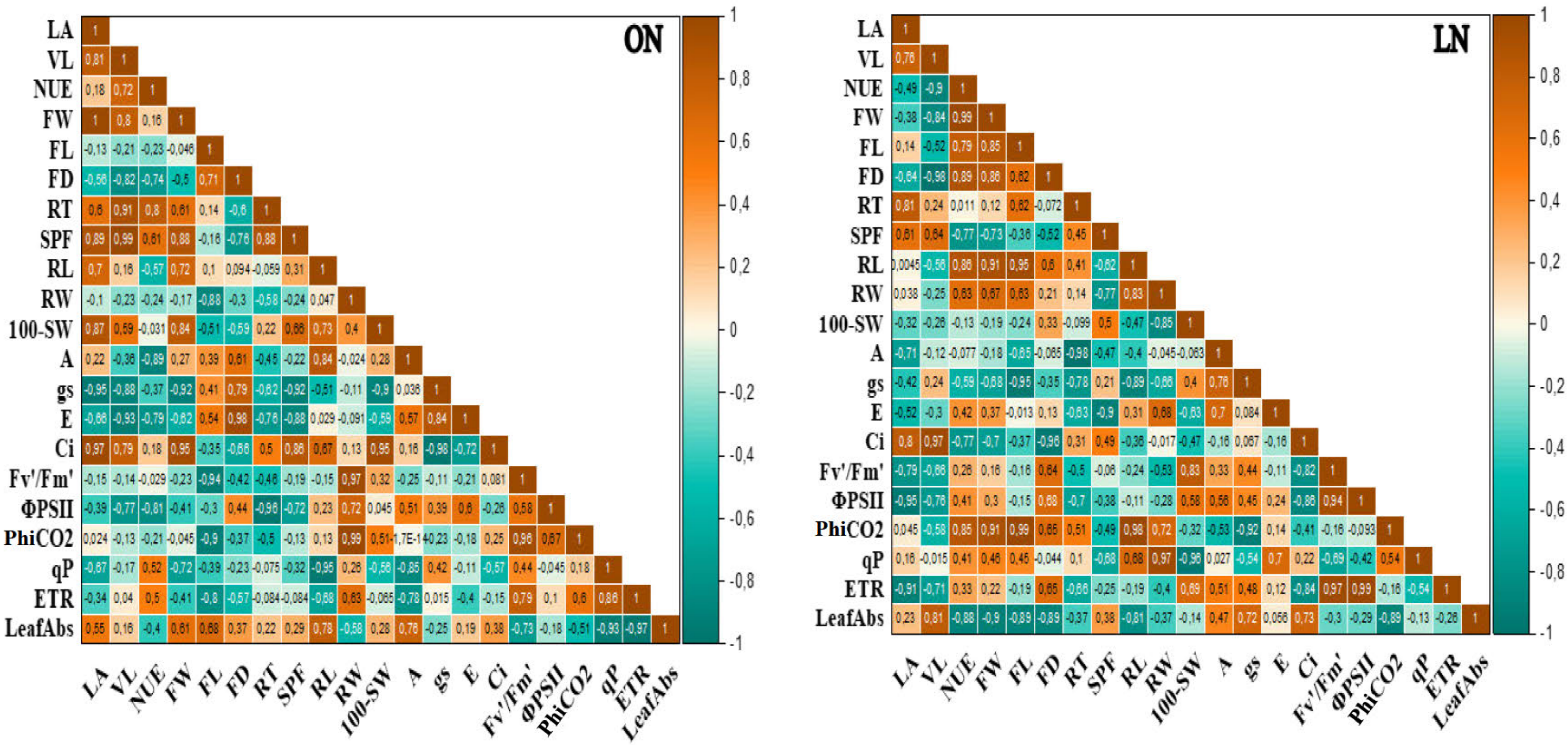
	ΦPSII		$PhiCO_2$ (mol mol^{-1})		qP		ETR (m^{-2} s^{-1})		LeafAbs	
Genotype	ON	LN	ON	LN	ON	LN	ON	LN	ON	LN
WWM-16	0.07	0.15	1.63E-4	1.41E-4	0.35	0.31	15458.04	31545.20	450.14	467.15
WWM-58	0.05	0.07	1.62E-4	1.49E-4	0.16	0.28	11429.09	15180.41	453.26	459.56
WWM-65	0.04	0.09	6.4E-4	1.37E-4	0.07	0.15	13274.36	20513.70	443.17	468.07
WWM-Ranoto	0.04	0.09	1.63E-4	1.41E-4	0.26	0.19	8637.09	20065.79	451.34	463.49
Grand mean	0.05	0.10	2.82E-4	1.42E-4	0.21	0.23	12199.65	21826.27	449.48	464.57
Min. mean	0.04	0.07	1.62E-4	1.37E-4	0.07	0.15	8637.09	15180.41	443.17	459.56
Max. mean	0.07	0.15	6.4E-4	1.49E-4	0.35	0.31	15458.04	31545.20	453.26	468.07
LSD (5%)	0.05		1.25E-4		0.09		9904.2		15.14	
$p \leq 0.05$	0.05		0.03		0.59		0.06		0.05	

ΦPSII; the effective quantum efficiency of PSII photochemistry, ***PhiCO₂***; partial pressure of CO_2, ***qP***; photochemical quenching, ***ETR***; electron transport rate, ***LeafAbs*****;** amount of light absorbed by the leaf.

3.5.3 Pearson's correlation analysis

Pearson correlation coefficients (r) showing bi-variate links between the studied morpho-physiological traits evaluated under ON and LN conditions in GH and SH production sites are presented in **Figures 3.3** and **3.4**. Positive bi-variate trait associations are important, allowing for direct and indirect trait selection in breeding programs. Under LN, the GH site showed significant correlation coefficients that were observed in *FW* with *NUE* (r = 0.99; $p \leq 0.05$), suggesting that genotypes with higher nitrogen-use efficiency tend to allocate more assimilates to fruit development, which was also observed in watermelon and melon under N stress (Chen et al., 2022; Nawaz et al., 2018; Ulas, 2022). The association of *VL* with *Ci* (r = 0.97; $p \leq 0.05$) suggests that longer vines may indicate high shoot biomass and enhanced CO_2 diffusion and photosynthetic capacity (Matimati et al., 2013; Nawaz et al., 2018), which could aid in maintaining suitable productivity levels under low-nitrogen stress. Additionally, the positive correlation of *FL* with $PhiCO_2$ (r = 0.98; $p \leq 0.05$) indicates that enhanced photosynthesis capacity increases fruit yield (Cheng et al., 2001). The positive association between *FW* with *NUE* (r = 0.99; $p \leq 0.01$) and *Ci* (r = 0.99; $p \leq 0.01$) signify that genotypes producing heavier fruit can better assimilate the limited nitrogen which in turn supports reproductive growth before nutrient depletion, confirming the connection between physiological efficiency and yield (Chen et al., 2022; Nawaz et al., 2018).

The positive correlation of *VL* with *Ci* (r = 0.96; $p \leq 0.05$) and with $PhiCO_2$ (r = 0.96; $p \leq 0.05$) suggests that longer vines enhance light capture and photochemical efficiency, thereby supporting photosynthetic resilience under N stress, which was also observed in the greenhouse (Matimati et al., 2013; Mu and Chen et al., 2021; Xu et al., 2025). The association of *FD* with *qP* (r = 0.98; $p \leq 0.05$) suggests that energy dissipation mechanisms are linked to fruit size development through enhanced carbon assimilation, which was also observed in the correlation of rice yield (Peng et al., 2021). The positive correlations of physiological attributes *Fv'/Fm'* with *ETR* (r = 0.97; $p \leq 0.05$), and *ΦPSII* with *ETR* (r = 0.99; $p \leq 0.01$) emphasises the crucial role of nitrogen in maintaining PSII efficiently and electron transport under N stress conditions (Cheng et al, 2001; Zhu et al., 2008). The physiological attribute *qP* was positively correlated with *RL* (r = 0.97; $p \leq 0.05$) and *RW* (r = 0.97; $p \leq 0.05$), and *ETR* with *Fv'/Fm'* (r = 0.96; $p \leq 0.05$). These relationships highlight the coordinated energy distribution between photosystems and root growth under nitrogen stress (Cheng et al., 2001; Ulas, 2022; Zhu et al., 2008). Negative and significant correlations were observed between *VL* with *FD* (r = -0.97; $p \leq 0.05$), *Ci* with *FD* ($r = -0.96$; $p \leq 0.05$) and *FL* with *gs* ($r = -0.95$; $p \leq 0.05$), these suggest that genotypes investing in vine elongation and CO_2 assimilation may compromise fruit diameter and stomatal conductance, this is a common stress adaptation strategy in citron melon (Mandizvo et al., 2022a). Under LN in the SH environment, positive and significant correlation coefficients were observed between *LA* and *RL* ($r = 0.97$; $p \leq 0.05$), suggesting that large leaves support root elongation for nutrient acquisition, which is a sign of adaptive strategies in low-nitrogen growing conditions where plants direct shoot and root growth so that they can capture resources better (Khan et al., 2019; Sun et al., 2020).



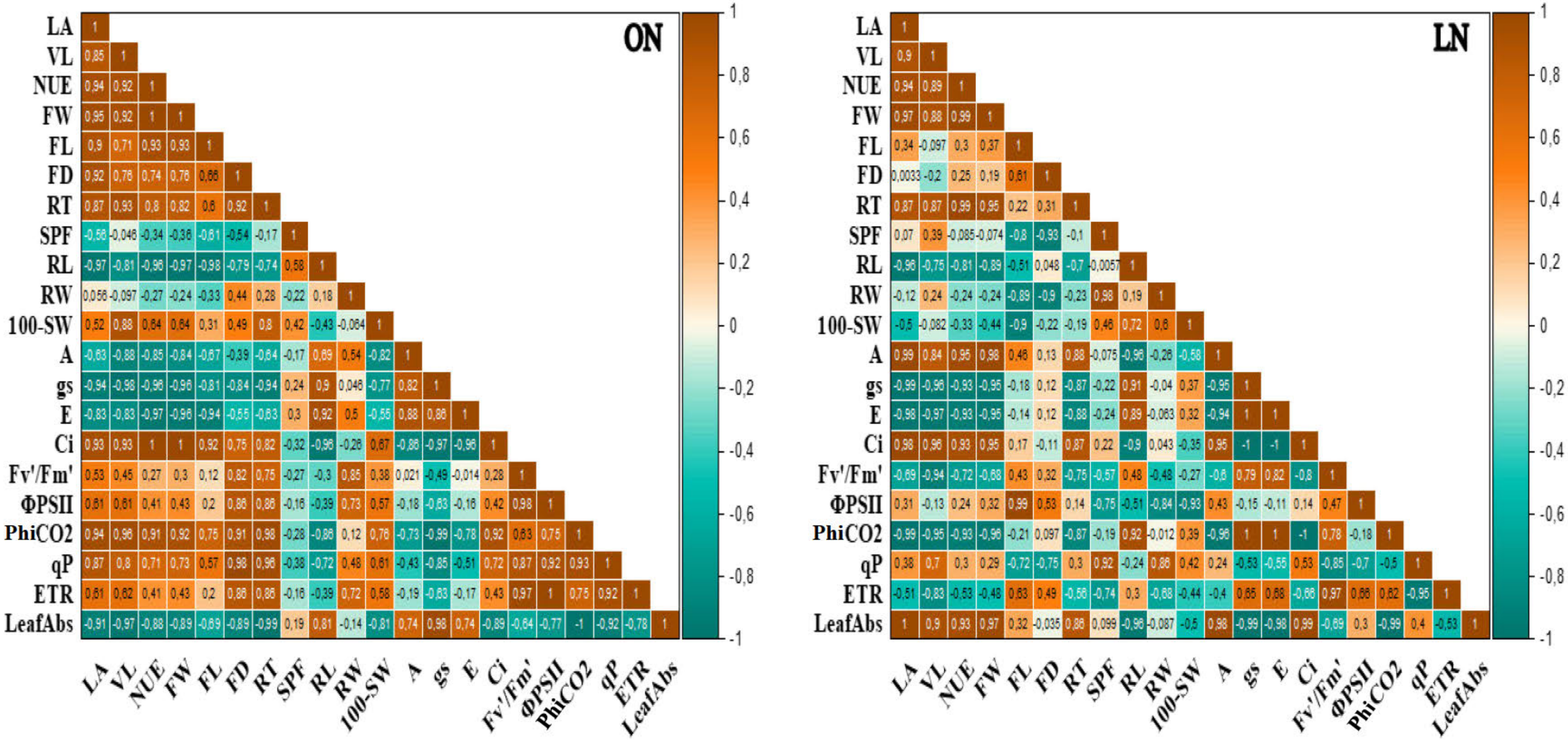
ON

	LA	VL	NUE	FW	FL	FD	RT	SPF	RL	RW	100-SW	A	gs	E	Ci	Fv'/Fm'	ΦPSII	PhiCO2	qP	ETR	LeafAbs
LA	1																				
VL	0,81	1																			
NUE	0,18	0,72	1																		
FW	1	0,8	0,16	1																	
FL	-0,13	-0,21	-0,23	-0,046	1																
FD	-0,56	-0,82	-0,74	-0,5	0,71	1															
RT	0,6	0,91	0,8	0,61	0,14	-0,6	1														
SPF	0,89	0,99	0,61	0,88	-0,16	-0,76	0,88	1													
RL	0,7	0,16	-0,57	0,72	0,1	0,094	-0,059	0,31	1												
RW	-0,1	-0,23	-0,24	-0,17	-0,88	-0,3	-0,58	-0,24	0,047	1											
100-SW	0,87	0,59	-0,031	0,84	-0,51	-0,59	0,22	0,66	0,73	0,4	1										
A	0,22	-0,36	-0,89	0,27	0,39	0,61	-0,45	-0,22	0,84	-0,024	0,28	1									
gs	-0,95	-0,88	-0,37	-0,92	0,41	0,79	-0,62	-0,92	-0,51	-0,11	-0,9	0,036	1								
E	-0,66	-0,93	-0,79	-0,62	0,54	0,98	-0,76	-0,88	0,029	-0,091	-0,59	0,57	0,84	1							
Ci	0,97	0,79	0,18	0,95	-0,35	-0,66	0,5	0,86	0,67	0,13	0,95	0,16	-0,98	-0,72	1						
Fv'/Fm'	-0,15	-0,14	-0,029	-0,23	-0,94	-0,42	-0,46	-0,19	-0,15	0,97	0,32	-0,25	-0,11	-0,21	0,081	1					
ΦPSII	-0,39	-0,77	-0,81	-0,41	-0,3	0,44	-0,96	-0,72	0,23	0,72	0,045	0,51	0,39	0,6	-0,26	0,58	1				
PhiCO2	0,024	-0,13	-0,21	-0,045	-0,9	-0,37	-0,5	-0,13	0,13	0,99	0,51	-1,7E-14	0,23	-0,18	0,25	0,96	0,67	1			
qP	-0,67	-0,17	0,52	-0,72	-0,39	-0,23	-0,075	-0,32	-0,95	0,26	-0,56	-0,85	0,42	-0,11	-0,57	0,44	-0,045	0,18	1		
ETR	-0,34	0,04	0,5	-0,41	-0,8	-0,57	-0,084	-0,084	-0,68	0,63	-0,065	-0,78	0,015	-0,4	-0,15	0,79	0,1	0,6	0,86	1	
LeafAbs	0,55	0,16	-0,4	0,61	0,68	0,37	0,22	0,29	0,78	-0,58	0,28	0,76	-0,25	0,19	0,38	-0,73	-0,18	-0,51	-0,93	-0,97	1

LN

	LA	VL	NUE	FW	FL	FD	RT	SPF	RL	RW	100-SW	A	gs	E	Ci	Fv'/Fm'	ΦPSII	PhiCO2	qP	ETR	LeafAbs
LA	1																				
VL	0,76	1																			
NUE	-0,49	-0,9	1																		
FW	-0,38	-0,84	0,99	1																	
FL	0,14	-0,52	0,79	0,85	1																
FD	-0,64	-0,98	0,89	0,86	0,82	1															
RT	0,81	0,24	0,011	0,12	0,82	-0,072	1														
SPF	0,81	0,64	-0,77	-0,73	-0,36	-0,52	0,45	1													
RL	,0045	-0,56	0,86	0,91	0,95	0,6	0,41	-0,62	1												
RW	0,038	-0,25	0,63	0,67	0,63	0,21	0,14	-0,77	0,83	1											
100-SW	-0,32	-0,26	-0,13	-0,19	-0,24	0,33	-0,099	0,5	-0,47	-0,85	1										
A	-0,71	-0,12	-0,077	-0,18	-0,65	-0,065	-0,98	-0,47	-0,4	-0,045	-0,063	1									
gs	-0,42	0,24	-0,59	-0,68	-0,95	-0,35	-0,78	0,21	-0,89	-0,66	0,4	0,76	1								
E	-0,52	-0,3	0,42	0,37	-0,013	0,13	-0,63	-0,9	0,31	0,68	-0,63	0,7	0,084	1							
Ci	0,8	0,97	-0,77	-0,7	-0,37	-0,96	0,31	0,49	-0,36	-0,017	-0,47	-0,16	0,067	-0,16	1						
Fv'/Fm'	-0,79	-0,66	0,26	0,16	-0,16	0,64	-0,5	-0,06	-0,24	-0,53	0,83	0,33	0,44	-0,11	-0,82	1					
ΦPSII	-0,95	-0,76	0,41	0,3	-0,15	0,68	-0,7	-0,38	-0,11	-0,28	0,58	0,56	0,45	0,24	-0,86	0,94	1				
PhiCO2	0,045	-0,58	0,85	0,91	0,99	0,65	0,51	-0,49	0,98	0,72	-0,32	-0,53	-0,92	0,14	-0,41	-0,16	-0,093	1			
qP	0,16	-0,015	0,41	0,46	0,45	-0,044	0,1	-0,68	0,68	0,97	-0,96	0,027	-0,54	0,7	0,22	-0,69	-0,42	0,54	1		
ETR	-0,91	-0,71	0,33	0,22	-0,19	0,65	-0,66	-0,25	-0,19	-0,4	0,69	0,51	0,48	0,12	-0,84	0,97	0,99	-0,16	-0,54	1	
LeafAbs	0,23	0,81	-0,88	-0,9	-0,89	-0,89	-0,37	0,38	-0,81	-0,37	-0,14	0,47	0,72	0,056	0,73	-0,3	-0,29	-0,89	-0,13	-0,26	1

Figure 3.3: Pearson correlation coefficients showing relationship among morpho-physiological parameters, under greenhouse environment and under both nitrogen levels ON and LN, ***LA***; leaf area, ***VL***; vine length, ***NUE***; nitrogen use efficiency, ***FW***; fruit weigh, ***FL***; fruit length, ***FD***; fruit diameter, ***RT***; rind thickness, ***SPF***; seed number per fruit, ***RL***; root length, ***RW***; root weight, ***100-SW***; 100 seed weight, ***A***; photosynthetic rate, ***gs***; stomatal conductance, ***E***; transpiration rate, ***Ci***; intercellular CO_2 concentration, ***Fv'/Fm'***; maximum quantum yield of photosystem II (PSII) in photosynthesis, ***ΦPSII***; the effective quantum efficiency of PSII photochemistry, ***PhiCO₂***; partial pressure of CO_2, ***qP***; photochemical quenching, ***ETR***; electron transport rate, ***LeafAbs***; amount of light absorbed by the leaf.



ON

	LA	VL	NUE	FW	FL	FD	RT	SPF	RL	RW	100-SW	A	gs	E	Ci	Fv'/Fm'	ΦPSII	PhiCO2	qP	ETR	LeafAbs
LA	1																				
VL	0,85	1																			
NUE	0,94	0,92	1																		
FW	0,95	0,92	1	1																	
FL	0,9	0,71	0,93	0,93	1																
FD	0,92	0,76	0,74	0,76	0,66	1															
RT	0,87	0,93	0,8	0,82	0,6	0,92	1														
SPF	-0,56	-0,046	-0,34	-0,36	-0,61	-0,54	-0,17	1													
RL	-0,97	-0,81	-0,96	-0,97	-0,98	-0,79	-0,74	0,58	1												
RW	0,056	-0,097	-0,27	-0,24	-0,33	0,44	0,28	-0,22	0,18	1											
100-SW	0,52	0,88	0,64	0,64	0,31	0,49	0,8	0,42	-0,43	-0,064	1										
A	-0,63	-0,88	-0,85	-0,84	-0,67	-0,39	-0,64	-0,17	0,69	0,54	-0,82	1									
gs	-0,94	-0,98	-0,96	-0,96	-0,81	-0,84	-0,94	0,24	0,9	0,046	-0,77	0,82	1								
E	-0,83	-0,83	-0,97	-0,96	-0,94	-0,55	-0,63	0,3	0,92	0,5	-0,55	0,88	0,86	1							
Ci	0,93	0,93	1	1	0,92	0,75	0,82	-0,32	-0,96	-0,26	0,67	-0,86	-0,97	-0,96	1						
Fv'/Fm'	0,53	0,45	0,27	0,3	0,12	0,82	0,75	-0,27	-0,3	0,85	0,38	0,021	-0,49	-0,014	0,28	1					
ΦPSII	0,61	0,61	0,41	0,43	0,2	0,86	0,86	-0,16	-0,39	0,73	0,57	-0,18	-0,63	-0,16	0,42	0,98	1				
PhiCO2	0,94	0,96	0,91	0,92	0,75	0,91	0,98	-0,28	-0,86	0,12	0,76	-0,73	-0,99	-0,78	0,92	0,63	0,75	1			
qP	0,87	0,8	0,71	0,73	0,57	0,98	0,96	-0,38	-0,72	0,48	0,61	-0,43	-0,85	-0,51	0,72	0,87	0,92	0,93	1		
ETR	0,61	0,62	0,41	0,43	0,2	0,86	0,86	-0,16	-0,39	0,72	0,58	-0,19	-0,63	-0,17	0,43	0,97	1	0,75	0,92	1	
LeafAbs	-0,91	-0,97	-0,88	-0,89	-0,69	-0,89	-0,99	0,19	0,81	-0,14	-0,81	0,74	0,98	0,74	-0,89	-0,64	-0,77	-1	-0,92	-0,78	1

LN

	LA	VL	NUE	FW	FL	FD	RT	SPF	RL	RW	100-SW	A	gs	E	Ci	Fv'/Fm'	ΦPSII	PhiCO2	qP	ETR	LeafAbs
LA	1																				
VL	0,9	1																			
NUE	0,94	0,89	1																		
FW	0,97	0,88	0,99	1																	
FL	0,34	-0,097	0,3	0,37	1																
FD	0,0033	-0,2	0,25	0,19	0,61	1															
RT	0,87	0,87	0,99	0,95	0,22	0,31	1														
SPF	0,07	0,39	-0,085	-0,074	-0,8	-0,93	-0,1	1													
RL	-0,96	-0,75	-0,81	-0,89	-0,51	0,048	-0,7	-0,0057	1												
RW	-0,12	0,24	-0,24	-0,24	-0,89	-0,9	-0,23	0,98	0,19	1											
100-SW	-0,5	-0,082	-0,33	-0,44	-0,9	-0,22	-0,19	0,46	0,72	0,8	1										
A	0,99	0,84	0,95	0,98	0,46	0,13	0,88	-0,075	-0,96	-0,26	-0,58	1									
gs	-0,99	-0,96	-0,93	-0,95	-0,18	0,12	-0,87	-0,22	0,91	-0,04	0,37	-0,95	1								
E	-0,98	-0,97	-0,93	-0,95	-0,14	0,12	-0,88	-0,24	0,89	-0,063	0,32	-0,94	1	1							
Ci	0,98	0,96	0,93	0,95	0,17	-0,11	0,87	0,22	-0,9	0,043	-0,35	0,95	-1	-1	1						
Fv'/Fm'	-0,69	-0,94	-0,72	-0,68	0,43	0,32	-0,75	-0,57	0,48	-0,48	-0,27	-0,6	0,79	0,82	-0,8	1					
ΦPSII	0,31	-0,13	0,24	0,32	0,99	0,53	0,14	-0,75	-0,51	-0,84	-0,93	0,43	-0,15	-0,11	0,14	0,47	1				
PhiCO2	-0,99	-0,95	-0,93	-0,96	-0,21	0,097	-0,87	-0,19	0,92	-0,012	0,39	-0,96	1	1	-1	0,78	-0,18	1			
qP	0,38	0,7	0,3	0,29	-0,72	-0,75	0,3	0,92	-0,24	0,86	0,42	0,24	-0,53	-0,55	0,53	-0,85	-0,7	-0,5	1		
ETR	-0,51	-0,83	-0,53	-0,48	0,63	0,49	-0,56	-0,74	0,3	-0,68	-0,44	-0,4	0,65	0,68	-0,66	0,97	0,66	0,82	-0,95	1	
LeafAbs	1	0,9	0,93	0,97	0,32	-0,035	0,86	0,099	-0,96	-0,087	-0,5	0,98	-0,99	-0,98	0,99	-0,69	0,3	-0,99	0,4	-0,53	1

Figure 3.4: Pearson correlation coefficients displaying relationship among morpho-physiological parameters, under shadehouse environment and under both nitrogen levels ON and LN, ***LA***; leaf area, ***VL***; vine length, ***NUE***; nitrogen use efficiency, ***FW***; fruit weigh, ***FL***; fruit length, ***FD***; fruit diameter, ***RT***; rind thickness, ***SPF***; seed number per fruit, ***RL***; root length, ***RW***; root weight, ***100-SW***; 100 seed weight, ***A***; photosynthetic rate, ***gs***; stomatal conductance, ***E***; transpiration rate, ***Ci***; intercellular CO_2 concentration, ***Fv'/Fm'***; maximum quantum yield of photosystem II (PSII) in photosynthesis, ***ΦPSII***; the effective quantum efficiency of PSII photochemistry, ***PhiCO₂***; partial pressure of CO_2, ***qP***; photochemical quenching, ***ETR***; electron transport rate, ***LeafAbs***; amount of light absorbed by the leaf.

3.5.4 Principal component analysis (PCA) for the evaluated morpho-physiological parameters

Principal component analysis (PCA) showing variation explained for the studies morphological and physiological traits in ON and LN conditions under GH and SH sites is presented in **Table 3.5**. Under GH in ON, PCA revealed three principal components PCs (i.e., PC1, PC2 and PC3), which accounted for 44, 32 and 24% of total variation observed for the studied morpho-physiological traits. Under GH in LN, the three significant PCs (i.e., PC1, PC2 and PC3) explained 41, 35 and 24% of the total variation observed. Under SH in ON, the three significant PCs (i.e., PC1, PC2 and PC3) explained 61, 32 and 7% of the total variation observed. Under SH in LN, the three significant PCs (i.e., PC1, PC2 and PC3) explained 70, 20 and 10% of the total variation observed. In LN across all test sites, morpho-physiological traits *LA*, *VL*, *NUE*, *FW*, *RT*, *SPF*, *100-SW*, *Ci*, *PhiCO*$_2$, *qP* and *LeafAbs* recorded high loading scores (≥ 0.30) under PC1.

In LN across GH and SH sites, morpho-physiological traits *NUE*, *FL*, *FD*, *FW*, *VL*, *Ci*, *qP* and *PhiCO*$_2$ recorded high loading scores under PC1. This means these traits collectively contribute to a major portion of variation under N stress, suggesting a strong and significant interdependency between agronomic and fruit traits. Moreover, these dominant morpho-physiological traits coordinate to stabilise nitrogen use efficiency and fruit yield (Peng et al., 2021; Zhang et al., 2017). The PC biplots, which show the grouping of studied citron melon genotypes based on morpho-physiological attributes under ON and LN conditions in GH and SH environments, are presented in **Figures 3.5** and **3.6** Under LN conditions in GH, citron melon genotype WWM-58 was passively associated with high *RT*, *qP*, *RW*, *FL*, *RL*, *PhiCO*$_2$, *FW* and *NUE*, indicating its strong adaptability through enhanced root traits and photochemical efficiency, such associations suggest that root vigour and efficient energy conversion are critical for sustaining fruit development under nitrogen stress, this was also observed in cucurbit studies, where root architecture and PSII performance were driven by stress resilience (Nawaz et al., 2018; Li et al., 2023; Lopez et al., 2023; Wu et al., 2025).

Moreover, these results are consistent with the literature on cucurbits and other crops, where increased biomass and root length under nitrogen deficit are thought to be adaptive processes to enhance nitrogen uptake (Gruber et al., 2013; Lynch, 2019). Morpho-physiological attributes *FD*, *E*, *ETR* and *Fv'/Fm'* associated with citron melon genotype WWM-65 under LN and GH environment imply that this genotype relies more on photochemical adjustments and water relations than on vegetative growth. Similar genotype-specific strategies have been reported in melon studies, where PSII efficiency and electron transport dominate variance under nutrient stress (Flexas et al., 2006; Pal et al., 2018; Tajaragh et al., 2022; Zhao et al., 2017). These are known as key indicators of low nitrogen tolerance in cucurbits. Under LN in the SH site, citron melon WWM-16 was associated with *RW*, *Fv'/Fm'*, Φ*PSII*, *ETR*, *FD*, *FL*, *qP*, $PhiCO_2$, *100-SW* and *LA,* suggesting a combined reliance on root development, photochemical efficiency, and fruit traits for adaptation. This aligns with findings that the main variation was captured in integrated physiological and reproductive traits, which are key contributors to stress tolerance in cucurbits (Maghfiroh et al., 2023; Aydın & Yetişir, 2025; Sharma & Leskovar, 2020).

The Citron melon genotypes WWM-58 and WWM-Ranoto were linked to high *LA*, *VL*, *FW*, *NUE*, *Ci*, and *FL* under LN conditions at the SH production site, emphasising their shoot biomass development, fruit yield and nitrogen-use efficiency. This grouping is similar to the greenhouse environment (**Figure 3.6**). Such clustering reflects the role of vegetative structure and carbon assimilation in maintaining yield under low-input systems, consistent with PCA-based genotype differentiation in melon and watermelon breeding programs aimed at improving abiotic stress tolerance (Tembo & Maphosa, 2025; Maghfiroh et al., 2023). These genotype-specific trait associations offer a framework for selecting genotypes for breeding programs targeting nitrogen efficiency (Donald, 1968; Xu et al., 2012).

Table 3.5: Summary of factor loadings, eigenvalue, percent and cumulative variation for morpho-physiological parameters assessed in selected genotypes in the greenhouse and shadehouse and under optimum (ON) and low nitrogen (LN).

	Greenhouse						Shadehouse					
	ON			LN			ON			LN		
Trait	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3
LA	**0.31**	0.08	0.10	-0.14	**0.31**	-0.17	0.2	0.06	-0.07	**0.25**	-0.02	-0.18
VL	**0.32**	-0.06	-0.11	-0.29	0.18	0.03	**0.27**	-0.10	0.07	**0.25**	-0.06	0.19
NUE	0.16	-0.21	-0.30	**0.34**	-0.01	0.03	**0.28**	0.07	0.22	**0.25**	-0.16	-0.04
FW	**0.31**	0.11	0.09	**0.34**	0.03	0.01	**0.27**	0.09	0.11	**0.25**	-0.15	-0.05
FL	-0.07	**0.32**	-0.22	**0.27**	0.18	-0.16	0.05	**0.37**	-0.15	**0.21**	-0.21	-0.27
FD	-0.24	**0.26**	0.01	**0.29**	-0.15	-0.13	-0.02	0.29	**0.52**	**0.24**	0.17	-0.16
RT	**0.26**	-0.004	-0.27	0.02	**0.26**	-0.31	0.25	0.05	**0.34**	**0.25**	0.12	0.11
SPF	**0.33**	-0.02	-0.07	-0.22	0.03	-0.34	0.05	-0.35	-0.28	-0.09	-0.03	**0.65**
RL	0.15	0.23	**0.29**	**0.29**	0.18	0.02	-0.25	-0.11	0.27	-0.24	0.14	**0.22**
RW	-0.05	-0.24	**0.34**	0.19	0.20	**0.28**	0.002	-0.37	-0.21	0.02	**0.48**	-0.09
100-SW	**0.25**	-0.02	**0.28**	-0.04	-0.24	-0.34	-0.09	-0.29	**0.44**	0.19	-0.01	**0.48**
A	-0.03	**0.30**	0.27	-0.04	-0.21	**0.36**	**0.27**	0.11	-0.04	-0.19	**0.26**	-0.28
gs	-0.32	0.08	-0.11	-0.20	-0.27	0.14	-0.28	0.01	0.05	-0.26	0.05	-0.06
E	-0.28	0.19	0.06	0.10	-0.03	**0.42**	-0.28	0.02	0.02	-0.21	**0.28**	0.05
Ci	**0.31**	0.01	0.17	-0.25	**0.24**	0.09	**0.28**	-0.01	-0.04	**0.25**	-0.16	-0.02
Fv'/Fm'	-0.04	-0.30	**0.26**	0.08	-0.34	-0.15	-0.22	**0.21**	-0.19	0.15	**0.39**	-0.02
ϕPSII	-0.20	-0.01	**0.35**	0.12	-0.35	0.02	0.04	**0.36**	-0.23	0.18	**0.34**	0.08
$PhiCO_2$	-0.01	-0.23	**0.35**	**0.29**	0.18	-0.08	-0.28	-0.01	0.05	**0.26**	0.03	0.04
qP	-0.14	-0.31	-0.19	0.12	**0.22**	**0.32**	0.14	-0.33	-0.08	**0.24**	**0.20**	-0.04
ETR	-0.04	-0.38	-0.01	0.09	-0.35	-0.04	-0.18	**0.29**	-0.14	0.18	**0.34**	0.08
LeafAbs	0.12	**0.36**	0.04	-0.29	-0.02	**0.22**	**0.28**	0.05	-0.09	-0.26	-0.05	-0.10
Eigenvalue	9.14	6.80	5.05	8.66	7.27	5.07	12.74	6.75	1.51	14.64	4.25	2.12
Percentage of Variance (%)	43.55	32.39	24.06	41.26	34.62	24.12	60.65	32.15	7.20	69.71	20.22	10.07
Cumulative (%)	43.55	75.94	100.00	41.26	75.88	100.00	60.65	91.79	100.00	69.71	89.93	100.00

LA; leaf area, ***VL***; vine length, ***NUE***; nitrogen use efficiency, ***FW***; fruit weigh, ***FL***; fruit length, ***FD***; fruit diameter, ***RT***; rind thickness, ***SPF***; seed number per fruit, ***RL***; root length, ***RW***; root weight, ***100-SW***; 100 seed weight, ***A***; photosynthetic rate, ***gs***; stomatal conductance, ***E***; transpiration rate, ***Ci****;* intercellular CO_2 concentration*,* ***Fv'/Fm'***; maximum quantum yield of photosystem II (PSII) in photosynthesis, Φ***PSII***; the effective quantum efficiency of PSII photochemistry, ***PhiCO*** $_2$; partial pressure of CO_2, ***qP***; photochemical quenching, ***ETR***; electron transport rate, ***LeafAbs***; amount of light absorbed by the leaf.

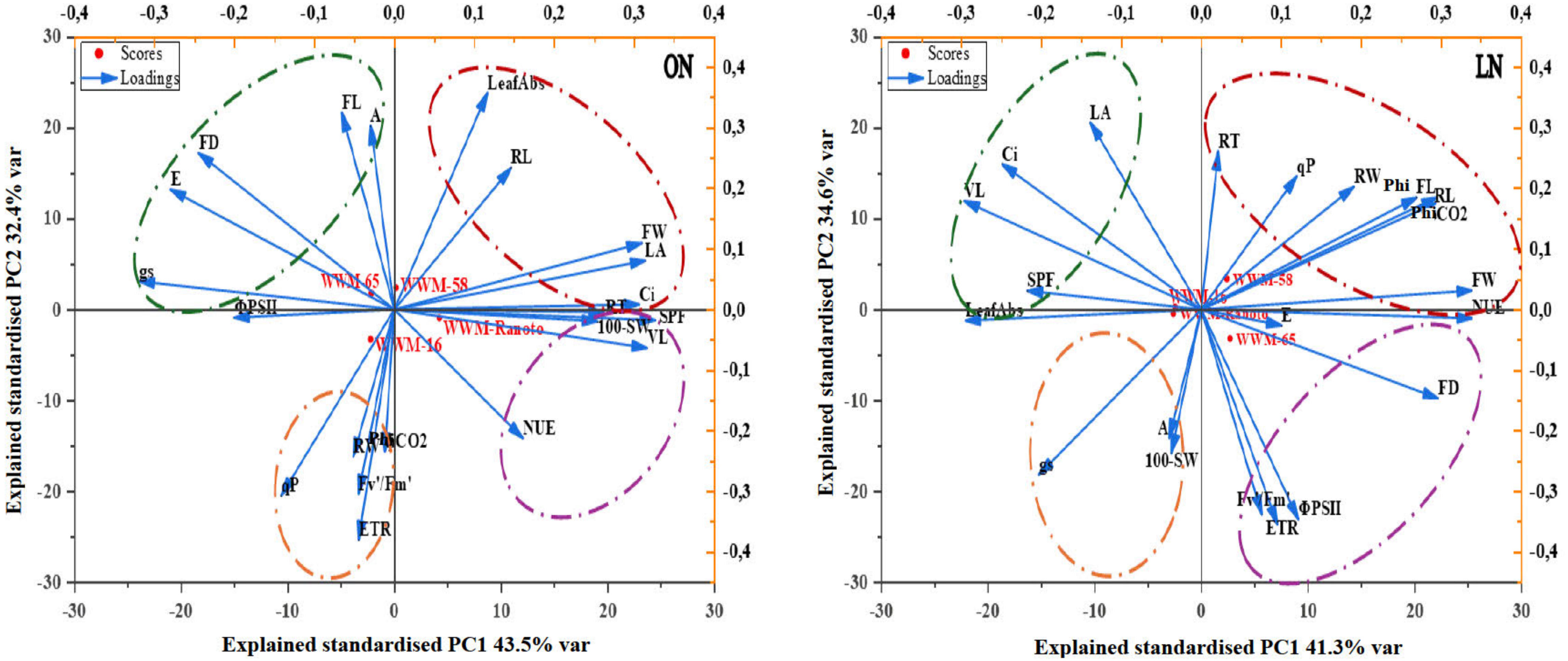


Figure 3.5: Biplot PCAs showing relations among morpho-physiological traits and selected genotypes (WWM 02, WWM16, WWM 58 and WWM 65) under greenhouse (ON and LN): ***LA***; leaf area, ***VL***; vine length, ***NUE***; nitrogen use efficiency, ***FW***; fruit weigh, ***FL***; fruit length, ***FD***; fruit diameter, ***RT***; rind thickness, ***SPF***; seed number per fruit, ***RL***; root length, ***RW***; root weight, ***100-SW***; 100 seed weight, ***A***; photosynthetic rate, ***gs***; stomatal conductance, ***E***; transpiration rate, ***Ci;*** intercellular CO_2 concentration, ***Fv'/Fm'***; maximum quantum yield of photosystem II (PSII) in photosynthesis, Φ***PSII***; the effective quantum efficiency of PSII photochemistry, ***PhiCO₂***; partial pressure of CO_2, ***qP***; photochemical quenching, ***ETR***; electron transport rate, ***LeafAbs***; amount of light absorbed by the leaf.

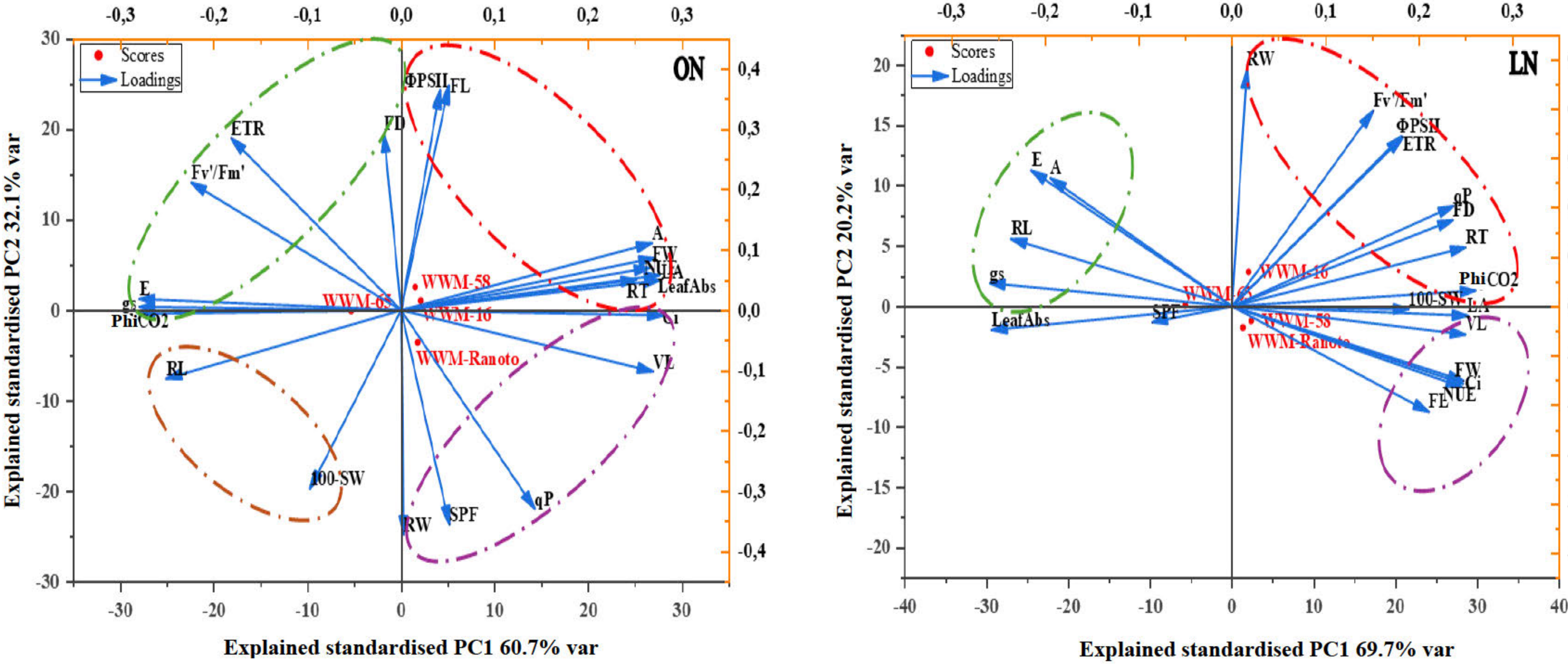


Figure 3.6: Biplot PCAs showing relations among morpho-physiological traits and selected genotypes (WWM 02, WWM16, WWM 58 and WWM 65) under shadehouse (ON and LN): ***LA***; leaf area, ***VL***; vine length, ***NUE***; nitrogen use efficiency, ***FW***; fruit weigh, ***FL***; fruit length, ***FD***; fruit diameter, ***RT***; rind thickness, ***SPF***; seed number per fruit, ***RL***; root length, ***RW***; root weight, ***100-SW***; 100 seed weight, ***A***; photosynthetic rate, ***gs***; stomatal conductance, ***E***; transpiration rate, ***Ci;*** intercellular CO_2 concentration, ***Fv'/Fm'***; maximum quantum yield of photosystem II (PSII) in photosynthesis, Φ***PSII***; the effective quantum efficiency of PSII photochemistry, ***PhiCO₂***; partial pressure of CO_2, ***qP***; photochemical quenching, ***ETR***; electron transport rate, ***LeafAbs***; amount of light absorbed by the leaf.

3.6 Conclusion

In this study, four citron melon genotypes were investigated for their morphological and physiological responses under optimum and low nitrogen conditions across greenhouse and shadehouse environments. Overall, the results showed that most of the studied traits were significantly affected by both genotype and environment. The following genotypes, WWM-58 and WWM-16, were classified as tolerant due to their strong morphological resilience under low N stress, whereas genotypes WWM-Ranoto and WWM-65 were physiologically tolerant to N stress. Therefore, genotypes WWM-58 and WWM-16 are promising candidates and have been selected for further breeding, as they exhibited higher yields despite the limitation of N supply. Future research can focus on the molecular mechanisms of promising candidates, such as gene expression under low-nitrogen conditions.

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CHAPTER 4

The biochemical response of selected citron melon (*Citrullus lanatus var citroides*) genotypes evaluated under low-nitrogen growing conditions

Abstract

There is limited research investigating the extent of phenotypic variation for fruit quality attributes in response to cultivation under low-nitrogen conditions in wild watermelon (*Citrullus lanatus var citroides*). The crop is composed of minerals, proteins, lipids, vitamins, and carbohydrates, which are found in its leaves and fruits, serving as food resources for human and animal consumption. Bioactive compounds possessing medicinal properties, such as antioxidants, are also present in citron melon. Therefore, the objective of this study was to determine accumulated metabolites in citron melon genotypes cultivated under low and optimal nitrogen applications to aid selection of suitable genotypes for cultivation. Four genetically diverse citron melon genotypes (WWM-Ranoto, WWM-16, WWM-58, and WWM-65) were evaluated for fruit compositional attributes under optimal-nitrogen (ON) and low-nitrogen (LN) conditions in greenhouse (GH) and shade house (SH) production sites using a randomised complete block design. The compositional parameters, including total soluble solids (*TSS)*, titratable acidity (*TA*), sugar-acid ratio (*TSS/TA*), total carotenoids (*TC*), sucrose (*S*), glucose (*G*), fructose (*F*), ascorbic acid/ vitamin C (*AA*), and water content (*WC*), were recorded. Analysis of variance revealed a significant ($p \leq 0.01$) genotype effect for *TSS, TA*, *TSS/TA*, *TC*, G, *AA*, and *WC*. The contents of all evaluated metabolites differed significantly based on nitrogen application, except for *TC*. Pearson's correlation revealed a positive correlation between *AA* and *TC* ($r = 0.96$) in LN conditions. Negative bi-variate correlations were observed for *TSS/TA* with *TA* ($r = -0.97$), and for *AA* with *G* ($r = -0.96$). Principal components analysis associated with citron melon genotypes revealed that genotypes WWM-65 and WWM-58 exhibited high *TA*, *TSS*, *TSS/TA*, *TC*, and *AA*. These citron melon genotypes have the potential for commercialisation in low-input agricultural systems and can be selected for low-nitrogen stress tolerance and fruit quality improvement in breeding programs.

Keywords: Antioxidants, biochemical attributes, citron melon, composition, low-nitrogen stress, metabolites, nutrition, sugars

4.1 Introduction

Citron melon (*Citrullus lanatus* var. *citroides*) is a significant source of important compositions, including micro- and macro-nutrients, i.e., vitamins (i.e., vitamin A, vitamin C, etc.), minerals (i.e., potassium, calcium, iron, etc.), proteins (i.e., globulins, albumins, glutelins, etc.), lipids (i.e., linoleic acid, oleic acid, stearic acid, etc.), and carbohydrates (i.e., fructose, glucose, cellulose, malic acid, etc.), which are present in its edible above-ground vegetative structures as food resources for human and animal consumption. Bioactive substances possessing medicinal properties, such as flavonoids and cucurbitacin, are also present in citron melon (El-Sayed et al., 2021; Masoko et al., 2022; Semenya & Maroyi, 2019). These nutritional and anti-nutritional attributes are important for combating malnutrition and improving health and immunity. These make citron melon a promising food crop for cultivation, enhancing global access to adequate and healthy food while improving human health Status.

The cultivation of citron melon is characterised by limited productivity under low-input agricultural production systems (Tembo & Maphosa, 2025). Also, the crop is under-researched, lacking production data and guidelines. There is a need for increased research to investigate production factors in citron melon cultivation. The response to its major yield traits, including compositional attributes, in relation to various production factors needs to be investigated to inform cultivation and management guidelines. Environmental stress due to abiotic factors presents with significant changes in the compositional attributes of citron melon (Kurtar et al., 2024). Environmental stress due to limited soil nitrogen, a fundamental macronutrient required for growth by terrestrial crop plants, affects the biosynthesis of proteins, nucleic acids, chlorophyll, and other critical biomolecules (e.g., amino acids, nucleotides) (Farrante et al.,2008; Kusano et al., 2011; Zayed et al., 2023).

Therefore, changes in N availability can rapidly alter carbon-nitrogen partitioning and thus the biosynthesis and accumulation of metabolites, including nutrients and phytochemical compounds (Farrante et al., 2008; Huang et al., 2022; Liu et al., 2024; Mishra et al., 2024). For instance, nitrogen supply influences the accumulation of total soluble solids (*TSS*), vitamin C, and carotenoids (Boskovic-Rakocevic et al., 2012; Huang et al., 2022; Liao et al., 2019). The findings signify that cultivation under variable nitrogen conditions may influence productivity levels and reduce the efficiency of selection due to the genotype-by-environment interaction effect in selection programs.

Superior citron melon genotypes should accumulate high contents of essential compositions in the face of limited soil nitrogen. Therefore, the genetic potential of citron melon for increased accumulation of key compositional attributes under low soil nitrogen conditions is expected to increase. This will extend the crop's role in food and nutrition security and enhance genetic advances for these attributes in cucurbit breeding. The profiling of fruit compositional traits in crop plant tissues offers an opportunity to understand the adaptability of crop plants to environmental stress.

This allows researchers to capture comprehensive snapshots of primary metabolites (i.e., amino acids, organic acids, sugars) and secondary metabolites (i.e., phytochemical compositions) that change in response to environmental stress (Huang et al., 2022). These metabolic signatures often reflect the underlying biochemical pathways that are activated or suppressed in response to stress and can serve as biomarkers of tolerance or sensitivity. For instance, in potatoes under nitrogen deficiency, integrative transcriptomic and metabolomic analyses identified specific sugars, amino acids, and regulatory genes associated with enhanced nitrogen-use traits (Barandalla et al., 2025). Similarly, carrots accumulated high vitamin C, low nitrate, and modest β-carotene levels under low-nitrogen growing conditions, signifying antioxidant preservation under limited soil fertility stress (Boskovic-Rakocevic et al., 2012).

In cucurbits, studies that pair metabolite profiling with genotype evaluation demonstrate that compositional differences often lead to phenotypic differences in fruit yield and quality and can therefore accelerate the selection for fruit quality traits (i.e., TSS, TSS/TA, vitamin C, carotenoids) in breeding programs (Ali et al., 2025; Ngwepe et al., 2023). Despite the promise of metabolomics, a significant knowledge gap remains for citron melon. There are limited, if any, studies that have profiled its metabolome under abiotic stress, especially nitrogen stress, and even fewer (if any) have leveraged metabolite data to select genotypes for improved nitrogen use efficiency. Given the crop's potential importance in low-input, nutrient-limited farming systems, further research is still needed on this topic. By analysing the profiles of TSS, TA, vitamin C, carotenoids, and water-related metabolites under low versus optimum N, one can identify biochemical markers of genotype performance in fruit quality.

Genotypes that preserve high sugar content, optimal acid levels, favourable TSS/TA, robust ascorbic acid, and key carotenoids under N limitation are likely to combine resilience with market-desirable fruit quality. Therefore, the aim of this study is to determine accumulated metabolites in citron melon genotypes cultivated under low and optimal nitrogen applications for selecting suitable genotypes for production and breeding.

4.2 Materials and methods

4.2.1 Genetic materials

Four citron melon genotypes sourced from different districts of Limpopo province in South Africa, selected based on the ability to withstand abiotic stresses (Mashilo et al., 2017 b, c; Ngwepe et al., 2023). The genotype panel was limited to four lines due to germplasm availability; while adequate for contrasting responses, this may constrain broad generalisability.

4.2.2 Study site and trial layout

The study was conducted in the Controlled Environment Facility (CEF) at the University of KwaZulu-Natal (UKZN), Pietermaritzburg, South Africa, at 29°37′37.5″S and 30°24′10.4″E. The experiment took place in the under greenhouse (GH), which maintained a temperature range of 25 to 32 ºC, Air temperature was actively regulated using a wet-wall evaporative cooling system with extraction fans during hot periods and shadehouse (SH) (**Figure 3.1 A & B**). Conditions reflected ambient outdoor weather moderated by the SH, therefor, no active climate control. four selected citron melon genotypes, WWM-Ranoto, WWM-16, WWM-58, and WWM-65 were assessed for their tolerance to low nitrogen conditions. The experiment followed a randomised complete block design (RCBD) with three replications. Each environment had two nitrogen levels: Low Nitrogen (LN) and Optimum Nitrogen (ON). Each genotype was grown in a 10-L pot filled with Duzi-turf compost-based growth medium, as described in Chapter 3.

4.3 Data collection

4.3.1 Fruit preparation

All the fruits collected from the field were prepared for lab analysis. The fruits were cut immediately into small cubes and stored in the deep freezer to slow down any enzymic and biochemical activities. The analyses were conducted on the pulp of the fruit because the pulp represents the primary edible and commercially relevant portion of citron melon. The samples were freeze-dried using a Virtis freeze dryer (#6KBTES-55, SP Industries, USA) over a period of 5 days until they were completely dry. Once completely dried, the samples were ground into a fine, homogeneous powder using a clean mortar and pestle. The powdered samples were stored in airtight ziplock plastic bags in the deep freezer and protected from light until further biochemical analyses were conducted, including the determination of vitamin C, Titratable acid and total sugar content, which were recorded in mg/g of dry weight.

4.3.2 Water content (WC)

To determine citron melon's water content, the fresh weight was weighed before freeze-drying the samples, and after they were completely dry, dry weight was recorded. The total water content was calculated using equation 3 (Kusumiyati et al., 2018):

$$\boldsymbol{WC\ in}\ \% = \frac{\boldsymbol{fresh\ weight - dry\ weight}}{\boldsymbol{fresh\ weight}} \times \mathbf{100} \qquad \text{Equation 3}$$

4.3.3 Sugar content (*S, G, F*)

Soluble sugars were quantified following the procedure described by Tesfay et al. (2016) and Ngceni (2019), with minor modifications. Approximately 0.20 g of powdered citron melon sample was combined with 10 mL of 80% (v/v) ethanol and homogenised for 60 seconds. The mixture was then incubated in a hot water bath at 80°C for 60 minutes and subsequently stored at 4°C overnight. After centrifugation at 12100 rpm for 15 minutes at 4°C, the supernatant was evaporated to dryness to remove alcohol using a Genevac personal evaporator (EZ-2.3, SP Scientific, Genevac Ltd, Ipswich, England). The dried residue (sugars) were reconstituted in 2 mL of ultra-pure water, filtered through 0.45 µm nylon filters, and analysed using an HPLC system (LC-20AT, Shimadzu Corporation, Kyoto, Japan) equipped with a refractive index detector (RID-10A) and a Rezex RCM–RCM-monosaccharide column (8-mm pore size; Phenomenex, Torrance, CA, USA). Individual sugar concentrations were determined by comparison with authentic standards of sucrose (*S*), glucose (*G*), and fructose (*F*), each showing calibration curves with $R^2 = 0.999$ (Ngceni, 2019).

4.3.4 Total soluble solids (TSS)

Total soluble solids (TSS) content was measured as °Brix using a digital refractometer (RFM340+, Bellingham + Stanley Ltd, Tunbridge Wells, UK), as explained by Luthuli et al. (2024) with adjustments. A citron watermelon frozen fruit sample was allowed to thaw at room temperature until it was soft. Once it was soft, it was transferred into a clean filtering mortar and gently macerated using a pestle to facilitate juice release. Using a micropipette, an aliquot of the expressed juice was carefully collected from the homogenate and dispensed onto the prism surface of the digital refractometer. The refractometer was calibrated before taking any measurements by washing the prism with distilled water, followed by the measurement of a zero sample. Each sample was analysed in triplicate, and the mean value will be used to report TSS results.

4.3.5 Titratable acidity (TA)

Titratable acidity was determined following the procedure by Bambalele et al. (2023) with modifications. A citron watermelon frozen fruit sample was thawed at room temperature until it was soft. Once softened, it was transferred into a clean filtering mortar and gently macerated using a pestle to facilitate juice release. A total of 5 mL of the juice was used to determine TA levels and mixed with 45 mL of distilled water (v/v). Titratable acid was measured using an automated titration system (Rondolino G20 Compact Titrator, Mettler Toledo, Schwerzenbach, Switzerland). Before titration, the electrode was calibrated with buffer solutions of pH 7 and pH 4 to adjust the slope and zero point (offset). Samples were titrated with 0.1 M sodium hydroxide (NaOH) to an endpoint of pH 8.1, and results were expressed as % malic acid equivalents using the following equation 4

$$\boldsymbol{TA = 1.34 \times Titre \times NaOH \times 0.1 \times 10} \qquad \text{Equation 4}$$

Where **Titre** is the volume of NaOH consumed during the titration. The constant 1.34 was derived from the equivalent weight of malic acid (67.045 g/mol), the molarity of NaOH (0.1 M), and the sample volume (5 mL). The factors 0.1 and 10 account for the NaOH molarity and unit conversion to g/100 mL of juice, respectively. This formula was used for all analysed samples.

The **solid-acid ratio (TSS/TA)** was calculated using Equation 3.

$$\boldsymbol{Solid\ acid\ ratio = \frac{TSS}{TA}} \qquad \text{Equation 5}$$

4.3.6 Total carotenoids (TC)

Leaf absorbance was calculated according to the equations of Lichtenthaler (1987), following the approach used by Dlamini et al. (2024). Citron melon powder samples weighing 0.3 g were suspended in 4 ml of 100% acetone, vortexed for 30 seconds, and centrifuged for 5 minutes at 23,500 rpm at 4 °C. In a spectrophotometer (IRMECO GmbH, Lütjensee, Germany, Model U2020), the absorbance of the supernatant was measured at 663.2 and 646.8 nm for chlorophyll a and b, and at 470 nm for carotenoids. Total chlorophyll and carotenoid concentrations per ml extract were calculated using the following equations:

$$\boldsymbol{Chlorophyll\ a\ (Ca) = 12.25\ A663.2 - 2.79A646.8} \qquad \text{Equation 6}$$

$$\boldsymbol{Chlorophyll\ b\ (Cb) = 21.50A646.8 - 5.10A663.2} \qquad \text{Equation 7}$$

Total carotenoids (xanthophylls and carotenes)

$$\boldsymbol{(Cx + c) = \frac{1000A470 - 1.82Ca - 85.02\ Cb}{198}} \qquad \text{Equation 8}$$

4.3.7 Ascorbic acid (AA/Vitamin C)

With modifications for the use of citron melon, the ascorbic acid content was calculated using the methodology of Soliva-Fortuny et al. (2004) and Boonkasem et al. (2015). Here, 10 mL of an extraction solution containing 10% metaphosphoric acid was used to homogenise 0.5 grams of citron melon powder. Following homogenization, the mixture was centrifuged for 15 minutes at 4°C at 1890 rpm. After filtering the supernatant, 1 mL was combined with 5 mL of 0.05 mM 2,6-Dichlorophenolindophenol (DCPIP) for 15 seconds. A spectrophotometer (UV-1800, Shimadzu Corporation, Japan) was used to analyse the materials, and absorbance measurements were made at 515 nm in comparison to a blank. Ascorbic acid concentrations ranging from 0 to 3200 µg/ml were used for measurement ($R^2 = 0.6218$).

The intrinsic instability of ascorbic acid, which oxidises quickly even in cold or low-light handling settings, limited the achievement of good linearity in the *AA* standard curve. The $R^2 = 0.6218$ found in this study illustrates how this chemical sensitivity might lower the accuracy of colorimetric calibration curves. As a result, *AA* levels were interpreted with caution, and this analytical restriction is identified as a drawback.

4.4 Data analysis

Data analysis was generated using RStudio software version 4.5.2, which was employed to conduct all statistical analyses for the study (R Core Team, 2025). Normality and homogeneity of variance were assessed using residual diagnostics in RStudio, and the data met the assumptions required for ANOVA. The ANOVA was performed using the base R aov() function. Fisher's Least Significant Difference (LSD) was utilised to compare the significant effects of genotype, site, nitrogen level, and their interactions on the measured biochemical traits at a probability of $p \leq 0.05$. Post hoc mean comparisons were conducted using the emmeans package, which allowed calculation of estimated marginal means and pairwise comparisons. The analysis enabled the identification of significant main effects and interactions, providing a basis for further graphical and tabular representations of the results.

4.5 Results and discussion

4.5.1 Genotype, environment, nitrogen and interaction effects on studied biochemical traits

Analysis of variance (ANOVA) for biochemical parameters evaluated among the studied citron melon genotypes, nitrogen application, and test environments is presented in **Table 4.1**. The genotype effect was highly significant ($p \leq 0.01$) for most of the studied metabolic traits, except *S* and *F*. The test site significantly affected ($p \leq 0.01$) the response of most studied traits except for *TSS/TA*, *TC* and *%WC*. Nitrogen application was significant ($p \leq 0.05$) for most evaluated traits except *S* and *%WC*, with significant interactions between these factors. This suggests a wide genetic variation in acidity, sweetness, water retention, vitamin C, and carotenoid content. The significant interactions indicate that site and nitrogen levels affect genotype responses. This interaction highlights the importance of selecting genotypes suited to specific nutritional regimes and habitats. Whereas, the Genotype × Nitrogen × Site interaction effect was highly significant for *TSS*, *TSS/TA* and *AA*. This suggests that these traits are highly influenced by genotype, treatment and type of environment.

Table 4.1: Analysis of variance table shows the effect of genotype, site, and nitrogen on the biochemical traits of selected citron melon genotypes.

Source	d.f.	TSS (˚Brix)	TA g/100mL	TSS/TA	TC mg/g	S mg/g	G mg/g	F mg/g	AA mg/g	%WC
Genotype (G)	3	4.25**	0.06**	612.8**	634.1**	3.17E-5^{ns}	0.04**	1.75E-10^{ns}	961224**	3.86**
Site (S)	1	1.11**	0.08**	12.2^{ns}	0.5^{ns}	5.88E-4**	1.0E-5*	8.52E-09**	855468**	0.77^{ns}
Nitrogen (N)	1	6.38**	0.01*	467.1**	31.7**	2.43E-5^{ns}	0.04**	3.69E-09*	121344**	0.25^{ns}
G × S	3	3.77**	0.27**	2944.2**	96.3**	3.55E-5^{ns}	0.00^{ns}	8.31E-09**	1211780**	2.43**
G × N	3	1.65**	0.002^{ns}	147.8**	2.0*	2.90E-5^{ns}	0.04**	1.66E-09^{ns}	248906**	0.44^{ns}
S × N	1	0.39*	0.001^{ns}	23.9^{ns}	1.4^{ns}	2.1E-6E-5^{ns}	4E-5**	3.99E-09*	745457**	0.97^{ns}
G × S × N	3	0.89**	0.01^{ns}	343.6**	5.9*	4.17^{ns}	0.00^{ns}	1.48E-09^{ns}	185343**	0.36^{ns}
Residual	30	0.081	0.00278	15.4	0.6	0.0000187	0.00	8.050e-10	1178	0.323^{ns}

d.f; degrees of freedom, ***TSS***; total soluble solids, ***TA***; titratable acid, ***TSS/TA***; sugar to acid ratio, ***TC***; total carotenoids, ***S***; sucrose, ***G***; glucose, ***F***; fructose, ***AA***; ascorbic acid/ vitamin C, ***WC***; water content, ×; interaction between factors, ******; $p \leq 0.01$ Highly significant, *****; $p \leq 0.05$ significant and ***ns*** not significant.

4.5.2 Mean performances of biochemical traits among the studied citron melon genotypes

Mean values for metabolomic parameters of the tested citron melon genotypes evaluated in low-nitrogen (LN) and optimal-nitrogen (ON) conditions across GH and SH production sites are presented in **Table 4.2**. For metabolic traits observed under low-nitrogen conditions, the following ranges were recorded for *TSS* (2-3 °Brix), *TA* (0.1-0.3 g/100 mL), *TSS/TA* (13-31), *TC* (5-20 mg/g), *AA* (470-729 mg/g), *S* (0.21-0.22 mg/g), *G* (0-0.23 mg/g), *F* (0.18 mg/g), and *%WC* (96-98 %) (Tables 4.2. The observations of low *TSS*, moderate *TA,* and high stable water content are typical in citron melon genotypes; however, the decrease suggests that LN conditions have an effect among genotypes, highlighting a genotype-specific response (Kaźmińska et al., 2024). Nitrogen limitation reduces leaf N, inhibits N-dependent enzymes such as Rubisco and protein synthesis, and frequently reduces photosynthetic capacity because it weakens the key storage organs (fruits) for assimilates (Ferrante et al., 2008; Grasso et al., 2022; Ruiz-Vera et al., 2017).

Consequently, less carbon is allocated to fruit sugar synthesis and unloading (Lu et al., 2021; Wu et al., 2025). However, plants under N stress can also remobilise stored N from vegetative organs into reproductive sinks and increase some free amino acids and antioxidant pools (i.e., *AA*), a trend seen in transcriptomic responses to N deficiency and cucurbit studies (Lu et al., 2021; Nawaz et al., 2018). Therefore, elevation of *TC* and *AA* accumulation indicates the presence of stress-induced antioxidants, which differ by genotype; this same observation was reported by Hermans et al. (2020) and Oleszkiewicz et al. (2025). The following ranges were observed for the same biochemical traits under optimum-nitrogen conditions, i.e., *TSS* (2.47-4.48 °Brix), *TA* (0.18-0.36 g/100 mL), *TSS/TA* (15.19-36.42), *TC* (5.02-22.89 mg/g), *%WC* (96.32-97.52 %), *S* (0.22 mg/g), *G* (0.23 mg/g), *F* (0.18 mg/g) and *AA* (250.55-1102.87 mg/g) (Tables 4.2). These results reflect a biochemical profile under favourable N conditions; they align with the response of other cucurbits observed under sufficient nitrogen supply (Grasso et al., 2022).

In contrast to low-N environments, optimal N levels often promotes better vegetative growth, increase carbon assimilation, and supports higher levels of chlorophyll and Rubisco synthesis. This strengthens the source-sink gradient, which favours sugar biosynthesis and transport to the fruit, corresponding with trends in cucumber and melon, where N sufficiency enhances soluble solids and sweetness compared to N-deficient plants (Nawaz et al., 2018; Lu et al., 2021; Wu et al., 2025). The fruits maintained the typical mild sourness of citron melon, as indicated by the balanced *TSS/TA* ratios and modest *TA* levels under ON. Sugar profiles remain stabilised under ON with no significant difference from LN conditions, suggesting that sugars are not affected by N limitation (Nawaz et al., 2018). Under LN, genotype WWM-58 showed the numerically highest *TSS* and *TA*, signalling the maintenance of carbohydrate supply and organic-acid metabolism that support flavour, since traits *TSS* and *TSS/TA* ratio are standard quality indices strongly associated with perceived sweetness-acidity in fruit quality trials (Cavalcante et al., 2019; Dike et al., 2024; Ferrante et al., 2008; Irshad et al., 2014). Genotype WWM-65 recorded high *TC*; this indicates that *TC* is not N-sensitive, but it is a genotype-controlled determinant of fruit antioxidant value, and its levels in cucurbits (such as watermelon and related species) are documented to be regulated by genetic factors and G × E interactions (Ferrante et al., 2008; Lu et al., 2024; Tembo & Maphosa, 2025).

Moreover, genotype WWM-Ranoto recorded the highest *TSS/TA*, and *AA* under low nitrogen, suggesting a favourable taste balance, good tissue hydration/juiciness, and enhanced antioxidant status at measurement (Cavalcante et al., 2019; Ferrante et al., 2008; Yuan et al., 2015). Higher TSS and starch levels indicate improved carbohydrate accumulation under nitrogen stress. Meanwhile, higher *TA* and *TSS/TA* levels contribute to balanced flavour and metabolic stability (Nerson, 2007). Increased water content promotes cellular hydration and fruit firmness, while an increased ascorbic acid concentration boosts antioxidant capacity and nutritional value (Cavalcante et al., 2019). These responses signify superior metabolic stability potential and strong physiological resilience in these genotypes when N supply is limited (Amzeri et al., 2022; Wu et al., 2025).

Table 4.2: Mean values of evaluated genotypes for biochemical traits under optimum nitrogen and low nitrogen across environments.

	TSS (◦Brix)		TA (g/100 mL)		TSS/TA		TC (mg/g)		%WC	
Genotype	ON	LN	ON	LN	ON	LN	ON	LN	ON	LN
WWM-16	3.30	3.07	0.31	0.27	15.19	18.51	10.28	8.45	96.32	96.92
WWM-58	3.73	3.27	0.35	0.32	31.01	19.89	15.65	13.89	96.47	96.50
WWM-65	4.48	2.65	0.36	0.28	18.08	12.81	22.88	20.46	96.62	96.31
WWM-Ranoto	2.47	2.05	0.18	0.14	36.42	30.72	5.02	5.04	97.52	97.92
Grand mean	3.49	2.76	0.29	0.25	25.18	20.48	13.46	11.96	96.73	96.91
Min. mean	2.47	2.05	0.18	0.14	15.19	12.81	5.02	5.04	96.32	96.31
Max. mean	4.48	3.27	0.36	0.32	36.42	30.72	22.89	20.46	97.52	97.92
LSD (5%)	0.48		0.09		6.64		1.31		0.91	
$p \leq 0.05$	< 0.01		0.03		< 0.01		< 0.01		0.13	

TSS; total soluble solids, ***TA***; titratable acid, ***TSS/TA***; sugar to acid ratio, ***TC***; total carotenoid, ***WC***; water content, **LSD;** least significant differences of means (5% level), **$p \leq 0.05$;** significance, **ON;** optimum nitrogen, **LN;** low nitrogen.

Table 4.2: Continued.

	S (mg/g)		G (mg/g)		F (mg/g)		AA (mg/g)	
Genotype	ON	LN	ON	LN	ON	LN	ON	LN
WWM-16	0.22	0.22	0.23	0.23	0.18	0.18	250.55	512.58
WWM-58	0.22	0.22	0.23	0.23	0.18	0.18	592.75	469.87
WWM-65	0.22	0.22	0.23	0.23	0.18	0.18	289.28	713.22
WWM-Ranoto	0.22	0.22	0.23	0.00	0.18	0.18	1102.87	728.8
Grand mean	0.22	0.22	0.23	0.17	0.18	0.18	558.86	606.12
Min. mean	0.22	0.22	0.23	0.00	0.18	0.18	250.55	469.87
Max. mean	0.22	0.22	0.23	0.23	0.18	0.18	1102.87	728.80
LSD (5%)	0.01		2E-3		4.8E-5		5.82	
$p \leq 0.05$	0.26		< 0.01		0.04		< 0.01	

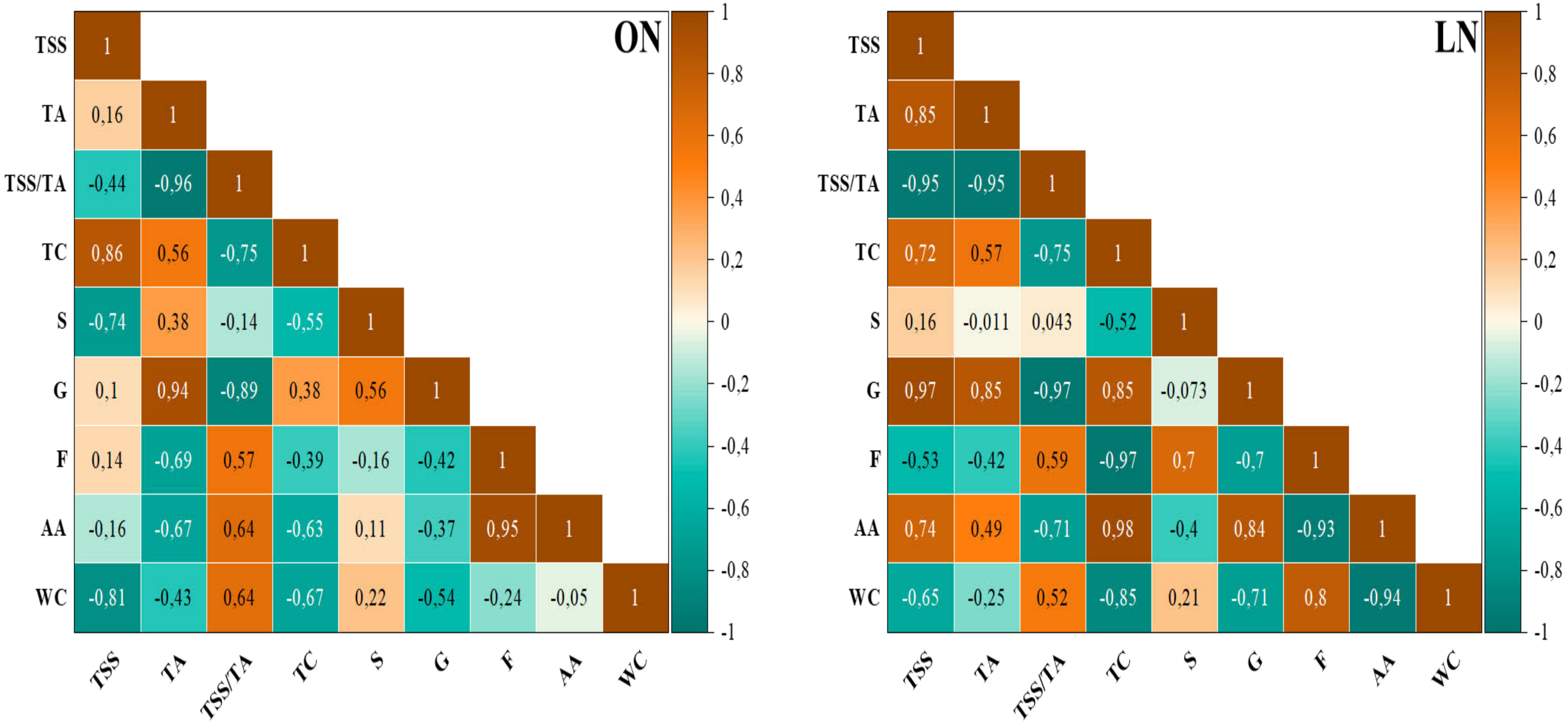
S; sucrose, ***G***; glucose, ***F***; fructose, ***AA***; ascorbic acid/ vitamin C.

4.5.3 Pearson’s Correlation analysis among biochemical traits

Pearson’s correlation coefficients (r) showing bi-variate links between the studied biochemical traits evaluated under ON and LN conditions in GH and SH production sites are presented in **Figures 4.1** and **4.2**. Under LN in the GH site, positive and significant correlation coefficients were observed between *G* with *TSS* (r = 0.97; $p \leq 0.05$) and *AA* with *TC* (r = 0.98; $p \leq 0.05$). Under N stress, sugar accumulation is primarily driven by the accumulation of easily soluble sugars, implying that when more glucose accumulates, *TSS* rises, which may indicate stress-induced remobilisation of growth requirements and allow sugars to accumulate in the fruit (Liao et al., 2019; Wu et al., 2025). This is a within-LN association; the mean of *TSS* remained lower under LN than under ON, so the correlation does not imply that LN raised TSS overall (Table 4.2). This pattern of *AA* and *TC* association, indicating corresponding antioxidant and carotenoid production in response to nitrogen stress, is also noted in studies showing that nitrogen constraint tends to lead to metabolic changes that preserve redox balance and photoprotection through improved antioxidant systems and carotenoid pathways (Farhan et al., 2024; Zhou et al., 2023).

On the contrary, negative and significant co-variate correlations were observed between *TSS/TA* with *TSS* (r = -0.95; $p \leq 0.05$), *TSS/TA* with *TA* (r = -0.95; $p \leq 0.05$), *G* with *TSS/TA* (r = -0.97; $p \leq 0.05$), *F* with *TC* (r = -0.97; $p \leq 0.05$). This is an indication of genotype compromise between sugar accumulation, acidity balance, and carotenoid metabolism under N stress, high carotenoid synthesis may compete with sugar or acid metabolism for the structure of carbon, which leads to absolute sugar buildup may compromising the maintenance of an ideal sugar-to-acid balance (TSS/TA) (Ren et al., 2023; Villwock et al., 2024). Notably, in the SH under LN, negative and significant ci-variate correlation were observed between *TSS/TA* with *TA* (r = -0.97; $p \leq 0.05$) and *AA* with *G* (r = -0.96; $p \leq 0.05$) confirming stress-induced metabolic restructuring toward sugars and antioxidants, which was recognised with adaptive responses in other cucurbits (Huang et al., 2022; Mishra et al., 2024).

Nitrogen limitation reduces the demand for protein and structural growth, weakening the biochemical pathways in vegetative tissues and redirecting carbon into soluble sugars, free amino acids, antioxidant metabolites (such as ascorbate and carotenoids), or storage compounds (Liao et al., 2019; Oleszkiewicz et al., 2025). This stress-adaptation strategy can maintain or increase the nutritional and antioxidant value of fruit despite decreased growth.



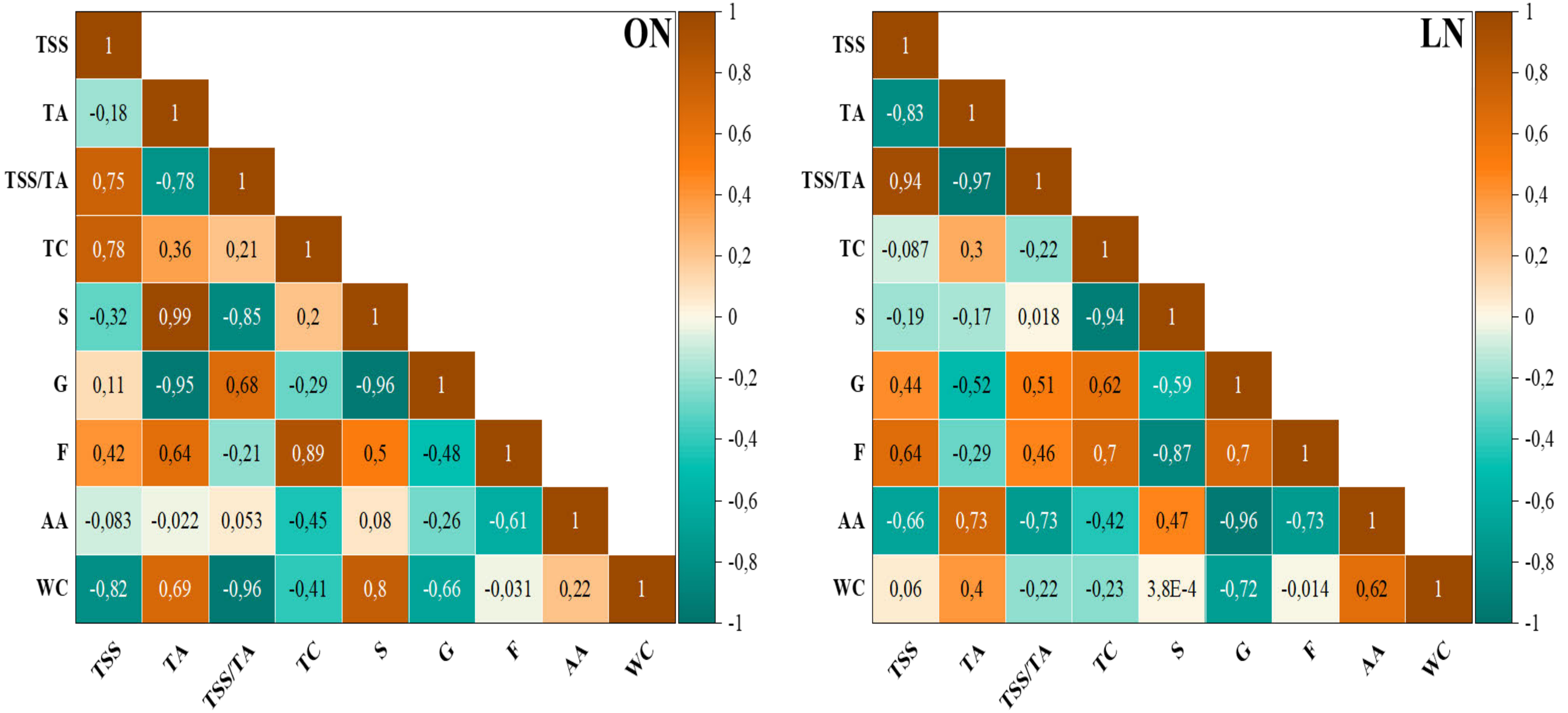
ON

	TSS	TA	TSS/TA	TC	S	G	F	AA	WC
TSS	1								
TA	0,16	1							
TSS/TA	-0,44	-0,96	1						
TC	0,86	0,56	-0,75	1					
S	-0,74	0,38	-0,14	-0,55	1				
G	0,1	0,94	-0,89	0,38	0,56	1			
F	0,14	-0,69	0,57	-0,39	-0,16	-0,42	1		
AA	-0,16	-0,67	0,64	-0,63	0,11	-0,37	0,95	1	
WC	-0,81	-0,43	0,64	-0,67	0,22	-0,54	-0,24	-0,05	1

LN

	TSS	TA	TSS/TA	TC	S	G	F	AA	WC
TSS	1								
TA	0,85	1							
TSS/TA	-0,95	-0,95	1						
TC	0,72	0,57	-0,75	1					
S	0,16	-0,011	0,043	-0,52	1				
G	0,97	0,85	-0,97	0,85	-0,073	1			
F	-0,53	-0,42	0,59	-0,97	0,7	-0,7	1		
AA	0,74	0,49	-0,71	0,98	-0,4	0,84	-0,93	1	
WC	-0,65	-0,25	0,52	-0,85	0,21	-0,71	0,8	-0,94	1

Figure 4.1: Pearson correlation coefficients showing relationship among biochemical parameters, under greenhouse environment and under both nitrogen levels ON and LN, ***TSS***; total soluble solids, ***TA***; titratable acid, ***TSS/TA***; sugar to acid ratio, ***TC***; total carotenoids, ***S***; sucrose, ***G***; glucose, ***F***; fructose, ***AA***; ascorbic acid/ vitamin C, ***WC***; water content.



ON

	TSS	TA	TSS/TA	TC	S	G	F	AA	WC
TSS	1								
TA	-0,18	1							
TSS/TA	0,75	-0,78	1						
TC	0,78	0,36	0,21	1					
S	-0,32	0,99	-0,85	0,2	1				
G	0,11	-0,95	0,68	-0,29	-0,96	1			
F	0,42	0,64	-0,21	0,89	0,5	-0,48	1		
AA	-0,083	-0,022	0,053	-0,45	0,08	-0,26	-0,61	1	
WC	-0,82	0,69	-0,96	-0,41	0,8	-0,66	-0,031	0,22	1

LN

	TSS	TA	TSS/TA	TC	S	G	F	AA	WC
TSS	1								
TA	-0,83	1							
TSS/TA	0,94	-0,97	1						
TC	-0,087	0,3	-0,22	1					
S	-0,19	-0,17	0,018	-0,94	1				
G	0,44	-0,52	0,51	0,62	-0,59	1			
F	0,64	-0,29	0,46	0,7	-0,87	0,7	1		
AA	-0,66	0,73	-0,73	-0,42	0,47	-0,96	-0,73	1	
WC	0,06	0,4	-0,22	-0,23	3,8E-4	-0,72	-0,014	0,62	1

Figure 4.2: Pearson correlation coefficients showing relationship among biochemical parameters, under shadehouse environment and under both nitrogen levels ON and LN, ***TSS***; total soluble solids, ***TA***; titratable acid, ***TSS/TA***; sugar to acid ratio, ***TC***; total carotenoids, ***S***; sucrose, ***G***; glucose, ***F***; fructose, ***AA***; ascorbic acid/ vitamin C, ***WC***; water content.

4.5.4 Principal component analysis (PCA) for evaluated metabolomic parameters

Principal component analysis (PCA) showing variation explained for the studied metabolomic traits in ON and LN conditions under GH and SH sites is presented in **Table 4.3.** Components were retained based on eigenvalues, percentage of variance and the cumulative percentage, variable interpretation emphasised physiological plausibility with loading score of ≥ 0.40 treated as primary. Under GH in ON, PCA revealed three principal components PCs (i.e., PC1, PC2 and PC3), which accounted for 52, 30 and 18% of the total variation observed for the studied metabolic traits. Under GH in LN, the three significant PCs (i.e., PC1, PC2 and PC3) explained 71, 20, and 9% of the total variation observed, respectively. Under SH in ON, the three significant PCs (i.e., PC1, PC2 and PC3) explained 52, 34 and 13% of the total variation observed, respectively. Under SH in LN, the three significant PCs (i.e., PC1, PC2 and PC3) explained 54, 30 and 16% of the total variation observed, respectively. Across all test sites, LN showed high loading scores (≥ 0.40) for biochemical traits *TSS*, *TC*, *G*, *F*, *TSS/TA* and *AA* under PC1.

In LN across all GH and SH sites, biochemical traits *G*, *F* and *TC* recorded high loading scores under PC1. The PC biplots, which show the grouping of studied citron melon genotypes based on metabolomic attributes under ON and LN conditions in GH and SH environments, are presented in **Figures 4.3 and 4.4** Under LN conditions in GH, the citron melon genotypes WWM-16 and WWM-58 are classified based on their strong positive effects on *TA*, *TSS*, and *G,* signifying that these genotypes may contain a balance of sugar sweetness and acidity, which is crucial for fruit quality (Amzeri et al., 2022; Wu et al., 2025). In contrast, antioxidant traits *AA* and *TC* are positively associated with genotype WWM-65, indicating potential genotype-specific stress resilience. This suggests that this genotype will accumulate more carbohydrates and antioxidants as a self-preservation mechanism under nitrogen stress; similar observations have also been seen in tomatoes under nitrogen stress (Bénard et al., 2009).

Additionally, stress-driven improvements in sweetness and antioxidant capacity were observed for genotype WWM-65, aligning with a previous study by Henan et al. (2016). The Citron melon genotype WWM-65 was linked to high *TC, F* and *G* under LN conditions at the SH production site. The metabolic traits *TSS* and *TSS/TA* were clustered by citron melon genotypes WWM-58 and WWM-16 under LN in the SH production site, singling it out as a genotype for stress resilience and maintaining fruit quality, these results imply that WWM-65 and WWM-58 have desired traits for both nutritional quality and commercial appeal under low-input systems (Henan et al., 2016). Genotype WWM-65 is showing promise for breeding efforts aimed at achieving high carotenoid and antioxidant content or health-promoting traits under LN conditions in a controlled environment, and genotype WWM-58 possesses more sugars and sweetness to acid balance under N stress, indicating palatability for consumption.

Table 4.3: Summary of factor loadings, eigenvalue, percent and cumulative variation for biochemical parameters assessed in selected genotypes in the greenhouse and shadehouse and under optimum (ON) and low nitrogen (LN).

	Greenhouse						Shadehouse					
	ON			LN			ON			LN		
Trait	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3
TSS	0.23	**0.53**	0.01	**0.35**	0.34	0.12	-0.22	**0.44**	0.37	**0.34**	-0.28	**0.39**
TA	**0.42**	-0.22	0.11	0.29	**0.38**	-0.48	**0.44**	0.17	0.09	-0.32	**0.42**	0.05
TSS/TA	-0.45	**0.05**	-0.12	-0.36	-0.29	**0.22**	-0.43	0.14	**0.25**	**0.35**	-0.38	0.14
TC	**0.38**	0.31	-0.17	**0.38**	-0.21	-0.003	0.03	**0.57**	0.07	0.20	**0.54**	-0.04
S	-5.17E-4	-0.53	**0.39**	-0.12	0.60	0.56	**0.45**	0.08	0.11	-0.25	-0.48	**-0.22**
G	0.37	-0.22	**0.38**	**0.38**	0.19	-9.32E-4	-0.41	-0.12	-0.35	**0.41**	0.13	-0.28
F	-0.29	0.32	**0.45**	-0.34	**0.36**	0.08	0.19	**0.51**	-0.18	**0.38**	0.24	0.34
AA	-0.34	0.15	**0.49**	**0.37**	-0.19	0.22	0.03	-0.29	**0.78**	-0.44	0.02	**0.16**
WC	**-0.29**	-0.35	-0.44	-0.32	**0.19**	-0.58	**0.41**	-0.26	-0.07	-0.21	0.02	**0.74**
Eigenvalue	4.72	2.67	1.62	6.38	1.84	0.78	4.73	3.09	1.19	4.87	2.69348	1.43
Percentage of Variance (%)	52.40	29.65	17.95	70.90	20.401	8.69	52.54	34.28	13.19	54.15	29.93	15.92
Cumulative (%)	52.40	82.05	100.00	70.90	91.31	100.00	52.54	86.81	100.00	54.15	84.08	100.00

TSS; total soluble solids, ***TA***; titratable acid, ***TSS/TA***; sugar to acid ratio, ***TC***; total carotenoids, ***S***; sucrose, ***G***; glucose, ***F***; fructose, ***AA***; ascorbic acid/ vitamin C, ***WC***; water content.

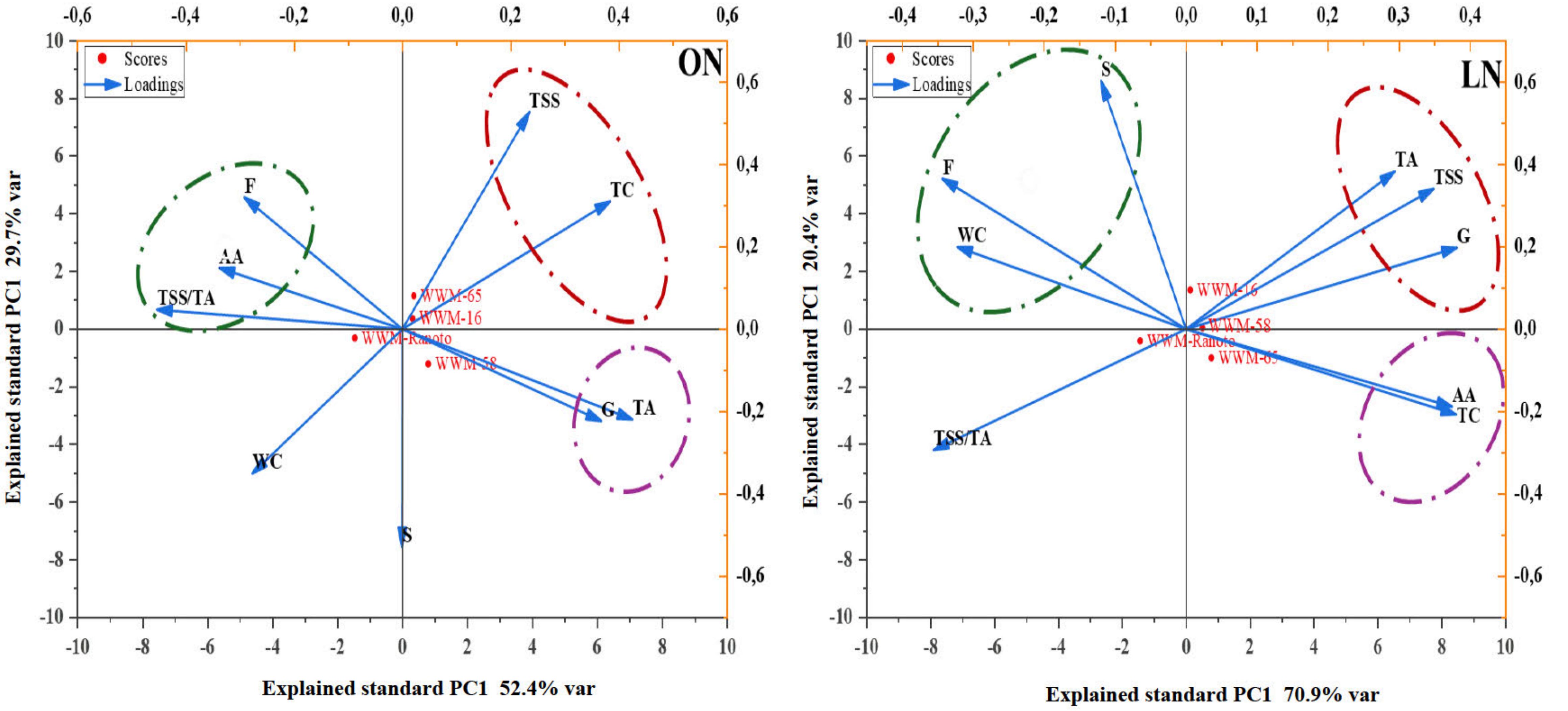


Figure 4.3: Biplot PCAs showing relations among biochemical traits and selected genotypes (WWM- Ranoto, WWM16, WWM 58 and WWM 65) under greenhouse (ON and LN): ***TSS***; total soluble solids, ***TA***; titratable acid, ***TSS/TA***; sugar to acid ratio, ***TC***; total carotenoids, ***S***; sucrose, ***G***; glucose, ***F***; fructose, ***AA***; ascorbic acid/ vitamin C, ***WC***; water content.

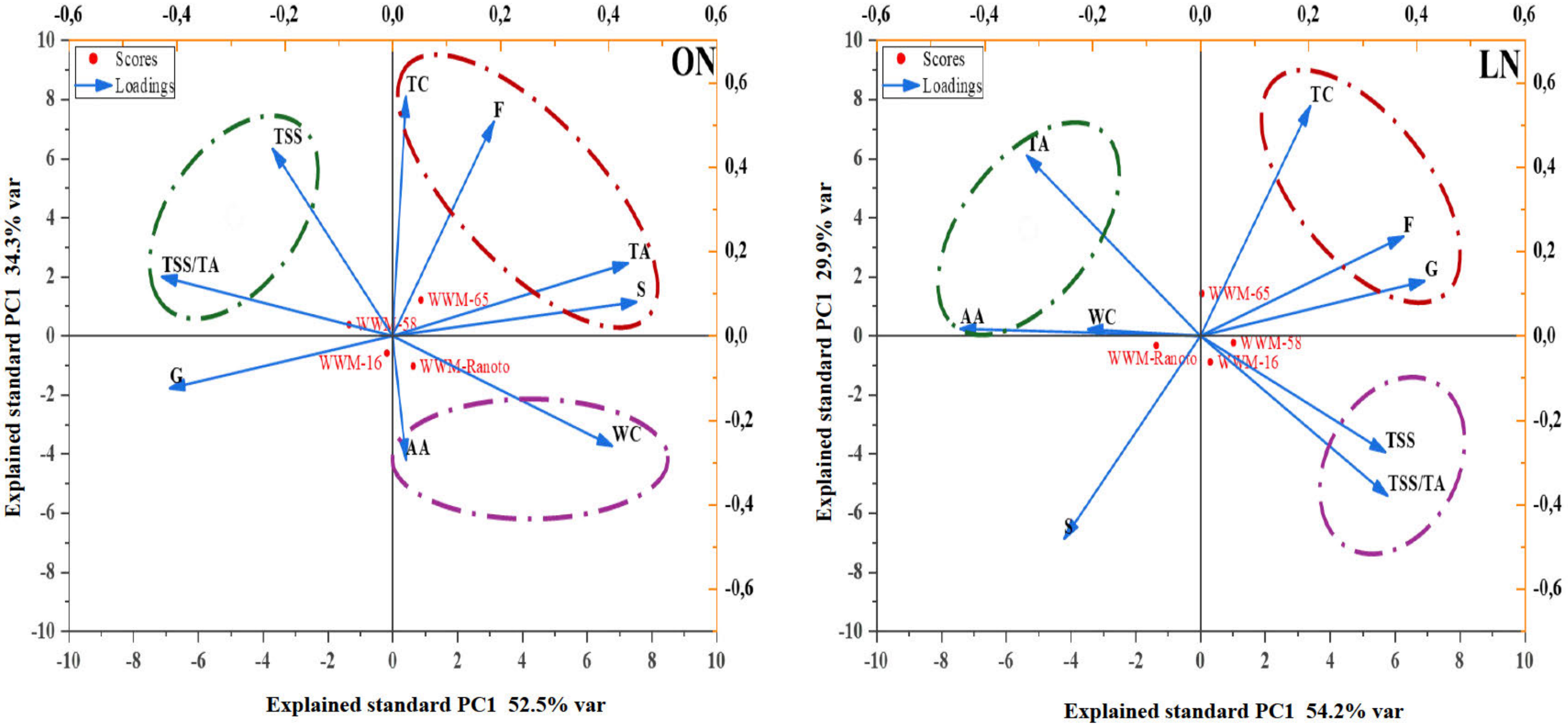


Figure 4.4: Biplot PCAs showing relations among biochemical traits and selected genotypes (WWM- Ranoto, WWM16, WWM 58 and WWM 65) under shadehouse (ON and LN): ***TSS***; total soluble solids, ***TA***; titratable acid, ***TSS/TA***; sugar to acid ratio, ***TC***; total carotenoids, ***S***; sucrose, ***G***; glucose, ***F***; fructose, ***AA***; ascorbic acid/ vitamin C, ***WC***; water content.

4.6 Conclusion

Nitrogen stress increases total soluble solids (*TSS*), antioxidant activity (*AA*), and carotenoid content (*TC*), traits that are widely sought after by health-conscious consumers (Bénard et al., 2009). Due to their superior nutritional and sensory profiles under LN, genotypes WWM-65 (high *TC*, *AA*) and WWM-58 (high *TSS/TA*) have the potential for commercialisation in low-input systems (Henan et al., 2013). To accelerate the development of fruit quality cultivars, breeding strategies should utilise molecular markers for nitrogen-use efficiency and metabolites. Citron melon's ability to retain its sweetness and antioxidant activity under LN makes it a vital crop for sustainable agriculture and specialised markets, especially considering the growing consumer demand for functional foods. Additionally, there is evidence that the crop promotes food and nutrition security while maintaining sustainable agricultural practices.

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CHAPTER 5

General discussion

5.1 Introduction and objectives of the study

Citron melon (*Citrullus lanatus var citroides*), also known as wild watermelon, is an underutilised crop that belongs to the family Cucurbitaceae and originated from Sub-Saharan Africa. The crop is consumed as a vegetable, fruit and forage, and is cultivated for its nutritional composition and health benefits. The interest in the crop has increased due to its ability to thrive under abiotic stress, primarily drought stress, making it an ideal candidate for addressing soil nutrient deficiencies, particularly in terms of nitrogen stress tolerance. Despite its versatility, little is known about the crop's morpho-physiological and metabolomic response in nitrogen-limited conditions, which is a significant limitation in many areas where the crop is grown. Climate-related issues, including high temperatures that cause volatilisation and high rainfall events that result in leaching, along with the growing cost of fertilisers, aggravate nitrogen limitation. These limitations highlight the need for crops that can remain productive even with reduced nitrogen inputs or low-input systems. Hence, citron melon is a promising ideotype with potential for improvement. Moreover, there is still limited, if any, literature available about citron melon's metabolic resilience, biochemical modifications, and nitrogen utilisation efficiency, which is crucial for fruit quality and holds potential for identifying biochemical markers. Therefore, this study aimed to address this gap by analysing the crop's biochemical, physiological, and agronomic responses under low-nitrogen versus optimum nitrogen conditions to establish its potential for commercialisation in low-nitrogen environments. This chapter summarises the specific objectives of the study, key findings and their implications.

The specific objectives of the study were:

- To identify suitable citron melon genotypes possessing enhanced adaptive attributes for cultivation under low-nitrogen availability for production and breeding
- To determine accumulated metabolites in citron melon genotypes cultivated under low and optimal nitrogen applications to aid selection of suitable genotypes for cultivation.

5.2 Research findings in brief

The morpho-physiological responses of selected citron melon (*Citrullus lanatus var citroides*) genotypes evaluated under low nitrogen conditions

Four genetically distinct citron melon genotypes were evaluated using morphological and physiological traits under optimum and low nitrogen stress conditions in greenhouse and shadehouse production environments. The following morphological attributes were assessed: Leaf area (*LA*), fruit weight (*FW*), fruit length (*FL*), fruit diameter (*FD*) and rind thickness (RT), seed number per fruit (*SPF*) and 100-seed weight (*100-SW*). The following physiological traits were measured: photosynthetic rate (*A*), stomatal conductance (*gs*), transpiration rate (*E*), intercellular CO_2 concentration (*Ci*), maximum quantum yield of PSII in photosynthesis (Fv'/Fm'), effective quantum efficiency of PSII photochemistry (*ΦPSII*), partial pressure of CO_2 ($PhiCO_2$), photochemical quenching (*qP*), electron transport rate (*ETR*) and amount of light absorbed by the leaf (*LeafAbs*). Analysis of variance, correlation analysis, principal component analysis, and principal component biplot analysis were conducted. The main findings were as follows:

- Nitrogen stress generally reduced morpho-physiological traits, the differentiation depends largely on both genotype and production environment.
- The Pearsons' correlation analysis revealed significant associations *FW* and *NUE*, *VL* and *FD*, *FL* and $PhiCO_2$, *LA* and *RL*, *VL* and *Ci*, *FL* and *gs*, *ΦPSII* with *ETR*, *Fv'/Fm'* with *ETR* and *FW* and *E* under low nitrogen conditions.
- Genotypes WWM-58 and WWM-16 exhibited resilience towards nitrogen stress; therefore, they are promising candidates for yield production in low-input systems and for further breeding.

The metabolomic response of selected citron melon (*Citrullus lanatus* var *citroides*) genotypes evaluated under low-nitrogen conditions

For biochemical traits, the four citron melon genotypes were evaluated using fruit quality traits under optimum and low nitrogen stress conditions in greenhouse and shadehouse production environments. The fruit yield collected was carefully prepared for analysis of fruit quality. The following metabolic attributes were assessed: Total soluble solids (*TSS*), Titratable acidity (*TA*), solid-acid ratio (*TSS/TA*), total carotenoids (*TC*), Sucrose (*S*), glucose (*G*) and fructose (*F*), ascorbic acid (*AA/Vitamin C*) and water content (*WC*). Analysis of variance, correlation analysis, principal component analysis, and principal component biplot analysis were conducted. The main findings were as follows:

- Do you mean: The accumulation of most biochemical traits was induced by nitrogen stress and significantly influenced by genotype × nitrogen interactions. The mean values suggest the variation among metabolic traits is stress-induced and genotype-specific.
- The Pearsons' correlation analysis revealed significant associations between *G* and *TSS*, *AA* and *TC*, *TSS/TA* and *TSS*, *TSS/TA* and *TA* and *AA* and *G*.
- Genotypes WWM-58 and WWM-65 were selected as they exhibited both nutritional quality and health-promoting metabolites under nitrogen stress, which qualifies them for commercial appeal.

5.3 Implications of the study

1. These findings offer cost-effective production alternatives for farmers facing high fertiliser costs and nitrogen loss due to climate change difficulties, i.e., Nitrogen leaching and volatilisation.

2. Citron melon genotypes exhibiting higher nitrogen use efficiency and strong biochemical characteristics under nitrogen stress, could serve as promising parent lines for breeding programmes targeting nitrogen resilience.

3. Additionally, the study provides data on citron melon for developing enhanced cultivars and other cucurbits suited for low-input environments.

4. This supports citron melon as a viable commercial crop for a low-cost, climate-smart production strategy.
5. The research project identified morpho-physiological and metabolomic trends under nitrogen stress that warrant further scientific exploration to validate across seasons and at the molecular or genomic level.

6 The study contributes to knowledge on an underutilised crop, which will be helpful to extension services; moreover, citron melon supports small-holder farming and sustainable cropping principles.

5.4 Conclusion

This study indicated that citron melon genotypes exhibit a wide range of morpho-physiological and metabolomic responses to low-nitrogen environments. Stronger antioxidant buildup, more pronounced metabolic changes, and more consistent morpho-physiological parameters, including fruit weight and size, as well as overall plant performance under nitrogen stress, were observed in genotypes with higher nitrogen use efficiency. Consistent trends across biochemical and agronomic attributes were confirmed by biplot and correlation analysis, which further distinguished efficient and inefficient groups. Overall, the results indicate that citron melon shows promising adaptation responses to nitrogen limitation under the controlled environments evaluated in this study. While these findings highlight potential for low-input and climate-resilient production systems, further multi-season and field validations are required to confirm this adaptability at a broader scale. Therefore, genotype WWM-58 have been identified as nitrogen-efficient plants and have been selected for future breeding observation and possibly commercialisation.

5.5 Recommendations

Repeat the trial across multiple seasons and environments to confirm nitrogen-use efficiency rankings and trait stability, and to validate the commercial potential of WWM-58 (and any promising lines such as WWM-65). Future research should investigate the molecular and transcriptome mechanisms underlying nitrogen stress tolerance in citron melon to identify critical regulatory genes that are expressed during metabolic and morpho-physiological alterations. A more detailed biochemical examination is required to validate the metabolic pathways involved in antioxidant accumulation and nitrogen absorption revealed in this study. Expanding genotype screening and integrating the most resilient genotypes into breeding programmes would further assist the development of nitrogen-efficient cultivars suitable for low-input farming systems. Lastly, combining metabolomic, physiological, and agronomic approaches in future studies will lead to a better understanding of how these variables interact under nitrogen constraint, and ultimately result in more targeted crop development efforts.